

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 & 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 & Gross, 1990; Kirkpatrick & 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.*, 2003).

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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 pre-zygotic 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 sympatry, 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 & 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 & 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 lifecycle. 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 subordinate 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. bicollis*) flycatchers hybridize at a low frequency throughout zones of sympatry in central Europe. However, by having a large proportion of their offspring sired by extra-pair, 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, 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, 1993). It is therefore difficult to infer differences in the niches of the two species by comparing populations. One previous study (in the Czech Republic) has investigated the diets of the two species in sympatry (Bures, 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).

Materials 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, 1988; 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 & 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 environment (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 & 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 & 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 (Araneae). 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, 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 & 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 1 is depicted in Fig. 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

Table 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.

