Hybridization - interbreeding between individuals of two different species - often entails a fitness loss for the parents due to reduced fertility or viability of hybrid offspring. Why does hybridization still occur, even though species recognition appears to be well developed? The aim of this thesis is to increase our understanding of mating decisions in hybrid zones, by investigating the costs and benefits of hybridization between two passerine species, the pied (*Ficedula hypoleuca*) and collared flycatcher (*F. albicollis*). The fitness consequences of hybridization turn out to be highly context dependent and choosing a heterospecific male may under certain conditions be a much better choice for a female than expected at first sight.
Mating decisions in a hybrid zone
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Mating decisions in a hybrid zone

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In memory of
Richard Watson
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Introduction
Introduction

In this thesis I will investigate the factors determining the mating decisions of two hybridizing bird species: the collared flycatcher (*Ficedula albicollis*) and the pied flycatcher (*F. hypoleuca*). These two species hybridize despite the fact that their ability to recognize a mate of their own species appears to be well developed and hybrid offspring represent a fitness loss compared to pure-species offspring. In order to understand why individuals still engage in a mixed-species pairing, I will try to quantify the most important costs and benefits of hybridization.

For a proper understanding of this thesis it is important to first get an idea of the general context in which my study fits. Wherever possible, avian examples will be given to clarify general mechanisms, but examples of other taxa will be provided as well. After this broad introduction, I will focus on the study system, giving general information on the morphology, ecology and behaviour of the two flycatcher species and motivate the research compiled in this thesis.

Hybridization and species integrity

When individuals of two previously isolated populations meet, two situations may occur: either the two populations are reproductively isolated from each other or not. Individuals of both populations (for ease of use referred to as species) can be reproductively isolated from each other as a result of behavioural and genetic differences accumulated in allopatry. The mate choice processes which often involve elaborated ornaments and courtship behaviour can be so diverged between the species that neither species sees a potential mate in a heterospecific individual. Genetic differences between the species accumulated during their time in allopatry may lead to inherent developmental defects in hybrid offspring making them inviable. Another alternative is that the two species hybridize but no gene flow occurs because hybrids are instantly reproductively isolated from both parental species (e.g. due to polyploidy of hybrids), so called ‘hybrid speciation’ (Coyne and Orr 2004; Mallet 2007). The second possible outcome of contact between the species is interbreeding (hybridization) resulting in gene flow between the species. This can have severe consequences for the species’ integrity. If hybridization takes place freely, it could lead to the merging of the two species into one large interbreeding population.

In nature hybridization occurs in a wide variety of taxa, such as mammals (Arnold and Meyer 2006), insects (Mavárez et al. 2006), birds (Grant and Grant 1992) and plants (Ellstrand et al. 1996). In the latter two groups, hybridization has been found to occur in many species. For instance, 9% of all bird species are known to have hybridized at least once. In plants, of five major flora, 6-16% of
all genera are known to have hybridized (Ellstrand et al. 1996). An important observation is that hybridization does not necessarily lead to the collapse of species integrity. In hybridizing birds for example, interbreeding has been reported to occur in relatively low, but stable frequencies in sympatric breeding areas. It is interesting to ask why hybridization frequencies would be stable. It is even more interesting though to ask the question why hybridization takes place at all, because it often results in less fit offspring.

**Why hybridize?**

Hybrids often have reduced viability or fertility compared to individuals belonging to the parental species (see below). This makes it puzzling why individuals would hybridize at all. An obvious explanation is that hybridization simply is the result of an error in mate recognition (e.g. Grant and Grant 1997a; Arnold et al. 1999; Randler 2002). Such errors could be caused by factors intrinsic to the mate recognition mechanism. Recognition mechanisms are generally assumed to involve learning and this is subject to errors stemming from occasional exposure to inappropriate templates i.e. the wrong species. This could happen when an individual is raised in the nest of a heterospecific as a result of egg-dumping (Randler 2005) or in a situation where one species is present among many heterospecific neighbours. Nestlings of one species could then easily get imprinted on, for example, the song of a heterospecific male (e.g. Grant and Grant 1997a).

A different type of error could arise when environmental circumstances interfere with the recognition process and thereby affect the accuracy with which mate choice could be executed. For many species visual cues are important in mate choice and reduced visibility could negatively affect the accuracy of the species recognition process. For example, in cichlid fishes assortative mating decreases when the water gets more turbid (Seehausen et al. 1997). Errors in mate choice are a plausible explanation for hybridization and might be common in nature.

Hybridization is generally believed to be costly, but in some situations their may be benefits to hybridizing. Consider a scenario where conspecific males are scarce or unavailable, in such a case it may be better to accept a heterospecific male, than not to mate at all (making ‘the best of a bad job’). However, many other factors are also likely to affect the mating decision. Examples include fitness trade-offs between current and future reproduction, offspring quality and costs of searching for a conspecific mate and these need to be considered besides availability of suitable (conspecific) mates. Consequently, the fitness trade-offs (factors which might be costly or beneficial to hybridizing individuals), need to be carefully considered and quantified to obtain an understanding
of the mating decisions. It is the aim of this thesis to derive such estimates in order to better understand the mating decisions between the hybridizing collared and pied flycatchers. The next section is devoted to presenting an overview of the most important factors affecting the fitness of hybridizing individuals. Table 1.1 provides a summary of these factors, but this table is also useful to read early on as it outlines the structure of the next section.

**Costs and benefits of hybridization**

**Mate choice**
Searching for and selecting a suitable mate may have associated costs (e.g. Jennions and Petrie 1997). For many avian species, the female is believed to be the most choosy sex and for convenience I adopt this. This does not mean that male choice is not important, but for simplicity I largely neglect it for now. Females need to invest time and energy into finding a mate and searching could result in elevated predation risk (Milinski and Bakker 1992; Gibson and Langen 1996; Byers et al. 2005). There could also be physiological costs resulting from encountering mates, such as elevated hormone levels (see Price 2007) for a review). All these costs are likely to increase if a female spends more time searching or if she checks more potential partners before deciding on a mate. How quickly a ‘suitable’ partner is found depends on mate availability. The availability of suitable partners in a hybrid zone can also be seen as the availability of conspecific males. When conspecifics are rare, a female faces a trade off between search time for finding a conspecific mate and accepting a heterospecific mate with the additional decrease of fitness due to hybrid offspring (see below). This is nicely illustrated theoretically by Wilson and Hedrick (1982), who found that individuals change mating strategy from ‘keep searching for a conspecific’ to ‘accept first male encountered’ when fitness loss associated with a heterospecific pairing was low and/or there were relatively few conspecifics available. The cost of choice described above could be circumvented by not being choosy at all, which in a mixed-species population might lead to hybridization. Whether or not this choice strategy will lead to a net fitness benefit for an individual depends on the magnitude of cost reduction by not being choosy relative to the additional costs resulting from hybridization.

A more direct physical cost of heterospecific mating might arise if the two species differ from each other to such an extent that one individual damages the other during courtship or mating. Such differences can be in body size (too heavy a male mounting a female) or in the mating apparatus of the two sexes (physical damage during mating).

Lastly, a female can have a heterospecific male as a partner, but seek extra-pair copulations with a conspecific male to father (part of) her offspring. This
has been found for female collared flycatchers paired with a male pied flycatcher (Veen et al. 2001) and this will be discussed in more detail later on when hybridization in flycatchers will be treated in more detail.

**Fertilisation**
The period between insemination and fertilisation is of significant importance because sexual selection (or cryptic female choice) can differentially favour one sperm type over the other (Birkhead and Pizzari 2002). In the case of a mixed-species pair, there are several barriers which could decrease the chances of fertilisation (Birkhead and Brillard 2007). One important barrier is the transfer of sperm through the female reproductive organ. Heterospecific sperm might not be well suited for such transfer or not be recognised by the biochemical recognition mechanisms involved in the fusions between egg and sperm, resulting in low fertilisation rates (Vacquier 1998; Price and Bouvier 2002; Coyne and Orr 2004; Birkhead and Brillard 2007). This incompatibility between species has been hypothesised to result from sexually antagonistic co-evolution. Sexually antagonistic co-evolution arises when selection favours different reproductive interests in the two sexes, which can result in rapid evolution of species-specific reproductive organs (Birkhead and Pizzari 2002). Lower fertilisation rates could also be caused by a difference in sperm production by the male when mated to a heterospecific partner (Aspbury and Gabor 2004). Lower fertilization rates may be an important cost for both males and females engaged in heterospecific pairings. The birds invest time and energy, in the form of courtship, sperm or eggs, resulting in a lower reproductive output as would be the case with similar investments in pure-specific pairings.

**Direct costs and benefits of mixed-species pairing**
Species often differ from each other in various characteristics some of which have direct effects on reproductive success. For this reason it might be beneficial for a female to mate with a heterospecific male. Territory quality can provide direct benefits, which has been put forward as a reason for preferring a heterospecific male in birds (Bronson et al. 2003b) and in mammals (Goldsworthy et al. 1999). A heterospecific male might invest more in a breeding attempt compared to a conspecific male (Pierotti and Annett 1993), which can occur in various ways of which working harder and being better in defending the territory are two examples. Many closely related species utilise different food resources. If the food resources of a heterospecific partner are more abundant or complementary such that it increases the survival of the offspring, it might pay to be engaged in a mixed-species pair. Not all benefits are as obvious as the ones mentioned above. For example, a female mallard benefits from being mated to a male of a more dominant species as she will be less harassed by males of the less dominant species (Brodsky et al. 1988).
Many of the direct benefits work one way: only the females of a particular species obtain the benefits that the heterospecific male provides. Females from the other species would incur a cost if they would choose for a heterospecific male which, for example, invests less in the brood or does not defend the nest against predators in comparison to a conspecific male.

**Hybrid viability and fecundity**

Hybrids inherit a mixture of the genetic material of both parental species. Such a novel genetic make-up can be advantageous as it could result in an individual outperforming either parental species, a phenomenon known as hybrid vigour or heterosis (Rieseberg and Carney 1998; Arnold et al. 1999). Hybrid vigour can be the consequence of a higher level of heterozygosity in the hybrid (Burke and Arnold 2001). This could result in higher viability of the offspring if both parental species are severely inbred. In a more general setting, it could result in a lower expression of negative recessive alleles (expressed in the homozygote parental species) (Johansen-Morris and Latta 2006). Additionally, several other effects have been put forward such as the formation of a novel beneficial genetic combination (Burke and Arnold 2001). Hybrid vigour is often apparent at the phenotypic level; hybrid plants are for example well known to be good at colonising new environments (e.g. Arnold et al. 1999). The effects on the phenotypic level will however not be discussed here, but in the section ‘intermediate hybrid phenotype’.

Hybrid vigour has traditionally been thought to be especially important in hybridizing plants (Rieseberg and Carney 1998). In vertebrates, the genetic make-up of hybrids is generally believed to result in the opposite effect, a strong decrease in fitness (Seehausen 2004). A well known example of this is the strongly reduced viability and sterility of hybrid offspring (e.g. Coyne and Orr 2004). Hybrid sterility and inviability has been widely studied in a variety of organisms (Coyne and Orr 2004), which is aided by the fact that genetic incompatibilities can be studied in artificial crosses (McCarthy 2006). The viability and fertility reductions are mostly caused by genetic incompatibilities between the species and are referred to as *intrinsic post-zygotic* costs of hybridization. The most simple model of such incompatibilities is based on a single gene of one species negatively interacting with a single gene of the other species when they are combined in a hybrid this results in a decrease in viability and/or fertility (Wu and Palopoli 1994; Burke and Arnold 2001). There are several variants of this basic model, of which I will discuss a prominent one, often referred to as the Dobzhansky-Muller model (Coyne and Orr 2004), in a bit more detail to clarify the idea of genetic incompatibilities. This model is based on the functional divergence of genes in two different populations (see figure 1.1). Once the two populations come in secondary contact and interbreed, the hybrids will have a mixture of the two genotypes. Some allele combinations (ab in figure 1.1) have
never co-occurred during evolutionary history and have therefore never been subject to natural selection. These allelic combinations can have strong negative effects on fitness. Only recently, the first direct empirical evidence for a pair of Dobzhansky-Muller genes was found in *Drosophila* (Brideau et al. 2006). The fertility and viability of hybrids decreases with an increase of separation time between both species prior to hybridization, i.e. the older the allopatric taxa, the more severe the reduction in fertility and viability of hybrids. As a general rule, fertility decreases more rapidly than viability with separation time, and this has been found in many different groups of organisms (Coyne and Orr 2004), including birds (Price and Bouvier 2002; Lijtmaer et al. 2003). Reduced fertility and viability are most severely expressed in the heterogametic sex (females in birds, ZW), a phenomenon known as Haldane's rule (Haldane 1922). This effect has received considerable attention over the years and has been confirmed for a wide variety of organisms (Orr 1997), including birds (Price and Bouvier 2002; Bronson et al. 2003a; Price 2007). The basic idea is that if a gene from one species does not function properly in the genetic-background of a hybrid, the gene from the other species might mask this effect. In this way, the expression of the negative effects of a gene might be suppressed or avoided. This will only work if the gene is present in ‘two-fold’ which is the case for autosomes and the sex chromosomes of the homogametic sex. Thus, on the sex chromosomes of the heterogametic sex a detrimental effect might be expressed much more easily (for a review see Coyne and Orr 2004).

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**Figure 1.1.** Illustration of the Dobzhansky-Muller model for genetic incompatibility. An ancestral population is split into two allopatric populations (as indicated by the horizontal line). Mutations occur in both populations and, if they are under positive selection, fixation could take place. When the two populations come in secondary contact and hybridization occurs, the hybrid genotype is heterozygote on both loci. Some of the allele combinations (e.g. Aa and Bb) have been together within a locus, but the allelic combination ab (indicated by the ellipse) has never been and this could have detrimental effects on the fitness.
Intermediate hybrid phenotype
The mixed-species ancestry of the hybrid is not only apparent at the genotypic level but extends to the phenotypic level. Hybrids are often intermediate compared to the parental species in characters such as morphology and behaviour. Species are typically adapted to their environment and hybrids, due to their intermediate phenotype, might fall in between the parental optima and consequently do less well (e.g. Hatfield and Schluter 1999). Costs resulting from such maladaptations are referred to as extrinsic post-zygotic costs (Coyne and Orr 2004). In birds, the Darwin finches provide an interesting example. The bills of the parental species are adapted to different seed types and hybrid fitness is reduced as a consequence of their intermediate bill size being suboptimal to consume these seed types (Grant and Grant 1996 but see a later section for context dependence of this effect). Another compelling example concerns hybrids between two mimetic butterfly species which suffer higher predation rates as a consequence of their non-mimetic intermediate colouration (McMillan et al. 1997 and references therein). These are just two of many different situations in which the intermediate phenotype is suboptimal compared to either parental species.

In contrast to the above, hybrids could also have a higher fitness as was mentioned in the previous section. Hybrids are believed to be better in colonising new environments and this is ascribed to their deviant phenotypes (e.g. Arnold and Hodges 1995; Arnold et al. 1999). Similarly, hybrid zones often occur in contact zones between the two different ecological environments to which the parental species are adapted, and an intermediate, hybrid phenotype may be well adapted to such transitional areas (Moore 1977; Good et al. 2000). Finally, hybrids may not acquire an intermediate phenotype but obtain complementary characters from both parental species and thereby get 'the best of both worlds'. An example is parasite resistance; hybrids could be resistant against more parasites than either parental species and might therefore perform better in certain circumstance (Fritz et al. 1999 and references herein).

Hybrid attractiveness
The attractiveness of hybrids might affect fitness. Sexually selected traits of hybrids are often intermediate and therefore may not match the preferences of either parental species resulting in reduced reproductive success (Price 2002 and references therein). The courtship behaviour of hybrids can be intermediate compared to the parental species and not found attractive by individuals of either pure species (Hobel and Gerhardt 2003). Song is a display character used during courtship and territory defence. In general, intermediate hybrid song elicits intermediate responses in both contexts and therefore does not result in an advantage for the hybrids (see den Hartog et al. 2007 and references therein).
Hybrid phenotypes might also confer a sexually selective advantage. This could arise because the females have a pre-existing bias in their sensory system preferring a certain colour or pattern (e.g. Endler and Basolo 1998 and references therein). It is also possible that hybrids are preferred due to the ‘rare male effect’. In this situation, the rare male type has a mating advantage over the common types (Singh 1999). Two hybridizing species of manakins in Central America provide an interesting example in which a sexually selected trait (yellow collar) of one species introgressed (through hybridization) into the other species (see Stein and Uy 2006 and references therein).

There could be several explanations for patterns such as those described above in the manakins. It could be due to a sensory bias but it might also be a side effect of interference between (intra)sexual selection processes and species recognition. The interaction between sexual selection processes and species recognition is particularly important and deserves more attention (Pfennig 1998; Phelps et al. 2006). Mate choice for individuals in a hybrid zone is particularly challenging as potential suitable partners need to be both of the right species (species recognition) and when of the right species preferably of good quality (sexual selection). These two processes may interfere with each other if both processes make (partial) use of the same characteristics, and it is important to consider such interference when trying to understand mating decisions in a mixed-species population.

**Feedback between pre and post-zygotic processes**

So far I have treated the various costs and benefits of hybridization in relation to different causal factors (such as genetic incompatibilities versus adaptation to environment) occurring in the various stages of the life-cycle. This might create the impression that the costs and benefits of hybridization occurring in different times during the life-cycle act independently of each other, which is not the case. Factors acting in the post-zygotic period, such as reduced hybrid fitness, will influence mate choice decisions. If, for example, a female has a preference for a very low quality male which results in none of her offspring surviving till reproduction, then her preference will be strongly selected against and will decrease in frequency in the population. The reduced fitness of hybrid offspring has been hypothesised to have a similar result and lead to a divergence of male traits and female preferences for these traits between the two species. This process is better known as reinforcement (Noor 1999; Servedio and Noor 2003; reviewed in Coyne and Orr 2004). In order to increase pre-zygotic isolation between the species, a tight linkage between male trait and female preference within each species is required. A major obstacle against the evolution of this association is recombination as this breaks up the beneficial gene combinations. Even low levels of hybridization are enough to strongly reduce the linkage disequilibrium (Felsenstein 1981). Theoretical models showed that this obstacle can
be circumvented in several ways, of which sex-linkage of both trait and preference is one (e.g. Servedio and Sætre 2003).

**Context dependence**

In the section ‘direct costs and benefits of mixed-species pairing’ a clear example of context dependence is given, as often only one species benefits from mating with the other and not vice versa. The same asymmetry can apply to the two sexes. Males frequently invest less in a reproductive attempt (as little as only sperm) whereas females need to invest more (e.g. zygote production). This can lead to sexual conflict, where the male only wants to inseminate, but the female wants more in return (e.g. paternal care). Differences in investment between the sexes of two species can also facilitate hybridization (Parker and Partridge 1998). Forced copulations with a heterospecific partner as found in several bird species, or the ‘sneaker strategy’ (attempting to inseminate a female when she is not paying attention) observed in fish species are examples of this (Wirtz 1999 and references therein).

Some of the costs and benefits described are relatively fixed, such as the reduced viability of hybrid offspring. I used ‘relatively fixed’ because the magnitude of viability reduction differs markedly between different species, which could also be seen as context dependence. The context dependence I am interested in here is within the same species pair and applies to many of the above described situations. For example, the reduced fitness of hybrid Darwin’s finches as a consequence of their intermediate bill size changes into an advantage in some years due to a sudden abundance of a different seed type (Grant and Grant 1996). Similarly, the increased parasite resistance of hybrids depends highly on the environment, and in other species the effect could be reversed (increased hybrid susceptibility) (reviewed in Fritz et al. 1999). Many of the extrinsic post-zygotic factors are, by definition, at least partly environmentally determined. The environment in turn is often variable and thus the magnitude of the costs and benefits are often equally variable. One way to visualise this is to imagine a fitness landscape consisting of two peaks (with each parental species adapted to one) and a valley in between (the intermediate hybrid). Fluctuations in the height of the peaks are determined by the environment, and so are the relative costs the hybrids face (the difference between peak and valley).

The mating decisions of an individual are determined by the relative strengths of the costs and the benefits (e.g. Jennions and Petrie 1997; Cotton et al. 2006 and references therein). Because of the context dependence of this trade-off, the optimal mating decisions are predicted to vary with context. To illustrate this, imagine a population of seed eating birds whose bill size is adapted to different seed sizes, resulting in small-billed and large-billed individuals. The average seed size declines through the season. This effects the benefits the different males can provide to a female as a mismatch between seeds size...
and bill size will reduce the potential to provide direct benefits (solid thick and thin black line in figure 1.2). The female’s mating decisions early in the season (large-billed individuals provide more direct benefits) is predicted to differ from the one made at the end (small-billed individuals provide more direct benefits). This is a very simple example but it can easily be expanded to a more complex situation. Assume now that the two groups with different bill sizes represent different species. The fitness return for a female (belonging to the large-billed species) that mates with a male of the small-billed species now changes as an additional cost (reduced viability of their hybrid offspring) has to be taken into account (grey line in figure 1.2). It might still be adaptive for a female to switch her mate preference through the season but the timing of this has changed. This illustrates that an individual faces qualitatively similar cost-benefit trade-offs when having to choose from a conspecific to a heterospecific partner have been found in nature (Lesna and Sabelis 1999; Pfennig 2007).

The above illustrates that getting a good quantitative estimate of the costs and benefits of hybridization is not trivial. One way to get such estimates, and the one taken in this thesis, is to focus on one system. Before I introduce the

**Figure 1.2.** Direct benefits provided by two types of males differing in bill size through the breeding season (time). Both males are adapted to feed on different seed sizes (large-billed (thick black line) for large seeds and small-billed (thin black line) for small seeds). The abundance of seed sizes changes through the season, such that the proportion of large seeds declines through the season (dashed line). Selection on female mating decisions is caused by the relative fitness return received from the male she chooses. In this simple example it is expected that her choice changes from large-billed males early in the season to small billed males later in the season. The thin grey line indicated the direct benefits returned by a small-billed male including the reduction of hybrid offspring (see text for details).
empirical system in more detail, the most important processes influencing the costs and benefits attached to hybridization are summarized in table 1.1.

**Table 1.1.** A summary of the consequences of hybridization on fitness in birds as discussed in this introductory chapter. The table gives, for the pre and post-zygotic period, various aspects of reproduction through the life cycle of an individual and over multiple generations. In the subsequent columns the potential costs and benefits related to these aspects are given. Certain consequences have been listed under costs as well as under benefits, because they may act either way, dependent on the context.

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<td>pre-zygotic</td>
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<td>mate choice</td>
<td>- physical damage</td>
<td>- reduced cost of choice</td>
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<td>fertilisation</td>
<td>- reduced fertilisation rates</td>
<td>- conspecific extra-pair matings</td>
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<td>post-zygotic</td>
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<td>direct costs &amp; benefits of mixed-species pairing</td>
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<td>- diet differences</td>
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<td>hybrid viability &amp;</td>
<td>- genetic incompatibilities</td>
<td>- hybrid vigour</td>
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<td>fecundity</td>
<td>(e.g. Dobzhansky-Muller)</td>
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<td></td>
<td>- Haldane’s rule</td>
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<td>intermediate</td>
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<td>- ‘best of both worlds’</td>
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<td>hybrid phenotype</td>
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<td>hybrid attractiveness</td>
<td>- mismatching courtship behaviour</td>
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Hybridization in *Ficedula* flycatchers

In order to understand hybridization, quantitative estimates of the costs and benefits related to it are needed. However, costs and benefits are manifold and often context-dependent as discussed in the previous sections. This means that one needs to select a study object where it is possible to obtain detailed information on the behaviour, ecology and genetics of the species involved. It is also of prime importance to be able to relate the various aspects of pair formation and reproduction to components of fitness. In my opinion, the hybridizing collared (*Ficedula albicollis*) and pied flycatcher (*F. hypoleuca*) provide an excellent opportunity to conduct such a study. Both species are well studied which provides good basic knowledge on which further investigations can build.
Furthermore, the species are easy to work with because they readily breed in nest-boxes. Lastly, the presence of an already established long-term database of a population of pied and collared flycatchers, and their hybrids, on the island of Gotland, Sweden is a major benefit to working on this system.

**The study species**
The collared and pied flycatchers are insectivorous passerines that breed in cavities. Male collared flycatchers have a distinctive black and white plumage pattern and are readily distinguished from the closely related pied flycatcher by their white collar, larger white patch on the forehead and wings and several other characters (see plate 1A and B). Furthermore, the collared flycatcher is the larger of the two (in most biometric measurements). It is worth noting that some male pied flycatchers have more brown instead of black upperparts. Females of the two species are much more drab-coloured and harder to distinguish (see plate 2A and B). The female collared flycatcher is on average larger and has several species specific characters (such as more white at the base of the primaries and a different pattern on the neck feathers). The species-specific alarm call is a useful identification tool in the field (Lundberg and Alatalo 1992; Cramp and Perrins 1993). Hybrids look intermediate between the two species (see plate 1C and 2C), but plumage characteristics can vary extensively. In males, a broken collar, which makes them look more similar to the closely related semi-collared flycatcher (*F. semitorquata*), is a good indication of a hybrid male (Sætre et al. 1999a). Not surprisingly, the females are especially hard to tell apart. Good indicators, in the field, to suspect a hybrid female are infertile clutches and a mixed alarm call. The identification of hybrids in this thesis was verified using pedigrees and genetic analyses.

Both species are long distant migrants that winter in sub-Saharan Africa (see plate 3A and B). The collared flycatcher has an eastern migratory route and winters in central Africa, whereas the pied flycatcher follows a westerly route spending the winters in west Africa (Cramp and Perrins 1993). The breeding distributions of the two species differ. The pied flycatcher occupies large parts of western Europe, extending eastwards into Russia (see figure 1.3) and in general these breeding areas contain more coniferous tree species (see plate 4A) and are colder, which might have severe consequences if the weather changes (especially further north). The collared flycatcher occupies more deciduous habitat in central and southern Europe and extends less further east into Russia (see figure 1.3, and plate 4B and C). The breeding ranges of the two species overlap in central Europe (e.g. Czech Republic) and on the Baltic islands of Gotland and Öland (Cramp and Perrins 1993). The collared flycatcher is believed to have arrived on Gotland and Öland recently (Lundberg and Alatalo 1992). Collared flycatchers show strong natal philopatry (Pärt 1990) but whether or not this is the case for pied flycatchers is less clear.
Plate 1. A. A male collared flycatcher.  
B. A male pied flycatcher.  
C. A male hybrid flycatcher.
Plate 2. A. A female collared flycatcher. 
B. A female pied flycatcher. 
C. A female hybrid flycatcher.
In spring, both species arrive at their breeding grounds at the end of April/beginning of May (Gotland) (Part 1994) (see plate 3C). Males search for a territory, defend this against intruders and try to attract a female (Part and Gustafsson 1989) (see plate 5A and B). The females are believed to arrive a bit later and inspect several males before choosing a mate (Dale and Slagsvold

**Figure 1.3.** The breeding grounds in Eurasia and the wintering grounds in sub-Saharan Africa of the pied (grey) and collared flycatcher (hatched). Note that the breeding grounds of the Iberian pied flycatcher (sub)species ‘iberiae’ have been left out.
After pair formations and nest building, the female collared flycatcher lays a clutch of on average 6.1 eggs (6.3 for the pied flycatcher) and incubates it for approximately 13 days (see plate 5C and D). After hatching, both sexes share the parental care (Sheldon and Ellegren 1998) (see plate 6A and B). Fledging takes place after around 14-15 days after hatching (see plate 6C). On average 81% of the nestlings of the collared flycatcher and 88% of the nestlings of the pied flycatcher survive to fledge. After fledging, parents and young stay together on the breeding grounds for a couple of weeks until autumn migration (starting in August) which is assumed to be undertaken separately based on the different migration peaks of first-year individuals and adults (Lundberg and Alatalo 1992; Cramp and Perrins 1993). Only a small percentage (11% for collared and 6% for pied flycatchers) of the fledglings is found in subsequent years to recruit to the natal breeding area. Breeding collared flycatcher live on average 2.3 years and pied flycatchers 2.8 years.

The hybrid zone
The two species hybridize in areas where their breeding distributions overlap. The species composition on the sites where the interactions between the two species has been studied is strongly skewed towards collared flycatchers (proportion of flycatchers that is collared is 0.96 on Gotland, 0.70 on Öland and 0.85 in the Czech Republic).

The species mate assortatively: females of both species recognise and prefer to mate with conspecifics (Sætre et al. 1997b) whereas males apparently show no mate discrimination (Dale and Slagsvold 1994; Sætre et al. 1997a). Earlier experiments showed that plumage characteristics of males of the two species play an important role in assortative mating (Sætre et al. 1997b). Despite the fact that the species mate assortatively and seem to have a well developed species recognition system, they do hybridize. At the study sites on Gotland and in the Czech Republic, 2-3% of all breeding pairs were mixed-species pairs (Sætre et al. 1997b; Veen et al. 2001). On Öland, slightly higher proportions were found (5%) (Anna Qvarnström, pers. comm.).

The hybrid zones in the Czech Republic and on the Baltic islands differ from each other in several aspects. An important difference is that in Czech Republic the hybrid zone is situated on an altitudinal gradient, with collared flycatchers occupying the more deciduous valleys and lower slopes and the pied flycatchers the higher more coniferous areas (Sætre et al. 1999a). It has been suggested that the competitively dominant collared flycatcher forces the pied flycatcher to these areas but that the pied flycatchers can better cope with these conditions, which are more similar to a large part of their allopatric breeding range (Sætre et al. 1999a).
Plate 3. A. A male collared flycatcher during spring migration in Tunisia. Photo René Pop.
B. A female collared flycatcher during spring migration in Tunisia. Photo René Pop.
C. A male pied flycatcher just after arrival at Faludden (Gotland).
Plate 4. A. The habitat surrounding a nest-box occupied by a pied flycatcher on Öland. B. The habitat surrounding a nest-box occupied by a collared flycatcher on Öland. C. Typical breeding habitat of collared flycatchers on Gotland. A caterpillar frass trap is being installed (see also chapter 4). Photo Johan Träff.
Motivation and outline of this thesis

One of the great advantages of choosing pied and collared flycatchers for a study of hybridization is the presence of a long-term database of the populations of both species on Gotland. This opens up the possibility of estimating the fitness consequences of hybridization. In an earlier study, Veen et al. (2001) used this approach to take a first step in trying to elucidate the mating decision in the flycatchers’ hybrid zone. This study inspired many of the questions asked in this thesis and I therefore believe that it is useful to summarize its main results.

In the aforementioned study, we tried to quantify the fitness consequences of hybridization by first validating the presumed costs of producing hybrid offspring, then estimating the actual proportion of hybrids found in mixed-species broods and finally calculating the predicted reproductive output of mixed and pure-species pairs. The data were collected on Gotland and in the Czech Republic. As mentioned above, the pied flycatcher is the scarcer breeding species and it is therefore easier to understand why a female pied flycatcher would end up with a male collared flycatcher, as she might not have much other choice (engaging in a polygynous pair bond might be a possibility, but secondary broods in such pairings have strongly reduced reproductive success (Huk and Winkel 2006)). For female collared flycatchers, pairing with a pied flycatcher male is more difficult to understand, as she is surrounded by conspecifics. We therefore focused on mixed-species pairs with a female collared flycatcher. As a first step, the very low fertility of hybrid females was confirmed as a severe cost of hybridization. In the next step, it turned out that the proportion of hybrid female young in the mixed-species broods was much less than expected. The sex ratio was skewed towards sons (see figure 1.4).

More importantly, we found that on average 56% of all nestlings in mixed-species broods did not originate from the social (heterospecific) male but were sired by an extra-pair father (figure 1.5A). Most importantly, this extra-pair father was in all case, and for both pairing types, conspecific to the female (figure 1.5B) which means for the mixed-species pairs that the production of unfit hybrids is strongly avoided. Both factors combined to strongly reduce the estimated cost for a female collared flycatcher to accept a male pied flycatcher. Nevertheless a cost still remained as a sizeable proportion of the broods consisted of hybrids. Lastly, we estimated the reproductive success of a brood by the fledging success of its nestlings. It turned out that the number of fledged young through the breeding season differed markedly between mixed-species and pure collared flycatcher pairs. At the end of the breeding season, female collared flycatchers that mated with a male pied flycatcher enjoyed a higher fledging success, even after subtracting the fitness loss as a consequence of producing hybrids. Our study suggested that under some circumstances female
collared flycatchers could have a fitness advantage by choosing a male pied flycatcher as a mate. It remained unknown what mechanisms underlie the paternity and sex ratio patterns described. Could it be that females adaptively change their sex allocation and their strategy for searching for extra-pair copulations?

**Figure 1.4.** The sex ratio in pure collared flycatcher broods (CF x CF) and mixed-species broods of a male pied flycatcher paired with a female collared flycatcher (PF x CF). Data are presented from the hybrid zone in Gotland and in the Czech Republic. Numbers above the bars indicate the sample sizes. Note that the sample sizes are slightly larger compared to Veen et al. 2001 as some additional samples from the same period have been added.

**Figure 1.5.** Proportion of nestlings fathered by a different male than the social male (extra-pair nestlings) for pure collared flycatcher broods (CF x CF) and mixed-species broods of a male pied flycatcher paired with a female collared flycatcher (PF x CF) (A). The proportion of these extra-pair nestling which is fathered by a male conspecific to the female is given in the figure B. Data are presented from the hybrid zone in Gotland and in the Czech Republic. Numbers above the bars indicate the sample sizes.
Plate 5.  A. A male collared flycatcher singing.
   B. A male collared flycatcher displaying when a female approaches.
   C. A collared flycatcher nest with eggs.
   D. An incubating female collared flycatcher.
Plate 6. A hybrid male entering the nest-box to feed the nestlings. Photo Johan Träff.
B. A female collared flycatcher with a caterpillar. Photo Johan Träff.
C. A fledged pied flycatcher nestling. Photo Johan Träff.
Or could the high levels of conspecific extra-pair young be the results of some interspecific compatibility problem such as conspecific sperm precedence? Furthermore, the cause of the differences in reproductive output from pure and mixed pairs remained to be elucidated.

In this thesis I will try to elucidate the factors affecting the mating decisions in the flycatcher hybrid zone. This requires the acquisition of good estimates of the different costs and benefits involved, and to obtain these I used a variety of approaches, including field experiments, database analyses, stable isotope analyses, and theoretical modelling. For similar reason as outlined earlier, the main focus will be on the female (collared flycatcher) side of the mating decisions.

Engaging in a mixed-species pair may under some circumstances entail a fitness benefit for female collared flycatchers. The high levels of extra-pair paternity and sex ratio skew are two crucial components, but what we do not know is what mechanism causes these effects. In chapter 2 an experimental approach was used to try to find out whether the high levels of conspecific extra-pair young were the result of an adaptive female strategy or a compatibility problem. In the field, male collared flycatchers were caught upon arrival at the breeding grounds and divided in two groups. The white parts of the male plumage of one group were manipulated with a black marker pen to make them resemble a pied flycatcher male and the other group functioned as a control. The pairing success and speed of pair formation of the experimental males was determined in the field. Blood samples collected from the nestling in broods attended by an experimental male were used to determine the sex ratio and level of extra-pair paternity within the broods.

In chapter 3 my colleagues and I investigate the potential origin of direct benefits a female might gain from a heterospecific mate. We looked at two direct benefits the female could experience by being mated to a heterospecific male: heterospecific males could occupy a superior territory or use it in a different way to extract resources for the offspring. The ‘quality’ of the territory was investigated using the long-term database. All nest-boxes were divided in two categories: nest-boxes in which a pied flycatcher male had been recorded breeding and nest-boxes in which only collared flycatcher males had bred. Only the reproductive success of pure collared flycatcher broods was compared between these two categories to see if the location of the nest-box per se influenced the reproductive success. The idea that males might utilise the territories differently was tested by examining if males of the two species differed in their food provisioning rate and whether they brought in different prey items.

Chapter 4 follows up on the previous chapter. Here, we tried to unravel the causes underlying the differences in reproductive output between territories occupied by the two species and focus on habitat characteristics. First we characterised the tree species and vegetation density around the nest-boxes of the
two flycatcher species. In a second step, this was linked with the temporal abundance of an important food resource, caterpillars, to predict the food abundance in territories of the two species through the breeding season.

The inherent developmental defects resulting in hybrid fertility and sterility are often regarded as the most influential costs of hybridization. However, hybrids might suffer a reduced fitness as a consequence of suboptimal performance due to their intermediate phenotype not being optimised to either parental niche. Intermediate migratory behaviour of hybrids has been put forward as one such ‘extrinsic’ cost of hybridization. In chapter 5, we investigated whether hybrids followed an intermediate migratory route and ended up at suboptimal wintering grounds. We tested if hybrids had a higher winter mortality compared to the parental species, which would be predicted if they would take an intermediate route. Second, we characterised the different wintering locations of the two parental species with the use of stable isotope signatures extracted from feathers moulted on the wintering sites. These signatures were then compared with those of the hybrids to infer where they spent the winter.

Pre-zygotic isolation is a key component in reproductive isolation. A major obstacle in the evolution of pre-zygotic isolation is that the required association between a species specific trait and preference for it is dissociated rapidly through recombination. There are several ways to circumvent this, of which physical linkage of the trait and preference on the same part of the genome is one. It is known for flycatchers that the species specific male trait is located on the Z chromosome, but it is currently unknown how species recognition is determined. In chapter 6, we used a combination of database analyses, molecular techniques and experimental work in the field to establish how female flycatchers determined which species to prefer.

In order to fully understand the mating decisions in a hybrid zone, an integration of both sexual selection and species recognition is required. While trying to achieve this, we noticed that sexually selected signals are frequently used in different contexts, namely in male-male competition and female choice. Such ‘dual-function signals’ are common in nature and must be understood before an attempt can be made to combine sexual selection theory and species recognition. In chapter 7, we derived a theoretical framework for the evolutionary dynamics of dual-function signalling and described several surprising results.

Chapter 8 is the synthesis of this thesis. In this chapter our current knowledge of the costs and benefits of hybridization in the flycatchers system will be reviewed.
Reduced costs of mixed-species pairings in flycatchers: by-product or female strategy?

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submitted to Behavioral Ecology and Sociobiology
Abstract

Heterospecific matings are generally assumed to be unconditionally disadvantageous due to reduced viability or fertility of hybrid offspring. For female collared flycatchers (*Ficedula albicollis*) mated to male pied flycatchers (*F. hypoleuca*) the cost of heterospecific pair formation is reduced due to high levels of conspecific extra-pair paternity and a male-biased offspring sex ratio. In order to investigate whether these cost-reducing mechanisms are the result of female mating strategies, rather than being a by-product of species incompatibilities, we manipulated the plumage of male collared flycatchers before pair formation to make them resemble male pied flycatchers. Since species incompatibilities are absent in this design, any systematic effect of manipulation on sex ratio or paternity would indicate a role of female mating strategy. Paternity was determined by means of a likelihood approach that controls the errors made in assigning a chick to be ‘within-pair’ or ‘extra-pair’. Neither the sex ratio, nor the male share of paternity, was affected by the manipulation in a systematic manner. We therefore conclude that our experimental data provide only very limited support for the suggestion that female behavioural strategies are adjusted in response to formation of mixed pairs.
Introduction

Mate choice can be an important factor determining an individual’s reproductive success, and incorrect mate choice can have large negative fitness consequences, especially when an individual chooses a partner of another species. Such mixed-species pairings often entail substantial fitness costs since hybrid offspring typically have strongly reduced viability and/or fertility (e.g. Barton and Hewitt 1985; Coyne and Orr 2004). One would therefore expect strong selection against such maladaptive mate choice. Birds are no exception when it comes to the fertility costs of hybridization (Price and Bouvier 2002). Considering this, it is surprising that mixed-species pairings are regularly observed in natural bird populations (Grant and Grant 1992).

Several hypotheses have been put forward to explain the occurrence of mixed-species pairings (e.g. Grant and Grant 1997a). An obvious explanation is that they result from a mistake in mate choice. This could be due to intrinsic problems linked with species recognition systems due to the fact that learning processes, on which species recognition systems are often based, are error prone (e.g. imprinting on the wrong species (for a review: Irwin and Price 1999 and references herein)) or due to an environment-induced ‘malfunctioning’ of mate recognition (e.g. water turbidity causing difficulties for cichlid fish to recognise conspecifics (Seehausen et al. 1997)). If only one sex bears the costs of a hybridization event, sexual conflict might be an important factor (Parker and Partridge 1998). An example is forced copulations, where in the absence of paternal care, the male benefits from any extra fertilization while the female has to bear the costs of raising hybrid offspring with a low fitness (Randler 2005).

Obtaining reliable estimates of the costs involved in hybridization is of crucial importance for understanding the evolutionary implications of mixed-species pairings. There are some indications that these costs are lower than expected. First, there may be no costs at all (or even net benefits) if hybrid offspring profit from synergistic effects. For example, hybrid Galapagos finches with an intermediate bill size were more successful in certain environmental conditions compared to the parental species due to their intermediate diet (Grant and Grant 1996). Secondly, the costs of hybridization may be relatively small since few other options with higher fitness benefits are available. In such a situation, hybridization may correspond to ‘making the best of a bad job’. For example, late in the breeding season, with little choice of conspecific mates, an individual might be better off accepting a heterospecific partner than not to breed at all. Lastly, the estimation of the costs may be biased in the upward direction, since the costs only apply to part of the offspring. For example, hybrid offspring of the heterogametic sex typically suffer a much more severe fitness reduction than those of the homogametic sex (Haldane’s rule (Haldane 1922)).
These processes are not mutually exclusive, and multiple factors may well work at the same time. In hybridizing pied (Ficedula hypoleuca) and collared flycatchers (F. albicollis) this is the case and all three of the mechanisms mentioned above are thought to be important (Veen et al. 2001; Qvarnström et al. 2005). Female collared flycatchers mated with male pied flycatchers gain fitness in comparison to pure collared flycatcher broods due to an increased number of fledged young per brood (‘synergistic effects’) (Veen et al. 2001). This does not, of course, decrease the costs of having hybrid offspring per se. In pure collared flycatcher broods there is a selective advantage to early breeding (Sheldon et al. 2003), so that female collared flycatchers arriving relatively late in the season are faced with a lower quality breeding situation, and therefore might be more prepared to accept a heterospecific mate (‘the best of a bad job’). Furthermore, the costs resulting from reduced fertility of hybrid offspring are in fact much lower than they seem to be at first sight (‘less severe costs’), primarily due the fact that only a minority of the nestlings raised by a mixed-species pair are actually the product of hybridization. In fact, on average 56% of all offspring in a brood are conspecific extra-pair young (Veen et al. 2001). Additionally, the costs are reduced due to a sex ratio skew towards male offspring, i.e. towards the sex that is least affected by hybridization. These three components combined create a situation in which late in the season mixed-species pairs even have higher expected reproductive success than pure collared flycatcher pairs (Veen et al. 2001).

It has been suggested (Veen et al. 2001) that the sex ratio skew and the high frequency of conspecific extra-pair young in the nests of mixed-species pairs could both represent an adaptive mating strategy of female collared flycatchers. First, when mated to a male pied flycatcher, a collared flycatcher female might adjust the sex ratio of the brood towards the more fertile (male) sex. This is not completely implausible, since several examples are known where birds show a remarkable flexibility in adapting the sex ratio to the local circumstances (Komdeur et al. 1997; Badyaev et al. 2006). Second, collared flycatcher females paired with a pied flycatcher male might actively seek extra-pair copulations with a conspecific. This does not necessarily need to be a newly evolved adaptation. Male collared flycatchers with a small forehead patch lose paternity compared to large patched males (Sheldon et al. 1997) and this might have a particularly large consequence for the very small patched pied flycatcher.

The alternative explanation for the patterns described above is that the sex ratio bias and the high frequency of conspecific extra-pair offspring might both be the result of species incompatibilities. First, the sex ratio bias in favour of sons might represent sex-biased abortion and mortality in line with Haldane’s rule (Haldane 1922): female offspring (i.e. the heterogametic sex) might just suffer more from early embryonic death than male offspring. Secondly, the high
frequency of conspecific extra-pair paternity could be caused by an inherent advantage of conspecific sperm in sperm competition (conspecific sperm precedence: (Price 1997; Fricke and Arñqvist 2004; Harper and Hart 2005).

It was the central aim of the present study to discriminate between these alternative explanations. To this end, we manipulated the white plumage parts of free-ranging male collared flycatchers with a black marker pen before pair formation to resemble that of a male pied flycatcher. Then, the pairing success, the male’s share of paternity in the brood, and the sex ratio of the offspring were studied. Earlier studies have shown that females of both species are capable of distinguishing con- and heterospecifics, and that experimental alteration of the plumage is perceived as a change of species identity (Sætre et al. 1997b). Furthermore, other studies found clear effects of manipulations of sexually selected characters on mate choice decisions and parental care (Qvarnström 1997; Qvarnström et al. 2000). Our experiment thus excludes the possibility that species incompatibilities cause a systematic difference in sex ratio or paternity (since all individuals were genetically collared flycatchers): hence any effect of the manipulation would suggest a female mating strategy.

Material and methods

Study species and study population
Flycatchers in the genus *Ficedula* are migrant sexually dimorphic insectivorous passerine birds. Both pied and collared flycatchers are cavity-nesting species, readily accepting nest-boxes, and in both species the male provides an approximately equal amount of parental care as the female. We used populations breeding in nest-boxes on the island of Gotland (Sweden) that have been monitored for over 25 years by JT or by biologists based at the University of Uppsala. On Gotland, pied and collared flycatchers breed in sympatry, with the latter species being numerically dominant (>95% of all breeding birds); the two species hybridize in low numbers 2–3% of the breeding pairs (Veen et al. 2001).

Previous studies of the mating system of the collared flycatcher have shown that extra-pair paternity occurs in around 1/3 of all broods, and that on average about 15% of all young are extra-pair sired (Sheldon and Ellegren 1999; Veen et al. 2001). Both the sex ratio and the level of extra-pair paternity are correlated with the size of the sexually selected characters (the size of the white patch on the forehead and the amount of white on the base of the primaries (Ellegren et al. 1996; Sheldon and Ellegren 1999; Michl et al. 2002)). The two species differ in several morphological characteristics, these differences being most pronounced in males. Males of the two species differ in the amount of white on the forehead, the base of the primaries and in the neck, with pied flycatchers always having less white (Svensson 1992).
Experimental procedures and field data collection

Male collared flycatchers were caught upon arrival at the breeding grounds from the beginning of May onwards in three consecutive years (2002–2004) using traps inside the nest-boxes, which were triggered when males entered nest boxes to inspect them (something they do frequently when newly arrived and establishing a breeding territory). In each year a new area was used for the experiment to avoid using the same individuals multiple times. In 2002, we aimed to catch all newly arrived males each day. These males are often very conspicuous and actively singing (pers. obs.), we believe that most males were caught within a day or two after arrival. In 2003 and 2004 we changed catching strategy since we had the possibility to trap very efficiently in many boxes at the same time. Every second day, we placed traps in half of all the nest-boxes in our experimental area for an extensive period of time (up to 5 hours per day with frequent checks), creating a situation in which newly arrived males were very likely to be caught within 2 days after arrival. The nest-box areas used had the following characteristics: 2002 at Sproge: 224 boxes with 50 breeding pairs; 2003 at Grötlingbo: 172 boxes with 74 breeding pairs; 2004 at Grötlingbo: 158 boxes with 70 breeding pairs.

We assigned male collared flycatchers randomly to treatment groups within the experiment. In the ‘experimental’ group (from now on referred to as ‘E’) the three main differences in male plumage between the two species were manipulated to match the plumage of a male pied flycatcher. In practice this meant that the white patch on the forehead, the base of the primaries and the neck were painted with a black marker pen (COPIC 100 Black) to match the mean values of a male pied flycatcher for the above mentioned traits (forehead: height 3.6 mm, width 7.1 mm, white on the primaries (p), measured from the tip of the primary covert: p2–5 no white, p6; 4mm, p7; 5mm (primaries numbered ascendently from outside of the wing inwards)). The white collar was painted by eye to resemble the collar of a pied flycatcher male. Males in the ‘control’ group (from now on referred to as control 1 ‘C1’) received the same treatment as the E males but were painted with a marker pen without ink (COPIC colourless blender) so as not to change the colour or extent of the white plumage areas (thus males in this group resembled a normal collared flycatcher).

Previous studies have shown that differences in reflectance of ultraviolet (UV) wavelengths can be of importance for mate choice (e.g. Bennett et al. 1996). In order to exclude the possibility that the manipulation changed the reflectance of wavelengths shorter than visible for humans (human range 400-700 nm), we tested whether the marker pens changed the reflectance in the UV part of the colour spectrum (320–400 nm). This was done by measuring the reflectance of white and black parts of 10 male median tertial feathers using a USB-2000 spectrophotometer with illumination by a DH-2000 deuterium-halogen light source (both Avantes, Eerbeek, The Netherlands). The white parts
of five feathers were then treated with the colourless blender marker pen and the other five with black marker pen. The reflectance of each feather was measured again after manipulation. The reflectance curves were in all cases very similar to the curves prior to the manipulation of the white and black parts respectively, and we therefore assume that our treatment did not change the reflectance of the manipulated areas in the UV region.

In 2003 and 2004 a third group was added to the experiment. Males in this group (from now on referred to as control 0 ‘C0’) were only measured, and a blood sample taken, before being released. The purpose of adding this group was to function as control for painting per se and aims to control for unforeseen effects of painting (for example due to olfactory cues (Moore and Moore 1999)). For this reason, the C0 and C1 group will be compared at the end, after the comparison between E and C1.

From the start of the breeding season, all nest-boxes were checked daily to record onset of nest-building, start of egg laying and clutch size. Because we could not record the start of pair formation directly, we used the onset of nest-building as the moment of pair formation. Once building of the nest started (i.e. the bottom of the nest-box was covered with nesting material) the nest was, in the vast majority of cases, finished and followed by a breeding attempt (JT, pers. obs.). Adults were caught and measured, and ring numbers were recorded during incubation (females only) and nestling feeding (both sexes). Only at this stage we could decide on whether or not to include a brood in the experiment. We had to discard all males that had been caught and manipulated after pair formation, since for these males the manipulation was unrelated to female mate choice. Nestlings were ringed and measured at day 12, where hatching is day 0 (for more details on methods see Pärt and Gustafsson 1989). A blood sample (3–10 µl) was collected by brachial venipuncture from all adults and nestlings and stored in 96% ethanol.

Sex determination and paternity analysis
The sex of the offspring in each brood was determined using primers P2 and P8 followed by standard PCR procedures (Griffiths et al. 1998). The products were made visible by silver-staining on a 6% polyacrylamide gel and scored manually. Paternity in all broods was determined on basis of allele-sharing at eight polymorphic microsatellite markers: FhU2, FhU3, FhU4, FhU5, Pdou5, Pca3, Phtr1 and Phtr2. Different sets of markers were used for different years (data from 2002 with Fhu2, Fhu3, Fhu4, Fhu5 and Pdou5 and data from 2003 and 2004 with all markers except Fhu5). DNA from all blood samples was extracted using Chelex extraction followed by standard PCR procedures (Ellegren 1992; Primmer et al. 1996; Sætre et al. 2001). In all years the PCR products were arranged in family groups. In 2002 these products were run out on a 6% polyacrylamide gel with size standard, made visible using silver-staining and after
drying were scored manually. The 2003 and 2004 PCR products were run on an ABI PRISM 377 Sequencer with size standards and scored using GENESCAN and GENOTYPER software packages. Egg dumping is thought to be rare in collared flycatchers (Gelter et al. 1992) and our data support this (only eight nestlings had a single mismatch with their mother, making it likely that these mismatches either reflect typing errors or mutations). We therefore assume the female caught at the nest to be the genetic mother of the offspring. In appendix 2.1, we describe in detail how paternity was determined using a likelihood approach performed by the program Cervus 3.0 (Kalinowski et al. 2007). For a given offspring and the given social mate of the known mother, Cervus calculates a LOD-score (corresponding to the logarithm of a likelihood ratio) that corresponds to the relative likelihood of obtaining the offspring genotype under the assumption that the social mate is the genetic father. These LOD-scores are based on the allele frequencies at the marker loci of all adult individuals found in the study population and therefore take account of differences in discriminatory power between the markers. The offspring was considered a ‘within-pair young’ (WPY) if the LOD-score was above a certain threshold value $T$, and it was considered an ‘extra-pair young’ (EPY) if the LOD-score was lower than this threshold. As described in appendix 2.1, the threshold was determined in such a way that the probability of both types of error inherent in a paternity analysis could be controlled: (1) the misclassification of a WPY as an EPY (type I error) and (2) the misclassification of an EPY as a WPY (type II error). It turned out that in all three study years each type of error was smaller than 2.4% (see appendix 2.1 for details).

**Statistical analysis**

**PAIRING SUCCESS**

If females indeed prefer conspecific over heterospecific partners as predicted, we would expect the ‘pied-like’ (E) males to have a lower pairing success. We defined the ‘pairing success’ of a given category of males as the proportion of males caught on a given day which were found breeding later on in the season with respect to the total number of males caught on that given day. The effect of experimental treatment on pairing success (the dependent variable) was analysed using a logistic regression (binomial error distributions and logit link function). Independent variables included in the full model, besides experimental treatment, were the relative date caught (relative to the first capture date for each year) as a covariate, and year as a factor. An interaction term between treatment and relative date caught was included for the comparison between E and C1 to test for a seasonal change of mate preference (Qvarnström et al. 2000; Veen et al. 2001).
SPEED OF PAIR FORMATION
A second way to test for an effect of experimental treatment is to look at pairing speed, which is defined as the time in days between experimental treatment of the male and pair formation (onset of nest-building). If female preferences change through the season, as suggested above, one would expect a difference in pairing speed between the two groups through the season. A General Linear Model (full factorial) procedure was used, with pairing speed as dependent variable, relative capture date as explanatory variable and treatment as factor.

MALE SHARE OF PATERNITY AND SEX RATIO
The effect of the experimental treatment on the dependent variables male share of paternity (number of nestling sired in a brood) and sex ratio (number of male nestlings in a brood) was analysed using a logistic regression (binomial error distributions and logit link function). Independent variables included in the full model, beside treatment and year, were laying date and the size of the male’s forehead patch (width × height in mm) because previous studies have shown an influence of both on male share of paternity (Sheldon and Ellegren 1999) and sex ratio (Ellegren et al. 1996; Sheldon and Ellegren 1999).

For all the logistic regressions, non-significant variables were excluded from the full model in a backwards elimination process to get the final model. Values for each variable given in the results were calculated by adding the variables individually to the final significant model. Where informative, the coefficient and standard error (between brackets) for covariates is provided to assess the direction and magnitude of the effect. In all cases, overdispersion was, if necessary, accounted for by scaling the deviances. All analyses were conducted using Statistica 7.

Results

Data selection
Over the three seasons, a total of 177 males were caught early in the season and assigned to one of the treatment groups. Of these males, we retrapped 105 males while feeding nestlings. Only those males experimentally manipulated before pair formation could be used. In other words, males which had already paired up (measured as the start of the nest-building in the nest-box they were found feeding nestlings) and were caught and manipulated after this date, were excluded. This was the case for 33 broods. Four broods were excluded from the analyses as data on the start of nest-building (and hence the date of pairing) was lacking. Moreover, one E male in 2004 paired with a female pied flycatcher and was also excluded. In six cases males were polygynous (one C0 male, two C1 males and three E males); the secondary broods of all bigamists were
excluded to avoid pseudoreplication. The paternity analysis for three broods failed. Table 2.1 presents an overview of sample sizes for all three treatment categories for all three years, together with mean laying date and pairing speed.

**Pairing success**

A little more than half (59%) of all males caught early in the season were found attending a brood later in the season. The other males (31%) may have been unable to attract a mate or bred outside our study area. In line with the prediction based on the selective advantage of early breeding (Sheldon et al. 2003), we found a high pairing success for both E and C1 early in the season which decreased through the breeding season (figure 2.1; relative capture date, Wald $\chi^2 = 11.44$, coefficient = –0.128 (0.038), df = 1, p<0.001). Previous work suggested that later in the season male pied flycatchers might be preferred by female collared flycatchers, which would be revealed in our experiment by an interaction between treatment and relative capture date. This suggestion is weakly supported by our data, as the pairing success of the E group declines less steeply through the season and is higher than the C1 group at the end of the season (figure 2.1; Wald $\chi^2 = 2.772$, df= 1, p = 0.096). Differences in paternity between years was near-significant (Wald $\chi^2 = 5.298$, df = 2, p = 0.071) but the treatment effect (Wald $\chi^2 = 2.351$, df = 1, p = 0.125) was not significant. In conclusion, the pairing success of an individual decreases through the

![Figure 2.1](image_url). The proportion of males of the C1 and E treatment groups that successfully paired relative to their day of capture. Day of capture is relative to the first catching day of the season. Data have been pooled in categories of 3 days and sample sizes for each category are presented next to the plotted data. The fitted curves are predicted proportion of males mated from a logistic regression on the original (non-pooled) data (dotted line is C1, solid line is E).
breeding season and an interaction between treatment and capture date suggests a less steep decline for the E group.

**Speed of pair formation**

It is immediately apparent from table 2.1 that the pairing speed shows great variation both between years and treatment groups, but not in a consistent way. This is reflected by the results of the GLM, as none of the variables in the models had a significant effect (GLM: relative capture date $F_{1,54} = 1.363, p = 0.248$, treatment $F_{1,54} = 0.011, p = 0.916$, relative capture date $\times$ treatment $F_{1,54} = 0.028, p = 0.868$).

**Table 2.1.** For each year and each experimental group the numbers of males caught early in the season and subsequently found breeding are presented. Sample sizes differ between columns because not all individuals were found paired at the end of the season, some males were caught after pair formation and some paternity analyses failed (see respective columns for sample sizes). The mean and standard error (se) of laying date and pairing speed (day found nest-building – day caught) are given in the last columns.

<table>
<thead>
<tr>
<th>year</th>
<th>treatment</th>
<th>males caught</th>
<th>paired</th>
<th>caught before pair formation</th>
<th>established</th>
<th>laying date</th>
<th>se</th>
<th>pairing speed</th>
<th>se</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>C1</td>
<td>17</td>
<td>14</td>
<td>11</td>
<td>11</td>
<td>17.27</td>
<td>0.74</td>
<td>3.36</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>18</td>
<td>11</td>
<td>11</td>
<td>10</td>
<td>20.64</td>
<td>1.48</td>
<td>6.18</td>
<td>1.31</td>
</tr>
<tr>
<td>2003</td>
<td>C0</td>
<td>22</td>
<td>11</td>
<td>5</td>
<td>5</td>
<td>21.40</td>
<td>2.91</td>
<td>9.00</td>
<td>3.36</td>
</tr>
<tr>
<td></td>
<td>C1</td>
<td>21</td>
<td>15</td>
<td>8</td>
<td>7</td>
<td>21.88</td>
<td>1.53</td>
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<td>1.42</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>22</td>
<td>11</td>
<td>9</td>
<td>9</td>
<td>23.11</td>
<td>1.69</td>
<td>5.11</td>
<td>1.54</td>
</tr>
<tr>
<td>2004</td>
<td>C0</td>
<td>26</td>
<td>15</td>
<td>4</td>
<td>4</td>
<td>19.75</td>
<td>3.45</td>
<td>3.75</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>C1</td>
<td>25</td>
<td>13</td>
<td>9</td>
<td>9</td>
<td>19.33</td>
<td>1.19</td>
<td>3.77</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>37</td>
<td>28</td>
<td>10</td>
<td>28</td>
<td>20.67</td>
<td>0.82</td>
<td>5.30</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>all years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.00</td>
<td>0.55</td>
<td>5.39</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>177</td>
<td>105</td>
<td>67</td>
<td>64</td>
<td>20.07</td>
<td>0.55</td>
<td>5.39</td>
<td>0.52</td>
</tr>
</tbody>
</table>
Share of paternity
The male share of paternity of the C1 group is similar to previous studies (85%, Sheldon and Ellegren 1999) and fluctuates little over the years. The differences found between the C1 and the E groups were small and showed no significant effect of experimental treatment or size of the male’s forehead patch on the male share of paternity (figure 2.2A and table 2.2A). Year effects were marginally significant, but are hard to interpret. Laying date had a highly significant negative effect on male share of paternity. These results indicate that the experimental treatment did not change the level of extra-pair paternity. This differs significantly from the pattern found in nature, where broods with a male pied flycatchers mated with a female collared flycatcher had much higher rates of extra-pair paternity (logistic regression comparing E pairs with female collared flycatcher mixed-species pairs (data from Veen et al. 2001): Wald $\chi^2 = 28.434$, df = 1, p < 0.001).

Sex ratio
The results of the sex ratio analysis again show a lot of variation among groups and no really consistent pattern between the different treatment groups (figure 2.2B). None of the factors contributed significantly to the final model, which contained as last factor the experimental treatment (table 2.2B). The lack of effect differs from the patterns found in ‘real’ mixed-species pairs where the sex

Figure 2.2. Male share of paternity (A) and sex ratio (B) for the different experimental groups for the three years in which the experiment was conducted (mean with standard errors and sample sizes above bars). The three experimental groups are C0 (male only caught, no plumage manipulation treatment), C1 (male painted with colourless blender marker pen to resemble collared flycatcher) and E (male painted with black marker pen to resemble male pied flycatcher).
ratio was skewed towards sons. A direct comparison between the E group and male pied flycatchers engaged in a mixed species pair (data from (Veen et al. 2001)) is not significant (logistic regression: Wald $\chi^2 = 0.88$, df = 1, $p = 0.349$), but this is not surprising due to the small skew found in the latter pairs and the large variation in the E group.

**Comparison between C0 and C1**

In order to test whether the treatment per se might have affected individual behaviour, we compared the ‘painted like a collared flycatcher’ (C1) group with the controls that were only caught (C0). From table 2.1 it is clear that a surprisingly large proportion of the C0 group was caught after pair formation and had to be excluded. As we assigned the newly caught males randomly to the treatment groups, the only plausible explanation we can think of is that this reduced pairing success of the C0 group is due to chance. As a consequence of the low sample sizes we cannot draw firm conclusion from the C0 – C1 comparison and will only discuss the results briefly. The pairing success decreased through the season for both the C0 and C1 groups, but also differed significantly between the years. Speed of pair formation was not influenced significantly by any of the variables included in the model. The male share of paternity decreased significantly through the season for both groups and was furthermore significantly lower in the C0 group, for which we have no explanation. Sex ratios did not differ between the groups.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Wald $\chi^2$</th>
<th>coefficient</th>
<th>standard error</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>treatment</td>
<td>1</td>
<td>0.057</td>
<td></td>
<td></td>
<td>0.811</td>
</tr>
<tr>
<td>year</td>
<td>2</td>
<td>5.419</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>laying date</td>
<td>1</td>
<td>6.132</td>
<td>-0.086</td>
<td>0.035</td>
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</tr>
<tr>
<td>forehead patch size</td>
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<td>0.005</td>
<td>0.009</td>
<td>0.581</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>treatment</td>
<td>1</td>
<td>1.894</td>
<td></td>
<td></td>
<td>0.169</td>
</tr>
<tr>
<td>year</td>
<td>2</td>
<td>2.358</td>
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<td></td>
<td>0.308</td>
</tr>
<tr>
<td>laying date</td>
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<td>0.146</td>
<td>0.010</td>
<td>0.026</td>
<td>0.702</td>
</tr>
<tr>
<td>forehead patch size</td>
<td>1</td>
<td>0.902</td>
<td>0.006</td>
<td>0.007</td>
<td>0.342</td>
</tr>
</tbody>
</table>

**Table 2.2.** Logistic regression of the effects of treatment, year, laying date and size of the male’s forehead patch on male share of paternity (A) and sex ratio (B).
The aim of this study was to experimentally mimic the situation where a female collared flycatcher pairs with a male pied flycatcher, and to determine whether the high levels of extra-pair paternity and sex ratio skew towards males found in natural mixed-species pairs might be explained by an adaptive female mating strategy. We found very little evidence that this was the case, as broods with a ‘pied-like’ (E) male did not differ with respect to extra-pair paternity or sex ratio compared to control (C1) pairs.

The interpretation of the results from studies in which no effect of the experimental treatment was found, like this study, are problematic because this can occur for two reasons. The lack of a treatment effect can either be due to the female not having perceived the treatment or alternatively, because although the treatment was perceived the behaviour was not changed accordingly. From a scientific point of view the latter situation is interesting and neglecting such studies might have the unwanted side effect of causing a publication bias. We argue that in our study it is unlikely that the females did not perceive the treatment as earlier, similar, experimental treatments of species specific characters did change the behaviour (Sætre et al. 1997b). Furthermore, much less conspicuous manipulations of the size of the forehead patch of males affected the behaviour of females (Qvarnström et al. 2000). Weak support for the suggestion that females did perceive the experimental treatment comes from differences in seasonal change of pairing success between the ‘pied-like’ (E) and the control (C1) groups such that the ‘pied-like’ group was more successful at the end of the season. The direction of this change is in accordance with earlier studies (Veen et al. 2001) and can be seen as support for females perceiving the treatment but only weakly responding to it. Note that although this effect is in line with our predictions, the underlying mechanism remains unresolved. It might be explained by a change in mate choice, or by a side-effect of male-male competition if ‘pied-like’ males received less strong intraspecific competition compared to collared flycatcher males and consequently were less likely to leave the study area. The lack of a clear treatment effect might be due to the fact that some traits important for mate choice, like song, were left unmanipulated. The absence of a clear treatment effect limits the possibility of drawing firm conclusions.

The second explanation for the results is that the ‘adaptive female mating strategy’ hypothesis alone cannot explain the patterns found in naturally occurring mixed-species pairs. During sperm competition, conspecific sperm often has a competitive advantage over heterospecific sperm, also known as conspecific sperm precedence (Price 1997; Dixon et al. 2003; Fricke and Arnoqvist 2004; Harper and Hart 2005). For a collared flycatcher female in a mixed-species pair, this could mean that a single conspecific extra-pair mating may well result in a much higher fertilization rate due to the competitive disadvantage of the
heterospecific sperm of her social (pied) partner. Haldane’s rule (Haldane 1922) is by many best known as predicting reduced fertility of hybrids of the heterogametic sex, but it also applies to the viability of hybrids. Early and higher levels of embryonic death of the heterogametic sex (females) could thus explain the sex ratio skew towards sons.

Although our results did not support an important role for female mating strategy in determining paternity levels in mixed-species pairs, we cannot fully exclude it altogether for several reasons. First, in natural mixed-species pairs roughly 56% of all the nestlings are extra-pair and they are found in almost all broods (Veen et al. 2001). This means that every female collared flycatcher engaged in a mixed species pairing must have had at least one extra-pair copulation (excluding the possibility that they all still have sperm in their reproductive tract from a previous (failed) breeding attempt). We tested whether we found such a pattern in our data by comparing the number of broods with and without extra-pair young between the E and C1, but this did not differ (Fisher exact test; p = 0.403). However, this does not mean that females in a heterospecific pair changed their mating patterns. In our experimental set-up, sperm from extra-pair copulations competed with conspecific sperm, but in the case of mixed-species pairs the sperm competition was between heterospecific sperm. This might result in differences in fertilization rate of extra-pair copulations because of a conspecific sperm advantage, which could explain the observed high levels of extra-pair paternity in mixed-species pairs. A study by Michl et al. (2002) gives some support for this idea, as they experimentally showed, by preventing the social male to transfer sperm during copulation, that seven out of nine females engaged in extra-pair mating. This result indicates that the level of extra-pair copulations is higher than the rate of extra-pair fertilizations found in pure-species pairs. The remaining discrepancy between a large proportion (pure collared flycatcher pairs) and almost all broods containing extra-pair young (mixed-species pairs) could be explained by a (small) change of mating behaviour of females in heterospecific pairs. Alternatively, it could be due to the combined effect of an overall increase of extra-pair paternity through the breeding season (as indicated by the lower share of paternity found in this study) and the later breeding dates of mixed-species broods.

The two possible explanations for the cost reducing mechanisms put forward here, ‘adaptive female mating strategies’ and ‘species incompatibilities’, are not mutually exclusive, and an interaction of both might be present in nature. Such an interaction would be hard to detect with the set-up used here and could thus partly explain the lack of effect in our study. Imagine a situation where the mating strategy of a collared flycatcher female changed slightly through the season, such that (i) she more readily accepts (or even prefers) a male pied later in the season and that (ii) she has at least one extra-pair copulation. These rela-
tively small changes in female mating strategy in synergy with the effect of conspecific sperm precedence (resulting in non-hybrid offspring) and species incompatibilities (resulting in a bias toward sons) can have a large positive effect on the female fitness. A meta-analysis comparing extra-pair paternity levels of hybridizing and non-hybridizing bird species failed to find a difference between the two groups (Randler 2006). This of course does not exclude a potentially important role of extra-pair paternity for (some) hybridizing species as the occurrence of extra-pair paternity can have several other reasons. Studies specifically investigating paternity between hybridizing bird species are few and the results mixed, with two studies finding high levels of conspecific extra-pair young in mixed-species pairs (Veen et al. 2001; Solberg et al. 2006) and another not (Reudink et al. 2006). It is interesting to note that the effects might differ between different hybridizing species pair as phylogenetic relatedness is known to influence the strength of conspecific sperm precedence in Drosophila (Dixon et al. 2003).

Hybridization has traditionally been seen as resulting from mistakes during mate choice or as a means of trying to ‘make the best of a bad job’. A detailed analysis of costs and benefits of mixed-species pairing reveals a more complex situation than expected at first sight. In this study, we find no convincing evidence that females actively change their behaviour or sex allocation to counteract the negative fitness consequences of producing hybrid offspring. This does not mean mixed-species pairing is indeed maladaptive. Reduced costs through mechanistic side effects like conspecific sperm precedence causes the fitness consequences of mixed-species pairings to be less severe and lowers the selection pressure against such matings (pre-zygotic isolation) considerably. An interaction between adaptive female mating strategies and mechanistic side effects can have a pronounced effect on fitness calculation of apparently hybridizing species and should therefore not be excluded. As mixed-species pairings among birds are widespread, it will be of great interest to see whether the predicted negative fitness effects are really as high as expected, which in turn could put the high levels of interspecific mating frequencies in a different perspective.

Acknowledgements

We would like to thank Lars Gustafsson for giving the possibility to work in the Uppsala University nest-box areas. Tristan Marshall generously provided extensive help with the paternity analysis. Mårten Hjernquist, Anna Qvarnström, Chris Wiley, and Marco van der Velde for helpful discussions, practical help in the field and the molecular work (Katherine Thuman Hjernquist), and Oscar Vedder for testing the effect of experimental treatment on light reflectance. Financial support was obtained from the Netherlands Organization for Scientific Research (grant NWO-ALW 812.04.001) (TV). The plumage manipulation was approved by the Swedish National Board for Laboratory Animals.
Appendix 2.1
Type I and type II errors in determining paternity with molecular markers.

In many studies, the determination of paternity on the basis of molecular markers works is based on the following general procedure. After having obtained the allelic values (at various loci) for an offspring and a putative father, the degree of allele sharing is translated into a score that is related to the likelihood to obtain the offspring genotype given that the putative father is the genetic father. This score, to be defined below, will be called the ‘L-score’ in the following considerations. To determine paternity, the L-score is compared with a threshold value (to be specified below): if \( L \geq T \), the putative father is considered to be the genetic father, if \( L < T \), the putative father is discarded from being the genetic father. Let us for simplicity assume that the focus is at the social mate of the known mother. Then the offspring is considered to be a ‘within-pair young’ (WPY) if \( L \geq T \) and an ‘extra-pair young’ (EPY) if \( L < T \).

Two types of error can be made in case of a classification as the one described above: a within-pair young can falsely be considered to be an extra-pair young (type I error), or an extra-pair young can be falsely considered to be a within-pair young (type II error). As shown in figure A2.1.1, the magnitude of the errors can be kept in check by a proper choice of the threshold value \( T \). The probability distribution \( A \) corresponds to the expected distribution of L-scores.

![Figure A2.1.1](image)

Figure A2.1.1. Probability of errors of type I (\( \alpha_a \)) and type II (\( \alpha_b \)) in paternity analysis. Curve A is the probability distribution of L-scores given that the social mate of the known mother is the genetic father, while curve B represents the distribution where the social mate is not the genetic father. If paternity is determined on the basis of a threshold value \( T \), two errors can occur: with probability \( \alpha_a \), a within-pair young is considered extra-pair (type I error); with probability \( \alpha_b \), an extra-pair young is considered within-pair (type II error).

Reduced costs of hybridization
given that the social father is the true genetic father. Accordingly, the area $\alpha_A$ under this distribution to the left of $T$ corresponds to the probability to misclassify a WPY as being an EPY. In other words, $\alpha_A$ is the probability of making a type I error. The probability distribution $B$ corresponds to the expected distribution of L-scores of males that are not the genetic father. Now the area $aB$ under this distribution to the right of $T$ corresponds to the probability of making a type II error.

The magnitude of $\alpha_A$ and $\alpha_B$ reflects the degree of overlap of distribution $A$ and $B$, and hence the discriminatory power of the set of molecular markers used. In case of highly variable markers, the distributions will be more separated allowing to achieve low values of both types of errors. Given the distributions $A$ and $B$, the choice of $T$ determines the relative magnitude of $\alpha_A$ and $\alpha_B$. This means that the choice of $T$ should reflect the relative importance one wants to give to either error, which may strongly depend on the underlying research question. In our case, we consider both types of error equally important and therefore choose $T$ such that $\alpha_A = \alpha_B$.

In practise, we used the program Cervus 3.0 (Kalinowski et al. 2007) to determine our 'L-scores' and the distributions $A$ and $B$. For any given year, we entered the allele frequencies at the marker loci of all adult individuals found in the study population, allowing Cervus to take into account differences in discriminatory power between the markers. The L-scores calculated by Cervus are log-likelihood ratios, also called LOD-scores. Given the allelic pattern of the known mother, Cervus calculates two likelihoods (for details see (Kalinowski et al. 2007)): (1) the likelihood that the offspring genotype is obtained given that the social male is the true father; and (2) the likelihood that the offspring pattern is obtained from a random male in the population. The LOD-score then corresponds to the natural logarithm of the ratio of these likelihoods. The distributions $A$ and $B$ are obtained by a simulation approach. First, the parental population is generated by producing a large number of adults, whose genotype frequencies reflect the known allele frequencies at the marker loci found in our study population. Then, a large number (n = 100,000) of offspring is produced by randomly pairing a male and a female. The offspring genotypes are derived from the parental genotypes in a Mendelian way. To mimic the real data as closely as possible, the allelic values of the offspring were altered with a small probability (chosen to be 0.01) corresponding to typing errors and other mistakes. In this way, we obtained the LOD-scores of the 'genetic father'-offspring pairs, which correspond to distribution $A$ in figure A2.1.1. Similarly, LOD-scores of 'random male'-offspring pairs were generated, corresponding to distribution $B$.

We applied this procedure separately for the three study years, 2002, 2003 and 2004. For the year 2004, the results are represented in figure A2.1.2. In this case, the threshold value separating WPY and EPY is chosen such that the proba-
bilities of making a type I and a type II error turns out to be $\alpha_A = \alpha_B = 0.024$. In other words, the chance to falsely assign a WPY to be an EPY is 2.4%, and this is equal to falsely assign an EPY to be a WPY. The distribution of LOD-scores was very similar in the other two years. Type I and type II errors were limited to $\alpha = 0.022$ (in 2002) and $\alpha = 0.024$ (in 2003), respectively.

**Figure A2.1.2.** The distribution of LOD-scores generated by Cervus for ‘genetic father’-offspring pairs (curve A) and ‘random male’-offspring pairs (curve B). These curves correspond to the probability distributions A and B in figure A2.1.1. The threshold value (here $T= 0.176$) was chosen in such a way that the probability of type I and type II errors was equal (here $\alpha_A = \alpha_B = 0.024$).

NB: The seven peaks in distribution B reflect the fact that we had markers at seven loci. The peaks correspond to mismatches between random male and offspring at $k =1,..., 7$ loci.
Direct benefits and costs for hybridizing *Ficedula* flycatchers

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Abstract

It is well understood that females may gain direct benefits from breeding with attractive males. However, the direct fitness effects of mate-choice are rarely considered with respect to mating between different species (hybridization), a field dominated by discussion of indirect costs of producing unfit hybrid offspring. Hybridizing females may also gain by the types of direct benefits that are important for intraspecific mate choice, and in addition may have access to certain benefits that are restricted to mating with males of an ecologically diverged sister-taxon. We investigate possible direct benefits and costs female *Ficedula* flycatchers gain from breeding with a heterospecific male, and demonstrate that hybridizing female collared flycatchers (*F. albicollis*) breed in territories that do not suffer the seasonal decline in habitat quality experienced by females breeding with conspecifics. We exclude the hypotheses that heterospecific males provide alternative food-types or assume a greater amount of the parental workload. In fact, the diets of the two species (*F. albicollis* and *F. hypoleuca*) were highly similar, suggesting possible interspecific competition over food resources in sympatry. We discuss the implications of direct fitness effects of hybridization, and why there has been such a disparity in the attention paid to such benefits and costs with regard to intraspecific and interspecific mate-choice.
Introduction

Understanding selective forces causing speciation and the maintenance of species integrity often depends on an accurate estimation of the fitness consequences of hybridization between divergent populations. However, general tests for this, such as measuring fertility in hybrids (e.g. Gallant and Fairbairn 1997; Gooding 1997; Britton-Davidian et al. 2005; Christianson et al. 2005), often ignore other important costs and benefits of hybridization in natural situations. Choosing a genetically compatible mate (i.e. obtaining good genes for the offspring) is not the only role of mate-choice. Direct benefits and costs to choosy females (those that directly enhance or suppress the female's fecundity or lifespan) are widely acknowledged as important selective forces driving the evolution of intraspecific mate-choice (Thornhill 1976; Searcy 1979; Gwynne 1984; Reynolds and Gross 1990; Kirkpatrick and Ryan 1991).

Despite our knowledge of the importance of direct fitness effects for intraspecific mate-choice, our understanding of their role in determining selection for or against hybridization between different species is poor. If favourable resources (e.g. territories, parental care, nuptial gifts) tend to be provided by heterospecific males, females potentially stand to benefit from heterospecific pairing (Goldsworthy et al. 1999; Bronson et al. 2003a). The existence of direct benefits to females of hybridization may have important consequences for both post-zygotic isolation between species (through increased numbers of surviving hybrid offspring), and for prezygotic isolation (through relaxed selection against mating with heterospecifics). Direct costs, on the other hand, may further strengthen reproductive isolation between hybridizing taxa.

Not only may direct benefits widely recognized for intraspecific mate-choice be applicable for interspecific contexts, there are potential direct benefits that are primarily available to hybridizing females. Because of differences in past evolutionary history and character displacement in sympathy, hybridizing species are expected to utilize their environment in different ways, which can have important implications for choosing optimal mates. For example, males of one species may participate to a greater extent in parental duties, reducing costs for females of the 'lazy male'-species to pair with a heterospecific partner. An additional direct benefit for hybridizing individuals could arise if the two species extract different, complementary resources, such as food, from their environment. For the prolonged coexistence of two closely related species, they should typically occupy distinct niches (Gause 1934; Lack 1946; MacArthur and Levins 1964, 1967), and coexisting species might therefore be expected to exploit different food resources. Such niche differences are frequently implicated in post-zygotic isolation between taxa, as intermediate hybrids may be maladapted to either parental niche (Benkman 1993; Grant and Grant 1996; Rundle 2002). However, there are two reasons to expect that niche differences between
parental species may in fact directly benefit hybrids at earlier stages of the life-cycle. First, there is reduced competition over local resources within the territory from heterospecific social partners, such that each parent has access to more of their own resource than when with a conspecific partner. Secondly, niche differences may allow heterospecific parents to provide a wider diversity of food resources to the offspring. These could be important direct benefits of hybridizing, especially during times when the food-types on which one species specialize are limited. An important distinction between these novel ideas and the situation when two species differ in the average quality of their territories is that females of both species, as well as males, obtain such benefits from heterospecific pairing. These benefits may therefore be an important force reducing selection against hybridization. Other direct benefits tend to be unidirectional with respect to the species of the female, and can in fact increase direct costs of hybridization for females of the other species. For example, if males of two species differ in their ability to procure and defend the best territories, females of the subdominant species receive direct benefits from heterospecific pairing, whereas females of the dominant species receive direct costs.

Direct benefits of heterospecific pairing may be especially important in taxa where the genetic costs of hybridization are low, or where females are able to decrease these genetic costs by obtaining extra-pair copulations, which result in a substantial proportion of their offspring being pure (Goldsworthy et al. 1999; Veen et al. 2001). Pied (*Ficedula hypoleuca*) and collared (*F. albicollis*) flycatchers hybridize at a low frequency throughout zones of sympatry in central Europe. However, by having a large proportion of their offspring sired by extrapair, conspecific males (Veen et al. 2001), female collared flycatchers in heterospecific pairs apparently negate some of the indirect costs associated with hybridization (i.e. low fertility of hybrids: Alatalo et al. 1990; Gelter et al. 1992). Furthermore, nests reared by such heterospecific fathers actually fledged more offspring during certain conditions (the food-limited, latter part of the breeding season) than those in nests of collared flycatchers (Veen et al. 2001). Such a pattern suggests either that hybrid offspring experience enhanced survival to fledging through heterosis, which is only evident in stressful environments, or that there are direct benefits for female collared flycatchers associated with breeding with a heterospecific male late in season. The nature of these possible direct benefits is currently unknown, and is the focus of this study.

Studies of collared or pied flycatchers in various locations within their European breeding grounds indicate that the diet of both species varies greatly between regions (see review by Cramp and Perrins 1993 and more recent papers by Moreno et al. 1995; Siikamäki et al. 1998; Eeva et al. 2005) and between habitats within a particular region (Cramp and Perrins 1993). It is therefore difficult to infer differences in the niches of the two species by comparing popu-
lations. One previous study (in the Czech Republic) has investigated the diets of the two species in sympatry (Bureš 1995). Although that study indicated that dietary differences between the two species exist, it did not calculate the overlap in the diets, or relate dietary differences to the success of heterospecific nests. Furthermore, because pied flycatchers tend to be excluded from certain habitats by collared flycatchers (Sætre et al. 1993; Alatalo et al. 1994), subtle dietary differences may derive from differences in local food supply rather than differential usage of a common habitat. In addition, by not comparing variation between individuals within each species with that between species, the Czech study did not test whether significant dietary differences actually exist between pied and collared flycatchers.

In the current study, we investigate the types of direct benefits and costs female flycatchers potentially experience by breeding with a heterospecific male. Specifically, we examine whether heterospecific partners (i) occupy superior territories, or (ii) utilize these territories in different ways that convey direct fitness effects to hybridizing females (e.g. by adopting a greater proportion of the parental workload or extracting different food resources from the environment).

**Material and methods**

**Study system**
Pied and collared flycatchers are insectivorous, migratory passerines. Both species are primarily socially monogamous, and the success of each brood is highly dependent on the parental efforts of both sexes (Alatalo et al. 1981, 1988b; Lubjuhn et al. 2000; Garamszegi et al. 2004), as well as local food supply (Siikamäki 1998). The current study was carried out on the Swedish islands of Gotland and Öland, where intermixed populations of collared and pied flycatchers breed in nest-boxes installed in a large number of forest sites. The study population is monitored throughout the breeding season, by inspecting nests for the date that the first egg is laid, the date the chicks hatch, and by catching both parents to record their identity (all are individually marked) and take morphological measurements (see Pärt and Qvarnström 1997). On Gotland, where pied flycatchers are scarce, breeding data have been gathered since 1981. On Öland, where pied flycatchers are more common (although still a minority), breeding data were gathered since 2002.

**Do females paired with heterospecific males breed in better territories?**
Females can benefit from mixed pairing by choosing males that occupy superior territories. Territory quality is complicated to measure, as it entails aspects such as food availability, nest-site quality, risk of depredation, and the social environ-
ment (number of competitors). One way of summarizing all these aspects of territory quality is by investigating the success of other flycatchers breeding within the territory in other years (e.g. Pärt 1994; Both et al. 2005). We used breeding data from Gotland between 1981 and 2003 for doing such analyses.

We calculated the quality of territories occupied by heterospecific pairs by investigating how other flycatchers (excluding the actual heterospecific pair) performed in the same boxes within 5 years of the heterospecific pair breeding there. Investigating the success of collared flycatchers in the same territories allowed us to differentiate the effects of territory-quality from the benefits or costs of being raised by heterospecific parents. We used the total mass of each brood reared in each box as a measure of territory quality. Not only does total fledgling biomass strongly correlates with the number of fledglings ($R = 0.86$), but it also incorporates mean size of the chicks, a variable linked to local food availability. Analyses using fledgling number as an alternative response variable gave almost identical results. For each box used by a heterospecific pair, we repeated this procedure for a randomly selected box used by a pair of collared flycatchers within the same forest site, in the same year, and with the same laying date. Such a territory was thus possibly available to the choosing female at around the same time as she selected her heterospecific mate/territory. To control for the fact that there were several non-independent measurements of quality for each territory (in different years), territory was included as a random factor in the general linear mixed model. These models tested the null hypothesis that the territories occupied by heterospecific and collared pairs are of equal quality. Because the direct benefits of heterospecific pairing in flycatchers are potentially dependent on the time in the breeding season and on the species of the female (Veen et al. 2001), we included these predictor variables and interaction terms in the mixed models. If territory quality is an important direct benefit behind late-breeding females producing more fledged offspring when they are paired with a heterospecific male, heterospecific pairs should tend to occur in territories of higher quality than those occupied by pairs of collared flycatchers, and this should occur primarily late in the season.

**Are there direct benefits through dietary differences?**
Not only may heterospecific males defend territories of higher quality, they may also confer direct benefits through utilizing these territories in different ways. We explored the hypothesis that by utilizing different feeding niches, heterospecific males provide complimentary food to the brood. For such a hypothesis to be clearly tested, a number of different predictions must be fulfilled. First, the diversity of food offered to broods of heterospecific pairs is expected to be higher than that to nests reared by conspecifics of either species. Secondly, it must be shown that this higher diversity results from differences in the niches of the two species, and not resulting from confounding effects of the environments
in which heterospecific pairs tend to occur. Finally, high dietary diversity should have a positive effect on the success of the brood. We tested each of these predictions in 2004 and 2005 within the *Ficedula* hybrid zone on Öland, where frequencies of rare pair-types (pied and heterospecific) were higher than on Gotland.

To investigate the food brought by each parent, we installed infrared cameras (YOKO model YK-3045B; YOKO Technology Corp., Jhonghe City, Taiwan) into the nest-boxes when the chicks were 8–10 days old. This coincides with the age at which overall feeding rates are highest (Lundberg and Alatalo, 1992) and nutritional demands of the chicks are probably greatest. These cameras were connected to digital video recorders (JVC GR-D30; JVC Europe Ltd., London, UK), which were placed on the ground outside the nest-box. Cameras were installed within the boxes on the day prior to filming to allow parents to get accustomed to the presence of the novel object. Filming was carried out for 1 h, and this was normally replicated on the following day at a different time. Past studies suggest that feeding rates vary little throughout the day (Lundberg and Alatalo 1992; Moreno et al. 1995), and filming was variously carried out between 05:00 and 18:00 hours. Food-types were categorized according to whether they were (i) larval Lepidoptera, (ii) adult Lepidoptera and Diptera and (iii) ants (Hymenoptera: Formicidae) and spiders (Aranae). These three classes of prey are the most numerically dominant taxa in the diet of nestlings, and together constitute 60–95% of the diet, depending on habitat and region (Cramp and Perrins 1993). Furthermore, they incorporate prey types obtained from a range of feeding strategies (i.e. aerial, among foliage and on ground).

Dietary diversity was calculated according to the Shannon Index (H) (see Spellerberg and Fedor 2003), which assigns highest scores (ranging between 0 and 1.098 for three categories) to nests receiving equal numbers of all food types and lowest values if the nest receives only a single type. Only nests receiving 10 or more food deliveries were included in the analysis of dietary diversity to prevent scores of low diversity resulting from few feeding events (two out of 66 nests were excluded). The dietary diversity of heterospecific pairs was compared with conspecific pairs of both species. High dietary diversity of a brood can arise through two mechanisms: (i) the two parents can feed different types of prey, or (ii) individuals may each bring a greater diversity of prey. We tested whether dietary diversity in heterospecific pairs was influenced by differences between the species in their diets. The diet may be influenced by factors other than the foraging niche of the species. Competition among fledglings (hunger) in nests of the two species may vary (see Qvarnström et al. 2005), potentially causing different foraging tactics among parents of the two species. Furthermore, the two species may tend to breed in slightly different habitats. Because we wanted to test whether the two species differentially used a
common environment, the above two possible confounding factors were controlled for. First, analyses of dietary differences were carried out using only individuals in heterospecific pairs (both species are exposed to identical habitats and begging cues). Secondly, we compared nests of pure pairs of pied and collared flycatchers in which we artificially cross-fostered half of each brood between species (for details see Qvarnström et al. 2005). These broods contained roughly equal number of pied and collared nestlings, having the advantage that pied and collared parents were subjected to the same stimuli from nestlings. Differences between the species in their diet (i.e. feeding rates of the three food-categories) were examined using manovas; first, comparing conspecific pairs of either species, and then comparing individuals of either species within heterospecific pairs. Trials in which one parent failed to feed were excluded from analyses. This ensures that all nests where one parent had died, as well as most secondary nests of polygynous males, were not included.

To examine how dietary diversity influenced the success of the brood, we tested its effect on the total mass of chicks produced in the nest (the product of the number of chicks and their average mass). The mass of chicks (to 0.1 g) was measured when they were 13 days old (just prior to fledging) using a Pesola balance. Because time in the season potentially affects both the success of the brood and the positive influence of dietary diversity (H) (Siikamäki 1998), laying date (the date the first egg was laid) and its interaction term with H was included in the multiple regression analysis.

Do heterospecific males assume a greater proportion of the parental workload?
Heterospecific males may not only utilize the territory differently by providing different food-types to the chicks, but may also assume a greater proportion of the parental workload. We tested the relative effort of the two sexes in each type of pair to examine whether heterospecific males assume a larger role in feeding nestlings. In addition to the 66 nests filmed for the dietary analysis, 86 additional nests were observed on Gotland and Öland between 2000 and 2005 when the chicks were 8–10 days old. These observations were made with binoculars from a distance of approximately 20 m from the box, and the number of times that either sex entered the box during a 1-h period was recorded. To test whether certain males carried out a greater proportion of the workload, we applied generalized linear models (logit function) to examine the effect of species and pair-type (heterospecific vs. conspecific) on the number of feeds by the male, relative to the total number by both parents.
Results

Do females in heterospecific pairs gain superior territories?
We tested if female flycatchers gain direct benefits from pairing with heterospecific males with better territories by investigating nest-boxes occupied by pairs of collared flycatchers within 5 years of it being used by a heterospecific pair. There were a total of 181 territories on Gotland that had been occupied by heterospecific pairs (97 with a collared male and 84 with a pied male) once during the 22 years of study. Of these, 172 territories had also been used by a collared pair within 5 years of the heterospecific pair (93 of the heterospecific pairs with a collared male and 79 with a pied male). These nest-boxes were occupied by collared flycatchers an average of 2.45 times during the preceding and subsequent 5 years. Data on the mass of chicks was not available for all nests, and 161 territories of heterospecific pairs were included in the analysis of territory quality (85 with a collared male and 76 with a pied male). Data on the masses of chicks reared in these territories were available for a mean of 1.83 years per territory.

We used general linear mixed models to test whether territories differed in their quality (total mass of chicks produced), by examining the success of broods of collared flycatchers reared there in other years. Territories of female collared flycatchers with a male pied flycatcher differed from those of female collared flycatchers paired to conspecific males by not suffering from the same decline in quality during the season (significant three-way interaction in table 3.1 is depicted in figure 3.1). This interaction is a result of fitting all data across the entire season, and thus may be induced by high quality collared territories early in the season, rather than direct benefits of hybridizing late in the season. To test the more specific hypothesis that heterospecific pairs inhabit significantly better territories late in the season, we fitted a general linear mixed model (territory as a random factor) to half of our data (those nests with a laying date later than the median) on the territories of heterospecific pairs with male pied flycatchers, and their control territories. This model indicated that heterospecific territories were significantly better quality than control territories ($F_{1,39} = 4.17$, $p = 0.048$). Thus, in the latter half of the breeding season, territories that had once been used by heterospecific pairs with a male pied flycatcher were of higher quality than those that had been used by a control pair of collared flycatchers. The rarity of pure pairs of pied flycatchers on Gotland meant that it was not possible to do a complimentary test to see if female pied flycatchers that hybridize end up in territories of better or worse quality than pied females that breed with conspecific males. However, if collared flycatchers are used as controls, there is no suggestion that such heterospecific pairs (with a female pied flycatcher) breed in more stable environments (figure 3.1). Unlike female collared flycatchers in heterospecific pairs, pied females that paired with a
heterospecific male suffered a decline in territory quality through the season similar to that of pure collared flycatcher pairs (figure 3.1).

To elucidate whether this reduced decline in territory quality reflects low competition over food from neighbouring pairs, we compared the numbers of neighbouring pairs of flycatchers surrounding heterospecific pairs with male pied flycatchers and their control collared pairs. ‘Neighbours’ occupied a box adjacent to the focal nest. Because the boxes were roughly distributed in a grid pattern, the maximum number of possible neighbours was eight, although this was observed only once. The data were analysed using a general linear model (Poisson distribution) with ‘territory type’ (heterospecific or collared) as the sole fixed effect, and ‘number of neighbours’ as the response variable. Heterospecific pairs with male pied flycatchers had fewer neighbours (mean = 1.67, n = 83) than collared pairs (mean = 2.27, n = 83) (GLM, $\chi^2_1 = 4.90$, $p = 0.027$). The sample size was slightly less than expected from the numbers of heterospecific pairs reported above, because information was lacking about the nest-box arrangement in a previously managed forest site containing one heterospecific pair.

<table>
<thead>
<tr>
<th>Factor</th>
<th>d.f.</th>
<th>F</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Laying date* (A)</td>
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<td>21.05</td>
<td>&lt; 0.0001</td>
</tr>
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<td>Territory type† (B)</td>
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<tr>
<td>Type of heterospecific pair‡ (C)</td>
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<tr>
<td>B x C</td>
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<td>0.779</td>
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<td>Territory [random effect]</td>
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</tr>
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</table>

*Depending on year, breeding is earlier or later with regards to mean laying date. We therefore used residuals from the mean laying date of the year.
†Territory type refers to whether the territory had been occupied by a heterospecific pair or a control pair of collared flycatchers.
‡Type of heterospecific pair refers to whether the heterospecific pair involved a female collared or pied flycatcher. Collared pairs were assigned according to which type of heterospecific pair they were a control for.
§The three-way interaction between laying date, territory type and type of heterospecific pair on territory quality are presented in figure 3.1.

Table 3.1. General linear mixed model testing the factors affecting territory quality, as estimated by the total mass of each brood of collared flycatchers produced there.
Are there direct benefits through dietary differences?

The three categories of prey recorded in this study comprised 90.6% of the feeding visits by collared flycatchers and 89.4% of the visits by pied flycatchers. In accordance with predictions of the hypothesis that chicks reared by heterospecific parents gain access to a broader range of prey, the diversity of food-types brought to heterospecific nests was greater than to nests reared by conspecific pairs of either species (anova, F2,61 = 6.399, p = 0.003; see figure 3.2). However, these differences in dietary diversity were not induced by heterospecific parents bringing complimentary food-types. In fact, we found no significant differences between the diets of the two species (see table 3.2, figure 3.3). Pianka's (1973) index of dietary overlap was very high, regardless if this was calculated by comparing individuals in pure pairs (98.7% overlap) or when controlling for environment by only using individuals in heterospecific pairs (98.2% overlap). This compares with 89.4% dietary overlap in the Czech Republic, which we calculated from the raw data published by Bureš (1995),
which were collected by preventing chicks from swallowing. When comparing the diets of pure pairs of either species rearing mixed broods, the two species significantly differed in how their diets changed during the season (see table 3.2). Additional manovas for each species separately indicated that the significant interaction term in manova 1 in table 3.2 reflects the pattern that collared flycatchers increased their feeding rates of all food types during the season (correlation coefficients for laying date and feeding rate of Lepidopteran larvae, adult Diptera/Lepidoptera, and Formicidae/Araneae, were 0.42, 0.77 and 0.19). Pied flycatchers, however, reduced their feeding rates of larval Lepidoptera and Formicidae/Araneae during the season (correlation coefficients for laying date and feeding rate of Lepidopteran larvae, adult Diptera/Lepidoptera, and Formicidae/Araneae, were –0.343, 0.521 and –0.074). When individuals in heterospecific pairs were compared (the two species feeding in identical habitat), no such interaction between the effect of species and laying date on diet was observed (table 3.2). Unfortunately, manova does not allow for a paired analysis, taking territory into account. However, if the principle components are used as the ‘diet’ response variable in order to do a paired analysis, the

Figure 3.2. Panel A shows the mean diversity of food brought to nests reared by collared flycatchers, heterospecific pairs, and pied flycatchers. Panel B shows the diversity of food delivered by each individual parent within each pair. The dietary diversity of one individual per pair was used to calculate means and standard errors, although the statistics presented in the text refer to a nested anova using all data. For selecting one individual per pair, we chose to calculate the mean dietary diversity brought by males (open triangles) and females (closed triangles) separately. Sample sizes refer to number of nests (A) and number of individuals (B). Nests (A) or individuals (B) for which there were less than 10 food items recorded are not included in the analysis. This accounts for the differences in sample sizes shown in the two panels. The diversity of food was calculated using the Shannon Index.

![Figure 3.2](image-url)
Table 3.2. Results of manovas testing if collared and pied flycatchers differed in their diet (i.e. feeding rates of Lepidopteran larvae, adult Diptera/Lepidoptera and Formicidae/Araneae). manova 1 tested whether different diets were delivered to mixed nests (partially cross-fostered broods) reared by pure pairs of either species (combining data from the male and female). manova 2 compared individuals of the two species that were part of a heterospecific pair.

<table>
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<th>Factor</th>
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<th>F</th>
<th>p</th>
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<tbody>
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<td>(1) Comparing pure pairs</td>
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<tr>
<td>Species (A)</td>
<td>3,42</td>
<td>1.573</td>
<td>0.210</td>
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<td>Laying date (B)*</td>
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<tr>
<td>A x B</td>
<td>3,42</td>
<td>3.134</td>
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<tr>
<td>(2) Comparing individuals in heterospecific pairs</td>
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<td></td>
</tr>
<tr>
<td>Species (A)</td>
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<td>0.804</td>
</tr>
<tr>
<td>Laying date (B)*</td>
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<td>0.102</td>
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<tr>
<td>A x B</td>
<td>3,16</td>
<td>0.168</td>
<td>0.916</td>
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*Depending on year, breeding is earlier or later with regards to mean laying date. We therefore used residuals from the mean laying date of the year.

Figure 3.3. Principle components analysis illustrating the high degree of overlap between the diets of collared and pied flycatchers. All adults from heterospecific pairs and the swapped nests of pure pairs are included (n = 63 collared flycatchers and 69 pied flycatchers). The first two principle components explained 74.1% of the variation in the food types given to their chicks. Factor loadings for PC1 and PC2 were 0.551, 0.518, 0.654 and −0.661, 0.749, −0.036, respectively for Lepidoptera larvae, Formicidae/Araneae and adult Lepidoptera/Diptera.
two species do not significantly differ in their diet (PC1: $t_{10} = 0.383$, $p = 0.71$; PC2: $t_{10} = 0.826$, $p = 0.43$). Furthermore, the difference between the species in their principle component scores do not change throughout the season (PC1: $t_{10} = –0.46$, $p = 0.657$; PC2: $t_{10} = –0.56$, $p = 0.586$), indicating that the differences between the species in how their diet changes through the season that were observed when comparing pure pairs could not be detected among individuals within heterospecific pairs.

Instead of being caused by niche differences between the two species, the greater dietary diversity of hybrid broods resulted from the fact that individual parents in heterospecific pairs had higher dietary diversity than parents in conspecific pairs of either species (figure 3.2). 'Pair' nested within 'pair-type' was a significant predictor of dietary diversity (table 3.3), meaning that individuals within a certain pair tend to have similar dietary diversity. With this nested effect in an anova, ‘pair-type’ remained a highly significant predictor of dietary diversity (table 3.3).

Many heterospecific pairs of flycatchers involve 1-year-old males (Wiley et al. 2005). However, higher dietary diversity among hybridizing individuals was not a result of them being inexperienced (perhaps unspecialized) foragers. When age of the individual (1 year old vs. older) was included as a covariate in the nested anova, it did not account for a significant amount of variance in dietary diversity (table 3.3).

We found no evidence of any biological significance of high dietary diversity within the nests sampled. After controlling for pair-type (collared, pied and heterospecific) and lay-date, dietary diversity was not a significant predictor of the total mass of chicks successfully reared in the nest ($F_{1,48} = 0.41$, $p = 0.523$). As the previously reported direct benefits of heterospecific pairing are only apparent late in the season, we included the interaction-term between H and lay-date in the model. However, the effect of having high H on the success of the brood did not change during the season (interaction, $F_{1,47} = 0.49$, $p = 0.486$).

### Table 3.3

<table>
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<th>Factor</th>
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<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair [pair-type]</td>
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<td>0.033</td>
</tr>
<tr>
<td>Pair-type*</td>
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</tr>
<tr>
<td>Age-class†</td>
<td>1, 90</td>
<td>0.309</td>
<td>0.582</td>
</tr>
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</table>

*Pair-type refers to whether the pair was collared, pied or heterospecific.
†Age-class refers to whether the parent was 1-year-old or older. The age of five parents was unknown, accounting for the lower degrees of freedom than would be expected from figure 3.3.
Do heterospecific males assume a greater proportion of the parental workload?

We tested whether heterospecific males assumed a greater proportion of the total feeding rate of each nest by fitting generalized linear models (logit function). Males of the two species did not differ in the relative amount of the workload they carried out ($t_{151} = 0.037$, $p = 0.971$). Furthermore, males of neither species performed a greater or smaller proportion of the workload when they were paired with a heterospecific female than with a conspecific female ($t_{151} = 1.08$, $p = 0.282$).

**Discussion**

This study shows that direct benefits to hybridizing females are a likely reason behind previously reported patterns that heterospecific pairs involving female collared flycatchers successfully rear more chicks than collared pairs during food-limited conditions late in the breeding season (Veen et al. 2001). Our data suggest that the nature of these direct benefits stems from the fact that female collared flycatchers breed in territories that are more stable against the seasonal decline in food supply when paired with a pied male than when with a conspecific male. These direct benefits of heterospecific pairing were not a result of breeding with a partner that is differently able to utilize the territory. Heterospecific males neither carried out a greater proportion of the parental workload, nor provided food-types outside the dietary width of females. In fact, the two species fed highly similar diets to their chicks. What is currently unclear is whether or not the direct benefits to female collared flycatchers reported here outweigh the indirect costs of producing hybrid flycatcher offspring with low fertility. On average, across the season, this is unlikely. This is because while late-breeding collared females experience direct benefits, early breeders actually experience direct costs (lower territory quality than conspecifically paired females). The lack of an effect of ‘territory type’ or an interaction between ‘territory type’ and ‘type of heterospecific pair’ on territory quality (see table 3.1) suggest that on average across all laying dates, there are no direct benefits to collared females of heterospecific pairing. Our study, however, suggests a reason why heterospecific pairing may be adaptive for female collared flycatchers at specific times in the breeding season (Veen et al. 2001).

Our estimate of niche overlap is almost certainly an overestimate of the similarity of the diets of the two species. This is because there may be differences in the species of prey taken (this study was at the level of Order) or the foraging locations. However, there are several reasons to suspect that the two species of flycatchers do indeed have highly overlapping feeding niches. First, a past study on the foraging tactics (feeding heights and strategies) of the two species on
Gotland also suggested a high degree of similarity (Pianka's index = 91%) (Alerstam et al. 1978). This suggests that the two species not only focus on the same Orders of prey, but also collect these from similar locations. Secondly, our analysis of detailed, previously reported data (Bures 1995) collected in the Czech hybrid zone also revealed a high degree of niche overlap (89.4%). This figure may actually overestimate niche differences as differences in the habitat of the two species were not controlled for, and results from the current study suggest that once environment is controlled for, by comparing individuals in heterospecific pairs, dietary differences may cease to exist. Dietary differences because of differing habitats are by no means evolutionarily inconsequential, but they do not confer direct benefits to females pairing with a heterospecific male. Such benefits only arise if such males utilize the territory in a complimentary way. Overall, current evidence suggests that the two species of flycatchers have highly overlapping dietary niches, and interspecific competition over resources is a likely cause of previously reported patterns that populations of either species are highly regulated by densities of the other species (Sætre et al. 1999). However, directly inferring competition from niche overlap should always be done with caution. Highly overlapping diets indicate that chicks do not benefit more from receiving food from heterospecific parents than from conspecific parents. It does, however, also imply that there is unlikely to be extrinsic, post-zygotic isolation operating between the two species through hybrids with intermediate dietary niches being unable to utilize either parental niche.

In spite of a lack of marked dietary differences between the two species, offspring reared by heterospecific parents received a greater diversity of food types. This study failed to detect an adaptive significance of this higher diversity, although it is possible that such benefits are only expressed at certain times, such as when the optimal prey type is limiting. The fact that this pattern arises through both individuals in heterospecific pairs bringing more diverse diets supports the idea that heterospecific pairs settle in different types of habitat than pure pairs typically do.

The importance of direct benefits when pairing with heterospecific mates with complementary parental care is likely to be greatest in taxa that have undergone substantial divergence in niche utilization prior to secondary contact. Although this may not be the case in pied and collared flycatchers, this novel hypothesis may be important in other systems where niche divergence has occurred, bi-parental care is important, and food availability limits breeding success. Investigations of such systems will reveal how widespread such direct benefits may be.

In the Swedish flycatcher hybrid zone, the greatest direct benefits to late-breeding females in heterospecific pairs appear to arise not through differences between the species in parental care (amount and type of food), but rather
through differences in the breeding territories of female collared flycatchers paired with conspecific vs. heterospecific males. Territory quality is an important cue used by female flycatchers to select partners (Alatalo et al. 1986). Heterospecific pairs with male pied flycatchers occupied territories that were more stable against the seasonal decline in habitat quality observed in territories occupied by control pairs of collared flycatchers. In flycatchers, low reproductive success of later nests is known to be a result of declining food supplies, rather than a confounding effect of later breeding birds being poorer parents or occupying lower quality territories (Wiggins et al. 1994; Siikamäki 1998). That heterospecific pairs with male pied flycatchers occupy territories that are superior to those of collared flycatchers late in the season appears to be the underlying explanation for the previous finding that hybridizing female collared flycatchers produce more fledglings late in the season than pairs of collared flycatchers (Veen et al. 2001). However, we cannot exclude the possibility that other, untested, direct benefits of hybridization (e.g. predator defence) may also exist.

The reason why heterospecific pairs occupy territories that are more stable against this seasonal decline in food supply is unclear. This may reflect differences in the vegetation community, and associated peaks in insect abundance, in territories normally defended by males of the two species. Such a situation would arise if males of the two species have differing habitat preferences. If such a scenario exists, this suggests that the habitat preferences of either species are only adaptive during specific times in the breeding season, and are maladaptive at other times. An alternative explanation for the differences in territory stability during the breeding season might be that these reflect differences in social environment. For example, female collared flycatchers may only pair with male pied flycatchers when overall densities of birds are low and conspecific mates are unavailable. In such sites, the seasonal decline in the availability of food could be cushioned by low levels of competition with neighbouring pairs. As a result, the direct benefits of heterospecific pairing later in the season would be an incidental by-product of being paired with an undesired mate (i.e. one that is only chosen in habitats with low densities of competitors). The finding that heterospecific pairs tend to have fewer neighbours is consistent with both of these hypotheses, and it is currently unknown which of these two scenarios is the primary explanation for the patterns in flycatchers.

In flycatchers, as in the few other hybrid zones for which data exist (Goldsworthy et al. 1999; Bronson et al. 2003a), direct benefits of hybridization are asymmetrical. Although females of one species may receive direct benefits from heterospecific pairing, their partners do not, and, often, neither do females of the other species. It has long been recognized that costs of hybridization are not equal for males and females, due to differences between the sexes in their investment into each unfit hybrid offspring, and opportunities for re-mating
Asymmetrical direct benefits of heterospecific pairing are yet another reason why selection for species recognition may differ in strength between males and females.

It has been suggested that species recognition and choosing conspecific mates of the highest quality are not distinct processes, but are merely opposing ends of a continuum of mate-choice (West-Eberhard 1983; Ryan and Rand 1993; Boake et al. 1997; Phelps et al. 2006). It may therefore be surprising given the amount of attention that has been devoted to direct benefits of intraspecific mate choice that so little is known regarding the role of direct benefits and costs in hybridization. One clear distinction between the two contexts is that the genetic (indirect) costs of hybridization are frequently high compared with variation in genetic quality of offspring resulting from intraspecific mating. In flycatchers, female hybrids are sterile and male hybrids also have lower fitness than the parental species (Alatalo et al. 1990; Gelter et al. 1992). As in other hybrid zones where there is strong genetic incompatibility between the hybridizing taxa, direct benefits of having heterospecific mates are unlikely to supersede the indirect costs of producing unfit hybrid offspring, unless these costs can be reduced through means such as extra-pair copulations (Veen et al. 2001). However, this does not mean such benefits should be ignored. By countering some of the indirect costs of hybridization arising through genetic incompatibility, direct benefits may relax selection for species recognition. Furthermore, there may be certain hybrid zones (e.g. between species at early stages of divergence) where such direct benefits for choosing heterospecific partners over-ride the indirect costs of producing hybrid offspring. In such situations, direct benefits would be an important factor hindering reproductive isolation in sympathy. Although it is unlikely that direct benefits are the reason for hybridization by flycatchers, they are sometimes a consequence of it, and this may influence selection for/against hybridization in nature.

Acknowledgements
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Habitat segregation and seasonal patterns of food abundance in two regionally coexisting congeneric passerines

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Abstract

In nature, many species appear to share the same environment and utilize similar resources. This is surprising because based on the principle of competitive exclusion one would predict that the superior competitor would drive the other species to extinction. Interspecific competition is frequently reduced by differential habitat use, which results in spatial segregation. Here we use the closely related collared (Ficedula albicollis) and pied flycatcher (F. hypoleuca) to assess if habitat differences might have another effect, namely on the temporal food availability for the two species. We find that tree species composition of the breeding territories of the two species showed small, but consistent differences. Collared flycatchers occupied nest-boxes in hazel-rich habitats, while pied flycatchers bred in those surrounded by more pine trees. Furthermore, it turned out that the temporal distribution of a major food source for nestlings (caterpillars, as measured by frass-fall) differed markedly between the four deciduous and two coniferous tree species. The deciduous tree species showed an early and narrow peak in abundance, which contrasted with the steady increase in caterpillar abundance in the coniferous tree species through the season. The predicted relative food abundance for a typical territory of both flycatcher species showed great similarities between the two species. The most marked difference is at the end of the season where pied flycatcher territories had higher food abundance which was predicted to coincide with peak food demand of the nestlings. We conclude that habitat differences likely influence the temporal abundance of food for the two species especially late in the breeding season. More generally, small differences in habitat between coexisting species may have several, mutually reinforcing effects which reduce the effects of interspecific competition and thereby facilitate coexistence.
Introduction

A striking pattern in nature is the apparent coexistence of ecologically similar species. The principle of competitive exclusion would predict that differences in competitive ability, even if they are relatively small, should lead to the extinction of the less competitive species (Hardin 1960). This appears not to be the case in many natural systems and various different explanations have been put forward of which niche differentiation is one particular influential one (e.g. Chesson 2000; Wright 2002). The basic idea is that different species specialise on, for example, different food resources and thereby decrease the strength of interspecific competition. The observed differences are often rather small and appear at first sight unlikely to reduce the interspecific competition to such an extent that competitive exclusion is avoided altogether (e.g. Chesson 2000 and references therein). One explanation for this pattern, and the focus of the present study, is that the observed differences between the species have not only one, but several different (small) effects which reduce interspecific competition.

Habitat specialisation is frequent among ecologically similar bird species (e.g. Cody 1978; Forstmeier et al. 2001; Hudman and Chandler 2002). Differences in habitat occupation are predicted to decrease the frequency of interactions between heterospecific individuals and thereby reduce competition intensity. A second, but much less well studied, effect of habitat differences is the potential influence it could have on the temporal availability of food in the different habitats. Food availability is known to be an important factor influencing reproductive success, and birds typically match periods of high energetic demands of the nestlings with those of greatest food availability (Perrins 1970). The aim of this study is to investigate how habitat differences between two closely related passerines affect the temporal food abundance in their breeding territories and to explore the consequences this has on their coexistence.

The closely related collared (Ficedula albicollis) and pied flycatchers (F. hypoleuca) are a representative example of an ecologically similar species pair with overlapping breeding ranges. In the Czech Republic, previous work showed that the collared flycatcher is competitively dominant and the pied flycatcher is forced through interspecific competition to (potentially) less favourable breeding locations at higher altitudes (Sætre et al. 1999a; Sætre et al. 1999b). A recent study however indicates that the differences in habitat occupation might be (partly) explained by differences in habitat preferences between the two species ( contra Alerstam et al. 1978; Lundberg and Alatalo 1992; Adamik and Bureš 2007). Regardless of how it arises, differences in habitat occupation results in (local) spatial segregation between the species. An important question to be addressed is how the pied flycatcher can persist in such, especially for the collared flycatcher, unfavourable environment. The pied flycatcher is predicted to be better adapted to this harsher and more coniferous environment as it is
similar to large parts of the allopatric breeding environment (Sætre et al. 1999a). Such adaptation could be so strong that the pied flycatcher would actually prefer this habitat type (Adamik and Bureš 2007), but it remains unsure if this is the case as several studies found deciduous trees to be a considerably more rich food resource (see below) and Adamik and Bureš found higher prey catching rates in a deciduous tree species (oak).

Finding food is of crucial importance for survival and reproduction, and pied flycatchers might be better adapted in doing so in their native environments. Specialisation on different food resources might be such an adaptation, but unlikely to be very important for these species as their diets are very similar (Bureš 1995; Wiley et al. 2007). Not only food type, but also food availability is important. It remains untested if habitat differences result in differences in food abundance between breeding territories of the two species. If such effect benefits the competitively subordinate species (pied flycatcher), it could counteract the negative fitness effects of interspecific competition on reproductive success, and hereby facilitate coexistence.

In this study we test this hypothesis by linking food availability with habitat characteristics for the sympatric breeding population of collared and pied flycatchers on the Baltic Islands of Gotland and Öland (Sweden). The abundance through the breeding season of a major food resource (caterpillars) is predicted to differ between deciduous and coniferous trees (van Balen 1973). This together with the habitat differences found between the species (Alerstam et al. 1978; Sætre et al. 1999a) predicts a temporal differences in food availability in territories for the two species. The potential importance of breeding territory characteristics on reproductive success has been suggested by previous studies. Veen et al. (2001) found that the reproductive success of broods with a male pied flycatcher parent was higher late in the season compared to pure collared flycatcher pairs. A recent study showed that this can, at least partly, be attributed to differences in territory characteristics (Wiley et al. 2007). As in this study we use data from both Gotland and Öland, we will first validate if the differences in reproductive success between the two species found on Gotland is also present on Öland. Then, we set out to corroborate the previously described habitat differences on the Baltic Islands (Alerstam et al. 1978) with a finer-grained habitat classification method. Third, caterpillar abundance was recorded under six dominant tree species by measuring frass-fall. These two measures were then combined to predict the seasonal changes in food availability in an average territory of the two species.
Methods

Study species
Collared and pied flycatchers are migratory passerine bird species. The breeding range of the collared flycatcher covers south-eastern Europe and extends into Ukraine and Russia, whereas the pied flycatcher occupies most of western and northern Europe and extends further north into Russia (Cramp and Perrins 1993). The breeding ranges of the two species overlap in central Europe and on the Baltic islands of Gotland and Ōland. We used flycatcher populations breeding in nest-boxes on Gotland and on Ōland. At these locations, altitudinal gradients are effectively absent. During the breeding season standard reproductive data and parental identities were recorded for all breeding pairs as part of ongoing long-term study. In cases of identification uncertainty, the individual’s identity was checked using species specific differences in single nucleotide polymorphisms (SNPs), as described elsewhere (Sætre et al. 2003; Veen et al. unpublished).

Reproductive success on Ōland
In order to elucidate the role of territory characteristics in causing differences in reproductive success between the two flycatcher species through the breeding season as (Veen et al. 2001), we first need to establish if this pattern is present on both islands. On Gotland, the number of fledged young between pure collared flycatchers and mixed-species pairs consisting of a male pied and female collared flycatcher was plotted against the laying date (relative to the mean laying date) (Veen et al. 2001). We will do the same for Ōland using all available data from the period 2002-2007. Only pure species pairs were used because the territory characteristics of only a few mixed-species pairs were measured (see below). The mean clutch size of the two species differs slightly (6.1 and 6.3 eggs for collared and pied flycatchers, respectively) but this has been accounted for by adjusting the clutch size of the pied flycatcher broods. The laying dates are relative to the mean laying date of all breeding pairs combined (both species) and calculated for each year separately. A second order polynomial is fitted through the data for each species separately to visualise the relation between reproductive success and laying date.

Habitat measurements
Measuring the important habitat characteristics of a flycatcher’s breeding territory is not straightforward, as this should be done according to how an individual flycatcher utilises its surroundings, which is little known. One method used is to measure the vegetation in a circle around the point of interest (e.g. Hudman and Chandler 2002), which assumes that this area accurately reflects
the feeding habitat. We believe this method is insufficient, as it neglects important but distant food resources (e.g. a large oak tree). An alternative method, which emphasizes the habitat characteristics in the near vicinity while including important more distant, features is more appropriate. The ‘angle count’ method used in forestry (and also for ecological questions e.g. Edwards and Collopy 1988) uses the relative size of the tree trunks assessed from a point in the woodland (in our case the nest-box). Inclusion of a tree in the count depends on the trunk size of the tree and its proximity to the sampling point (here the nest-box), i.e. at a certain distance, small trees will not be included but larger ones will be. We adopted this method and the measurements were made using a ‘relaskop’, which is a measuring scale kept at a fixed distance from the eye. While standing next to the nest-box, each individual tree was classified, based on the size of the trunk relative to the scale, into one of three categories and assigned a value accordingly; (1) ‘too small’ (value 0), (2) medium sized (value 0.5) and (3) large (value 1). For each flycatcher territory measured, the values for all individual trees belonging to the same tree species were summed to give a measure of the total ‘volume’ (hereafter ‘relaskopic volume’). Most of our study sites were in relatively open woodlands and in the few cases where understorey vegetation blocked the view, the size of the obscured trunks were assessed by measuring the tree from a different angle while keeping the same distance. A prerequisite for a good estimator of caterpillar abundance is that it needs to reflect the quantity of leaves (the food resource for caterpillars), which is the case for the ‘relaskopic volume’ as the volume of tree trunks is highly correlated with the crown volume (Verner and Larson 1989).

The breeding territories to be included in the habitat measurements were selected in matched species pairs, comprising a collared flycatcher breeding pair and a pied flycatcher breeding pair. The division of the breeding pairs was based on the male species of the pair, as males are believed to arrive before the females on the breeding grounds and choose the territory. Because the pied flycatcher was the less numerous species, in practise this meant that a male pied flycatcher breeding pair was matched with a male collared flycatcher breeding pair with the same egg laying date. The matching took place in two different ways: 1) pairs were matched within the same woodland which ranged in size from 2 to 200 hectare (hereafter referred to as ‘woodland scale’) and 2) within the same island (hereafter ‘island scale’). Within the same woodland, 13 pairs on Öland and one pair on Gotland were matched. An additional 22 and one (Öland and Gotland respectively) were added by matching pairs within the same island. Using the two geographical scales allows us to get a better understanding of the scale at which habitat separation acts.

Among the 74 breeding territories sampled (37 matched species pairs), there were four territories of mixed-species pairs (one male pied flycatcher mixed-species pair on Öland, and two and one collared male mixed-species pair on
Öland and Gotland respectively). If the mixed pairs would affect the results at all, we predict it to make the results more conservative as previous work showed that females include habitat characteristics in their mate choice decisions (Alatalo et al. 1986) which would increase the likelihood that e.g. a female pied flycatcher chooses a male collared with a ‘pied-like’ habitat.

Analysing differences in habitat composition is not trivial as the dataset is a mixture of both presence/absence data and for the ‘presence’ data a unimodal distribution of recorded relaskopic volumes. We choose to transform the relaskopic volume data to presence/absence data and use a logistic regression (binomial error distributions and logit link function) to see if territory occupation by either flycatcher species (the dependent variable) could be explained by tree species composition (tree species, included as factors). Non-significant variables were excluded from the full model in a backwards elimination process to get the final model. The values for each variable (presented in table 1) were calculated by adding the variables individually to the final significant model. An interaction term island x tree species should ideally be included in the analysis to test for differences in territory composition between the species on the different locations, but this was not possible due to the many tree species (11) making the model over-complex. The sample sizes are so low for Gotland that even if it were possible, it would be unlikely to be very informative. The logistic regression could only be performed at the island level, as the data structure at woodland level was such that the models violated assumptions of the software we used. The comparison between woodland and island scale is therefore done without formal statistical testing.

The results of the presence/absence analysis should be treated with care: finding no differences in occurrence does not mean that the abundance of the tree species is similar and this is an important factor for food availability. To investigate such effects, we check, for each tree species, whether the mean relaskopic volumes differ between the two flycatcher species. The means were calculated using only those territories in which the tree species was recorded (so not the summed volume divided by the total number of territories)

**Food abundance**

Caterpillars are one of the main food items fed to nestlings in both species (Bureš 1995; Wiley et al. 2007). The phenology of caterpillar abundance was measured indirectly by collecting their faecal pellets (‘frass’) in so-called ‘frass nets’ (see plate 4C in chapter 1 of this thesis). A frass net was positioned underneath a tree, roughly 1m from the trunk, and consisted of a 0.25 m² funnel shaped piece of ‘cheese cloth’ mounted on a metal frame (Visser et al. 2006). In 2002, 2003 and 2004, 19 frass traps were placed on Gotland under the same individual trees. The traps were placed in two predominantly deciduous woodlands under four deciduous (‘sp’ refers to the unknown species name; 7x oaks
(Quercus sp), 2x birches (Betula pendula), 4x hazels (Corylus avellana) and 1x ash (Fraxinus excelsior) and two coniferous tree species (2x pines (Pinus sp) and 3x spruces (Picea sp)). These temporal patterns of caterpillar abundance are probably also representative of Öland, given its close proximity (approximately 50 km apart), and similar habitat composition (Alerstam et al. 1978). The greater amount of habitat sampling carried out on Öland reflects the higher abundance of pied flycatchers breeding there.

The tree species and the number of individuals sampled of each species were chosen at the start of the study in such a way to as closely as possible reflect the natural abundance of tree species in the study areas. The trees were selected at two different sites (approximately 5 km apart) and within each site, individual trees were selected in close proximity of each other (less than 50 m) to allow for easy access (e.g. in case of rain, see below). Frass traps were placed early in the season, the sampling started before bud burst, and the traps were emptied every 4th day for 7 weeks unless the weather conditions did not allow this. The time of sampling was recorded in days and day 1 is the 1st of May and numbered consecutively afterwards (hereafter ‘May days’). Both rain and temperature could influence the (measured) production of frass (Tinbergen and Dietz 1994). Rain can disintegrate the frass, but this effect was reduced by covering the traps during heavy rain. The effect of temperature could not be accounted for as the correlation between temperature and caterpillar frass production is unknown for this location. Both the effect of rain and temperature are predicted to be similar for all traps due to the close proximity of the samplings sites. Samples were dried indoors and stored at room temperature. The frass was separated from litter using Retsch test sieves (smallest sieve 600 µm) and weighed with an accuracy of 0.1 milligram. For each trap separately, the frass weight of each sampling event (normally 10 per season) was divided by the duration of the sampling period (normally four days), to get frass weight per trap per day. Because this is an average over the sampling period, we used the midpoint between two subsequent checks as the sampling date (hereafter ‘mid-sample date’) in the analyses.

The temporal variation of frass weight of the deciduous tree species had a unimodal distribution (figure 4.2A) and could be approximated by a normal distribution. The frass distribution of some traps showed large fluctuations, mostly likely due to random disturbance of the frass trap (e.g. the cloth came off). To account for such random effects, fitted curves with a R² of less than 0.8 were excluded from further analyses (one hazel in 2002, 2003 and 2004). After visual inspection of the fitted curves, one birch from 2002 was excluded as well. Although the R² of this curve was relatively high (0.82), the predicted peak value was more than four times the recorded maximum weight. For the remaining 38 frass distributions, the date and height of the peak frass weight were calculated using the fitted Gaussian distributions. The width of the frass
distribution can be used as a proxy for the duration of food availability and this measure is here conveniently captured by the standard deviation of the Gaussian curve.

The coniferous species (spruce and pine) were predicted to differ from the deciduous species by having a much later peak date (van Balen 1973). Our sampling period was not long enough to capture the declining part of the frass curve properly and for this reason fitting a Gaussian curve through the distribution was not very successful; only 6 out of the 9 spruce and 1 out of 6 pine frass distribution met the $R^2 > 0.8$ criteria. Furthermore, some of the estimates were obviously biologically unrealistic (e.g. a standard deviation of $>1300$) and we therefore choose to not use the curve fitting approach for the coniferous species. Instead, we included the frass distributions of all sampled trees and used the highest recorded weight as an estimate for the peak weight. Statistical analyses were performed using Statistica 7.0 and Sigmaplot 10.0.

Results

Reproductive success Öland
The fledging success of 545 collared flycatcher and 79 pied flycatcher pairs was included in the comparison of reproductive success (figure 4.1). The fitted curves show a very similar pattern as found on Gotland by Veen et al. (2001): the collared flycatchers showed a steady decline in number of fledged young through the season whereas the reproductive success of pied flycatchers showed an increase over a large part of the breeding season. Unfortunately, the database of Öland is too recent to do the territory comparison as conducted by Wiley et al. (2007). The data presented here show the great similarity in temporal patterns of reproductive success between the islands. Accordingly combining data from both locations is argued to be a reasonable approach.

Habitat differences
16 tree species could be identified down to species level, but other trees could not be and were only assigned to a genus ($Alnus$ sp, $Populus$ sp, $Sorbus$ sp, and $Ulmus$ sp). This could potentially bias the influence of certain tree species or genera (e.g. hard to identify species were all recorded as $Genus$ sp instead of several species), and we therefore pooled the data on the level of the genus. Throughout this paper we use the term ‘tree species’ for both such ‘genus groups’ as well as single tree species. Genera in which only one tree species was recorded are referred to by their common name (e.g. oak). Tree species with an occurrence of less than 1% of the total relaskopic volume were amalgamated into one category (termed ‘other’). This category consisted of the following species: beech ($Fagus sylvatica$), common juniper ($Juniperus communis$), lime
tree (*Tilia platyphyllos*), Norway maple (*Acer platanoides*), wild apple (*Malus sylvestris*) and ‘unidentified trees’.

The mean relaskopic volume of each tree species for pied and collared flycatcher territories have been summarised in table 4.1. The logistic regression showed that the occurrence (presence/absence) of tree species differs significantly between territories of the two flycatcher species on the island level. Birch and hazel were significantly more often present in collared flycatcher territories, compared to pied flycatcher territories in which the pine and ‘other’ group were more frequently encountered (table 4.1). To check how these results relate to differences in abundance of the tree species, we determined for which species the mean relaskopic volumes (from table 4.1) differed more than 50% between the two flycatcher species. Aspen sp abundance is much higher in pied flycatcher territories but its occurrence is relatively rare and is therefore relatively unimportant. Oak is an interesting case, although it occurs relatively frequent, especially in collared flycatcher territories, it is most abundant in pied flycatcher territories. Spruce occurrence was low, but if it occurred it was more abundant in pied flycatcher territories. Hazel and pine stand out, as they have the highest abundance in the territory type in which they occur most frequently. This creates a considerable difference between the territories, as hazel and pine

Figure 4.1. Fledging success on Öland of pure collared and pure pied flycatchers breeding pairs relative to the mean laying date. The fitted curves are second order polynomials and have been fitted for each species separately. The laying date is relative to the mean laying date of all breeding pairs combined and calculated for each year separately. The small differences in mean clutch size between the species (6.1 and 6.3 eggs for collared and pied flycatchers, respectively) have been accounted for by adjusting the clutch size of the pied flycatcher broods. Data from the period 2002-2007 were used.
Table 4.1. The mean relaskopic volume (with standard error between brackets) for the tree species recorded in breeding territories of collared and pied flycatchers on the islands of Gotland and Öland. This is the average over all territories in which the tree species was present (so not over all territories sampled). The percentage (%) of territories in which a tree species was recorded is presented separately. Pied and collared flycatchers breeding pairs were coupled on two different scales: within the same woodland (A: woodland scale) and within the same island (B: island scale). The results of a logistic regression analysing the difference in occurrence of tree species (presence/absence) between the territories of the two flycatcher species is presented in the last three columns (significant differences in bold). This analysis is only performed on the island scale. The degrees of freedom for the final model (four factors and the intercept) is 5, and for all other models df = 6 (see methods for more details on the statistical analyses). The sample sizes refer to the total number of broods used.

<table>
<thead>
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<th>tree species</th>
<th>A woodland scale (n= 28)</th>
<th>B island scale (n= 74)</th>
<th>logistic regression</th>
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<td>collared x (se) %</td>
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<td>birch</td>
<td>3.3 (0.8) 64</td>
<td>2.0 (0.5) 14</td>
<td>3.9 (0.7) 65 38</td>
</tr>
<tr>
<td>Alnus sp</td>
<td>4.7 (1.3) 21</td>
<td>5.8 (1.9) 43</td>
<td>6.7 (1.4) 30 22</td>
</tr>
<tr>
<td>Sorbus sp</td>
<td>1.7 (0.4) 36</td>
<td>0.8 (0.2) 21</td>
<td>1.5 (0.2) 32 19</td>
</tr>
<tr>
<td>hazel</td>
<td>5.9 (1.9) 64</td>
<td>4.2 (1.6) 36</td>
<td>8.3 (1.5) 62 35</td>
</tr>
<tr>
<td>oak</td>
<td>4.3 (1.0) 93</td>
<td>7.5 (3.4) 43</td>
<td>4.4 (0.8) 73 46</td>
</tr>
<tr>
<td>Ulmus sp</td>
<td>2.9 (1.2) 29</td>
<td>2.6 (1.0) 43</td>
<td>3.9 (0.7) 27 35</td>
</tr>
<tr>
<td>pine</td>
<td>1.0 (0.0) 14</td>
<td>4.4 (3.5) 29</td>
<td>1.2 (0.5) 19 49</td>
</tr>
<tr>
<td>spruce</td>
<td>2.50 7</td>
<td>1.5 (0.8) 21</td>
<td>2.6 (0.6) 14 19</td>
</tr>
<tr>
<td>other</td>
<td>0</td>
<td>1.3 (0.3) 14</td>
<td>2.0 (1.0) 5 24</td>
</tr>
</tbody>
</table>
combined accounted for 27% of the pied flycatcher’s and 30% of the collared flycatcher’s habitat (19% and 25% on woodland scale for pied and collared respectively).

The above indicates that there is quite some variation in habitat characteristics and there is quite some similarity between the territories. The measurements on the two different geographical scales show roughly the same general patterns. Important differences are (island scale relative to woodland scale): increase in both occurrence (although still low) and abundance of Aspen sp for both species, change in occurrence and abundance of Alnus sp such that both are now higher (instead of lower) for the collared flycatcher, increase of occurrence of pine for both species and a less marked difference in occurrence of spruce between both flycatcher species, but an increase of abundance for both.

Our results are in line with earlier reported differences (Alerstam et al. 1978) and we conclude that the habitat characteristics found in territories occupied by male pied and collared flycatcher differ from each other with respect to the occurrence and abundance of hazel and pine, but that there is also a large overlap in tree species between the two species.

Caterpillar abundance

DECIDUOUS TREE SPECIES

The distribution of frass weights of all four deciduous species had a unimodal distribution (figure 4.2A). The peak date differed between the tree species, with birch being on average almost seven days earlier than the other three species. The small standard errors for each species indicate that this was a consistent difference. The period during in which food is available is an important factor for an individual and this was captured by the width of the food peak (see figure 4.2A). The standard deviation of the fitted Gaussian curves reflects the width of the caterpillar distributions and showed only small differences between the species, with the exception of ash, which had a larger deviation compared to the rest.

It is evident that there were large differences between the four species in the absolute frass weight at the peak date (see figure 4.2A), and oak reached by far the highest weights among the sampled trees. This could be explained by the fact that the oaks in the study sites were much larger in diameter with very well developed crowns containing a large quantity of leaves (pers. obs.).

CONIFEROUS TREE SPECIES

The frass distributions of all sampled coniferous trees and the highest recorded weight are presented in figure 4.2B. In contrast to the deciduous species, the frass weight of the two coniferous species increased through the season (figure 4.2B), and showed no sign of declining towards the end of the sampling period.
Figure 4.2. The relative weight of caterpillar frass collected under four deciduous tree species (A) and two coniferous tree species (B) during the breeding season. The data are pooled for the three years as the annual check date between the years only differed slightly (≤1 day). The date is counted started from the 1st of May (May days). The mean peak weight (from the absolute frass weights), peak date and width (standard deviation) of the frass curve with the standard error between brackets are presented for each tree species in the right top of each panel. Only peak weight is given for the coniferous species. The sample sizes refer to the number of individual trees for a given tree species under which frass has been collected in the three consecutive years. Each data point represents the mean with standard error of all frass distribution for a given tree species (sample size times three years). Number of frass distribution used: oak (21), hazel (9), ash (3), birch (5), spruce (9) and pine (6), see the Methods for details on why four deciduous distributions have been excluded.
We believe it is valuable to make an estimate of the peak weight, as this is an informative measure because for an individual flycatcher the absolute amount of food available is important. As discussed above, this measure has its limitations but it at least gives an indication that caterpillars were less abundant in coniferous tree species (especially pine) compared to deciduous tree species (figure 4.2) confirming other studies (van Balen 1973). When comparing the deciduous and coniferous tree species, we can conclude that the relative abundance of caterpillars through the breeding season differed markedly between deciduous and coniferous species, with the latter increasing steadily through the season. Within the deciduous species, birch had a consistently early peak date and oaks had a lot of caterpillars (high absolute weight) during a short time period.

**Predicted caterpillar abundance based on tree composition**

In order to understand the effect of the habitat differences found between territories of the two flycatcher species on food abundance, we predicted the abundance of caterpillars for a typical territory of each species through the breeding season. This was done by multiplying the average frass weights of the tree species (as depicted in figure 4.2) with their relaskopic volumes. For this measure the mean relaskopic volume over all territories was used as both the

![Figure 4.3](image-url). The predicted temporal distribution of frass weights for typical collared (solid line) and pied flycatcher (dashed line) territories. This was done by multiplying the relative caterpillar abundance of each tree species (as depicted in figure 1) with their mean relaskopic volume. These were added up for all six tree species to give the total distribution. Note that the mean relascopic volumes is not that same as those presented in table 4.1; the mean is taken over all territories as both the occurrence and abundance needs to be captured in this measure. The time period of highest nutritional demand of flycatcher nestlings has been estimated to be between the age of 9 and 15 days and this period is marked relative to the mean laying date of the species by the two horizontal lines (solid and dashed) at the right bottom, for collared and pied flycatcher nestling respectively.
occurrence and abundance needs to be incorporated in this prediction. The frass contributions of all six tree species were then combined to give the predicted total distribution of caterpillar frass over the breeding season for a typical territory of the two flycatcher species (figure 4.3). The combined relaskopic volumes of the six tree species of which the frass distribution was known, accounted for 65% and 64% of the total relaskopic volume for collared and pied flycatchers territories, respectively. This small difference was accounted for in the calculations to avoid a bias in predicted frass abundance (as we do not know how much the remaining tree species contributed to the frass abundance).

An important consideration regarding the calculations above is that it uses the relative frass weight (maximum recorded weight set to 1), to compensate for the effect of tree size (which varied considerably) on absolute frass weight recorded. If the same calculations were done with the absolute weights (weight as collected from the frass traps), the shape of the curves in figure 4.3 looked similar, but the differences between the species were much smaller or even reverse. For example, at day 50, the pied flycatcher curve is 1.33 times the collared flycatcher curve in figure 4.3, but this was reversed to 0.96 (pied flycatcher territories had less caterpillars) when the absolute measures were used.

Discussion

The tree composition in breeding territories of collared and pied flycatcher showed small but consistent differences, with collared flycatchers inhabiting territories very rich in deciduous trees, especially with a lot of hazel, and pied flycatchers territories containing more coniferous species. This is in line with the results of earlier studies (Alerstam et al. 1978; Sætre et al. 1999a). These differences are present at a small geographical (woodland) scale, but were more distinct on a larger (island) scale. The abundance of caterpillars, as assessed by frass, differed strongly between deciduous and coniferous tree species. The former showed a distinct peak in abundance through the breeding season, whereas the coniferous species increased steadily throughout. An estimation of caterpillar abundance based on the mean tree composition of territories of the two flycatcher species revealed different patterns in seasonal food abundance. Pied flycatcher territories were calculated to contain more food late in the breeding season compared to collared flycatcher territories. The temporal variation in food abundance is predicted to result in less favourable breeding conditions for the collared in comparison to the pied flycatcher late in the season (when the food abundance was already low) and it could be an explanation for the relatively high reproductive output of the pied flycatcher during this period found on both islands. However, the data need to be interpreted with care. First
we will discuss how the results of this study affect our understanding of the coexistence of the two flycatcher species. Then, we will address the possible caveats in the data reported here.

Predicted caterpillar availability for the two flycatcher species, as depicted in figure 4.3, were lower for the pied flycatcher through most of the season, but conspicuously high late in the season. The main peak of caterpillar abundance is caused by the deciduous tree species, which were abundant in territories of both species and peak earlier in the season. The collared flycatcher territories consisted predominantly of deciduous species and therefore lack the relatively high food abundance late in the season caused by the increasing caterpillar abundance in coniferous species as found in pied flycatcher territories.

An important question is how this affects reproductive success. It is important that food availability is sufficient during the peak food demand of the nestlings (Perrins 1970). One way to determine this period is by using the parental feeding rates, which was found to be highest between day 9 to 15 for pied flycatcher nestlings (Lundberg and Alatalo 1992). We assume this to be the same for collared flycatcher nestlings, and based on the average hatching dates in period 2002–2004, this corresponds to the period between day 47 and 53 (indicated by the solid line in figure 4.3). This period fell at the end of the declining part of the food peak, indicating that collared flycatchers might face food constraints (see Both and Visser 2001 for discussion as to why this might arise; see Both et al. 2006). The pied flycatcher bred on average 2.7 days later (Qvarnström et al. 2005) and the period of high food demand is therefore later (dashed line in figure 4.3). Importantly, for pied flycatchers the predicted food availability is higher during this period, suggesting that they are potentially less severely affected by low food availability late in the season compared to collared flycatchers. Our results suggest that this difference could be due to higher caterpillar abundance in coniferous trees (common in pied flycatcher territories).

The suggestion that coniferous habitat might be better late in the season is supported by a higher fledgling weights in this forest type found for great tits (van Balen 1973) and a switch of great tit family groups (parents with fledged offspring) to feeding in coniferous habitat later in the breeding season (Verboven et al. 2001). Also, the smaller species within the same genera have been hypothesised to be better adapted to foraging in coniferous tree species (Forstmeier et al. 2001; Korner-Nievergelt and Leisler 2004). The pied flycatcher indeed appears to be better in foraging in coniferous environments as shown in a recent experimental study (Adamik and Bureš 2007). From an evolutionary point of view the pied flycatcher is likely to be better adapted to coniferous environments for other reasons than size alone, as this is the dominant environment in large parts of its allopatric breeding grounds, in contrast to the warmer and more deciduous environments of the collared flycatcher (Sætre et al. 1999a).
The collared flycatcher is likely to be more restricted to deciduous forests, whereas the pied flycatchers is able to occupy a wider range of different environments (Alerstam et al. 1978; Sætre et al. 1999a). The suggested better adaptation to harsh conditions is supported by previous work showing a less steep decline of pied flycatcher nestlings growth through the breeding season when compared to collared flycatcher nestlings (Qvarnström et al. 2005) and adult pied flycatchers being better parents for nestlings of both species later in the season (Qvarnström et al. 2007). So, despite the fact that the pied flycatcher is competitively subordinate, it appears to do better than predicted at first sight. We suggest this is aided by a combination of; 1) a higher availability of caterpillars late in the season and 2) an intrinsic capability of dealing better with harsh and more coniferous environments. Our data supports the hypothesis that habitat differences might explain, at least partly, the differences in reproductive output between the species found at the end of the breeding season (Veen et al. 2001; Wiley et al. 2007 and this study). Whether the habitat differences found in this study will suffice to facilitate stable coexistence in the long run (i.e. no long term trends in density of the species Chesson 2000) remains to be seen, but the decline of breeding pairs of the pied flycatcher in most of the study areas (especially on Gotland) indicates that this is not necessarily the case for the population on the Baltic islands.

When drawing conclusion from our results, one should bear in mind that most of the components have been measured indirectly. An important assumption is that caterpillars are an important food resource. This is shown to be the case e.g. by Wiley et al. (2007) but the same study shows the proportion Lepidoptera larvae in the diet decreases through the season. This contradicts with the increased frass weight and its importance as a resource especially at the end of the breeding season as suggested here. This contradiction could be explained by an increased size of caterpillars (and thereby mass per caterpillar) at the end of the season (pers. obs.), which is not captured by the methods used in Wiley et al. Dietary differences might be partly caused by differences in feeding behaviour between the species (Adamik and Buresš 2007) resulting in the utilisation of different food resources, but these patterns are still poorly described and understood. As mentioned earlier, we used the relative frass weight to try to adjust for differences in caterpillar numbers caused by differences in tree size. This approach should ideally be validated, as using the absolute weight changes the results. Considering previous work showing consistent natural selection on early breeding (Sheldon et al. 2003), it is surprising to see that both flycatchers show a mismatch between the predicted caterpillar abundance and the time of highest nestling demand, with many apparently breeding too late (figure 4.3). This could either indicate that the species are somehow unable to start breeding early enough as found in another population of pied flycatchers (Both and Visser 2001) or that timing is adjusted to other
periods in the breeding cycle. To validate the assumptions made, detailed knowledge of the behavioural ecology of the flycatchers is required, but this is currently lacking.

Differences in habitat occupation are a frequently observed outcome of interspecific competition. The competitively subordinate species is predicted to end up in less favourable habitat (for the dominant species), but might be able to adapt to this environment if it has a broader environmental tolerance level. Here we find that occupying such ‘sub-optimal’ habitat (in terms of highest frass weights) has the benefit of an increase of food availability at the end of the season when food is getting scarce. This could result in an increase in reproductive success of the subordinate species, reducing the negative consequences of interspecific competition. Typically, the various factors contributing to a reduction in interspecific competition are believed to be small and hard to detect. Our study shows that the divergence in a single factor, habitat composition, can have several (small) effects which may mutually reinforce each other and hereby facilitate coexistence on a regional scale.

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Does migration of hybrids contribute to post-zygotic isolation in flycatchers?

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Abstract

In the face of hybridization, species integrity can only be maintained through post-zygotic isolating barriers (PIBs). PIBs need not only be intrinsic (i.e. hybrid inviability and sterility caused by developmental incompatibilities), but also can be extrinsic due to the hybrid’s intermediate phenotype falling between the parental niches. For example, in migratory species, hybrid fitness might be reduced as a result of intermediate migration pathways and reaching suboptimal wintering grounds. Here, we test this idea by comparing the juvenile to adult survival probabilities as well as the wintering grounds of pied flycatchers (*Ficedula hypoleuca*), collared flycatchers (*F. albicollis*) and their hybrids using stable isotope ratios of carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) in feathers developed at the wintering site. Our result supports earlier observations of largely segregated wintering grounds of the two parental species. The isotope signature of hybrids clustered with that of pied flycatchers. We argue that this pattern can explain the high annual survival of hybrid flycatchers. Hence, dominant expression of the traits of one of the parental species in hybrids may substantially reduce the ecological costs of hybridization.
Speciation, i.e. the split of one species into two, is generally viewed as the formation of reproductive barriers between different populations. Reproductive barriers build up as side effects of natural selection or genetic drift in allopatric populations (Mayr 1942) or evolve in response to disruptive selection (e.g. Naisbit et al. 2001). Once populations have diverged genetically, hybridizing individuals experience a cost as a consequence of reduced hybrid viability and/or fertility (i.e. Hewitt 1989; Tegelström and Gelter 1990). These costs result in selection against heterospecific pairing and may lead to additional pre-zygotic or post-zygotic isolation (Dobzhansky 1937). Post-zygotic selection on hybrids may be the result of developmental instability (intrinsic post-zygotic isolation), which has been established in many hybridizing taxa (Coyne and Orr 2004 and references therein). Yet, it may also occur in a different way as an effect of their intermediate phenotype which induces them to fall between the parental niches with reduced fitness as a consequence (extrinsic post-zygotic isolation; Coyne and Orr 2004). For example, intermediate bill size of hybrid Darwin's finches is in most years disadvantageous compared to the resource optimized bill size of the parental species (Grant and Grant 1993, 1996). For migratory passerines, it has been argued that an intermediate migration route acts in a similar way (Helbig 1991a,b; Sutherland 1998; Bensch et al. 1999). Changed migration routes are generally suboptimal (Sutherland 1998) and can therefore enhance post-zygotic reproductive isolation. Bensch et al. (1999) suggested that intermediate suboptimal migration routes may explain the lack of recruitment of hybrids between two willow warbler subspecies (*Phylloscopus trochilus trochilus* and *P. t. acredula*). An intermediate migration route, potentially taken by hybrid willow warblers, would take them straight over the central Sahara desert where many may succumb as a result of food and water shortage (Bensch et al. 1999).

Here, we use pied (*Ficedula hypoleuca*) and collared (*F. albicollis*) flycatchers and their hybrids to investigate differentiation in wintering grounds. Pied and collared flycatchers are long-distance migrants with distinct migratory routes and sub-Saharan wintering grounds. In many species, including pied and collared flycatchers, migratory traits like flight direction and distance are at least partially genetically determined. The autumn migration peaks of pied flycatchers are different for adults and first-year migrants, meaning that first-year migrants undertake their journey without parental guidance and probably alone, as in many other nocturnal migrants (Helbig 1991a; Lundberg and Alatalo 1992; Mouritsen and Larsen 1998; Berthold 2003). Pied flycatchers fly a western route, following the Iberian Peninsula and through western Africa (Cramp and Perrins 1993). Mouritsen and Larsen 1998 showed experimentally that first-year pied flycatchers use the 'clock-and-compass' migration model, which appeared to be in accordance with ring recoveries (Mouritsen and Mouritsen 2000). The
orientation mechanism in collared flycatcher has not been studied in such detail, but field observations and ring recoveries indicate that they migrate through Italy or further east, passing the Sahara desert on the eastern side (Cramp and Perrins 1993). Based on ringing recoveries and visual observations, pied flycatchers winter in western to central Africa, while collared flycatchers winter in southeastern Africa (see figure 5.1). Observations of collared flycatchers in western Africa are generally considered to be vagrants (Cramp and Perrins 1993; Lundberg and Alatalo 1992). The migratory route of hybrid flycatchers is unknown, but if they were to follow an intermediate route this would entail the crossing of large stretches of unfavourable habitat (i.e. Mediterranean Sea and central Sahara desert) as has been suggested for hybrid willow warblers (Helbig 1991a,b; Bensch et al. 1999).

In order to characterize the wintering locations of the pied, collared and hybrid flycatchers, we used stable isotopes extracted from feathers developed at their wintering sites in Africa, a technique that has been increasingly used over the last decade (Rubenstein and Hobson 2004 and references therein). Stable isotope ratios of the feathers reflect an individual’s diet and environmental conditions at the wintering location during feather replacement.

We tested whether hybrid individuals take an intermediate route and thereby suffer from reduced survival as well as whether they reached intermediate wintering grounds between both parental species. The latter potential scenario could result in different carbon and nitrogen isotope ratios, as these areas probably have different precipitation and vegetation types. As alternative hypotheses, we tested whether the wintering grounds of hybrids were determined by sex-linked determination or a parental-species dominance effect. The results were used to infer the most likely migratory pattern of hybrids and to evaluate the implications for post-zygotic isolation.

Figure 5.1. Location of the wintering areas of pied and collared flycatchers based on ringing recoveries and visual sightings (after Lundberg and Alatalo 1992).
Material and methods

Study species and data collection
Collared and pied flycatchers are insectivorous passerine species with a breeding distribution covering most of Europe. Pied flycatchers breed in western and northern parts of Europe continuing eastwards into Russia. Collared flycatchers breed in Central and Eastern Europe. On the Baltic Islands of Gotland and Öland, both species breed sympatrically and hybridize in low numbers (approx. 3% of breeding pairs; Veen et al. 2001).

Juvenile to adult survival differences were estimated from fledglings recruited back as adults to the breeding population for pied and collared flycatchers as well as hybrids, using 26 years (1980-2005) of data from breeding flycatchers on Gotland (57°10’N, 18°20’E), Sweden (for data collection methods, see Gustafsson 1989; Pärt and Gustafsson 1989). Individuals born in 2004 and 2005 may not yet have recruited and nests from those years were therefore excluded. We collected the median tertial feather of breeding flycatchers. These feathers are fully moulted on the wintering grounds before spring migration (Cramp and Perrins 1993; Salewski et al. 2004) and since the flycatchers are most probably territorial (Salewski et al. 2002), we assume that the isotope ratios in these feathers reflect their diet and can be used to discern wintering location differentiation between the two species and their hybrids. The feathers were collected at the end of the breeding season, just before pre-migratory moult with permission from the CFN (number M 78–05). Blood samples from birds were collected to ensure correct species identification (see below). Blood samples (3–10µl) were taken from the brachial vein and stored in 96% ethanol at 4°C.

Species identification and molecular genetic methods
Species were identified in the field using biometrics (Svensson 1984), behavioural characteristics and vocalization. Feather samples were collected from all individuals with features that deviated from the normal species range and from pairs whose broods contained infertile eggs or low hatching success, since female hybrids (the heterogametic sex) have reduced fertility (Gelter et al. 1992). Species identity of all birds was genetically determined as part of a large-scale study on genetic introgression between the two species (G.-P. Sætre and A. Qvarnström 2006, unpublished data). These results were used to exclude backcrosses (made possible through fertile hybrid males) and eliminate identification errors. For the genetic analysis, the DNA was extracted and purified using standard phenol-chloroform extraction. Tag-array-based mini-sequencing assays were applied to genotype single nucleotide polymorphisms (SNPs) based on the methods described in Sætre et al. (2003), with the difference that in this study 45 SNPs instead of 20 SNPs were used (G.-P. Sætre and A. Qvarnström 2006, unpublished data). Individuals were assigned as either pied, collared or F1
hybrids using the model-based cluster method described by Pritchard et al. (2000) using the same approach and setting as in Sætre et al. (2003).

The maternal species of F1 hybrids was determined using the amplification of a species-specific 32bp indel in the mitochondrial DNA (Sætre and Moum 2000).

**Stable isotope analysis**
The ratios of the stable isotopes $^{13}$C/$^{12}$C and $^{15}$N/$^{14}$N in chloroform-washed feather samples were determined at the Centre for Limnology of the Netherlands Institute of Ecology. Carbon stable isotope ratios (parts per thousand, ‰, difference from the $^{13}$C/$^{12}$C ratio in Vienna PeeDee limestone; henceforth referred to as $\delta^{13}$C) and nitrogen stable isotope ratios (‰ difference from the $^{15}$N/$^{14}$N ratio in atmospheric N2; henceforth referred to as $\delta^{15}$N) were determined in a HEKAtech EuroEA elemental analyser coupled online through a Finnigan con-flo interface to a Finnigan Delta S isotope ratio mass spectrometer. Average reproducibility based on replicate measurements was less than 0.2‰ for $\delta^{13}$C and less than 0.1‰ for $\delta^{15}$N.

**Statistical analysis**
Survival probabilities were compared between collared flycatchers, pied flycatchers and the two hybrid types (depending on their parental species pairing) using a generalized linear model (GLM) with a logit-link function and number of fledged juveniles within a nest as the denominator. The variable including the categories, collared flycatcher, pied flycatcher and both hybrid types, will henceforth be referred to as 'species' for ease of use. Differences in isotope values between the two species and both types of hybrid depending on their parental species paring were analysed separately for $\delta^{13}$C and $\delta^{15}$N using a main effect anova. For all the models tested, we did not find a significant deviation from normality, or an inequality of variances in the data. Besides species, sex, year and lay date (day of first egg) were also included as explanatory variables in the model. Non-significant variables were excluded from the analysis in a backwards elimination process. Pairwise differences between the species categories were analysed using a Tukey’s HSD test.

To test whether hybrids fit an intermediate isotope ratio distribution, we compared the combined weighted mean of pied and collared flycatchers to the hybrids using a one-sample t-test for both $\delta^{13}$C and $\delta^{15}$N. Statistical tests were conducted using STATISTICA 7.0, GENSTAT 8.0 and JMP 6.0.

To verify our models selected above, we conducted model selection using the Akaike Information Criteria corrected for sample size (AICc; Burnham and Anderson 2002). AIC scores for all models are presented in appendix 5.1).
The fledged juvenile to adult survival probabilities differed between the species (GLM: $\chi^2_{32}=36.76, p<0.0001$). However, only the pied flycatcher had a substantially lower survival probability (mean (s.e.)=0.038 (0.014), n=129) compared with the other species (collared flycatcher: mean (s.e.)=0.107 (0.003), n=3308; father pied flycatcher, mother collared flycatcher: mean (s.e.)=0.107 (0.023), n=47; father collared flycatcher, mother pied flycatcher: mean (s.e.)=0.096 (0.021), n=59). The data were overdispersed (overdispersion parameter=1.193) and the scaling parameter was therefore adjusted accordingly.

A total of 78 feather samples collected in 2004 and 2005 were analysed. The analyses involved 24 pied (12 males and 12 females) and 39 collared flycatchers (20 males and 19 females). Feather samples from 15 hybrids were analysed, of which 10 had a pied (3 males and 7 females) and 5 had a collared flycatcher mother (all males).

Pied and collared flycatchers, as well as their hybrids, show a clear difference in the $\delta^{13}C$ distribution (anova: 'species': $F_{3,74}=16.93, p<0.001$; all other explanatory variables: $p>0.45$), whereas only a near significant year effect was found for $\delta^{15}N$ (anova: year: $F_{1,76}=3.26, p=0.075$; all other explanatory variables: $p>0.50$; figure 5.2A). Pied and collared flycatchers differed in $\delta^{13}C$, whereas hybrids had $\delta^{13}C$ values similar to those of pied flycatchers (Tukey's HSD test, $p<0.01$; figure 5.2B). The hybrids differed significantly from the combined weighted mean of pied and collared flycatchers for $\delta^{13}C$ ($t_{14}=-3.58, p=0.003$), but not for $\delta^{15}N$ ($t_{14}=-0.97, p>0.30$).

The isotope ratio results above are supported by analyses using model selection theory (see appendix 5.1).

**Figure 5.2.** $\delta^{13}C$ and $\delta^{15}N$ isotope ratios for (A) pure pied (PF) and collared (CF) flycatchers and (B) hybrid individuals with maternal species in brackets.
Discussion

Differences in stable isotope ratios in the feathers of collared and pied flycatchers, moulted at the wintering site, were consistent with the presumed largely segregated wintering grounds of the two species. The feather isotope data of the hybrids suggest that the hybrids, regardless of parental species pairing, fall within the range of pied flycatchers.

That the average isotope ratios for nitrogen are indistinguishable for pied and collared flycatchers is in accordance with the recent findings in willow warbler feathers collected in West and Central-East Africa (Bensch et al. 2006). Bensch et al. also found West African willow warbler feathers to have lower carbon isotope ratios than those from Central-East Africa. This exactly mirrors the isotopic findings in pied and collared flycatchers, which are supposedly wintering in West and Central-East Africa, respectively. Variation in carbon is often attributed to the relative abundance of plants using C3 or C4 photosynthetic pathways (Smith and Epstein 1971; Rubenstein and Hobson 2004 and references herein). When using global C3/C4 plant distributions (Still et al. 2003), the pattern found for the willow warblers and flycatchers is opposite to the predicted pattern. However, qualitatively identical patterns found by Bensch et al. 2006 not only validate our findings, but also show that there is need for a better mechanistic understanding of isotope distribution (e.g. Kelly 2000).

Since variation in isotope ratios in tissues is both influenced by environmental and dietary differences, it is important to assess which of these factors is determining the observed stable isotope patterns among the flycatchers in this study. In other words, could the flycatchers winter at the same geographical location but differ in diet, or are they wintering at different locations? There are various studies indicating that dietary choice of pied and collared flycatchers are very similar. Prey items taken by the two flycatcher species during the breeding period largely overlap (Bureš 1995). Moreover, nestlings of both species can be successfully raised by foster parents of the alternative species (Qvarnström et al. 2005). On the wintering grounds, the pied flycatcher is an opportunistic forager with a broad diet, which is a common feature of migratory species compared to residents (Salewski et al. 2002). These observations suggest that the differences in feather isotope signatures reflect geographical differences in wintering sites and are not an effect of differences in diet within a single geographical range. This, together with the previously described differences in wintering range between pied and collared flycatchers (Cramp and Perrins 1993; Lundberg and Alatalo 1992), which matches the isotopic findings in willow warbler feathers collected in these same ranges (Bensch et al. 2006) makes us confident in our conclusion that the two species have segregated wintering distributions. We acknowledge that one of the drawbacks of the now widely used isotope approach is that if no differences in isotope values are found, it does not neces-
sarily mean that the geographical areas are the same. In the future, research such as that conducted by Bensch and his colleagues (2006), resulting in detailed isotope-based maps, will make the linkage between isotope values and geographical area more accurate.

With respect to the hybrid’s determination of the wintering grounds, three obvious hypotheses could be put forward. Firstly, determination of the wintering grounds could be additive, which results in ‘intermediate’ wintering grounds of hybrids. This hypothesis can most likely be excluded, as the survival probabilities of the two hybrid types were as high as that of collared flycatchers and higher compared with pied flycatchers. Further, δ¹³C of hybrids significantly differed from the predicted intermediate isotope ratio of both parental species. However, the latter test assumes a clear-cut difference in habitat types at the contact zone of the parental species. Such assumptions can be tested and further insights gained, once a more accurate linkage between isotope ratios and geographical location becomes available.

Secondly, the determination of wintering grounds could be sex linked, in which case hybrids should have isotope ratios overlapping those of both parental species. In the case of Z-linkage, the wintering range would be paternally determined in daughters (ZW). Alternatively, wintering grounds could be maternally determined through the W chromosome (resulting in maternal inheritance in daughters) or cytoplasmic DNA inheritance (resulting in complete maternal inheritance). Neither of these hypotheses is supported by our data, but the sample sizes are small making firm conclusion difficult.

Thirdly, dominance of the determination of wintering grounds of one species would create the clustering of hybrids with one parental species, which is consistent with the observed pattern. The most parsimonious explanation for our data is that the pied flycatcher’s migration route is dominant and hence all hybrids follow this route. This would explain both the lack of a reduced survival among hybrids, since these hybrids do not follow an intermediate and potentially suboptimal migration route (Helbig 1991b; Bensch et al. 1999) and the clustering of hybrids with pied flycatchers based on isotope ratios.

Sutherland (1998) states that ‘the change (of migration routes) will be most rapid when the genetic system is simple, there is assortative mating or the new route is dominant...’ In this study, we find indications for dominance in migratory traits when two populations hybridize, but an important question is whether dominance can evolve within populations and whether it has done so. Recently, there has been much discussion on the evolutionary potential of migratory traits (Berthold 2003; Pulido and Berthold 2003; Pulido and Widmer 2005) and an increasing body of evidence suggests that maintaining genetic variation and hence the potential for a response to selection of these traits is adaptive (e.g. Berthold et al. 1992; Sutherland 1998; Bearhop et al. 2005). Dominance effects in migratory traits therefore need to be taken into consideration, since they can
have marked effects on the evolution of changes in migratory traits.

More recently, an interest in the potential effects of changes in migratory behaviour on reproductive isolation has arisen. Bearhop et al. (2005) argue that in blackcaps, a switch of wintering location can lead to temporal segregation of sympatric breeding populations and eventually lead to reproductive isolation and sympatric speciation. At the moment, the effect of an accelerated change of migration route on speciation is still poorly understood and remains a challenge to be solved.

To our knowledge, this is the first study to find indications of a dominance effect on the location of wintering grounds between two closely related species. By following one of the parental species' migration routes and using their wintering grounds, hybrids will not bear the potential costs of an intermediate migration route. Thereby, the dominance effect reduces the extrinsic post-zygotic isolation between the two flycatcher species, since hybrid individuals do not suffer from intermediate maladaptive traits. Further, species integrity is negatively affected by the reduced reproductive isolation between the two species. Not only can the observed dominance effect in migratory traits affect reproductive isolation, but also it can have implication for the speed with which migratory traits can change in response to a changing environment.

Acknowledgements

We would like to thank Harry Korthals for the stable isotope ratio determinations; Ben Sheldon, Franjo Weissing, Francisco Pulido, Thomas Alerstam, Lars Gustafsson and two anonymous referees for helpful comments and discussions during preparation of the manuscript; Reija Dufva, Glenn-Peter Sætre and Chris Wiley for help in the laboratory. Financial support was obtained from the Netherlands Organization for Scientific Research (grant NWO-ALW 812.04.001) (T.V.), the Swedish Research Council Formas (M.B.H.), Academy of Finland, project no. 202388 (J.T.F.), European Commission (Marie Curie Intra-European Fellowship, MEIF-CT-2003-500554, J.T.F.) and the Swedish Research Council (A.Q.). This is publication 3952 from The Netherlands Research Council (KNAW-N100).
Appendix 5.1: AICc scores

The AICc scores for all models analysed are presented in the table below. The model best supported by the data for $\delta^{13}$C consisted of species only. For $\delta^{15}$N the best fitting models included only sex, only year or sex and years with very similar AICc scores. The models selected based on AICc values are in accordance with the best fitting models using anovas. The sample size is 64 samples because 14 individuals had to be excluded due to missing values. To calculate the AICc score, the formula $2k + n \ln (RSS/n) + 2k(k+1)/(n-k-1)$ was used ($k$ = number of parameters, $n$ = sample size and RSS = residual sum of squares). The models have been sorted from lowest AICc (best fit) to highest value (poorest fit).

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Migration of hybrids  105
Sex chromosome-linked species recognition and evolution of reproductive isolation in flycatchers

Stein A. Sæther, Glenn-Peter Sætre, Thomas Borge, Chris Wiley, Nina Svedin, Gunilla Andersson, Thor Veen, Jon Haavie, Maria R. Servedio, Stanislav Bureš, Miroslav Král, Mårten B. Hjernquist, Lars Gustafsson, Johan Träff & Anna Qvarnström
Abstract

Interbreeding between species (hybridization) typically produces unfit offspring. Reduced hybridization should therefore be favored by natural selection. However, this is difficult to accomplish because hybridization also sets the stage for genetic recombination to dissociate species-specific traits from the preferences for them. Here we show that this association is maintained by physical linkage (on the same chromosome) in two hybridizing *Ficedula* flycatchers. By analyzing the mating patterns of female hybrids and cross-fostered offspring, we demonstrate that species recognition is inherited on the Z chromosome, which is also the known location of species-specific male plumage traits and genes causing low hybrid fitness. Limited recombination on the Z chromosome maintains associations of Z-linked genes despite hybridization, suggesting that the sex chromosomes may be a hotspot for adaptive speciation.

Note: minor changes have been made to this chapter in comparison to the published version (headings have been added to the text).
Introduction

Reproductive isolation is traditionally viewed as an incidental by-product of genetic divergence during geographic isolation. However, many diverged populations come into contact before complete reproductive isolation has evolved. In such cases, natural selection against maladaptive interbreeding (hybridization) may complete speciation by reinforcing a tendency to mate with one’s own kind ( assortative mating) (Dobzhansky 1940; Servedio and Noor 2003; Coyne and Orr 2004). This process, termed reinforcement, is of potentially great evolutionary significance because it suggests that reproductive isolation itself can be an adaptive response to natural selection. However, the empirical support for reinforcement is limited, and the conditions under which it can theoretically occur are sometimes strict (Kirkpatrick and Ravigné 2002; Servedio and Noor 2003).

Selection for assortative mating favors the buildup of genetic associations between components of assortative mating (species-specific traits and the preferences for these traits) and between such pre-zygotic barriers and low hybrid fitness (post-zygotic barriers). However, DNA recombination during hybridization breaks these associations necessary for speciation (Felsenstein 1981). Using a combination of field experiments, molecular techniques, and long-term breeding data from hybrid zones of wild birds in the Czech Republic and Sweden, we tested this central problem of reinforcement: How can the traits involved in reproductive isolation remain associated in the face of gene flow?

Two solutions to this problem have been suggested. First, species recognition can occur through a "one-allele mechanism": a single allele, established in both incipient species, can cause assortative mating (Felsenstein 1981; Servedio and Noor 2003; Ortíz-Barrientos and Noor 2005). For example, sexual imprinting is a widespread phenomenon in birds (ten Cate and Vos 1999), whereby females learn the traits of their fathers and later prefer similar males as mates. An allele causing sexual imprinting would make recombination irrelevant, because it would result in opposite mate preferences in the two species (Servedio et al. in press; Irwin and Price 1999; ten Cate and Vos 1999). Second, recombination can be suppressed through, for example, physical linkage of genes (Felsenstein 1981; Trickett and Butlin 1994; Servedio 2000; Ortíz-Barrientos et al. 2002; Servedio and Noor 2003; Servedio and Sætre 2003). Recent theoretical studies have highlighted the idea that sex linkage (placement on a sex chromosome) of species-recognition genes may enhance reinforcement when, as is often the case, genes causing low hybrid fitness are also sex-linked (Servedio et al. in press; Servedio and Sætre 2003; Hall and Kirkpatrick 2006; Lemmon and Kirkpatrick 2006). We tested whether species recognition is due to sex-linked preferences, sexual imprinting, or autosomally inherited preferences in an avian system with evidence for reinforcement (Sætre et al. 1997b).
**Flycatchers**

Where the breeding distributions of pied flycatchers (*Ficedula hypoleuca* (pied)) and collared flycatchers (*F. albicollis* (collared)) overlap (sympatry), the populations mate assortatively with respect to species (Veen et al. 2001). However, some interbreeding occurs (2 to 5% of pairs (Veen et al. 2001)), and the resulting hybrids have reduced fitness, female hybrids being sterile (Veen et al. 2001). Despite gene flow through male hybrids (Sætre et al. 2003), both male plumage and female mate preferences have diverged furthest in sympatric areas, presumably to facilitate avoiding hybridization (Sætre et al. 1997b).

Genes causing hybrid incompatibility and genes influencing the expression of diverged male plumage traits are both located on the Z sex chromosome in flycatchers (Sætre et al. 2003). Genes on the Z chromosome are in general likely to recombine less than if they were on autosomes; for instance, because sex chromosome recombination can occur only in the homogametic sex (supporting online text). In fact, previous studies have failed to detect any interspecific recombination between the Z chromosomes of hybridizing flycatchers, whereas autosomes recombine between the species (Sætre et al. 2003). If genes on the Z also determine species recognition by females, recombination between loci that are important for reinforcement would be greatly reduced.

**Mating patterns of hybrids**

In birds, females inherit half of their autosomal genes from either parent, but their single Z chromosome solely from their father. We used this fact to distinguish between whether species recognition is inherited on autosomes or is paternally determined (by Z linkage or learned by sexual imprinting on fathers). If mate preferences are paternally determined, the mate choice of female hybrids should vary according to the species of their father, corresponding to that of pure females of the paternal species. To determine the paternal and maternal species of female hybrids, as well as their status as F1 hybrids, we used species-specific genetic markers at the Z chromosome (paternally inherited) and in the mitochondrial genome (maternally inherited) (for more information see appendix 6.1).

**a) female hybrids**

We found that female hybrids having a pied father predominately mated with a pied male, whereas female hybrids having a collared father predominately mated with a collared male (figure 6.1A). Only 4 out of 31 female hybrids mated with a male of the maternal species. Thus, there was a nonrandom association between the species of a female hybrid's father and the species of her mate ($\chi^2 = 12.37$, exact $p = 0.001$, $n = 31$ hybrids); excluding matings with male hybrids (Fisher's exact test, $p = 0.001$, $n = 29$). This pattern was present in both Czech ($p = 0.048$, $n = 9$) and Swedish ($p = 0.062$, $n = 22$) sympatric areas.
b) male hybrids

Male hybrids, on the other hand, inherit a Z chromosome from both parental species and are unaffected by sexual imprinting because male flycatchers do not discriminate against heterospecific partners (Sætre et al. 1997a). As expected, there was no association between the paternal species of male hybrids and the species of their mate (Fisher's exact test, $n = 42$, $p = 1$, figure 6.1B). This implies that the pattern observed in female hybrids is not simply some artefact of hybrids but is consistent only with paternal inheritance (through Z linkage or sexual imprinting) of species recognition; this therefore eliminates autosomal inheritance as the mechanism behind species-assortative mating.

**Sexual imprinting or Z linkage?**

Flycatchers provide an ideal system in which to disentangle sexual imprinting from Z linkage, because hybridizing females often engage in extrapair copulations with conspecific males (Veen et al. 2001), resulting in purebred offspring being reared by cuckolded heterospecific males. We used multi-generational breeding data to analyze the mate choices of such pure female flycatchers that had been reared by a male of the other species. If assortative mating is due to sexual imprinting, these females should prefer heterospecific males as their mates. We also experimentally cross-fostered offspring between nests of the two species and recorded the mate choice of cross-fostered females that returned to breed. Under genetic inheritance of species recognition, cross-fostered females should mate as other females of their own species, whereas under sexual imprinting, they should mate as females of the foster father's species (for more information see appendix 6.1).
Figure 6.2. Mating patterns of female flycatchers reared by heterospecific foster fathers compared to expectations based on sexual imprinting or genetic inheritance of species recognition. In each panel, the first bar shows the mating pattern of females reared by heterospecific foster fathers. The upper panels (A and B) show mating patterns of females naturally raised in mixed-species nests by cuckolded heterospecific stepfathers (natural cross-fostered), and the lower panels (C and D) show mating patterns of females experimentally transferred to heterospecific nests (experimental cross-fostered). The left panels [(A) and (C)] refer to collared flycatchers, whereas the right panels [(B) and (D)] present pied flycatchers. The expected mating patterns (specific to the population in which the focal patterns were obtained) were constructed in two ways, using either the mating pattern in the population (all pairs) or using only the mating pattern of females born in nests of known pure pairs (recruits only). P values indicate the exact binomial two-tailed probability of obtaining the observed focal mating pattern of females reared by heterospecific fathers (number of such females mating with conspecific males versus other males) under the different expected proportions. N indicates number of breeding pairs.
In contrast to prevailing views on the development of sexual preferences in birds (ten Cate and Vos 1999), figure 6.2 shows that females did not become sexually imprinted on their social father. Instead, females with a heterospecific foster father mated with conspecific males to the same extent as other females of their own species did. This conclusion applies to females of both species, using both naturally (figure 6.2, A and B) and experimentally (figure 6.2, C and D) cross-fostered offspring and regardless of whether expected mating patterns were calculated from all breeding pairs or from recruits of known parents only. Although some of the sample sizes are small because of the rarity of these events, the evidence is overwhelming that species recognition does not develop by sexual imprinting in these birds. Instead, our results imply that species-assortative mating has a genetic basis.

**Discussion**

Assuming Mendelian inheritance, the different mate preference of the two kinds of female hybrids is solely consistent with Z linkage. A non-Mendelian epigenetic possibility is that the preference genes are autosomally inherited, but only the paternal allele is expressed (maternal genomic imprinting). However, such parent-of-origin effects have not been found in birds (O'Neill et al. 2000). Moreover, they are not expected to occur in birds according to one predominant theory of the evolution of genomic imprinting, and genes that are imprinted in mammals show ordinary bi-allelic expression in birds (O'Neill et al. 2000). We therefore conclude that species-assortative mating preferences in flycatcher hybrid zones are mainly due to Z-linked genes.

All three major components of reproductive isolation (species recognition, species-specific male traits, and hybrid incompatibilities) being Z linked in flycatchers should facilitate an evolutionary response to natural selection against hybridization. This is because genetic associations between the male and the female components of pre-zygotic barriers to gene flow, as well as between pre-zygotic and post-zygotic barriers, can easily be maintained (for more information see appendix 6.2). Our results suggest that some organisms may be prone to speciation through reinforcement because of the mediating role of the sex chromosomes. Compared to autosomally inherited species recognition, both sex linkage and sexual imprinting may allow incipient species to avoid a collapse in assortative mating during secondary contact and be less likely to succumb to gene flow and fusion (Servedio et al. in press). However, paternal sexual imprinting requires that females be socially exposed to their father, which is not always true even in birds. Conversely, because reduced hybrid fitness is commonly caused by sex-linked incompatibilities (Coyne and Orr 2004), sex linkage of species recognition might provide a general connection between key
components of reproductive isolation, which facilitates adaptive speciation in the face of gene flow.

Sex-chromosome linkage of species-assortative female mate preferences may be widespread, but few previous studies have explicitly investigated the mechanism of species recognition in hybrid zones. Even fewer studies have provided additional information on the genetics of hybrid fitness and the preferred traits, or evidence for reinforcement (Grula and Taylor 1980; Grant and Grant 1997b; Noor et al. 2001; Iyengar et al. 2002). Nevertheless, disproportionately many genes involved in reproductive isolation seem to be located on the sex chromosomes (Kirkpatrick and Hall 2004; Kaiser and Ellegren 2006; Lemmon and Kirkpatrick 2006). In Lepidoptera, which also have heterogametic females, sex-linked traits seem to be more associated with reproductive isolation than in other insects (Ritchie and Phillips 1998), and it has been suggested that ornaments and preferences for these ornaments evolve more readily in organisms with ZW than with XY sex chromosomes (Reeve and Pfennig 2003; Kirkpatrick and Hall 2004). Although speciation would benefit from any kind of linkage (or other recombination-suppressing mechanism) that can maintain these genetic associations, traits involved in pre-zygotic isolation may simply be more likely to occur on sex chromosomes than on autosomes and possibly more likely on Z than on X chromosomes (Kaiser and Ellegren 2006). Sex chromosomes in general, and the Z in particular, may therefore be hotspots for speciation genes.

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Appendix 6.1
Material and methods

We tested whether the female species recognition that causes assortative mating in flycatchers is due to 1) autosomally inherited preferences, 2) sexual imprinting, or 3) sex-linked preferences. We did this in two steps, both involving testing mutually exclusive pairwise predictions that enabled us to produce a unique combination of predictions for each of the three scenarios. First, we distinguished between autosomal inheritance versus paternal determination (i.e., 1 vs. 2 or 3) by examining mate choice of hybrid females differing in their parental combination. Then, we distinguished between sexual imprinting and genetic inheritance (i.e., 2 vs. 1 or 3) by cross-fostering offspring between the two species and identifying their subsequent mate choice.

Mate choice of hybrids

The fieldwork was conducted in Northern Moravia, Czech Republic from 1985-2005, and on the islands of Öland and Gotland, closely situated in the Baltic Sea, Sweden, from 1980-2005. We caught females during incubation and males when feeding nestlings. Since female flycatcher hybrids are sterile the nests are eventually abandoned when the eggs do not hatch, and the mates of female hybrids are therefore difficult to catch. This problem was solved by temporarily introducing nestlings from a nest-box nearby so we could catch the male when feeding the foster-nestlings. The paternal and maternal species identity of female hybrids was established with DNA analysis (see below). We genotyped a total of 31 female F1 hybrids with male partners of known species identity (14 from Öland, 9 from the Czech population, and 8 from Gotland). The three areas of sympatry are qualitatively similar in many respects. In all areas the collared flycatcher outnumbers the pied flycatcher (the proportion of Collared to Pied is about 0.70 on Öland, 0.85 in the Czech study areas and 0.96 on Gotland); F1 hybrids constitute a relatively low percentage of the breeding population (about 4% on Öland, 3% in the Czech population and 2% on Gotland) and heterospecific pairing is rare compared to within-species pairing (e.g. Sætre et al. 1999a; Veen et al. 2001) . Therefore, we pooled data on female hybrids from the three areas, but checked if patterns held within the Czech and the Swedish hybrid zones.

We also genetically identified the paternal species of 42 male hybrids from the same areas as the female hybrids and identified the species of their partner based on morphology and vocalizations. If assortative mating is due to paternally determined mate references, there should be a relationship between the parental combination of female hybrids and the species of their partner, but not for male hybrids. We expect absence of such a relation in male hybrids for two separate reasons. First, male birds inherit a Z chromosome from each parent,
and the parental combination of male hybrids should hence not matter if preferences were genetically inherited at the Z. Second, empirical data suggest that flycatcher males, unlike females, do not discriminate against mating with heterospecifics (Sætre et al. 1997a), and male hybrids are consequently expected to mate independently of their parental combination. Therefore, the mating pattern of male hybrids provides a nice test of whether mating of female hybrids according to their parental combination could be due to something else than paternally determined mate preferences (e.g. biased occurrence of certain hybrids in relation to the frequency of the two parental species).

**Genetic identification of hybrids and their parental species combination**

Females of suspected hybrid identity on basis of plumage and/or whole clutches of unhatched eggs were genotyped for species-specific markers. Since mitochondria are inherited through the female germ-line, the species identity of a hybrid’s mother was determined using a species-specific mtDNA marker. Similarly, since females are the heterogametic sex (ZW) in birds, the species identity of a hybrid female’s father was determined with a species-specific marker on the Z chromosome. In addition, F1 hybrid status was also confirmed for the majority of birds using autosomal species-informative SNPs and species-specific substitutions, altogether 34 autosomal markers from 23 different genes (Borge et al. 2005a). The two methods always agreed about F1 hybrid status.

Male hybrids are easier to identify as such based on plumage, but to be sure to exclude later generation backcrosses or aberrant individuals of either species, we identified suspected male F1 hybrids as such by autosomal species-specific substitutions and SNPs, in the same way as described above for females (Borge et al. 2005a). The species of their mother was then identified by a species-specific mtDNA marker as for female hybrids.

25 µL blood was collected from each individual by puncturing the brachial vein and the blood was suspended in 1mL Queen’s lysis buffer (Seutin et al. 1991) or in ethanol. DNA was extracted from the blood samples by overnight incubation at 45°C with 50 µL of Proteinase K solution (10 mg/mL) and 50 µL of 10% SDS. Each sample was extracted with two rounds of standard phenol/chloroform treatment before DNA was recovered by ethanol precipitation, dried, and re-dissolved in 500–1000 µL TE-buffer (10 mM Tris, 1 mM EDTA, pH 7.5).

We amplified a stretch of mtDNA containing a 32 bp species-specific indel as described in (Sætre and Moum 2000). We used 10 µL PCR reactions containing 2.5 mM MgCl2, 0.2 mM of each dNTP, 0.32 µM of each primer, 1 µg of Bovine Serum Albumine (BSA), 0.3 units of HotStar DNA polymerase (Qiagen), 1X PCR Buffer (Qiagen) and 20ng DNA. On a PTC 225 (MJ Research), 35 cycles of amplification with 94°C for 30 seconds (s), 60°C for 30 s and 72°C for one minute (min) were preceded by 15 min pre-denaturation at 95°C and followed
by a prolonged 10 min extension step at 72°C. The species-specific PCR fragments were compared on a 4% agarose gel fixed in ethidium-bromide and the species identity determined based on fragment length comparisons of reference samples (see Sætre and Moum 2000 for further detail).

We used the Z-linked locus CHDZ-20 that has several nucleotide positions with a fixed difference between the species (Borge et al. 2005b). The primers CHDZ-20F: 5´-GAA GAG AGC TGA AAC TCG G-3 and CHDZ-20R: 5´-TCA TCT TCA TCC ATA TTG G-3 were used with the same reaction mix and PCR protocol as described above except that the fragment was amplified with two amplification steps and with different annealing temperatures: first 5 cycles, then 30 cycles with annealing temperatures of 58°C and 50°C, respectively. The fragments were purified using ExoSAP-IT (Amersham Biosciences) and direct sequenced using original PCR primers with the DYEnamic cycle sequencing kit (Amersham Biosciences), and analyzed on a MegaBACE 1000 (Amersham Biosciences) instrument. Sequences from both directions were aligned and edited in Sequencher 4.1 (Gene Codes).

Mate choice of pure female recruits from mixed species pairs (naturally cross-fostered extra-pair recruits)

To separate the effects on mate choice of genetically inherited species recognition and preferences learned by sexual imprinting, we analyzed the mate choice of females reared by heterospecific foster fathers on the Swedish island of Gotland in the Baltic Sea (1980–2005). We assumed that female hybrid flycatchers are sterile, strongly supported by previous genetic studies (e.g. Gelter et al. 1992; Veen et al. 2001). Female recruits that did not show evidence of reduced fertility (i.e., had eggs that hatched) and that came from nests of mixed pairs were thus identified as offspring resulting from conspecific extra-pair copulations. In pairs with a male Pied and a female Collared, on average 56 % of offspring are fathered by conspecific extra-pair males, as shown using molecular markers (Veen et al. 2001).

Cross-fostering experiment

To further test if females are sexually imprinted on heterospecific males, we conducted cross-fostering experiments (on Öland) by reciprocal transfer of half the brood between nests of the two species. Nests were matched for clutch size (± 1 egg) and laying date (same date). Chicks were transferred when 3 days old, temporarily identified by toe nail clipping and later permanently identified by numbered leg rings. Since we wanted to track the origin of individual offspring, we did not transfer eggs and assumed instead that any sexual imprinting effects would be negligible before age 3 days old. This assumption is supported by the fact that flycatcher offspring do not fully open their eyes until 7 days old (Creutz 1955). In total, 361 offspring cross-fostered during 2002–2004
(from 138 experimental broods) survived to fledging. (A larger number was initially cross-fostered, but predation rates before fledging were sometimes extensive.) Data on the species of the breeding partner of females that had been cross-fostered were gathered in 2003-2005.

**Null-model expectations of how preferences translate into mate choice:**

**assortative mating of recruits from pure pairs**

Because of constraints of mate availability and other factors, female flycatchers are not expected to always mate with a male of the preferred species. We assumed that a female having a preference for a certain species would have a probability of mating with a male of that species equal to the proportion of females of the preferred species that mated conspecifically. For example, if a female prefers Collared males and 95% of Collared females mate with Collared males, then we assume that she has a 95% probability of mating with a Collared male, irrespective of whether she is herself a Pied or a Collared. Male flycatchers do not seem to discriminate against heterospecific females (Sætre et al. 1997a), so the species of a female per se should not influence whether or not a female actually obtains a mate of the preferred species. The overall pattern of assortative mating in each sympatric population may thus be used as a measure of the expected mate choice under conspecific mate preference. We therefore calculated, in each population, the proportion of females of either species mated to Collared males, Pied males and hybrid males. As the expected mate choice pattern of cross-fostered Collared females under genetic inheritance of species recognition, we used the overall mating pattern of Collared females in the population (proportion of breeding females paired to Collared males).

Similarly, we used the mating pattern of females of the foster father species (proportion of Pied females paired with Pied males) as the alternative mating pattern, expected under imprinted preferences. Expected mate choice of cross-fostered Pied females was simply the reverse of the expectations for the cross-fostered Collared females.

However, the overall mating pattern in the population might provide a biased expectation of how preferences translate into mate choice due to several potential pitfalls. First, immigrants from allopatric populations could potentially have different preferences than local recruits (and non-local recruits are more common among pied than collared flycatchers). Second, in the complete data-set, some pure females may have been raised by a heterospecific foster father (due to conspecific extra-pair copulations by females in mixed pairs) and could potentially have developed preferences for heterospecific males due to sexual imprinting (although our results show that imprinting was not important). And last, misidentification of female species could occasionally occur (Pied and Collared females look very similar, and Pied females mated to males of the numerically more common Collared may in particular have been overlooked).
To avoid these potential biases in expected values, we also calculated expectations based on the mate choice of a restricted sample of local female recruits from known pure pairs (excluding cross-fostered recruits). This should provide a more precise estimate of the probability that a female with a certain preference actually obtains a mate according to the preference, but we present results from both approaches since the sample sizes were sometimes small for the females of known pedigree. Since the proportion of the two species and the overall mating patterns differed somewhat among populations, the expected mating patterns were calculated specific to the population where the particular mating pattern of females reared by heterospecific fathers was collected (Gotland for naturally cross-fostered extra-pair recruits, and Öland for experimentally cross-fostered recruits).

**Appendix 6.2**

**Supporting text**

**Reduced recombination between loci located at the Z chromosome**

We base our assumption that physical linkage at the Z chromosome also imply genetic linkage (reduced recombination) on four lines of evidence: 1) there are reduced interspecific recombination rates at the Z chromosome compared to autosomes for crosses between these two particular species (i.e., the Z is sheltered against gene flow). 2) There are reduced intraspecific recombination rates at the Z in one of the species and in birds in general, as predicted by theoretical arguments concerning 3) the Z chromosome in particular and 4) physical linkage in general. These facts all imply that loci situated on the Z chromosome are genetically linked in our system, and taken together the evidence for reduced recombination is very strong. We outline each of these four arguments in more detail below.

1. From genetic analyses of flycatcher hybrids and backcrosses (Sætre et al. 2003)- using species-specific markers located at the Z chromosome and autosomes - we have strong evidence that there is very little or no recombination at the Z chromosome between the two species (whereas autosomes recombine between species, and there is some recombination at the Z within species). This clearly demonstrates that species-specific alleles at loci physically linked at the Z sex chromosome will also be genetically linked because there is no (or very little) introgression of genes on the sex chromosomes between the two gene pools - in contrast to the situation for autosomes. There is no reason to believe that this is unique to flycatchers, but no information is, to our knowledge, available from other organisms with a ZW sex chromosome system.

2. Empirical results from mapping of the chicken genome shows that recombination rates at the Z is only about 25-50 % of that found on autosomes (see
e.g. Sundström et al. 2004). Pedigree-based linkage-mapping in one of our
species shows that the Z chromosome has even lower recombination rates in
flycatchers than in the chicken (Backström et al. 2006a), and that the Z exhibit
high levels of long-range linkage disequilibrium between markers (at least up to
a distance of 500kb; Backström et al. 2006b).

3. Because females only have one Z, any two loci at the Z may only recom-
bine in males (outside the pseudo-autosomal region). From this consideration
alone, two Z-linked loci will therefore be expected to have only half the recombi-
nation rate of two loci at an autosomal chromosome.

4. Unless the probability for crossover is 1 (complete non-linkage), two loci
on the same chromosome will have a reduced recombination rate compared to if
they were at different chromosomes.

There does not have to be complete genetic linkage (zero crossover probability)
for our finding of a physical linkage between these genes to be beneficial for
speciation (Felsenstein 1981). Any crossover probability less than one (partial
linkage) would facilitate reinforcement (Servedio 2000). Compared to a situa-
tion where these loci are located at different chromosomes, our finding of a
physical linkage therefore already suggests that there is likely to be some genetic
linkage, and more probably so because we find not autosomal linkage, but Z-
linkage. Moreover, genetic linkage for the particular situation we are analyzing
is directly established by the finding that sex chromosomes do not recombine
between the two species (Sætre et al. 2003).

**Implications for the flycatcher system**

Although a priori unlikely (at least in the flycatcher system), the design of our
study also allowed us to exclude maternally determined species recognition (W-
linked, or learned by sexual imprinting on the mother). Maternal species recog-
nition predicts that female hybrids should mate with males belonging to the
same species as their mother, opposite to what was found. We also exclude the
possibility of random mating because assortative mating is very strong in the
hybrid zones (see expected mating patterns in figure 6.2 in the main text of this study,
Alatalo et al. 1990; Veen et al. 2001). Furthermore, the results support Z-linkage
in both flycatcher species.

Most female hybrids mated according to the paternal species, but a few did
not. This is not surprising even under strict paternal determination of partner
preferences given that 2–7% of Collared females and 15-30 % of Pied females in
sympatry are involved in heterospecific mating (Alatalo et al. 1982; Sætre et al.
1999a; Veen et al. 2001, this study). We have no strong evidence that flycatcher
females that mate with heterospecific males actually have a preference for such
males over conspecifics, since most of these females mate with conspecific males
at other breeding attempts (Qvarnström et al. 2006b). Instead, mate availability,
time and available territory quality may constrain the mate choice of females. Hence, a particular preference is not expected to always translate into a particular choice of mate (see calculation of expected values above).

Regarding the cross-fostering experiment, the final sample sizes became quite small due to the low return rate of females (only 9 females returned as breeding adults of the estimated $361/2 = 180.5$ female fledglings i.e., 5%). However, the mating patterns obtained are very unlikely to have occurred under sexual imprinting on the foster father since all cross-fostered female Collared recruits ($n = 7$) mated with Collared males (compared to 16% or less on Öland expected from imprinting, binomial $p < 0.001$) and the two female Pied recruits both mated with Pied males (compared to 3% or less expected from imprinting, binomial $p < 0.001$). We would have expected the usual mating patterns to become reversed under sexual imprinting, but they were clearly not.

Our results suggest that a previously reported divergence of mate preferences in female flycatchers in sympatry compared to allopatry (Sætre et al. 1997b) has a genetic basis and is not simply an accidental side-effect of a change in male plumage followed by sexual imprinting on different-looking males. This is important to establish since there is otherwise a danger of circularity if one argues that male plumage has changed in sympatry in response to reinforced female preferences. We still do not know the ultimate reason why male plumage and female preferences have changed in sympathy, but our results are consistent with reinforcement due to selection against hybridization, facilitated by physical linkage among the genes involved in reproductive isolation.

Recent work in the collared flycatcher has found low heritability of a female preference for males with larger forehead patches and low genetic correlation between this preference and the preferred trait (Qvarnström et al. 2006a). This finding is not in contradiction to our result of a genetic determination of preferences for species-assortative mating since only within-species variation in a mate preference was considered in that study. Entirely different evolutionary dynamics may be involved in generating genetic correlations between preferences and preferred traits within as compared to among species (see also Blows 1999).
The evolution of dual-function signals

Thor Veen, G. Sander van Doorn & Franz J. Weissing
Abstract

The evolution of ornaments has been studied extensively in the contexts of male-male competition and female choice. A single ornament can however be used in both contexts, and such ‘dual-function’ signals have been frequently found to occur in nature. The aim of this study is to develop a theoretical framework in order to investigate the occurrence and evolutionary implications of dual-function signalling. We consider a dynamical model where signalling may evolve in the context of male-male competition where males may differ in their fighting capabilities and in the context of female choice in situations where males vary in their ability to provide direct benefits. We find that ornaments can be used in both contexts simultaneously. Furthermore we show that the use of an ornament in one context can facilitate its use in another context, in other words, an ornament used in male-male competition can pave the way for female choice for ornamented males and vice versa. Dual-function signalling does affect the equilibria attained in comparison to single function contexts. Analysing complex signalling system like attempted here is not a trivial task and several restricting assumptions had to be made. Nevertheless, we believe this is a fruitful approach which can provide new insights in processes that were previously too complex to analyse.
Introduction

The use of signals in animal communication is widespread in nature and is evident in many different contexts (Maynard Smith and Harper 2003). Central to signalling is the transfer of information from a ‘sender’ to a ‘receiver’ who might choose to adapt its behaviour accordingly. Signals are frequently used in sexual selection and may function in male-male competition or female choice (e.g. Andersson 1994). The evolution of signalling is not trivial, as the evolutionary interests of the sender and receiver are often not the same. To illustrate this, consider a situation where males with different fighting abilities (or ‘resource holding potential’; Parker 1974) compete over a resource and where engaging in an escalated fight is costly. Males with high fighting abilities could reduce the costs of fighting by signalling their quality to their opponents. If an opponent is of low quality, it should retreat. However, once such a signalling convention is adopted, it might pay for a male with low fighting capabilities to ‘cheat’, i.e. to use the same signal and thereby increasing its chances of getting the resource without an escalated fight. Similarly, for a female it would be beneficial to be able to single out males with high parental ability, since this would increase her reproductive success. Accordingly, it is in the interest of the high-quality males to signal their parental ability, since this would increase their mating chances. However, low-quality males could also benefit from producing the same signal which will result in a reduction of information content of the signal.

Several types of signals are robust against cheating, allowing for reliable signalling to persist (Maynard Smith and Harper 2003; Hurd and Enquist 2005). Indices, for example, are signals that simply cannot be faked. An index (e.g., body weight) thus necessarily indicates the quality of an individual with high reliability. Alternatively, the sender and receiver may have some joint interests, for example avoiding escalated fights, which prevent individuals from cheating (Maynard Smith and Parker 1976; Eshel and Sansone 2001; Maynard Smith and Harper 2003). The badge-of-status is a well-known example of such a convention (Krebs and Dawkins 1984). A third mechanism that allows for evolutionarily stable, honest signalling is the handicap principle (Zahavi 1975; Grafen 1990). A signal is a handicap if the cost of signal production depends on the individual’s quality, so that low-quality individuals have no or reduced ornament expression.

Traditionally, the evolution of signalling has been investigated separately in the contexts of female choice and male-male competition (Andersson 1994). Substantial empirical evidence for female preference for ‘handicap’ ornaments has lead to the common wisdom that female choice is based on costly traits (such as carotenoid-based feather ornaments; Olson and Owens 1998). In contrast, research on signalling in male-male competition has predominantly...
focused on conventional signals, which can be very cheap to produce (e.g. melanin based ornaments; Badyaev and Hill 2000). Whether there is indeed a dichotomy in the cost and function of signals used in inter- versus intra-sexual selection has been questioned (e.g. Maynard Smith 1994). For example, for feather pigmentations it was found that carotenoids might not be as costly as previously assumed (Jawor and Breitwisch 2003; Griffith et al. 2006). Another argument against a mechanistic link between cost and function comes from the observation that in nature, the same ornaments is frequently used in both male-male competition and female choice. Examples of such dual-function signals are the red belly of stickleback (Candolin 2000), the black facial mask of the common yellowthroat (Tarof et al. 2005) and the eye span of the stalk-eyed fly (Cotton et al. 2004 and references therein).

The idea that signals can have a function in both sexual selection contexts is not new (Kodric-Brown and Brown 1984; Andersson 1994) and has considerable empirical support (see Berglund et al. 1996 and references therein). Interactions between female choice and male-male competition may have important evolutionary implications, for example, when speciation is driven by sexual selection (Seehausen and Schluter 2004; van Doorn et al. 2004). There has been an increased awareness and interest among empiricists in dual-function signals (e.g. Candolin 2000; Wong and Candolin 2005; Griggio et al. 2007).

The theoretical interest in this topic has been limited. We are only aware of a single theoretical study that addressed this problem under highly simplifying assumptions (Johnstone and Norris 1993). The lack of a good theoretical understanding of dual-function signalling is not surprising, as male-male competition itself is complex and difficult to fully comprehend. Combining this with female choice increases the difficulties even further. Still we think that an attempt to integrate both contexts has to be made. This paper is a first step in this direction. It is well conceivable that interactions between inter- and intra-sexual selection change signal evolution and lead to fundamentally different predictions compared to a situation where such interactions are absent.

The issue of dual-function signalling presents a number of interesting questions. The first one relates to the establishment of new signals. The evolution of a new signal is not trivial, and may require special conditions, such as a sensory bias in the receiver of the signal. Signal evolution might be less problematic if the signal is used in another context and therefore already present in the population. Second, conventional signals in male-male competition are cheap to produce, but will such conventional signalling system remain stable when expressing the signal provides an additional benefit in a different context (e.g. increased mating chance)? Third, and more generally, should one expect that signals used in one context become integrated in another context, or should one rather expect the evolution of separate signals for different contexts?
The aim of this study is to develop a dynamical model incorporating male-male competition and female choice with the possibility to study both processes in isolation and in concert. Male-male competition is based on an asymmetrical Hawk-Dove game played in a population of individuals with different fighting abilities. Female choice may evolve as a consequence of differences in parental ability between the males. Males may invest in an ornament to signal their abilities. We will first describe male-male competition and female choice separately. Importantly, the model structure we use allows for reciprocal interaction between the two processes. This opens up the possibility for a signal with a single function to attain a dual function, i.e., a signalling function in both male-male competition and female choice. However, due to the inherent complexity of such a complex signalling model, we have to make several simplifying assumptions. For example, our model currently incorporates phenotypic quality differences but no heritable variation in quality as in good-genes models. The first aim of this study is to find out if signals with a combined function can arise. Second, to what extent do male-male competition and female choice work synergistically? Does female choice facilitate or hamper the use of signals in male-male competition? Lastly, does a dual function have an effect on signal-dependent behaviour in each of the two contexts? Do males play other condition-dependent strategies if signals have a dual function?

**Model overview**

**Quality differences and ornamentation**
We are interested in the evolution of signals that are used in different contexts, in this case male-male competition and female choice. We ask ourselves whether reliable signals can evolve either for fighting ability in male-male competition or parental ability in the context of female choice. For simplicity we assume that the differences in male ‘qualities’ (i.e. fighting ability and/or parental ability) are not heritable and assigned at random. Accordingly, we do not address indirect benefits of signalling systems that are related to heritable quality differences. Each male is faced with the decision whether or not to invest in an ornament. Ornament investment is costly and can, for simplicity, not be made dependent on the male’s quality. One might think of a system where investment decisions are made before quality differences arise. Whether investment results in the expression of the ornament depends on the quality of the male: the probability that an ornament is actually expressed is higher for a high quality male. In a single function context, ornament expression is dependent on either fighting or parental ability. In a dual-function context, an individual’s ‘overall’ quality is determined by the combined effect of both fighting and parental ability.
We will discuss these assumptions in more detail later on. Figure 7.1 provides an overview of the model structure. We will now continue to briefly introduce the different parts of the model (male-male competition, female choice and both combined). A more detailed explanation will be given when the results of these models are presented.

### Male-male competition

Each male is involved in a single contest, which is modelled by an asymmetrical Hawk-Dove game where the players differ in fighting ability (e.g. Maynard Smith and Price 1973; Maynard Smith and Parker 1976; Hammerstein 1981). It is well known that external cues can and should be used to settle a contest, since such cues allow the avoidance of escalated conflicts. The question we pose is whether such cues (i.e. ornaments) will evolve from scratch if they are costly to produce. Every individual knows his own fighting ability, but not that of his opponent. Accordingly, strategic decisions with respect to the conflict can only
be based on one’s own fighting quality and whether or not the opponent has an ornament. For simplicity we neglect the possibility that an individual can also adjust its strategy on whether or not he actually expresses the ornament. Whether or not the opponent expresses the ornament is informative with respect to the expected outcome of an escalated conflict, since the ornament is assumed to be a revealing handicap. Each male can decide to invest in the ornament, but this will come at a cost (reduced viability). Among the individuals that chose to invest in the ornament, individuals with low fighting capability express the ornament with a lower probability than high-quality individuals. One could easily imagine such a condition-dependent ornament to serve as a signal, and one would expect an individual with low fighting capabilities to be more reluctant to escalate and fight when faced with an ornamented opponent, since such an opponent is likely to have high fighting capabilities. In a first step we want to address the question if males will invest in such costly ornaments and make their strategy dependent on it.

**Female choice**
Each female samples two males and is more likely to mate with the more attractive one of them (best-of-two; Bulmer 1989). The males differ in their ability to provide direct benefits, i.e. mating with a high quality male will increase the reproductive success of a female (e.g. Hoelzer 1989). Males can invest in a costly ornament and ornament expression is condition dependent such that males with low parental ability have a lower probability of expressing the ornament. The question is whether and under which circumstances females will express a costly preference for ornamented males, and whether males will invest in ornament expression.

**Interaction between male-male competition and female choice**
Whether investment in an ornament results in expression depends on the fighting quality and parental quality of the males, which so far has acted in isolation because we focussed on one of the two contexts. This situation changes when combining both models and male ornamentation can potentially acquire a function in both contexts simultaneously. A very important consideration is that ‘quality’ now becomes a two dimensional attribute and ornament expression is in various different ways correlated with the two underlying qualities. A second important component of the model is that the payoff of the Hawk-Dove conflict affects the reproductive success of brood, thus both male and female profit from these (potential) benefits. The reproductive success of a brood is therefore determined by a joint effect of the payoff from the Hawk-Dove game and parental qualities of the male. The sequence of events is as follows. At the start of a generation, each male will meet one opponent and may use his own fighting ability and the presence/absence of an ornament of the opponent to determine
which strategy to play in this Hawk-Dove game. Each female will then choose a partner from two candidates, and her choice may be affected by a preference. After the choice process, the pair will reproduce.

**Model implementation**

In order to accommodate all aspects as described, we need six variables for the full model:

- $i$ frequency of ornament investment
- $p$ frequency of preference for ornamented males
- $h_{+,1}$ frequency of playing Hawk for a male with high fighting ability (+) confronted with an ornamented opponent (1).
- $h_{+,0}$ frequency of playing Hawk for a male with high fighting ability (+) confronted with a non-ornamented opponent (0).
- $h_{-,1}$ frequency of playing Hawk for a male with low fighting ability (−) confronted with an ornamented opponent (1).
- $h_{-,0}$ frequency of playing Hawk for a male with low fighting ability (−) confronted with a non-ornamented opponent (0).

We assume that individuals are haploid and carry six gene loci, with free recombination between all loci. Each of the six loci corresponds to one of the six variables. At each locus, an allele can take the value 0 or 1. The allele on a given locus can change by mutation with a low probability $\mu$. Generations are discrete and non-overlapping. The life-cycle of an individual is as follows: during reproduction the gametes of both parents fuse, after which recombination and meiosis takes place resulting in an individual with a haploid genotype. This individual is then assigned a fighting ability (±) and a parental ability (±), both with probability $\frac{1}{2}$. The individual may invest in an ornament (only males) but by doing so he pays a viability costs. The fighting and parental ability influences ornament expression, such that individuals with low abilities have reduced ornament expression. Similarly, a female may invest in expressing a preference, which also entails a viability cost. In the next step, all males engage in one contest which outcome will influence their reproductive success. In the last step, females will choose a male and reproduce.

With some additional assumptions it is possible in principle to translate the above described information into a population genetic model and derive recurrence equations describing the change of genotype frequencies over time. In practice this is a forbidding task since even for six diallelic loci the number of possible genotypes is very large ($2^6 = 64$). For this reason we make use of the quasi-linkage equilibrium technique (QLE; e.g. Kirkpatrick et al. 2002), which under the assumption of weak selection characterises the evolutionary dynamics by recurrence equation for the allele frequencies of the six loci:
Ornament investment: \[ \Delta i = \frac{1}{2}i(1 - i)(\epsilon \tilde{\sigma}_i + \epsilon \tilde{\tau}_i) \] 

Preference: \[ \Delta p = \frac{1}{2}p(1 - p)[\epsilon^2(\tilde{\sigma}_p + \tilde{\omega}_p) + \epsilon \tilde{\sigma}_{i,p} \Delta i] \]

Strategy \( h_{+,1} \): \[ \Delta h_{+,1} = \frac{1}{2}h_{+,1}(1 - h_{+,1})\epsilon \tilde{\sigma}_{h_{+,1}} \]

Strategy \( h_{+,0} \): \[ \Delta h_{+,0} = \frac{1}{2}h_{+,0}(1 - h_{+,0})\epsilon \tilde{\sigma}_{h_{+,0}} \]

Strategy \( h_{-,1} \): \[ \Delta h_{-,1} = \frac{1}{2}h_{-,1}(1 - h_{-,1})\epsilon \tilde{\sigma}_{h_{-,1}} \]

Strategy \( h_{-,0} \): \[ \Delta h_{-,0} = \frac{1}{2}h_{-,0}(1 - h_{-,0})\epsilon \tilde{\sigma}_{h_{-,0}} \]

Notice that the allele frequency dynamics all have the same basic structure: the allele frequency change \( \Delta x \) is equal to \( \frac{1}{2} \) (corresponding to sex limitation of the traits) times \( x(1-x) \) (corresponding to the variance in allele frequencies) times the selection differential at the corresponding locus. The term \( \epsilon \) quantifies the strength of selection. The QLE approximation (1) to (6) is valid for \( \epsilon \ll 1 \). See appendix 7.1 for a detailed justification and the derivation of the selection differentials.

### Male-male competition

**The model assumptions**

Depending on the allele at the investment locus, each male will decide on whether to invest in the ornament or not. Investing in the ornament comes at a cost \( c_i \) (an overview of all parameters and their meaning is given in table 7.1). Males with a high fighting ability who invest in the ornament will always express the ornament whereas males with a low fighting ability who invest in the ornament only express it with a probability \( \eta_f \).

**Table 7.1.** Overview of the parameter used in the full model and a short description of their meaning.

<table>
<thead>
<tr>
<th>symbol</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V )</td>
<td>value of resource in Hawk-Dove game</td>
</tr>
<tr>
<td>( C )</td>
<td>cost of losing escalated fight in Hawk-Dove game</td>
</tr>
<tr>
<td>( b )</td>
<td>direct benefits provided by the male</td>
</tr>
<tr>
<td>( c_i )</td>
<td>viability cost of investing in an ornament for males</td>
</tr>
<tr>
<td>( c_p )</td>
<td>viability cost of preference expression for females</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>fighting asymmetry: probability of winning an escalated fight is ( \frac{1}{2} + \alpha ) for the high fighting ability male</td>
</tr>
<tr>
<td>( \gamma )</td>
<td>strength of female preference: probability to choose a preferred male is ( \frac{1}{2} + \gamma )</td>
</tr>
<tr>
<td>( \eta_f )</td>
<td>probability for a male with low fighting quality that ornament investment leads to ornament expression</td>
</tr>
<tr>
<td>( \eta_p )</td>
<td>probability for a male with low parental quality that ornament investment leads to ornament expression</td>
</tr>
</tbody>
</table>
Each male is involved in one contest with a randomly assigned opponent. The choice of strategy played depends on one’s own quality and ornament expression of the opponent. If both competitors play different strategies, the Hawk will get the resource of value $V$ and the Dove will get 0. In case both play Dove, the resource is shared, and each male accrues a payoff of $\frac{1}{2}V$. The probability to win a Hawk-Hawk contest is determined by differences in fighting ability. If both competitors have the same fighting ability, each will have a probability of $\frac{1}{2}$ to win, but if they differ in fighting ability, the high-quality male will win with a probability $\frac{1}{2}+\alpha$ and the low quality male with probability $\frac{1}{2}–\alpha$. Hence, $\alpha(0 \leq \alpha \leq \frac{1}{2})$ is a measure for the asymmetry between high- and low-quality males. The payoff for the winner of the Hawk-Hawk conflict is $V$ and for the loser $C$. In the special case of $\alpha=0$, the differences in ‘fighting ability’ do not have any physiological meaning and signalling evolving in this context is purely conventional (e.g. Maynard Smith and Parker 1976; Hurd 2006). The payoff acquired in the contest affects the individual’s reproductive success and that of his breeding partner.

**Signalling and non-signalling equilibria**

It is conceivable that this system can attain different types of equilibria. To illustrate this, assume first that no individual invests in the ornament ($i=0$), hence no ornaments are being developed. This means that males can only base there strategic decisions on their own fighting capability and selection only acts on the strategy loci $h_{+,0}$ and $h_{-,0}$. A game theoretical analysis (see appendix 7.2) reveals that, depending on the relation between the fighting asymmetry $\alpha$ and the benefit-cost ratio $V/C$, three types of equilibria do exist which are illustrated in figure 7.2A. For $V/C < \frac{1}{2}$ the unique Nash equilibrium is given by $\hat{h}_{+,0} = \frac{2V}{C}$ and $\hat{h}_{-,0} = 0$. From now on we refer to an equilibrium with $i=0$ as a ‘non-signalling’ equilibrium because the males base their decision on their own quality and no ornaments are expressed.

For a second extreme scenario imagine that all individuals invest in the ornament ($i = 1$). In this situation, all the four strategy loci will be affected by selection because there are both ornamented males (all high quality and a fraction $\eta_f$ of the low quality individuals) and non-ornamented males (a fraction $1–\eta_f$ of the low quality individuals). Now it is no longer the case that the strategic behaviour at such ‘signalling’ equilibrium is uniquely determined by the model parameters. In fact, ‘paradoxical’ strategies (like individuals with low fighting ability playing Hawk when confronted with an ornamented opponent) may coexist with ‘reasonable’ strategies where high-quality individuals have a higher tendency to escalate then low-quality individuals. The unique Nash equilibrium with $\hat{h}_{-,1} = 0$ is illustrated in figure 7.2B. Notice that $\hat{h}_{+,1} = 1$ for all values of $V/C$ while $\hat{h}_{-,0}$ and $\hat{h}_{+,1}$ are mixed strategies for small to moderate values of $V/C$. Furthermore notice that $\hat{h}_{-,0} \geq \hat{h}_{+,1}$.
Evolutionary dynamics

To explore the dynamics of the model we iterated system (1) to (6). As illustrated in figure 7.3, the full system can indeed evolve to either a signalling or a non-signalling equilibrium. If we start at $h_{+,1}=h_{+,0}=h_{-,1}=h_{-,0}=V/C$, the evolutionary stable strategy of the symmetric Hawk-Dove game, the system will typically end up at the non-signalling equilibrium (figure 7.3A). The system will only evolve to the signalling equilibrium if the vast majority of the initial population invests in the signal (i.e. $i$ close to 1) (figure 7.3B). The parameter settings for these and other simulations presented in this paper can be found in table 7.2.

Table 7.2. Parameter values used in the figures.

<table>
<thead>
<tr>
<th>parameter</th>
<th>figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V$</td>
<td>7.2</td>
</tr>
<tr>
<td>$C$</td>
<td>0.04</td>
</tr>
<tr>
<td>$c_i$</td>
<td>0.1</td>
</tr>
<tr>
<td>$c_p$</td>
<td>0.01</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>-</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.25</td>
</tr>
<tr>
<td>$b$</td>
<td>-</td>
</tr>
<tr>
<td>$\eta_f$</td>
<td>0.5</td>
</tr>
<tr>
<td>$\eta_p$</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 7.2. The equilibrium values for the situation where no male invests in an ornament (‘non-signalling’ equilibrium, A) and where all males invest in an ornament (signalling equilibrium, B). See appendix 7.2 for details.
To get a more complete overview of the model behaviour we performed a bifurcation analysis with the program CONTENT (Kuznetsov and Levitin 1997). Figure 7.4 shows how the equilibrium pattern changes with a change in the parameter $\alpha$ (the asymmetry in fighting ability). It turns out that the non-signalling equilibrium (here the initial $i$ is 0.8) (A). The threshold value for this particular model is very high; the system only evolves to the signalling equilibrium if $i$ starts very close to 1 (B).

To get a more complete overview of the model behaviour we performed a bifurcation analysis with the program CONTENT (Kuznetsov and Levitin 1997). Figure 7.4 shows how the equilibrium pattern changes with a change in the parameter $\alpha$ (the asymmetry in fighting ability). It turns out that the non-signalling equilibrium is a stable equilibrium for each value of $\alpha$ ($0 < \alpha \leq \frac{1}{2}$; figure 7.4A). The signalling equilibrium is an alternative stable outcome (figure 7.4C) if $\alpha$ is below a certain threshold value (here $\alpha \leq \frac{1}{2} + \alpha \leq 0.88$). For values
of \( \alpha \) above a certain threshold value (here \( \frac{1}{2} + \alpha \geq 0.69 \)) there exists a third, ‘ambiguous’ equilibrium in which only a fraction of the population invests in the ornament (figure 7.4B). At this equilibrium a pure Dove strategy is played by low-quality individuals (i.e. \( \hat{h}_{-,0} = \hat{h}_{-,1} = 0 \)) while a pure Hawk strategy is played by high-quality individuals confronted with a non-ornamented opponent (\( h_{+,0} = 1 \)). In none of the runs of our model a paradoxical signalling equilibrium was observed.

Figure 7.4. The three types of stable equilibria for male-male competition with the possibility of signal use. (A) For all values of the fighting asymmetry, \( \alpha \), the non-signalling equilibrium characterised by \( i = 0 \) is stable. (B) For large values of \( \alpha \), an ‘ambiguous’ investment strategy is an alternative stable equilibrium. Here, only a fraction of the population invests in the ornament and all low-quality males play Dove. (C) For small to moderate values of \( \alpha \) a signalling equilibrium is an alternative stable outcome.
Female choice model

Model assumptions
Each female will mate once and has the choice between two randomly drawn males. Each female is either choosy or she mates at random. When a choosy female is confronted with two males differing in ornament expression, she mates with the ornamented male with a probability $\frac{1}{2} + \gamma$ and with the non-ornamented male with a probability $\frac{1}{2} - \gamma$. The variable $p$ corresponds with the relative frequency of choosy females in the population. Expression of the preference is costly: females with a preference have to pay a viability cost $c_p$. Females benefit from mating with a male with high parental abilities, since in that case her reproductive success is increased by $b(b > 0)$.

As before, males can invest in an ornament and the probability of this resulting in the expression of the ornament depends on the parental quality of the males. For simplicity we assume that investing males of high parental quality always express the ornament, while investing males of low parental quality will express the ornament with probability $\eta_p$.

Evolutionary dynamics
Direct benefit models of mate choice as considered here are relatively easy to understand (e.g. Kokko et al. 2003). For females it is beneficial to invest in a

\[ \text{Figure 7.5. Bifurcation diagram showing the co-occurrence of the three types of equilibria depending on the model parameters } \alpha \text{ and } \eta_f. \text{ The numbers refer to the number of coexisting equilibria while the types of equilibria are indicated in brackets (n = non-signalling, a = ambiguous and s = signalling). The dotted line indicates the } \eta_f \text{ setting used in figure 7.4.} \]
Figure 7.6. The two types of stable equilibria for female choice. (A) For all values of the direct benefits, $b$, the no preference equilibrium characterised by $p = 0$ is stable. (B) For large values of $b$, a second ‘preference’ equilibrium is an alternative stable equilibrium.

Figure 7.7. Bifurcation diagram showing the co-occurrence of the two types of equilibria for female choice depending on the model parameters $b$ and $\eta_P$. The numbers refer to the number of coexisting equilibria while the types of equilibria are indicated in brackets ($n =$ no preference and $p =$ preference). The dotted line indicates the $\eta_P$ setting used in figure 7.6.
preference for ornamented males if the costs of choice are outweighed by the benefits of this choice (i.e. a higher reproductive success due to mating with a male providing direct benefits). Iteration of the model shows that there are two types of equilibria which are illustrated in figure 7.6. For all values of \( b, \hat{p} = 0 \) (i.e. the absence of a preference) is a stable equilibrium. Obviously, the investment level of the males in the ornament is equal to zero when females do not exert preference for the ornament. If the direct benefits are larger than a threshold value, ‘preference equilibrium’ with \( 0 < \hat{p} \leq 1 \) and \( \hat{i} = 1 \) is an alternative stable strategy.

### Dual-function signalling

**Dual quality and ornament expression**

After these preparations, we are now able to link signalling in the contexts of male-male competition and female choice in an integrative model. In comparison to the constituent models, we now have to face the difficulty that male quality is a two-dimensional trait, consisting of fighting ability and parental ability. It is therefore no longer straightforward to translate a male’s investment into the ornament into quality-dependent expression of the ornament. We chose to implement this in a multiplicative way, corresponding to the assumption that both quality components affect ornament expression in the same way, and that there are no synergistic effects between the quality components. To be more explicit, we assumed that the probability that an investing male actually expresses the ornament is given by:

\[
\begin{align*}
1, & \text{ if male has high fighting and high parental ability;} \\
\eta_p, & \text{ if male has high fighting and low parental ability;} \\
\eta_f, & \text{ if male has low fighting and high parental ability;} \\
\eta_p \eta_f, & \text{ if male has low fighting and low parental ability.}
\end{align*}
\]

Notice that the joint effect of both quality components on ornament expression has important implications on both male-male competition and female choice, since the ornament becomes a less reliable indicator of each of the constituent quality components. For example, an investing male with high fighting ability does no longer automatically express the ornament (if it has low parental ability). As a consequence, in a population with maximal investment (i.e. \( \hat{i} = 1 \)), the fact that one’s opponent does not have an ornament does not imply that the opponent has low fighting ability. In other words, in the combined model the information content of the signal is inherently lower than in the constituent models, and this in turn should impede the evolution of costly signalling.
Analysis of the integrated model

We can now investigate whether, and under what conditions, signals with a dual function can evolve. In particular, we are interested in the question whether female choice affects signalling in male-male competition, and vice versa. Moreover, we will address how the strategic behaviour in the Hawk-Dove game (i.e. the strategies $\hat{h}_{+,1}$, $\hat{h}_{+,0}$, $\hat{h}_{-,1}$, $\hat{h}_{-,0}$) and/or the frequency of female preference ($\hat{p}$) is affected when a signal gets an additional function.

To study these questions, we first perform an equilibrium analysis in the combined model for each of the contexts (i.e. male-male competition or female choice) in isolation. This is necessary because in the combined model signals are inherently less reliable than in each constituent model. Starting from a male-male competition (respectively female choice) equilibrium, we then add the possibility for signalling to evolve in the complementary context.

We choose the following parameter combination: $V = 0.04$, $C = 0.1$, $c_i = 0.01$, $c_p = 0.001$, $\gamma = 0.05$, $\alpha = 0.2$, $b = 0.05$, $\eta_f = 0.6$ and $\eta_p = 0.4$. In line with figure 7.6, a female preference would not evolve as a single function for these parameters. Based on figure 7.4, one might expect three types of equilibria in the male-male competition context. However, it turns out that the signalling equilibrium ceases to exist in the combined model, because now the signal is less informative than in the constituent model on which figure 7.4 is based. In other words, only the non-signalling equilibrium and the ambiguous equilibrium are feasible as single-function equilibria in the male-male context.

Evolution of a dual-function signal

Let us start with the ambiguous signalling equilibrium in the male-male context. Figure 7.8 shows that allowing female preferences to evolve does indeed have important implications. First, the females express a preference for the same parameters for which this was not feasible in the single-function context. Second, the ambiguous male-male signalling equilibrium is turned into a ‘full-fledged’ signalling equilibrium where all the males invest into the signal. This example clearly demonstrates that dual-function signals can evolve, and that signalling in one context can facilitate the evolution of signalling in the other context.

Getting signalling off the ground

In the previous example, we started with a situation where already some males invested into the developing an ornament. It is less straightforward to study the effect of female choice on the non-signalling equilibrium in male-male interaction, and the effect of male-male competition on the no preference equilibrium in female choice. In fact, we know already that non-signalling, respectively no preference, is a stable equilibrium in the constituent models for all parameter combinations. This remains to be the case in the combined model: preference
cannot spread in the non-signalling equilibrium and signalling cannot spread in the non-preference equilibrium.

Still, we can study the question whether the stability of the no preference/non-signalling equilibrium is affected by allowing the signal to attain a dual function. To this end, we perform a perturbation analysis that will provide us with insights in whether signalling can get ‘off the ground’ more easily if the signal has a dual function.

Evolution of male-male signalling through female preference
We tested the stability of the non-signalling male-male equilibrium in the absence of female choice by repeatedly perturbing this equilibrium by introducing a higher frequency of investing males. In each case, the population rapidly returned to its initial state. This is in line with figure 7.2, which illustrates that in the male-male competition model alone, it is extremely difficult to get signalling off the ground. The situation changes if female preferences can

Figure 7.8. In the first part of the figure (A), male-male competition evolves towards the ambiguous signalling equilibrium. At a certain moment (indicated by the dashed lines) the female preference is introduced and a preference evolves (B). The ornament investment and preference frequencies are depicted in the upper panel, the allele frequencies of the four strategy loci in the lower panel.
Evolution of female preference through male-male signalling

For the parameters chosen, female preference cannot evolve if the signal only functions in a female choice context. This was tested by perturbing the by introducing a large frequency of females expressing a preference. Similarly to the male-male equilibrium, all frequencies returned to the initial conditions. This situation did not change when signal use in male-male competition was set free to evolve. Perturbations of the initial frequencies of investing males did not change the female preference equilibrium, nor the non-signalling equilibrium in male-male competition (figure 7.9B).
Conclusions and Implication

In this paper we provide proof of principle that dual-function signalling can evolve. Importantly, we find that male-male competition and female choice synergistically affect the evolution of signalling, i.e. signalling equilibria are more easily attained when the signal can have a dual function. A dual function of a signal affects the equilibria reached in comparison to a single function context. We find that ornament investment by males increases with a dual function and the strategic choices made in male-male competition change accordingly. Similarly, the female preference increases in the population. However, our results also emphasize the difficulties of destabilising the non-signalling equilibrium; in other words, the use of signals in either context is not trivial. Although our results show that the possibility of signal use in multiple contexts might facilitate the commencement of signal use, it remains to be quantified how significant this effect is.

We are aware that with this analysis, we have not more than scratched the surface of signal use in more multiple contexts. The kind of models as used in this study is notoriously intricate and not easy to analyse. For this reason we have, for this first attempt, made a large number of simplifying assumptions:

- the quality components interact multiplicatively, which assumes that both components have a similar effect on ornament expression and don’t, for example, work synergistically.
- only direct benefits are considered. This excludes models based on heritable quality components such as ‘good genes’ models which are widely used in sexual selection theory.
- females can profit twice; from direct benefits obtained by males in the contest and parental quality of the male. An obvious alternative is that contests between males only influence their mating chance.
- the ornament can only be present or absent; it cannot take on an intermediate value which would be required if the ornament would be a revealing handicap.
- males cannot base their investment strategies on their own quality. Such condition dependent investment can create complex non-equilibrium dynamics (van Doorn and Weissing 2006) and was therefore excluded.
- male contest strategy can only depend on own quality, but not on own ornament expression.

Even with these simplifications, the analysis of the resulting models is difficult. Here we made use of the QLE technique, which is only applicable in cases of weak selection, but which has the huge advantage that the dimensionality of the problem is reduced enormously (from $2^6$ to 6 recurrence equations). Individual based simulations can be used to test how robust our results are against
violations of the weak selection assumption. Furthermore, these models allow us to explore the effects of stochastic processes, such as drift, on the dynamics of the systems. Parts of our model have been analysed using individual based simulations and this validated the results obtained by the QLE technique.

The model presented here should be seen as a first step trying to link signalling processes in different sexual selection contexts. If successful, this might lead towards a more unifying theory of sexual selection.

Acknowledgements
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Appendix 7.1

The Evolution of Dual-Function Signals

Appendix - derivation of population-genetic equations

1 - Introduction

This appendix outlines the derivation of the population-genetic recurrence equations presented in the main text. Rather than deriving equations for genotype frequencies, as one would normally do, we derive equations for the change of genetical associations (i.e., statistical moments of the genotype frequency distribution) from one generation to the next. For an explanation of this technique we refer to


This appendix consists of four parts. After this introductory part, we define Mathematica procedures to calculate how selection and sexual reproduction affect the genetical associations (part 2 - the toolbox). Next, we calculate the selection coefficients that act on individual allele frequencies or on genetical associations between sets of alleles (part 3 - selection coefficients). In the final part of this appendix, we derive approximations for the change of allele frequencies from one generation to the next.

This appendix was written in Mathematica (Wolfram, 2003), and is provided in two versions. This printed version is a summary of a complete Mathematica worksheet that is available from the authors on request or at http://www.rug.nl/biologie/onderzoek/onderzoeksgroepen/theoreticalbiology/index. The complete version can be used to verify the calculations.

2 - The Toolbox

- Preliminaries - definition of positions and reference values

The evolving characters of our model are encoded by six loci, one locus for each character. Each locus segregates for two alleles. We keep track of the allele frequencies (denoted $f_k$ for locus $k$) and of the statistical associations between allele frequencies at different loci (denoted by $D[A]$, where $A$ may represent any set of loci).

In the procedures defined below, we use $P = \{ p \}$, $I = \{ i \}$ and $H = \{ h_1, h_2, h_3, h_4 \}$ to denote the sets of loci influencing female mating preference, male ornament investment and the male's
strategy in the Hawk-Dove game, respectively. Furthermore, we define \( M = I \cup H \) and \( \mathcal{W} = M \cup P \).

Genetical associations are defined with respect to a set of reference values that can be arbitrarily chosen. We choose the reference values equal to the allele frequencies at the zygote stage since this causes first-order associations at the zygote stage to vanish.

- Procedures

In the following section we implement five procedures that are used to derive our model equations. The first one is needed to expand associations with repeated indices.

```plaintext
removeDupl[Asctn_, A_List, B_List] :=
Module[{AB, dupl, k},
  AB = Join[A, B];
  If[Length[AB] > 0,
    If[dupl = Do[
      If[Count[AB, AB[[k]]] > 1, Return[AB[[k]]],
      , {k, 1, Length[AB]}]
    ]; MemberQ[AB, dupl],
    Return[\[rho]dupl (1 - \[rho]dupl) removeDupl[Asctn, DeleteCases[
      A, dupl], DeleteCases[B, dupl]] + (1 - 2 \[rho]dupl)
    removeDupl[Asctn, A, DeleteCases[B, dupl]]],
    Return[Asctn[Sort[Join[A, B]]]]],
  Return[Asctn[{}]]]
]
```

Viability selection changes the genetical associations. We use the following procedure to express genetical associations in female and male adults (denoted by \( D_f[A] \) and \( D_m[A] \), respectively) in terms of genetical associations in zyogotes (denoted as \( D_0[A] \)).

```plaintext
selection = \{ D_f[A_List] \} :=
Module[{C, dsym = D_0[A], k},
  C = Subsets[P];
  Do[
    dsym += \[omega]_C[k] (removeDupl[D_0, A, C[[k]]] -
    D_0[A] D_0[C[[k]]]), {k, 1, Length[C]}];
  dsym /. reference values
  ];
D_m[A_List] :=
Module[{C, dsym = D_0[A], k},
  C = Subsets[I];
  Do[
    dsym += \[tau]_C[k] (removeDupl[D_0, A, C[[k]]] -
    D_0[A] D_0[C[[k]]]), {k, 1, Length[C]}];
  dsym
  ];
```

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The coefficients $\tau_A$ and $\omega_A$ represent selection coefficients that capture the effect of selection on the loci in the set A. The values of these selection coefficients will be calculated in part 3 below.

The surviving adults form mating groups and reproduce sexually. The process of sexual selection, i.e., the conflict between the males and the subsequent choice of a mate by the female, is modelled by a single step of selection at the level of the mating group. A mating group is defined by the genotype of the female (loci with subscript $f$) and the genotypes of the two males (loci with subscripts $m_1$ and $m_2$) between whom she chooses. The fitness of a mating group reflects the probability that the female chooses to mate with the first male. The next procedure is used to map the genetical associations in the eventual mating pair back to associations in males and females after viability selection.

\[
\text{reproduction} = D_p[A\_List] \Rightarrow
\]
Module[\{Af, Aml, S, dsum, k, Uf, Uml, Um2\},
Af =
Complement[A, Replace[W, x_ \rightarrow x_n, 1]] /.
{x_ \rightarrow x};
Aml = Complement[A, Replace[W, x_ \rightarrow x_f, 1]] /.
{x_ \rightarrow x};
S = Subsets[Join[Replace[F, x_ \rightarrow x_f, 1], Replace[
M, x_ \rightarrow x_n, 1], Replace[M, x_ \rightarrow x_m, 1]], 2];
dsum = D_f[Af] D_n[Aml];
Do[
Uf = DeleteCases[S[[k]], _, _] /.
{x_ \rightarrow x};
Uml = DeleteCases[
DeleteCases[S[[k]], _, _], _, _] /.
{x_ \rightarrow x};
Um2 = DeleteCases[DeleteCases[S[[k]], _, _], _, _] /.
{x_ \rightarrow x};
dsum += \sum_{\text{oct}} S[[k]] \{\text{removeDup1}[D_f, Af, Uf]
\text{removeDup1}[D_n, Aml, Uml] D_n[Um2] -
\{k, 1, Length[S]\}];
dsum /.
{D_f[\{\}], D_n[\{\}] \rightarrow 1}\];

After mating pairs are formed, the female and male in the pair produce offspring. To calculate the values of association in the offspring, we use the following procedure. For simplicity, we assume single recombination between loci.

\[
\text{transmission} = D_s[A\_List] \Rightarrow
\]
Module[
\{B = Subsets[A], Af, Am, k, tc = 1 / 2^{\text{Length}[A]}, dsum = 0\},
Do[
Af = Replace[B[[k]], x_ \rightarrow x_f, 1];
Am = Replace[Complement[A, B[[k]]], x_ \rightarrow x_n, 1];
dsum += tc D_p[Sort[Join[Af, Am]]], \{k, 1, Length[B]\}];
dsum /.
D_p[\{\}] \rightarrow 1\];
The value of any given association can now be expressed in terms of associations at earlier phases of the lifecycle. In particular, by applying the following substitution rule, which traces its way back through a complete lifecycle, we can calculate how an association changes from one generation to the next.

\[
\text{lifeCycle} = \text{eq}_\Rightarrow (\text{eq} \text{/} . \text{changeReferenceValues} \text{/}. \text{transmission} \text{/}. \text{reproduction} \text{/}. \text{selection} \text{/}. \text{referencevalues});
\]

From here on, we assume that selection is weak. For example, we assume that \( \tau_{ij} \) can be written as \( \epsilon \bar{\tau}_{ij} \), where \( \epsilon \ll 1 \) and \( \bar{\tau}_{ij} = \mathcal{O}(1) \). An analogous situation applies to all other selection coefficients. As is common in sexual selection models we assume moreover that direct selection on female preference is weak relative to other selection pressures. Hence, \( \omega_{p_f} = \mathcal{O}(\epsilon^2) \). Since selection is weak relative to recombination, the genetical associations between loci will tend to be small. This allows us, later on, to drastically simplify the model by means of a Quasi-Linkage-Equilibrium (QLE) approximation.

### 3 - Selection coefficients

So far we have treated the selection coefficients as unspecified coefficients \( \sigma_A, \tau_A \) and \( \omega_A \). In this section we calculate the actual values of these coefficients. To do that, we need to define procedures to express the fitness of a genotype as a sum of contributions from individual loci and associations between loci, as detailed in Kirkpatrick, Johnson & Barton (2002). We will first calculate the viability selection coefficients, and then proceed to calculate the more intricate sexual selection coefficients.

Only a single locus is exposed directly to viability selection in males. The following procedure expresses the fitness of the two possible genotypes at this single locus in terms of allele frequencies and the selection coefficient \( \tau_{ik} \).

\[
\text{eqTAv}\{G, \text{List}\} := \\
\text{Module}\{\{A = \text{Subsets}[I], B, \text{sms} = 1, j, k, \xi\}, \\
\text{Do}[ \\
\xi = 1; \\
B = A[[k]]; \\
\text{Do}[\xi * = \\
(G[[\text{First}[\text{Flatten}[\text{Position}[I, B[[j]]]]]] - \varphi_{s[I][j]}), \\
\{j, 1, \text{Length}[B]\}); \\
\text{sms} += \tau_{A[k]} (\xi - D_0[A[[k]]], \{k, 1, \text{Length}[A]\}); \\
\text{sms} /\text{. referencevalues} /\text{. qle}
\]}

To calculate the value of the selection coefficient \( \tau_{ik} \), we equate the above to expression to their corresponding fitness values, and solve the resulting system of equations. Ignoring terms of order \( \epsilon \) and above, we find
\[
\text{tauSubs} = \text{Solve}\left[
\begin{array}{l}
edq\text{Tau}[\{0\}] = \frac{1}{\tilde{w}_i}, \\
edq\text{Tau}[\{1\}] = \frac{1 - e^2 c_i}{\tilde{w}_i}
\end{array}\right], \{e_{\{1\}}, \tilde{w}_i\}, \{e \to 0\} // \text{Flatten}
\]

\[
\{\tilde{e}_{\{1\}} \to -c_i, \tilde{w}_i \to 1\}
\]

where \(w_i\) represents the mean viability of males.

An analogous procedure is used to solve for the selection coefficients that are involved in female viability selection.

\[
\text{omegaSubs} = \text{Solve}\left[
\begin{array}{l}
edq\text{Omega}[\{0\}] = \frac{1}{\tilde{w}_p}, \\
edq\text{Omega}[\{1\}] = \frac{1 - e^2 c_p}{\tilde{w}_p}
\end{array}\right], \{e_{\{p\}}, \tilde{w}_p\}, \{e \to 0\} // \text{Flatten}
\]

\[
\{\tilde{e}_{\{p\}} \to -c_p, \tilde{w}_p \to 1\}
\]

The procedure for calculating the sexual selection coefficients \(\sigma_A\) is slightly more complicated, but is based on the same principle. First, we define a procedure to decompose fitness into contributions attributable to individual alleles and associations of alleles.

\[
\text{eqSigma}[\text{Gf\_List, Gml\_List, Gm2\_List, order\_}] := \\
\text{Module}[\{S, j, k, Uf, Uml, Um2, sms = 1, \xi\}, \\
S = \text{Subsets}[\text{Join}[\text{Replace}[\text{P}, x_\_ \to x_{\_1}, 1], \\
\text{Replace}[\text{M}, x_\_ \to x_{\_2}, 1], \text{Replace}[\text{M}, x_\_ \to x_{\_3}, 1]], 2]; \\
\text{Do}[ \\
Uf = \text{DeleteCases}[S[[k]], _\_\_ ] /. \{x_{\_2} \to x\}; \\
Uml = \text{DeleteCases}[ \\
\text{DeleteCases}[S[[k]], _\_\_ ], _\_\_ ] /. \{x_{\_2} \to x\}; \\
Um2 = \text{DeleteCases}[\text{DeleteCases}[S[[k]], _\_\_ ], _\_\_ ] /. \\
\{x_{\_3} \to x\}; \\
\xi = 1; \\
\text{Do}[\xi *= \\
(Gf[[\text{First}[\text{Flatten}[\text{Position}[\text{P}, Uf[[j]]]]]]] - \nu_{\text{Uf}[[j]]}), \\
\{j, 1, \text{Length}[Uf]\});}
\]
To calculate the fitness of this mating group, we define a procedure 'fitness', which implements the details of our biological model.

```plaintext
fitness[Gf_List, Gml_List, Gm2_List, order_] :=
Module[{qp1, qp2, qf1, qf2, probt1, probt2, t1, t2, h1, h2, pwinfight, plosefight, pwin, plose, pequal, offspring, pt1, pt2, ftns, cpf1, cpf2},
  probt1 = Gml[[5]] \eta_0 \eta_f^1 \eta_D^1 \eta_p^{1-qf1};
  probt2 = Gm2[[5]] \eta_0 \eta_f^1 \eta_D^1 \eta_p^{1-qf2};
  h1 = Gml[[1]] qf1 t2 + Gml[[2]] qf1 (1 - t2) +
      Gml[[3]] (1 - qf1) t2 + Gml[[4]] (1 - qf1) (1 - t2);
  h2 = Gm2[[1]] qf2 t1 + Gm2[[2]] qf2 (1 - t1) +
      Gm2[[3]] (1 - qf2) t1 + Gm2[[4]] (1 - qf2) (1 - t1);
  pwin = h1 (1 - h2);
  plose = (1 - h1) h2;
  pwinfight = h1 h2 (1/2 (qf1 qf2 + (1 - qf1) (1 - qf2)) +
                   \alpha (1 - qf2) qf1 + (1 - \alpha) (1 - qf1) qf2);
  plosefight = h1 h2 (1/2 (qf1 qf2 + (1 - qf1) (1 - qf2)) +
                    \alpha (1 - qf1) qf2 + (1 - \alpha) (1 - qf2) qf1);
  pequal = (1 - h1) (1 - h2);
  offspring = \frac{1 + \varepsilon \gamma Gf[[1]] t1}{2 + \varepsilon \gamma Gf[[1]] t1 + \varepsilon \gamma Gf[[1]] t2}
               (pequal (1 + \varepsilon V / 2) + (pwin + pwinfight) (1 + \varepsilon V) +
               (plose + plosefight (1 - \varepsilon C)));
  pt1 = 1 - t1 - (1 - 2 t1) probt1;
  pt2 = 1 - t2 - (1 - 2 t2) probt2;
  cpf1 =
         qp1 qf1 + (1 - qp1) (1 - qf1) - qp1 (1 - qf1) - qf1 (1 - qp1);
  cpf2 = qp2 qf2 + (1 - qp2) (1 - qf2) -
         qp2 (1 - qf2) - qf2 (1 - qp2);
  Series[Sum[pt1 (1 + \rho cpf1) pt2 (1 + \rho cpf2) offspring
             (1 + \varepsilon b (qp1 - 1/2)), {qp1, 0, 1}, {qp2, 0, 1},
             {qf1, 0, 1}, {qf2, 0, 1}, {t1, 0, 1}, {t2, 0, 1}] /
             (8 (1 + \varepsilon W)), {\varepsilon, 0, order}] // Normal]
```
Equations for the sexual selection coefficients

By averaging over different combinations of genotypes, we obtain equations for each of the sexual selection coefficients that are needed for our analysis.

For example, this is the equation that determines the value of $\bar{\sigma}_{[h_{1\text{st}}]}$:

$$\text{eq3} = \text{averagingProc}([\{0, 1\}, \{0, 1\}, \{0, 1\}, \{0, 1\}], \{\{0, 1\}, \{0, 1\}, \{0, 1\}, \{0, 1\}, \{0, 1\}, \{0, 1\}, \{0, 1\}, \{0, 1\}, \{0, 1\}, \{0, 1\}, \{0, 1\}, \{0, 1\}, \{0, 1\}, \{0, 1\}, \{0, 1\}, \{0, 1\}, \{0, 1\}], 1)$$

$$- \frac{1}{32} \varepsilon (-1 + f_{h_1}) f_{h_1} (f_{i_1}^2 \eta_0^2 (-1 - \rho + (1 + \rho) \eta_p) +$$

$$(C f_{h_1} (-1 - \rho + (-1 + \rho) \eta_p)) + c f_{h_2} (1 + \rho + \eta_p - \rho \eta_p) +$$

$$(-2 C - V + 2 (C + V) \alpha) (f_{h_3} - f_{h_4}) \eta \varepsilon_1 (-1 - \rho + (1 + \rho) \eta_p)) +$$

$$2 f_{i_1} \eta_0 (C f_{h_2} (-1 - \rho + (-1 + \rho) \eta_p)) + V (1 + \rho + \eta_p - \rho \eta_p) +$$

$$(V + (-2 C - V + 2 (C + V) \alpha) f_{h_4}) \eta \varepsilon_1$$

$$(1 - \rho + (1 + \rho) \eta_p)) + 32 \bar{\sigma}_{[h_{1\text{st}}]} \varepsilon_1)$$

Thirteen different selection coefficients appear in the QLE approximation or model (see the next section). In order to solve for these coefficients, we derive fourteen equations like the one above. We solve separately for the first and second order components of the selection coefficient acting on the female preference allele (see the online version of this worksheet) and then solve for the remaining selection coefficients.

$$\text{selCoeff} =$$

$$\text{Solve}([\text{eq1} == 0, \text{eq2} == 0, \text{eq3} == 0, \text{eq4} == 0, \text{eq5} == 0, \text{eq6} == 0, \text{eq7} == 0, \text{eq8} == 0, \text{eq9} == 0, \text{eq10} == 0, \text{eq11} == 0, \text{eq12} == 0, \text{eq13} == 0, \text{eq14} == 0], \{\bar{\sigma}_{(p)}, \bar{\sigma}_{(i_{1\text{st}})}, \bar{\sigma}_{(i_{2\text{nd}})}, \bar{\sigma}_{(h_{1\text{st}})}, \bar{\sigma}_{(h_{2\text{nd}})}, \bar{\sigma}_{(h_{3\text{rd}})}, \bar{\sigma}_{(h_{4\text{th}})}, \bar{\sigma}_{(h_{5\text{th}})}, \bar{\sigma}_{(h_{6\text{th}})}, \bar{\sigma}_{(h_{7\text{th}})}, \bar{\sigma}_{(h_{8\text{th}})}, \bar{\sigma}_{(h_{9\text{th}})}, \bar{\sigma}_{(h_{10\text{th}})}, \bar{\sigma}_{(h_{11\text{th}})}, \bar{\sigma}_{(h_{12\text{th}})}, \bar{\sigma}_{(h_{13\text{th}})}, \bar{\sigma}_{(h_{14\text{th}})}\}) // \text{Flatten} // \text{FullSimplify}$$

$$\{\bar{\sigma}_{(h_{1\text{st}})}, \bar{\sigma}_{(h_{2\text{nd}})}, \bar{\sigma}_{(h_{3\text{rd}})}, \bar{\sigma}_{(h_{4\text{th}})}, \bar{\sigma}_{(h_{5\text{th}})}, \bar{\sigma}_{(h_{6\text{th}})}, \bar{\sigma}_{(h_{7\text{th}})}, \bar{\sigma}_{(h_{8\text{th}})}, \bar{\sigma}_{(h_{9\text{th}})}, \bar{\sigma}_{(h_{10\text{th}})}, \bar{\sigma}_{(h_{11\text{th}})}, \bar{\sigma}_{(h_{12\text{th}})}, \bar{\sigma}_{(h_{13\text{th}})}, \bar{\sigma}_{(h_{14\text{th}})}\}$$
\[ \sigma_{(h_{1,2})} \rightarrow \frac{1}{32} \eta \eta \eta (1 - \rho + (1 + \rho) \eta_p) \]
\[ 2 \, C \, f^2_{h4} \, \eta_f + 2 \, C \, \rho \, f^2_{h4} \, \eta_f + 4 \, C \, f^2_{h4} \, \eta_f - 4 \, \rho \, f^2_p \, \eta_f + \]
\[ C \, f^2_{h3} \, f^2_{h4} \, \eta_f + 2 \, C \, \rho \, f^2_{h3} \, f^2_{h4} \, \eta_f + C \, f^2_{h3} \, \eta_f \, \eta_r - 2 \, C \, \rho \, f^2_{h4} \, \eta_f \, \eta_r + 2 \, C \, \rho \, f^2_{h4} \, \eta_f \, \eta_r - 2 \, C \, \rho \, f^2_{h4} \, \eta_f \, \eta_r + C \, \rho^2 \, f^2_{h4} \, \eta_f \, \eta_r - 2 \, (-1 + \rho) \, \gamma \, \eta^2_p + \]
\[ (1 + \rho) \, \langle \eta_{h3} + \eta_{h4} \rangle \, (V - C \, \alpha \, \eta_f) - \gamma \, \eta_f + \]
\[ C \, (1 + \rho) \, \eta_f \, \eta_r \, \eta_f + \]
\[ C \, f^2_{h3} \, f^2_{h4} \, \eta_f \, \eta_r + \]
\[ C \, f^2_{h3} \, f^2_{h4} \, \eta_f \, \eta_r + \]
\[ C \, f^2_{h3} \, f^2_{h4} \, \eta_f \, \eta_r + \]
\[ 2 \, f^2_{h3} \, (V + C \, \alpha \, \eta_f) + C \, f^2_{h4} \, (1 + \rho) \, \eta_f - \gamma \, \eta_f \, \eta_r \, \eta_f - \gamma \, \eta_f \, \eta_r \, \eta_f + \]
\[ 2 \, (C + V) \, \alpha \, \eta_f \, \eta_f - \gamma \, \eta_f \, \eta_r \, \eta_f - \gamma \, \eta_f \, \eta_r \, \eta_f + \]
\[ C \, f^2_{h3} \, f^2_{h4} \, \eta_f \, \eta_r \, \eta_f + \]
\[ 4 - \text{Quasi-linkage-equilibrium analysis} \]

When selection is weak relative to recombination, alleles will be only weakly associated with each other. Accordingly, the values of the genetical associations are of the same order of magnitude as the selection coefficients. After a few generations of selection and recombination, the associations converge on quasi-linkage values that change slowly as the allele frequencies evolve. We now calculate the quasi-equilibrium values for the associations, and will substitute these into the equations that describe the change of allele frequencies in the final section of this appendix.

- **Quasi-Linkage equilibrium values for the associations**

We are now ready to calculate quasi-linkage-equilibrium values for all the associations that are needed. For example, the QLE value of \( \mathcal{D}([i, p]) \) can be solved from

\[ \text{eqip} = \text{eqQLE}([i, p]) : 0 \]
\[ C \left\{ \begin{array}{l}
\frac{1}{4} \, (f_i - f_p) \, (f_p - f_i) \, \alpha \, \delta_{[i_1, p]} \, \eta_f \, \eta_r \, \eta_f - \frac{1}{2} \, \mathcal{D}([i, p]) \end{array} \right\} : 0 \]

After defining similar equations for the other association, we find the following quasi-linkage-equilibrium values

\[ \mathcal{D}([i, p]) \rightarrow \frac{1}{2} \, (1 + f_i) \, f_i \, (1 + f_p) \, \delta_{[i_1, p]} \, \sigma_{[i_1, p]} \]
\[ \mathcal{D}([h_1, p]) \rightarrow \frac{1}{2} \, (1 + f_{h_1}) \, f_{h_1} \, (1 + f_p) \, \delta_{[h_1, p]} \, \sigma_{[h_1, p]} \]
\[ \mathcal{D} \{h_2, p\} \rightarrow \frac{1}{2} \ (1 + f_{h_2}) \ f_{h_2} \ (1 + f_p) \ f_p \ \sigma_{(h_2, p)} \]
\[ \mathcal{D} \{h_3, p\} \rightarrow \frac{1}{2} \ (1 + f_{h_3}) \ f_{h_3} \ (1 + f_p) \ f_p \ \sigma_{(h_3, p)} \]
\[ \mathcal{D} \{h_4, p\} \rightarrow \frac{1}{2} \ (1 + f_{h_4}) \ f_{h_4} \ (1 + f_p) \ f_p \ \sigma_{(h_4, p)} \]
\[ \mathcal{D} \{h_1, h_2, p\} \rightarrow 0, \mathcal{D} \{h_1, h_3, p\} \rightarrow 0, \mathcal{D} \{h_1, h_4, p\} \rightarrow 0, \mathcal{D} \{h_2, h_3, p\} \rightarrow 0, \mathcal{D} \{h_2, h_4, p\} \rightarrow 0, \mathcal{D} \{h_3, h_4, p\} \rightarrow 0, \mathcal{D} \{h_1, i, p\} \rightarrow 0, \mathcal{D} \{h_2, i, p\} \rightarrow 0, \mathcal{D} \{h_3, i, p\} \rightarrow 0, \mathcal{D} \{h_4, i, p\} \rightarrow 0 \]

- **Equations for allele frequency change**

The final step of the analysis is to derive equations for the change of allele frequencies from one generation to the next.

The equations for the male characters are calculated up to first order in \( \epsilon \).

\[ \Delta i = \text{eqFreq\{i, 1\} / . selCoeffSimpl} \]
\[ -\frac{1}{2} \ \epsilon \ (1 + f_i) \ f_i \ (\sigma_{(i,p)}) + \tau_{(i)} \]

\[ \Delta h_1 = \text{eqFreq\{h1, 1\}} \]
\[ -\frac{1}{2} \ \epsilon \ (1 + f_{h_1}) \ f_{h_1} \ \sigma_{(h_1,p)} \]

\[ \Delta h_2 = \text{eqFreq\{h2, 1\}} \]
\[ -\frac{1}{2} \ \epsilon \ (1 + f_{h_2}) \ f_{h_2} \ \sigma_{(h_2,p)} \]

\[ \Delta h_3 = \text{eqFreq\{h3, 1\}} \]
\[ -\frac{1}{2} \ \epsilon \ (1 + f_{h_3}) \ f_{h_3} \ \sigma_{(h_3,p)} \]

\[ \Delta h_4 = \text{eqFreq\{h4, 1\}} \]
\[ -\frac{1}{2} \ \epsilon \ (1 + f_{h_4}) \ f_{h_4} \ \sigma_{(h_4,p)} \]

The equation for the dynamics of the female preference allele is given up to second order in \( \epsilon \), since all first order terms vanish, i.e., selection on the female mating preference is weak. At quasi-linkage equilibrium,

\[ \Delta p = \text{eqFreq\{p, 2\} / . DuQle / . selCoeffSimpl / / FullSimplify} \]
\[ \frac{1}{4} \ \epsilon^2 \ (1 + f_p) \ f_p \]
\[ (-2 \ \sigma_{(p)} + (1 + f_i) \ f_i \ \sigma_{(i,p)} \ (\sigma_{(i,p)} + \tau_{(i)}) - 2 \ \sigma_{(p)}) \]
Appendix 7.2
The asymmetric Hawk-Dove game

Local payoffs:
The expected payoff of an individual with strategy \( p \) (= probability to play Hawk) in a contest with an opponent with strategy \( q \) is given by:

\[
f_x(p|q) = pg \cdot ((1/2 + x)V - (1/2 - x)C) + p(1-q) \cdot V + (1-p)(1-q) \cdot 1/2 V,
\]
where \( 1/2 + x \) \((-1/2 \leq x \leq 1/2) \) is the probability that the focal individual wins a Hawk-Hawk confrontation. In our model, \( x \) can take on the values \( +\alpha, -\alpha \) and 0. Simplification of the local payoff function yields

\[
f_x(p|q) = \frac{1}{2}V \cdot (1 + p - q) - \frac{1}{2}Cpq + (V + C)xpq.
\]

Accordingly, the local selection gradient is given by:

\[
\frac{\partial f_x}{\partial p} (p|q) = \frac{1}{2}V - \frac{1}{2}Cq + (V + C)xq.
\]

It will be useful to rewrite this gradient in terms of \( v = V/C \), which corresponds to the ESS in the classical Hawk-Dove game without fighting asymmetries. In fact, up to the factor \( 1/2C \) (which will henceforth be neglected, since it does not affect the analysis in any way), the local selection gradient is of the form:

\[
\frac{\partial f_x}{\partial p} (p|q) = v - q + 2x(1 + v)q.
\]

Non-signalling equilibria:
Let us assume that there are two types of individuals in the population (indicated by ‘+’ and ‘-’) that differ in fighting ability. In confrontations of two individuals differing in fighting ability, the + type wins a Hawk-Hawk confrontation with probability \( 1/2 + \alpha \), while the other individual wins with probability \( 1/2 - \alpha \). Individuals know their own fighting ability, but they do not signal it to their opponents. Accordingly, individual strategies are only conditional on their own type: \( p = (p_+, p_-) \). Let \( \hat{p} = (\hat{p}_+, \hat{p}_-) \) denote the population strategy. Then the expected payoff of an individual of type + resp. – is given by:

\[
\begin{align*}
F_+(p|\hat{p}) &= \frac{1}{2}f_0(p_+|\hat{p}_+) + \frac{1}{2}f_\alpha(p_+|\hat{p}_-), \\
F_-(p|\hat{p}) &= \frac{1}{2}f_0(p_-|\hat{p}_+) + \frac{1}{2}f_-(p_-|\hat{p}_-).
\end{align*}
\]
In view of (9), the selection gradients in both situations are given by:

\[
\begin{align*}
\frac{\partial F_+}{\partial p_+} (p|\hat{p}) &= v^{-1/2}(\hat{p}_+ + \hat{p}_-) + \alpha (1 + v) \hat{p}_- \\
\frac{\partial F_-}{\partial p_-} (p|\hat{p}) &= v^{-1/2}(\hat{p}_+ + \hat{p}_-) - \alpha (1 + v) \hat{p}_+ 
\end{align*}
\]  

(12)

Every Nash strategy \( \hat{p}_i \) (where \( i \in \{+,\} \)) has to satisfy the following condition:

\[
\frac{\partial F_i}{\partial p_i} (p|\hat{p}) = 0 \quad \text{if} \quad 0 < \hat{p}_i < 1 \\
\text{and} \quad \frac{\partial F_i}{\partial p_i} (p|\hat{p}) \leq 0 \quad \text{if} \quad \hat{p}_i = 0 \\
\text{and} \quad \frac{\partial F_i}{\partial p_i} (p|\hat{p}) \geq 0 \quad \text{if} \quad \hat{p}_i = 1 
\]  

(13)

For the special case \( \alpha = 0 \), (12) immediately implies that the asymmetric Hawk-Dove game has a line of equilibria that is given by \( 1/2(\hat{p}_+ + \hat{p}_-) = v \), which correspond to the (single) equilibrium of the classical Hawk-Dove game without differences in fighting ability. Let us from now on assume that \( \alpha > 0 \).

Notice that (12) implies:

\[
\frac{\partial F_+}{\partial p_+} (p|\hat{p}) > \frac{\partial F_-}{\partial p_-} (p|\hat{p}) ,
\]  

(14)

unless \( \hat{p}_+ = \hat{p}_- = 0 \). The latter possibility can be neglected, since it is obvious from (12) that \( (\hat{p}_+, \hat{p}_-) = (0,0) \) is not a Nash equilibrium. From (14) we can conclude that there is no fully mixed Nash equilibrium, i.e. no Nash equilibrium with \( 0 < \hat{p}_+, \hat{p}_- < 1 \). At such an equilibrium, both selection gradients in (12) would have to be zero and, hence, to be identical, which is precluded by (14). It is also obvious from (12) that \( (\hat{p}_+, \hat{p}_-) = (1,1) \) is no Nash equilibrium. Combining (13) and (14) we can therefore conclude that there are three possible types of Nash equilibria: (a) \( \hat{p}_- = 0 \) and \( 0 < \hat{p}_+ < 1 \); (b) \( \hat{p}_+ = 1 \) and \( 0 < \hat{p}_- < 1 \); or (c) \( (\hat{p}_+, \hat{p}_-) = (1,0) \).

Let us first consider the equilibria of type (a). The selection gradient for \( \hat{p}_+ \) has to satisfy

\[
\frac{\partial F_+}{\partial p_+} (p|\hat{p}) = v^{-1/2} \hat{p}_+ = 0 ,
\]  

(15)

yielding \( \hat{p}_+ = 2v \). This requires \( v \leq 1/2 \). In this case, \( (\hat{p}_+, \hat{p}_-) = (2v,0) \) is indeed a Nash equilibrium, since (14) implies \( \partial F_-/\partial p_- \leq 0 \)

Next consider the equilibria of type (b). The selection gradient for \( \hat{p}_- \) has to satisfy

\[
\frac{\partial F_-}{\partial p_-} (p|\hat{p}) = v^{-1/2}(1+ \hat{p}_-) - \alpha (1+v) = 0 ,
\]  

(16)
yielding \( \hat{\alpha} = 2(1-\alpha)v - (1+2\alpha) \). This requires \( v \geq (\alpha + 1/2)/(1-\alpha) \). If this is the case, \((\hat{\alpha}^+, \hat{\alpha}^-)\) is indeed a Nash equilibrium, since (14) implies \( \partial F_+/\partial \alpha^+ \geq 0 \).

Let us finally consider the pure-strategy equilibrium \((\hat{\alpha}^+, \hat{\alpha}^-) = (1, 0)\). A simple calculation shows that the requirement \( \partial F_+/\partial \alpha^+ \geq 0 \geq \partial F_-/\partial \alpha^- \) is satisfied for \( 1/2 \leq v \leq (\alpha + 1/2)/(1-\alpha) \).

Taking all this together, we obtain the following (unique) Nash equilibrium in the non-signalling context (see figure 7.2A in the main text):

\[
\begin{align*}
\hat{\alpha}^- &= 0 \quad \text{and} \quad \hat{\alpha}^+ = 2v & \quad \text{if} \quad v \leq 1/2 \\
\hat{\alpha}^- &= 0 \quad \text{and} \quad \hat{\alpha}^+ = 1 & \quad \text{if} \quad 1/2 \leq v \leq (\alpha + 1/2)/(1-\alpha) . \quad (17)
\end{align*}
\]

Notice that \( 1/2(\hat{\alpha}^+ + \hat{\alpha}^-) = v \) only holds for the equilibria of type (a) (i.e. if \( v \leq 1/2 \)). For the other equilibria, the average tendency to play Hawk is smaller than in the classical Hawk-Dove game without fighting asymmetries.

**Signalling equilibria:**

Let us now consider the situation where all individuals invest into the ornament. All individuals with high fighting ability develop the ornament, while individuals with low fighting ability develop the ornament with probability \( \eta \). Individual strategies are now dependent on own fighting ability (+ or –) and the presence or absence (1 or 0) of the ornament in the opponent: \( \mathbf{p} = (p^+, p^0, p^-) \). Again \( \hat{\mathbf{p}} = (\hat{\alpha}^+, \hat{\alpha}^0, \hat{\alpha}^-) \) denotes the population strategy.

A focal individual matched with an opponent without ornament 'knows' that the opponent has low fighting ability. If it is matched with an ornamented opponent, the probability that the opponent has high (resp. low) fighting ability is \( 1/(1+\eta) \) (resp. \( \eta/(1+\eta) \)). If the focal individual has high fighting ability, its opponent will always perceive the ornament; if it has low fighting ability, the opponent will perceive the ornament with probability \( \eta \). Based on these considerations, one can calculate the expected payoff of a \( \mathbf{p} \)-individual in a \( \hat{\mathbf{p}} \)-population for each of the four information situations:

\[
\begin{align*}
F_{+0}(\mathbf{p} \mid \hat{\mathbf{p}}) &= f_\alpha(p^+ \mid \hat{\alpha}^-) \\
F_{+1}(\mathbf{p} \mid \hat{\mathbf{p}}) &= 1/(1+\eta) f_\alpha(p^+ \mid \hat{\alpha}^+) + \eta/(1+\eta) f_\alpha(p^+ \mid \hat{\alpha}^-) \\
F_{-0}(\mathbf{p} \mid \hat{\mathbf{p}}) &= \eta f_\alpha(p^- \mid \hat{\alpha}^-) + (1-\eta) f_\alpha(p^- \mid \hat{\alpha}^+) \\
F_{-1}(\mathbf{p} \mid \hat{\mathbf{p}}) &= \eta/(1+\eta) f_\alpha(p^- \mid \hat{\alpha}^+) + (1+\eta) f_\alpha(p^- \mid \hat{\alpha}^-) + (1-\eta)/(1+\eta) f_\alpha(p^- \mid \hat{\alpha}^+) + \eta/(1+\eta) f_\alpha(p^- \mid \hat{\alpha}^-) . \quad (18)
\end{align*}
\]
In view of (10), the selection gradients in the four information situations are given by:

\[ \frac{\partial F_+}{\partial p_+} = v - \hat{p}_{-1} + 2\alpha(1+v)\hat{p}_{-1} \]  

(19)

\[ \frac{\partial F_1}{\partial p_1} = v - \left(1/(1+\eta) \hat{p}_{+1} + \eta/(1+\eta) \hat{p}_{-1}\right) + 2\alpha(1+v)\eta/(1+\eta) \hat{p}_{-1} \]

\[ \frac{\partial F_0}{\partial p_0} = v - (\eta\hat{p}_{-1} + (1-\eta)\hat{p}_{-0}) \]

\[ \frac{\partial F_{-1}}{\partial p_{-1}} = v - \left(\eta/(1+\eta) \hat{p}_{+1} + \eta^2/(1+\eta) \hat{p}_{-1} + (1-\eta)/(1+\eta) \hat{p}_{+0} + \eta(1-\eta)/(1+\eta)\hat{p}_{-0}\right) - 2\alpha(1+v) \cdot (\eta/(1+\eta) \hat{p}_{+1} + (1-\eta)/(1+\eta) \hat{p}_{+0}) . \]

In the absence of fighting asymmetries (i.e. \( \alpha = 0 \)) the unique Nash equilibrium is given by \( \hat{p}_{+1} = \hat{p}_{+0} = \hat{p}_{-1} = \hat{p}_{-0} = v \). In other words: the ESS of the Hawk-Dove game without fighting asymmetries is played in all information situations. More generally, there exists a completely mixed Nash equilibrium if (and only if) the fighting asymmetry \( \alpha \) is sufficiently small. In fact, all selection gradients in (19) are equal to zero if \( \hat{p} \) is given by:

\[ \hat{p}_{+1} = v, \quad \hat{p}_{-1} = \frac{v}{1-2\alpha(1+v)}, \quad \hat{p}_{+0} = \frac{1-\eta-2\alpha\eta(1+v)}{1-\eta} \cdot \hat{p}_{-1}, \]

\[ \hat{p}_{-0} = \frac{1-\eta-2\alpha(1+v)}{1-\eta} \cdot \hat{p}_{-1} . \]

(20)

and the strategic parameters in (20) are between zero and one if \( \alpha \) satisfies the inequality

\[ \alpha \leq \frac{1}{2} \cdot \min \left[ \frac{1-v}{1+v}, \frac{1-\eta}{1+v} \right] . \]

(21)

Notice that the equilibrium in (20) is ‘paradoxical’ in that \( \hat{p}_{-1} \) is larger than all the other strategic parameters. In other words, an individual with low fighting ability that is confronted with an ornamented (and, hence, potentially strong) opponent is more inclined to play the Hawk strategy than, say an individual with high fighting ability that is confronted with a non-ornamented opponent (which definitely has low fighting ability). For small values of \( \alpha \), there exist even more extreme paradoxical equilibria. In particular,

\[ \hat{p}_{-1} = 1, \quad \hat{p}_{+1} = \hat{p}_{+0} = \hat{p}_{-0} = 0 \]

(22)
is a pure-strategy Nash equilibrium if
\[ \alpha \leq \frac{1}{2} \cdot \frac{1 - v - v/\eta}{1 + v} \quad \text{and} \quad \frac{\eta}{1 + \eta} > v > \frac{\eta^2}{1 + \eta}. \]  
(23)

Let us from now on focus on the ‘common-sense’ equilibria, where \( \hat{p}_{-1} \) is small. If we assume \( \hat{p}_{-1} = 0 \), the selection gradient for \( p_{+0} \) is positive, implying that \( \hat{p}_{+0} = 1 \) at equilibrium. In other words, individuals with low fighting ability that are confronted with an ornamented opponent always play Dove, while individuals with high fighting ability that are confronted with a non-ornamented opponent always play Hawk. The selection gradient for \( p_{+1} \) (resp. \( p_{-0} \)) is positive at \( \hat{p}_{+0} = 0 \) (resp. at \( \hat{p}_{-0} = 0 \)). This implies that both strategies have to be positive at equilibrium. The selection gradient for \( p_{+1} \) (resp. \( p_{-0} \)) is equal to zero for \( \hat{p}_{+1} = (1 + \eta)v \) (resp. \( \hat{p}_{-0} = v/(1-\eta) \)). Nash equilibria with \( \hat{p}_{-1} = 0 \) therefore have to satisfy:

\[ \begin{align*}
\hat{p}_{-1} &= 0, \hat{p}_{+0} = 1, \text{ and } \\
\hat{p}_{+1} &= (1 + \eta)v \quad \text{and} \quad \hat{p}_{-0} = v/(1-\eta) \quad \text{if} \quad 0 \leq \eta \leq 1-\eta \\
\hat{p}_{+1} &= (1 + \eta)v \quad \text{and} \quad \hat{p}_{-0} = 1 \quad \text{if} \quad 1-\eta \leq v \leq 1/(1-\eta) \\
\hat{p}_{+1} &= \hat{p}_{-0} = 1 \quad \text{if} \quad 1/(1-\eta) \leq v
\end{align*} \]

(24)

Notice that in all cases \( \hat{p}_{+1} \leq \hat{p}_{-0} \). The strategy combination given by (24) is indeed a Nash equilibrium if the selection gradient for \( p_{-1} \) is negative or zero at \( \hat{p}_{-1} = 0 \). This is the case for

\[ v \leq \Psi = \frac{1 + \eta (1-\eta) + 2\alpha}{1 + \eta - 2\alpha}. \]

(25)

In other words, (24) does indeed give a Nash equilibrium if \( v \leq \Psi \). Notice that \( \Psi > 1/(1+\eta) \), i.e. (24) potentially loses the equilibrium property in situations where \( \hat{p}_{+0} = \hat{p}_{+1} = \hat{p}_{-0} = 1 \). In this case the equilibrium is given by:

\[ \begin{align*}
\hat{p}_{+0} &= \hat{p}_{+1} = \hat{p}_{-0} = 1 \quad \text{and} \quad \hat{p}_{-1} = 1 - \frac{1}{\eta^2} \cdot ((1 + \eta)(1-v) + 2\alpha(1+v))
\end{align*} \]

(26)

The ‘common-sense’ equilibria characterized by (24) and (26) are illustrated by Figure 7.2B in the main text.
CHAPTER 8

Synthesis
Synthesis

Hybridization has in many instances marked fitness consequences for individuals engaged in a mixed-species pairing as hybrid offspring might, for example, be less viable. The aim of this thesis is to understand the mating decisions of female flycatchers in a hybrid zone. This is predicted to be rather complex as a multitude of different factors affect these decisions. In the first chapter of this thesis I have given an overview of important factors influencing mating decisions in hybridizing species without focusing on one particular species pair. I argued that due to the fact that many factors might be influential, it is important to obtain in depth knowledge of a specific system in order to get a good understanding of mating decisions in a hybrid zone. I set-out to investigate several potentially important costs and benefits which might influence the decisions of who to mate with in a hybrid zone of the collared and pied flycatcher. In this chapter I will review both the results from earlier studies on flycatchers and the results presented in this thesis following the same structure as in the first chapter. These results will be summarised in table 8.2.

Species recognition & mate choice

Song and plumage characteristics play an important role in mate attraction and species recognition in flycatchers (e.g. Lundberg and Alatalo 1992; Sætre et al. 1997b; Qvarnström et al. 2006b). The song is of particular importance to attract putative mates from long distances. Once closer, plumage characteristics and other factors like quality of the nesting location become more important. Song is species-specific in the flycatchers, but an interesting situation is present on Öland, namely the occurrence of so called ‘mixed-singers’ (Alatalo et al. 1990; Haavie et al. 2004). Mixed-singers are always pied flycatchers and their song consists of elements of both species. One of the effects of this mixed-singing is an increased chance of ending up in a mixed-species pairing (Qvarnström et al. 2006b). The proximate mechanisms determining this apparently maladaptive mixed-singing of pied flycatchers is unknown. One explanation is that song copying has an advantage for pied flycatcher males on the allopatric breeding grounds and that immigrant male pied flycatchers simply behave the same way when ending up among collared flycatchers (Qvarnström et al. 2006b), but this hypothesis needs to be further investigated.

In both flycatcher species, the male’s plumage is important for conspecific mate choice (e.g. Lundberg and Alatalo 1992; Qvarnström et al. 2000) and species recognition (e.g. Sætre et al. 1997b). Aviary experiments performed in the Czech Republic have shown that the two species are able to discriminate between hetero- and conspecifics. A particularly important result of these experi-
ments was that species recognition was especially well developed in the sympatric breeding areas which is indicative of reinforcement (Sætre et al. 1997b).

The relation between morphological characteristics and species recognition is strengthened by a study showing that one-year old (second calendar year) male collared flycatchers, of which some plumage characteristics are more similar to pied flycatcher males, had a higher chance of hybridization (Wiley et al. 2005). Mate choice experiments indicated that these increased hybridization rates might at least be partly due to the plumage characteristics, especially when the female choice was constrained.

Earlier studies on Gotland and Öland found mixed results when analysing the effect of plumage colour of male pied flycatchers (which ranges from brown to black) on their probability to hybridize; no relationship between plumage colour and hybridization probability (Alatalo et al. 1990) and males intermediate in colour had a higher probability to hybridize (see Alatalo et al. 1994 for details).

Mate choice is more straightforward to study in allopatric breeding areas because one only needs to focus on the intraspecific fitness trade-offs. In sympathy, however, the intraspecific mate choice decisions get entangled with the potential of hybridization which changes the costs and benefits of mate choice. This makes elucidating mating decisions between hybridizing species a much more challenging task. The same characters important for female choice between males of the same species are often also involved in species recognition. For the female, her (allopatric) mating decisions are shaped by costs and benefits which do not include the effects of hybridization. It is not implausible that a very good choice in allopatry results in hybridization in sympatric breeding areas. I will illustrate this idea with an earlier found correlation between the size of the male’s white forehead patch and his share of paternity in collared flycatchers. Males with a small patch were shown to have a lower share of paternity, i.e. they had more extra-pair nestlings in the brood (e.g. Sheldon and Ellegren 1999). Now consider the case of a female collared flycatcher mated with a male pied flycatcher who on average has a much smaller white forehead patch compared to the collared flycatcher. In figure 8.1 I use the relation between paternity and patch size of the social male of collared flycatcher broods to predict the male share of paternity for a flycatcher with the mean patch size of a pied flycatcher male (27.8 mm², standard error of 0.8). The predicted male share of paternity (0.51) is not dissimilar to the observed level in mixed-species pairs with a female collared flycatcher (0.44; Veen et al. 2001). This seems an intuitively appealing explanation for the low male share of paternity in mixed-pairs broods with a female collared flycatcher, but it is not supported by our plumage manipulation experiment in chapter 2. In this experiment we did not find any effect of the experimental treatment, despite earlier studies where even
much more restricted plumage manipulation (Qvarnström 1997) evoked a response. On the other hand, a different study indicates that intraspecific mating strategies might have an effect outside the range of conspecific phenotypes. Males with a phenotype resembling a hybrid male (but genetically a collared flycatcher) had a reduced share of paternity (Svedin 2006). Genetic incompatibilities are unlikely to cause this observation, as these males appear to be genetically ‘pure’, which would suggest that females change their mating strategy.

A potentially important reason to engage in mixed-species pairings, so far not addressed, concerns the reduction of potential costs of mate choice, such as search time, predation risk and energy expenditure. Quantitative estimates of such measures are very hard to obtain. Previous work on the pied flycatcher indicated that females reduce choosiness if confronted with increased search time (Alatalo et al. 1988a). Costs attached to choice are strongly determined by the availability of suitable partners, and for hybridizing flycatchers a suitable male should (under most conditions) be conspecific. Therefore, the effects of the availability of conspecific males could, in an indirect way, provide insights in the importance of search costs for mating decisions. Availability of conspecific mates can vary in two ways, through the breeding season (within the breeding popula-

Figure 8.1. The male share of paternity in 53 collared flycatcher broods (open circles) in relation to the size of the white forehead patch of the social male. A linear regression (solid line) illustrates the negative relation between male share of paternity and forehead patch size. This relation is used to calculate the predicted male share of paternity for a flycatcher male with an average forehead patch size of a male pied flycatcher (black circle with standard errors of forehead patch size; N= 238). The predicted male share of paternity calculated in this way (0.51) resembles the observed paternity in female collared flycatcher mixed-species pairs (0.44; Veen et al. 2001)). The collared flycatcher paternity data used were collected by Sheldon in 1994 (Sheldon and Ellegren 1999).
Mate availability is assumed to decrease through the breeding season as an increasing proportion of the potential mates will be engaged in a pair bond. The less preferred males, either conspecific or heterospecific males, might be the only ones remaining offering the female limited choice. Females then face the decision to extend searching (which may come at a cost) to find a male of the highest possible quality, or accept a low quality male, which might include a heterospecific one. Earlier studies found that the occurrence of mixed-species pairing increased towards the end of the breeding season, perhaps suggesting that this phenomena might indeed be facilitated by mate availability (Veen et al. 2001).

The relative abundance of the two species differs markedly between the breeding population on Öland and too a lesser extent also on Gotland. Most sites have a higher abundance of collared flycatchers, but pied flycatcher dominated sites are present on Öland (Wiley 2006). The proportion of females engaged in a mixed-species pairing was negatively related to the relative species abundance of their own species. This pattern was found for both species, but was particularly clear for pied flycatcher females. The proportion of pied flycatcher females hybridizing was 0.4 in extremely collared flycatcher dominated areas and decreased to 0.05 in extremely pied flycatcher dominated areas (based on predicted values from a logistic regression, see paper 4 in (Wiley 2006).

The relative abundance of conspecifics appears to be a good direct predictor for the occurrence of mixed-species pairing. However, there is no evidence that this is an effect of increasing search costs, although this is a very plausible explanation (see e.g. Wilson and Hedrick 1982). Obtaining good estimates of the search costs for flycatchers in sympatric breeding populations could provide important information to increase our understanding of their mating decisions. These data will be hard to obtain but hopefully technical advances allowing small passerines to be individually tracked will change this in the future.

**Fertilisation**

The experiment presented in chapter 2 was conducted in an attempt to find out if the high level of conspecific extra-pair young and the male biased sex ratio found in mixed-species broods (Veen et al. 2001) is the consequence of an adaptive female strategy or a result of incompatibilities between the species. Unfortunately, the results are ambiguous and we cannot draw firm conclusions. Nevertheless, it is clear that something interesting happens during this period, as the paternity and sex ratio patterns in mixed pairs appear to be robust (a paper reanalyzing these patterns with additional data is in preparation). Given that we did not find much evidence for an adaptive female mating strategy, and assuming that the plumage manipulation was perceived by the females, I believe
it is justified to suggest that compatibility problems between the species resulting in, for example, conspecific sperm precedence, at least partly underlie the paternity and sex ratio patterns found in natural mixed-species pairs. Hopefully future research can give better insight in the relative importance of compatibility issues and whether or not females mating strategies are adjusted when mated to a heterospecific male.

There might be other pre-zygotic post-copulatory costs, such as reduced fertilisation rates of the eggs resulting in higher levels of infertile eggs in mixed-species broods (e.g. Birkhead and Brillard 2007). If indeed heterospecific sperm would increase the infertility rate, then this should be apparent in the hatching success of female pied flycatcher mixed-species broods because these broods almost entirely consisted of hybrid offspring (Veen et al. 2001). No such pattern was found. Furthermore, mixed-species pairs with a female collared flycatcher would be less severely or not at all affected by such cost because of the ‘normal’ fertilisation rate of the conspecific sperm.

Direct costs and benefits of mixed-species pairing

Species differ from each other and hybridizing individuals could benefit from such differences if the two species are complementary to each other (e.g. in diets) or provide any other benefit which would not be obtained from a conspecific partner. Such a synergistic effect might underlie the differences in reproductive success between pure collared flycatcher pairs and mixed-species pairs with a female collared flycatcher. Pure collared flycatcher broods show a steady decline in reproductive success through the season, whereas the later breeding mixed-species pairs showed an increasing and higher reproductive success over the later part of the breeding season (Veen et al. 2001). Several other studies confirmed this seasonal difference. An earlier study partially cross-fostered nestlings of both species between broods (i.e. each brood contained nestlings of both species) and found that pied flycatcher nestlings showed a less steep decline through the season in mass and fledging success. Furthermore, the results indicated that pied flycatchers were better parents at the end of the season, for nestlings of both species (Qvarnström et al. 2005). In chapter 3 of this thesis we used the long-term database from the Gotland breeding population to investigate whether differences in the breeding territories per se could cause the differences in reproductive success. To this end, the breeding territories in the database were subdivided in two groups based on whether a pied flycatcher male had ever bred in the nest-box or not. In order to control for species effects (e.g. different habitat use) only the reproductive success of pure collared flycatcher broods were used (see chapter 3 for details on methods). Also here we found a difference in reproductive success between the two terri-
tory categories. Although the differences in reproductive success are clear and found in several flycatcher studies (see above), an explanation for this observation was lacking. Several possible explanations have been explored and I will discuss these in turn.

First, the food provisioning rates may differ between the species as a result of being adapted to different environments. In case of the flycatchers, pied flycatchers might be adapted to work harder to be able to provide enough food to their nestling in their relative poor allopatric breeding environment. We compared the parental provisioning rates for the different species-pair combinations but we did not find any such difference (chapter 3).

A second possibility is that the species might differ in their diet in such a way that nestlings of mixed-species pairs get a higher diversity or a larger quantity of food which could increases their survival probability. An earlier study in Czech Republic indicated that the two species have similar diets (Bureš 1995) and this was confirmed for the hybrid zone on the Baltic islands (chapter 3).

A third possibility is that the breeding territories differ in microhabitat, which in turn affects the seasonal abundance of food (Veen et al. 2001). Habitat differences between the species have been shown in the Czech Republic (Sætre et al. 1999a) and for the Baltic islands (Alerstam et al. 1978) and this can affect the abundance of an important food resource (caterpillars) (van Balen 1973). In chapter 4 we present the results of a study which measured the habitat characteristics of the breeding territories and determined the abundance of caterpillars through the breeding season for six different tree species. The breeding territories of the two species consistently differed in tree species and the six tree species differed markedly in their temporal abundance of caterpillars (essentially a contrast between deciduous and coniferous species). These two results combined predicted relatively higher food abundance in pied flycatcher territories at the end of the breeding season. Although the predicted differences were relatively small, these results could partly underlie the differences in reproductive success between the two territory categories as found, for example, in chapter 3, especially because this period of relatively higher food abundance appears to coincide with the period of high food demand of the nestlings. For mixed-species pairs with a male pied flycatcher the effect of the habitat differences might be more important. Pied flycatcher territories typically consisted of more coniferous tree species, which is the dominant tree type in large parts of the allopatric breeding grounds. Therefore, pied flycatchers are predicted to be better adapted to utilising these ‘coniferous’ territory types which might result in the higher fledging success as found by Veen et al. (2001).

The above studies are a good start in trying to uncover the intricate interactions between the species and their adaptations to the environment but we are far from having a full understanding of these complex interactions. This becomes apparent when one tries to link the different components, as discrepan-
cies then become apparent. For example, the predicted differences in relative food abundance at the end of the season have only been studied for caterpillars. In the diet study (chapter 3) we find that the diet composition of the pied flycatcher changes through the season in such a way that caterpillars decrease in the diet. This appears to conflict with our suggestions that the increase of caterpillar abundance late in the season is beneficial for the pied flycatcher. One explanation is that the way we measured diet, the number of prey items, does not properly reflect the biomass provided to the nestlings. The relation between number of caterpillars and the total biomass they represent is unlikely to be the same through the season.

As stated above, the differences in adaptation to the allopatric breeding grounds of the two species might potentially play an important role. To fully understand the potential importance of such effects, the different species and the different breeding locations should all be combined in one framework. Important aspects to be elucidated are to what extent the species are adapted to the sympatric breeding area and how this affects the interactions between the two species? This sort of large scale approach might well take our understanding of the system to a new level, but this obviously requires a lot of effort.

The important take home message is that it is not at all straightforward to get good quantitative measures of the potential direct costs and benefits, that the mechanisms underlying the fitness trade-offs might be correlated and furthermore context dependent. The long-term database of the Gotland population proved to be crucial in, firstly establishing the potential importance of the location of the breeding territory on the reproductive success of the different pairs. Besides using the database, it is of great importance to acquire a very thorough knowledge of the ecology of the species if one really wants to unravel the ecological interactions influencing the fitness of both species. My personal impression is that the flycatcher system is one of the avian systems in which this is well studied, but we are currently still far from thoroughly understanding it.

Hybrid viability and fecundity

Vertebrate hybrids often suffer from reduced viability and/or fertility which obviously have a strong negative effect on the reproductive success of hybridizing individuals. Some of these effects are relatively easy to measure, for example infertility of eggs. Good estimates of viability are harder to obtain. In our study, however, we could make use of the existing long-term database with good pedigrees enabling us to quantify the viability and fertility of hybrids. We could further make use of the possibility to genetically check the identity of individuals of unknown origin which were suspected of being hybrids (e.g. Sætre and Moum 2000; Borge et al. 2005a). In this way more individuals could be
included in the samples providing information on female hybrid fertility. In the following sections I will make use of various sources of information, in particular Veen et al. (2001) and Svedin 2006 (paper 4 of her thesis), which both provide estimates of several components of hybrid reproductive success. I will use data from Svedin’s thesis, which will be presented in an adapted form to allow easy comparison with Veen et al. (2001).

Viability of hybrid flycatchers appears not to be reduced in comparison to collared flycatchers, at least after hatching. The survival from fledging to adulthood did not differ between hybrids and collared flycatchers, but was lower for pied flycatchers (chapter 5 of this thesis). The low recruitment rate of pied flycatchers does not necessarily need to be caused by increased mortality, but might result from higher natal dispersal of this species. It is difficult to get good estimates of the lifespan of breeding individuals, as this is measured on the basis of the last breeding record, which does not necessarily mean that the bird has died. If anything, hybrid males appear to have a longer lifespan (see table 8.1).

Female hybrids might have a slightly reduced viability early in the embryonic development which could cause the sex ratio bias in mixed-species pairs (Veen et al. 2001).

The situation is different with respect to fertility of the offspring. Female hybrids are nearly always infertile (Alatalo et al. 1990; Gelter et al. 1992; Veen et al. 2001; Svedin 2006). Males on the other hand appear to be, at least partially, fertile. I will expand on the reproductive success of male hybrids and not of female hybrids because, despite being viable, they contribute virtually nothing to future generations.

The clutch size is very similar for broods attended by males of either species or hybrids, but the proportion of eggs hatched is slightly lower for hybrids compared to either parental species (Veen et al. 2001; see table 8.1). This might indicate (weak) incompatibility problems between hybrid males mated with collared or pied flycatcher females. Such incompatibility can arise from hybrid sperm leading to reduced fertilisation rates, similarly as has been found for heterospecific species pairs (e.g. Birkhead and Brillard 2007). Another possibility is that ‘backcross’ embryos have a lower viability, resulting in a higher embryonic death rate and ultimately in less nestlings fathered by the hybrid male.

The proportion of fledged nestlings is lower for collared flycatchers compared to pied flycatchers and hybrids (table 8.1). The proportion of fledglings which recruited to the study population differs markedly between the species, with pied flycatcher broods recruiting significantly less (table 8.1). Surprisingly, this is quite different from previous results, in which the recruitment rates of fledglings from hybrid males was much lower compared to collared flycatchers (Veen et al. 2001). One explanation could be that the recruitment rates drastically increased over the last six years (as this is included in the estimate by Svedin) but this would be surprising. This discrepancy is
The flycatcher project on Gotland has been established in the end of the ‘70’s and encompasses a large number of breeding pairs each season. This makes it now possible to obtain relatively good measures of hybrid fitness, despite their rarity. The obvious next step is to assess the fitness of future generation (e.g. the backcrosses). The only study which has attempted this concluded that backcrosses have an even lower fitness than hybrids (Wiley 2006), but this was based on low sample sizes. Both the advances of molecular techniques which will allow identifying backcrosses with a higher accuracy and additional generations added to the database in the future should make these estimates more accurate.

### Intermediate hybrid phenotype

Species are adapted to the environment they live in and have evolved optimal phenotypes to do so. Many hybrids are phenotypically intermediate compared to the parental species. As a consequence they will often be maladapted to their environment, but the reverse can also be true if a hybrid inherits complementary adaptive traits from both parents. Below we discuss two studies investigating “extrinsic post zygotic selection” against hybrid flycatchers, dealing with the species’ migration behaviour and their parasite resistance, respectively.

Migratory routes are often quite well defined and rigid through time. It has been argued that deviating from such routes is suboptimal (Sutherland 1998). If

<table>
<thead>
<tr>
<th>‘species’</th>
<th>clutch size</th>
<th>share paternity</th>
<th>hatchability</th>
<th>lifetime fledged young</th>
<th>lifetime recruitment</th>
<th>reproductive attempts</th>
<th>lifespan</th>
</tr>
</thead>
<tbody>
<tr>
<td>collared</td>
<td>6.10</td>
<td>0.92</td>
<td>0.93</td>
<td>7.70</td>
<td>0.85</td>
<td>1.68</td>
<td>2.31</td>
</tr>
<tr>
<td>hybrid</td>
<td>6.16</td>
<td>0.37</td>
<td>0.85</td>
<td>7.23</td>
<td>0.99</td>
<td>1.54</td>
<td>2.79</td>
</tr>
<tr>
<td>pied</td>
<td>6.29</td>
<td>0.96</td>
<td>0.94</td>
<td>7.21</td>
<td>0.46</td>
<td>1.35</td>
<td>2.75</td>
</tr>
</tbody>
</table>

Clutch size is the number of eggs in a brood. Male share of paternity is the proportion of the nestlings sired by the social male in a brood. Hatchability is the proportion of the clutch hatching. Lifetime fledged young and lifetime recruitment is the total number of nestlings successfully fledging and the total number recorded in subsequent years over an individual’s lifetime. Reproductive attempts is the total number of times an individual is observed breeding and lifespan is the age of an individual when it was last caught.
two hybridizing species follow distinct migratory routes, the migratory behaviour of hybrids might be intermediate between those of the parental species which is predicted to lead to increased hybrid mortality (Helbig 1991a; Sutherland 1998; Bensch et al. 1999). The collared and pied flycatchers occupy different wintering areas in sub-Saharan Africa and are believed to follow different migratory routes (along the east and west side of Africa, respectively). If hybrid flycatchers would indeed follow an intermediate route, ending up at suboptimal wintering sites, we would expect a lower recruitment rate for hybrid fledglings at the breeding grounds. This was not the case as shown in chapter 5 of this thesis. In order to further investigate hybrid migration behaviour we characterised the wintering grounds of the two parental species using stable isotope signatures extracted from feathers grown in winter. We then did the same with the hybrids which showed that their isotope signatures closely resembled those of the pied flycatchers. The isotope technique does not allow us to accurately pinpoint the geographical location where the feather moulting took place and we therefore cannot fully exclude the possibility that they do exhibit intermediate migratory behaviour. However, the most parsimonious explanation is that they do indeed adopt the wintering grounds of the parental species.

An unpublished study investigated the idea that hybrids might benefit from an increased parasite resistance compared to the parental species. The author compared parasite infection rates and immune response to a novel antigen and found intermediate responses for the hybrids compared to the parental species. Later generation backcrosses between hybrids and collared flycatchers had a stronger immune response and less high rates of parasite infection. However, sample sizes of the latter category were very low which makes it hard to draw any firm conclusions (Wiley 2006).

**Hybrid attractiveness**

Courtship behaviour and plumage characteristics used in mate attraction differ markedly between species. Consequently, individuals which deviate from the established behavioural patterns or which have intermediate ornament expression might be less favoured by females. In this context, the intermediate phenotype of male hybrids could result in reduced mating success.

We have investigated whether this is the case for male hybrid flycatchers by comparing the pairing success of male hybrids with that of both parental species (Svedin 2006). In 2005, males were caught on Öland upon arrival and before pair formation. The males were classified into three groups: pied flycatchers, collared flycatchers and intermediate birds. The last group consisted of ‘intermediate’ looking males and hybrids and they were pooled because our focus was on the effects of sexual selection on the intermediate phenotype (and not the
Males which were successful in acquiring a female were caught later in the season when feeding the nestlings. The intermediate phenotype group had roughly half of the pairing success (0.263) compared to collared (0.498) and pied flycatchers (0.486), which was not significant owing to the small sample size of the intermediate group (sample sizes were 19, 223 and 35, respectively). The advantage of this method is the direct way of measuring pairing success, but it is hard to acquire large enough sample sizes for the intermediate phenotype group. This latter group should ideally be separated in ‘intermediate phenotype’ males and hybrid males as these two categories might differ in song or other behavioural aspects which can influence the pairing success.

A second way of looking at the pairing success is by comparing the total number of reproductive attempts over an individual’s life (i.e. number of times observed breeding) of males for collared and pied flycatchers and hybrids. This has been done by Veen et al. 2001 and the same measure can be derived from the data presented by Svedin 2006 which includes additional years. This results in the following average number of reproductive attempts: male collared flycatchers 1.68, male pied flycatchers 1.40 and hybrid 1.54 which is very similar to the average presented by Veen et al. 2001 (1.65, 1.42 and 1.58, respectively). The pairing success can be calculated by dividing the number of reproductive attempts by an individual’s lifespan. This is low for the hybrid males (0.552) compared to collared flycatcher males (0.729), but this is primarily due to a longer lifespan and not less reproductive attempts (table 8.1).

The two approaches described above both indicate a lower pairing success for hybrids per breeding season, but this does not seem to result in a reduction of reproductive attempts through an individual’s lifetime. Why hybrid males appear to live longer remains to be elucidated.

Finding a partner is a first step in reproducing, fertilising the eggs the second important step. Previous work has indicated that in flycatchers ‘being mated’ does not necessarily mean that you father (all) offspring. The male’s share of paternity in broods of pure collared flycatchers depends on the phenotype of the male (Sheldon and Ellegren 1999). Therefore, hybrid males with their intermediate phenotype, might loose paternity. Indeed, the proportion of extra-pair young in broods attended by a male hybrid and a female collared flycatcher was significant higher as compared to pure collared or pure pied flycatcher broods (Svedin 2006). This could be either caused by females adjusting their mating strategy in response to the male’s phenotype or, alternatively, by hybrid sperm having a disadvantage in sperm competition. In one of the preceding sections I already mentioned the reduced share of paternity of ‘intermediate’ males (pure flycatcher species with an intermediate plumage) which indicates that the females indeed might change their mating strategies. However, deficiencies in hybrid sperm should be regarded a plausible alternative explanation.
Species recognition and reproductive isolation

The production of unfit hybrids is predicted to increase assortative mating, hereby reducing the probability of interspecific pair formation, a mechanism known as reinforcement. Reinforcement requires a tight linkage between species specific male traits and female preferences for these traits. A major obstacle to this is recombination, which will dissociate the linkage between trait and preference even at low frequencies of hybridization (e.g. Felsenstein 1981).

The process of reinforcement has received a lot of attention in flycatchers as an earlier study showed that the male trait divergence and female preferences matches those predicted by reinforcement (Sætre et al. 1997b). The male traits between the species are more diverged on the sympatric breeding grounds in the Czech Republic such that collared flycatchers have larger white patches on the forehead and the primaries and pied flycatcher are brownish instead of black in the allopatric areas. With the use of mate choice experiments in aviaries, Sætre and colleagues showed that female preferences for conspecifics were much stronger in sympathy compared to allopatry (see Sætre et al. 1997b for more details).

Reinforcement might take place on the sympatric breeding grounds of the flycatchers, the more fundamental question remains of how it can happen in the first place, given the recombination problems described above. There are several ways in which recombination could be reduced. The physical linkage of the male trait, the female preference for this trait and the factors determining hybrid fitness is one such way. In flycatchers the genetic determination of both the expression of male traits used in species recognition and those underlying hybrid incompatibilities are located on the Z chromosome (a sex chromosome) (Sætre et al. 2003). In chapter 6 we showed with the use of the long term database, field experiments and molecular techniques that females have a preference for males of the same species as their father. This is not due to imprinting but is passed on through the Z chromosome (in birds females are the heterogametic sex (ZW) and therefore inherit their sole Z chromosome from their father). A combination of physical linkage of the three components together with the fact that it is located on the Z chromosome (which has reduced recombination rates (Sætre et al. 2003)) severely reduces the negative effects of recombination in flycatchers.

The reduced recombination rates, together with the female preference and male plumage divergence patterns, as predicted by the reinforcement theory (Sætre et al. 1997b), strengthen the idea that reinforcement took (or takes) place in flycatchers.

The Ficedula flycatchers are often considered to be one of the most convincing examples for reinforcement in nature, but the situation might not be as clear-cut as sometimes suggested.
First, previous studies found an effect of the plumage colour of the males on the intensity of interspecific competition. The idea is that brown males of the competitively subordinate pied flycatcher resemble female flycatchers more closely. As a result, these males receive less intense aggression from collared flycatcher males compared to darker pied flycatcher males; so called ‘female mimicry’ (Sætre et al. 1993; Alatalo et al. 1994). Selection is therefore predicted to increase the frequency of brown coloured male pied flycatchers in the population on the sympatric breeding grounds. This avoidance of interspecific competition will increase trait differences between the species which facilitates species recognition. Note that the brown colour is not the only trait which diverged between sympatric and allopatric populations, so female mimicry is unlikely to be the sole explanation for the character displacement patterns found (e.g. Sætre et al. 1997b). Female mimicry to avoid competition and reinforcement driven by the reduced hybrid fitness are both predicted to decrease hybridization rates and could therefore work synergistically.

Second, the divergence of plumage traits as found in the Czech Republic does not appear to be present in the hybrid zone in Russia (V. G. Grinkov pers. comm.). In this part of the distribution of the pied flycatcher, the colour of males gradually declines from very dark in the north to more brown towards the south. There appears however to be no larger divergence in the sympatric breeding areas. This indicates that other selective forces may influence the colour of males, which is currently under investigation. This observation suggests that if reinforcement is acting in a hybrid zone, it does not necessarily need to do so over its entire geographical range.

**Limitations of this study**

In this next section I would like to highlight and discuss some choices and assumptions made in this thesis.

My aim is to understand the mating decisions in a hybrid zone, but an important part of my work presented focuses on the female side of these decisions. Obviously, a pair consists of individuals of both sexes and therefore both sexes should be included if one wants to fully understand the mating process. Males engaged in a mixed-species pair suffer equally high or at times even higher costs compared to females (male pied flycatchers mated to a female collared flycatcher only father a small proportion of the brood). Although males might be the less choosy sex in intraspecific mate choice decisions, this does not mean that the same holds when confronted with a partner of a different species (because the costs of ending up with a heterospecific female can be very large). I believe that the approach taken in this thesis by first focussing on the choosy sex is a good one, but I feel that with our current knowledge of the system it is
important to now focus on the male side of the story. This furthermore opens
the possibilities to study the interactions between the sexes. Considering the
context dependence of many of the costs and benefits, as described in this
thesis, unravelling the male mating decisions and especially the interaction
between the sexes, might be challenging.

For the empirical work we made use of flycatcher populations breeding in
nest-boxes. However, the use of nest-boxes might affect the behaviour
flycatchers in relation to the questions addressed in this thesis. The estimates of
the reproductive success might have been affected by nest-boxes as shown in
earlier studies (Mitrus 2003; Purcell et al. 1997) and the results obtained might
therefore not accurately reflect the natural situation (natural cavity breeding
population). Furthermore, the availability of nesting sites might have been artifi-
cially changed, which could lead to unnaturally high densities of breeding pairs,
potentially affecting competition both within and between species. Earlier
studies emphasized the importance of differences in competitive strength
between the species over breeding territories, but this might strongly depend on
the availability of nesting sites. If there is a surplus of nest-boxes, pied flycatchers
might breed at places where they would have otherwise been outcompeted by
collared flycatchers and excluded from breeding. One way to quantify such a
potential effect would be to experimentally alter the relative abundance of nest-
boxes and record the effect this has on the abundance of each species.

**Synopsis**

Despite the fact that the species recognition abilities of the two flycatcher
species seem to be well developed, heterospecific pair formation does occur in
the face of reduced fitness of hybrids. To understand this apparent contradiction,
one may ask the question whether the costs are indeed as high as expected. In
this thesis the costs and benefits of hybridization have been investigated, mainly
from a female perspective.

The results presented in the preceding chapters together with earlier work
shows that a multitude of different factors may affect the reproductive success of
hybridizing flycatchers. Table 8.2 provides a summary of the results. (It has the
same structure as table 1.1 in chapter 1, which was based on literature data for
birds in general.). Our current knowledge indicates that under certain circum-
stances a mixed-species pairing might turn out to be beneficial for female collared
flycatchers. For female pied flycatchers the situation seems to be different. This is
mainly because conspecifics are rare in most breeding populations. For this
reason, pied flycatcher females do not have the possibility to benefit from high
rates of conspecific extra-pair paternity. Finally, it should be noted that males
engaging in a mixed-species pairing probably always suffer important losses.
### Table 8.2. A summary of the results obtained in this study, supplemented with literature data. The table lists the various aspects of reproduction affecting the reproductive success of hybridizing flycatchers following the same order as used in this chapter (first column). Factors affecting the probability to hybridize and the fitness consequences are summarised in the second column. The fitness consequences are often context dependent and may vary between the sexes and between species. In general, fitness consequences have been described from a female collared flycatcher’s perspective, as this has been the focus of this study. The names of the flycatchers have been abbreviated: CF is collared flycatcher and PF is pied flycatcher.

<table>
<thead>
<tr>
<th>Synthesis headings</th>
<th>Hybridization and fitness consequences</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td><strong>pre-zygotic</strong></td>
<td></td>
<td></td>
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<tr>
<td>Species recognition and mate choice</td>
<td>- plumage and age of CF males can affect hybridization probability</td>
<td>- Wiley et al. 2005</td>
</tr>
<tr>
<td></td>
<td>- mixed-singing of PF males can affect hybridization probability</td>
<td>- Qvarnström et al. 2006</td>
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<td></td>
<td>- choosing heterospecific male may reduce search costs</td>
<td>- Wiley 2006</td>
</tr>
<tr>
<td>Fertilisation</td>
<td>- conspecific sperm precedence increases proportion of non-hybrid offspring</td>
<td>- this thesis (chapter 2)</td>
</tr>
<tr>
<td><strong>post-zygotic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct costs and benefits of mixed-species pairing</td>
<td>- PF are better parents (raise more young) at end of season</td>
<td>- Qvarnström et al. 2005</td>
</tr>
<tr>
<td></td>
<td>- PF territories provide more food (caterpillars) at end of season</td>
<td>- this thesis (chapter 4)</td>
</tr>
<tr>
<td></td>
<td>- diet and feeding rates of species are similar</td>
<td>- this thesis (chapter 3)</td>
</tr>
<tr>
<td>Hybrid viability and fecundity</td>
<td>- no reduced viability of hybrid males &amp; females</td>
<td>- e.g. Veen et al. 2001</td>
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<tr>
<td></td>
<td>- hybrid females have low fertility</td>
<td>- e.g. Alatalo et al. 1990; Veen et al. 2001</td>
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<tr>
<td></td>
<td>- hybrid males have reduced sperm performance</td>
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<td></td>
<td>- backcrosses have reduced reproductive success</td>
<td>- Wiley 2006</td>
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<td>Intermediate hybrid phenotype</td>
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<td>- lifespan of hybrids is not reduced</td>
<td>- Svedin 2006</td>
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<td></td>
<td>- hybrids have an intermediate immune response</td>
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<tr>
<td>Hybrid attractiveness</td>
<td>- hybrids are less attractive and have lower mating chance</td>
<td>- Svedin 2006</td>
</tr>
<tr>
<td></td>
<td>- hybrid males may loose paternity</td>
<td>- Svedin 2006</td>
</tr>
</tbody>
</table>

1Based on circumstantial evidence: the effect of availability of conspecifics on the probability to end up in a mixed-species pairing.

2Male hybrids father on average only 38% of the nestlings in the brood they attend. Similarly to mixed-species broods, this could be due to a response of the female or e.g. a consequence of low quality of hybrid sperm.
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T


V


**W**


**Z**

Partnerkeus en hybridisatie
Het probleem

Bij hogere diersoorten is het kiezen van een partner veelal een ingewikkeld proces. Voor vogels wordt aangenomen dat het vooral de vrouwen zijn die een partner kiezen. Daarbij gaat het erom een man te vinden waarmee een zo groot mogelijk aantal nakomelingen kan worden geproduceerd van een zo hoog mogelijke kwaliteit (fitness). Bij het kiezen van een man is het voor de vrouw van belang rekening te houden met zowel de negatieve als de positieve gevolgen van de keuze. In de context van deze studie worden dat de kosten en de baten van de partnerkeuze genoemd.

Gewoonlijk wordt een partner gekozen van dezelfde soort. Het kiezen van een partner van een andere soort, hybridisatie, wordt door biologen vrijwel steeds als een slechte keuze gezien. De hybride nakomelingen van dit soort mengparen hebben namelijk meestal een kleinere overlevingskans of zijn minder vruchtbaar. Toch komt hybridisatie geregeld voor in de natuur en de vraag is hoe dit veroorzaakt wordt. Een mogelijke verklaring is dat de soorten elkaar niet goed kunnen herkennen en er een fout wordt gemaakt. Er zijn echter ook hybridiserende soorten die individuen van de eigen soort wel degelijk goed kunnen herkennen. Waarom hybridiseren twee soorten ondanks de oogschijnlijk hoge kosten die dat met zich meebrengt in zo’n situatie? In dit proefschrift wordt nader op deze vraag ingegaan voor twee hybridiserende vliegenvangersoorten, de bonte vliegenvanger (*Ficedula hypoleuca*) en de withalsvliegenvanger (*F. albicollis*).

Hybridiserende vliegenvangers

De mannen van beide vliegenvangersoorten verschillen van elkaar in diverse kenmerken, waarvan de zang en de hoeveelheid wit in het verenkleed (de withalsvliegenvanger heeft meer wit) het meest opvallend zijn. De vrouwen van beide soorten lijken sterk op elkaar. De broedgebieden van de bonte vliegenvanger strekken zich uit van West en Noord Europa tot diep in Rusland. De withalsvliegenvanger broedt vooral in Centraal en Zuid Europa. Het broedareaal van beide soorten overlapt in Tsjechië en op de Zweedse eilanden Gotland en Öland. De gegevens voor dit proefschrift komen uit alle genoemde gebieden, maar vooral van Gotland. In de broedgebieden waar beide soorten voorkomen is de bonte vliegenvanger in de minderheid (5-15%) en het aantal gemengde paren is gering (2-5%).

De vrouwen van beide soorten lijken de mannen van de eigen soort goed te herkennen. Toch vindt hybridisatie plaats. Soms lijkt dit het gevolg van een herkenningsfout, omdat sommige bonte vliegenvangermannen de zang van een withalsvliegenvanger imiteren. Maar ook wanneer dit niet het geval is kan een
mengpaar ontstaan. Om het ontstaan van dit soort paren te begrijpen moeten we de kosten en baten van de gemaakte keuze nader bestuderen. Hybridisatie zou een slechte partnerkeuze zijn als het sterke negatieve gevolgen zou hebben voor het aantal en kwaliteit van de nakomelingen. Dit lijkt op het eerste gezicht het geval te zijn daar hybride vrouwen onvruchtbaar zijn. Er blijkt echter meer aan de hand te zijn. Mijn onderzoek als doktoraal student naar paren bestaande uit vrouwelijke withalsvliegenvangers en mannelijke bonte vliegenvangers toonde aan dat de verwachte kosten van het produceren van hybriden juist opvallend gering waren. In de eerste plaats vonden we dat de mengparen meer zonen produceerden dan dochters. En zonen blijken vruchtbaar te zijn en zich gewoon voort te kunnen planten, terwijl dochters onvruchtbaar zijn. In de tweede plaats bleek een groot deel van de jongen in het nest niet door de sociale vader (de bonte vliegenvanger) bevrucht te zijn, maar door een withalsvliegenvanger. Deze jongen zijn dus pure withalsvliegenvangers en hebben geen lagere fitness. Tenslotte bleek dat mengparen laat in het seizoen zelfs meer uitgevlogen jongen produceerden dan zuivere withalsvliegenvangerparen. De resultaten van deze eerste studie leidden tot de conclusie dat het aangaan van een paarband met een andere soort niet per se een slechte keuze hoeft te zijn en dat natuurlijke selectie niet onder alle omstandigheden hybridisatie tegengaat. Mijn doctoraalstudie is het uitgangspunt geweest voor dit proefschrift waarin ik een beter beeld probeer te krijgen van de partnerkeuze van vliegenvanger vrouwen.

Paringsstrategieën van vrouwelijke withalsvliegenvangers

In hoofdstuk 2 wordt ingegaan op de vraag waarom er binnen de bovengenoemde mengparen van vrouwelijke withalsvliegenvangers en mannelijke bonte vliegenvangers meer buitenechtelijke jongen worden geboren. Hiervoor zijn twee mogelijkheden denkbaar. In de eerste plaats zouden vrouwelijke withalsvliegenvangers hun paringsstrategie kunnen aanpassen door bijvoorbeeld actief meer buitenechtelijke copulaties aan te gaan dan wanneer ze gepaard zou zijn met een withalsvliegenvanger man. Anderzijds zouden ook zogenaamde intrinsieke verschillen tussen de soorten een verklaring kunnen vormen, bijvoorbeeld wanneer het sperma van de eigen soort een hogere bevruchtingskans heeft dan dat van de andere soort. Bij een gelijkblijvend aantal buitenechtelijke copulaties zal dat automatisch leiden tot meer buitenechtelijke nakomelingen.

Om beide mogelijkheden te onderzoeken hebben we het volgende experiment gedaan: we hebben withalsvliegenvangermannen gevangen op het moment dat ze uit Afrika in het broedgebied terugkeerden en zodanig geverfd dat het bonte vliegenvangers leken. De geverfde vogels raakten gepaard met vrouwelijke withalsvliegenvangers en het percentage buitenechtelijke nakomelingen (en
ook het percentage zonen) week niet af van dat van normale withalsvliegen-vanger paren. Dit suggereert dat de vrouwen hun paringsstrategie niet zouden hebben aangepast, maar dat intrinsieke verschillen tussen de soorten verantwoordelijk zouden zijn voor het hoge percentage buitenechtelijke jongen zoals gevonden bij mengparen. Omdat we echter geen effect vonden van ons verf-experiment dringt zich de vraag op of de vrouwelijk withalsvliegenvangers de geverfde soortgenoten wel voor bonte vliegenvangers hebben aangezien. Helaas hebben we hierover geen zekerheid, waardoor op basis van ons experiment geen eenduidige uitspraak gedaan kan worden.

Analyse van kosten en baten

Om de partnerkeuze bij vliegenvangers te kunnen begrijpen is het belangrijk om de kosten en de baten van een bepaalde keuze zo goed mogelijk in kaart te brengen. Bij de door ons bestudeerde mengparen zijn de kosten voor de withalsvliegenvangervrouwen sterk verlaagd door het grote aantal buitenechtelijke jongen en de productie van veel zonen. Andere factoren kunnen echter ook een rol spelen. Enkele hiervan worden in hoofdstuk 3, 4 en 5 nader onderzocht.

Een mannelijke partner van een andere soort zou een goede keus kunnen zijn als deze meer energie stopt in het grootbrengen van de jongen of gespecialiseerd is in het vinden van andere, voor de jongen belangrijke, voedselbronnen. De resultaten in hoofdstuk 3 laten zien dat de soortensamenstelling van het voedsel van beide vliegenvangersoorten weinig verschilt en dat de mannen van beide soorten evenveel bijdragen aan het grootbrengen van de jongen.

Vervolgens hebben we gekeken naar mogelijke verschillen in territoriumkwaliteit tussen beide soorten. Door gebruik te maken van gegevens over de vliegenvangerpopulatie op Gotland over de laatste 25 jaar, laten we zien dat er verschil is in het broedsucces (aantal uitgevlogen jongen) tussen nestkasten waar minimaal éénmaal bonte vliegenvangers in hebben gebroed en nestkasten waar alleen withalsvliegenvangers gebruik van hebben gemaakt. Wanneer we withalsvliegenvangerparen uit beide typen nestkasten vergeleken (die broedden namelijk ook in de “bonte vliegenvanger-nestkasten”), dan bleek het broedsucces aan het eind van het seizoen in de “bonte vliegenvanger-nestkasten” hoger te zijn. Zo konden we aantonen dat we niet te maken hadden met een verschil tussen de soorten maar met iets dat verband hield met de lokatie van de nestkasten. Ons vermoeden ging uit naar verschillen in habitat en, daaraan gerelateerd, de beschikbaarheid van voedsel.

In hoofdstuk 4 gaan we verder in op habitatverschillen tussen de territoria van beide vliegenvangersoorten. Bij de vliegenvangers bezetten de mannen direct na aankomst in de broedgebieden een territorium. Vervolgens kiezen de vrouwen een partner. Deze keuze kan gevolgen hebben voor het soort habitat
waarin de vrouw gaat broeden, als het habitat in de territoria van de twee soorten mannen verschilt. Dit blijkt het geval te zijn; de bomensamenstelling (frequentie van voorkomen) in de territoria van beide soorten verschilt. Vervolgens laten we zien dat een belangrijke voedselbron van de vliegenvangers, namelijk rupsen, boomsoortafhankelijk is. Door habitatkarakteristieken en voedselbeschikbaarheid met elkaar in verband te brengen tonen we aan dat de territoria van de bonte vliegenvangers aan het eind van de broedtijd inderdaad meer rupsen bevatten dan die van de withalsvliegenvangers.

In hoofdstuk 3 en 4 lag de nadruk op directe voor- en nadelen van het vormen van een mengpaar. Het is echter ook van belang om het succes van de (hybride) nakomelingen zo nauwkeurig mogelijk te meten. Soorten zijn aangepast aan hun omgeving en een hybride kan intermediaire kenmerken hebben waardoor het de optimale aanpassingen van beide ouders mist. In hoofdstuk 5 bekijken we of hybriden een nadeel ondervinden tijdens de jaarlijkse trek naar de Afrikaanse overwinteringsgebieden. De withalsvliegenvanger trekt via een oostelijke route naar Zuidoost Afrika, terwijl de bonte vliegenvanger een meer westelijke route volgt en in West Afrika overwintert. Er wordt aangenomen dat grote veranderingen in de trekroutes van vogels over het algemeen tot een verhoogde sterfte leiden. Een voorbeeld van zo’n grote verandering is een intermediaire trekroute van hybride vliegenvangers. Echter, we vinden geen verhoogde sterfte en de vraag rijst of de hybriden wel een intermediaire route volgen of dat ze de route van een van beide ouders kiezen en in dezelfde gebieden overwinteren. Dit hebben we onderzocht door gebruik te maken van een moderne techniek, namelijk het karakteriseren van de wintergebieden met behulp van stabiele isotopen. Stabiele isotopen (in ons geval van koolstof en stikstof) komen van nature overal op aarde voor, maar zijn per gebied verschillend. Deze isotopen worden opgeslagen in vogelveren op het moment dat deze worden gevormd. We weten welke veren in de wintergebieden geruild worden en ter plaatse door nieuwe worden vervangen. Een analyse van deze nieuwe veren (die later in het broedgebied verzameld kunnen worden) verschaft ons dus een gebiedseigen kenmerk, de “isotope signature”. De resultaten toonden aan, dat de veren van bonte vliegenvangers en withalsvliegenvangers verschillende isotopenwaarden hadden. Dit bevestigt dat beide soorten in verschillende gebieden overwinteren. De waarden van de hybriden waren echter niet intermediair, maar kwamen overeen met die van de bonte vliegenvanger.

**Vrouwelijke voorkeur**

Om de juiste partner te kiezen moet deze herkend worden en dit is met name belangrijk als je potentieel voor de verkeerde soort (met alle gevolgen van dien) kunt kiezen zoals voor de nauw verwante vliegenvangers het geval is. In hoofd-
stuk 6 onderzoeken we hoe de voorkeur van vrouwelijke vliegenvangers voor een bepaalde soort is vastgelegd. Eerst kijken we naar de partnerkeuze van hybride vrouwen (de dochters uit een gemengd paar). Die blijken een sterke voorkeur te hebben voor een man van dezelfde soort als de vader. Deze voorkeur zou het gevolg kunnen zijn van een zogenaamd inprentingproces tijdens het opgroeien in het nest, maar zou ook een genetisch basis kunnen hebben op het geslachtschromosoom. Bij vogels zijn de vrouwen namelijk de heterogametische sexe (aangeduid als ZW); ze krijgen het Z chromosoom altijd via de vader. Om beide mogelijkheden verder te onderzoeken hebben we nestjongen van bonte vliegenvangers en withalsvliegenvangers onderling verwisseld. Zo hebben we een situatie gecreëerd waarin jonge bonte vliegenvangers bij withalsvliegenvangers opgroeiden en omgekeerd. In de jaren na het experiment hebben we deze jongen gevolgd en vonden dat de vrouwen een paarband aangingen met een soort die overeenkwam met de genetische vader en niet degene door wie ze waren opgevoed. We concluderen dan ook dat de sociale vader niet bepalend is voor de latere soortskeuze van de dochters, maar dat daarvoor een genetisch basis bestaat die gelegen is op het Z chromosoom.

Signalen & partnerkeuze

Bepaalde signalen kunnen bij vogels voor verschillende doeleinden worden gebruikt. Zo speelt de witte voorhoofdsvlek bij bonte- en withalsvliegenvangers een rol bij conflicten tussen mannen, maar ook bij de partnerkeuze. De vraag is, of het hebben van een dubbele functie ook van invloed is op het gebruik van het signaal in de afzonderlijke situaties. In hoofdstuk 7 ontwikkelen we een mathematisch model met als doel het onderzoeken van het bestaan en de evolutionaire implicaties van signalen met een dubbele functie. We gaan uit van een dynamisch model waarbij de signaalfunctie zich op twee manieren kan ontwikkelen: (1) bij competitie tussen mannen met verschillen in vechtkracht en (2) bij de paarvorming waarbij de vrouwelijke vogel kiest uit mannen met verschillende kwaliteiten. We vinden dat het signaal gelijktijdig in beide situaties gebruikt kan worden. Ook tonen we aan dat het gebruik van het signaal in de ene situatie het gebruik in de andere situatie kan vergemakkelijken. Met andere woorden, het gebruik van de witte voorhoofdsvlek bij conflicten tussen mannen kan ertoe leiden dat vrouwen dit kenmerk gaan gebruiken bij het kiezen van een partner en omgekeerd. De door ons gebruikte modelmatige aanpak is slechts een eerste stap bij het ontrafelen van een ingewikkeld probleem. We zijn echter van mening dat de door ons gebruikte methode veelbelovend is omdat het nieuwe inzichten kan geven in processen die tot nu toe te complex waren om geanalyseerd te worden.

Nederlandse samenvatting

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Synthese

In het laatste hoofdstuk van mijn proefschrift geef ik een samenvatting van alle relevante studies met betrekking tot hybridiserende bonte- en withalsvliegenvangers. Een belangrijke conclusie is dat veel kosten en baten van de partnerkeuze context afhankelijk zijn. Zo verandert bijvoorbeeld de beschikbaarheid van het voedsel in de broedterritoria in de loop van het seizoen. Hierdoor worden ook de kosten en baten van de keuze voor een bepaalde territoriumhoudende man seizoensafhankelijk.

In dit proefschrift heb ik me beperkt tot het bestuderen van de partnerkeuze van hybridiserende vrouwelijke vliegenvangers. Hiervoor is gekozen omdat bij vliegenvangers deze sexe het sterkst de partnerkeuze lijkt te bepalen. Uiteraard ondervinden de mannen eveneens de “fitness consequenties” van de gemaakte keuze. Voor het verkrijgen van een compleet beeld van de gevolgen van hybridisatie dient toekomstig onderzoek zich dan ook mede op de mannelijke vogels te richten. De belangrijkste conclusie van dit proefschrift is dat de kosten van hybridisatie voor een vrouwelijke vogel veel minder groot kunnen zijn dan algemeen wordt aangenomen. Onder bepaalde omstandigheden blijkt hybridisatie in reproductief opzicht zelfs voordelig te zijn.
Acknowledgements
Through the years I realized that most people start with reading the acknowledgements. And quite some don’t go any further (although you’ll miss something). As I don’t want to discriminate against foreigners, they are the majority in this world after all, I will write this in English.

The Dudes

I’d like to thank Ben ‘Slender-billed’ Sheldon for supervising my second master project which has been an important step to get this PhD. Most likely, initial acceptance was partly based on the fact that I had bins, a scope, driver’s license and was keen to go birding... After this project you continued to supervise me during my PhD; thanks for all your advice, help and great days birding (sorry, about the Blyth’s reed at Faludden). Cheers.

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Thor
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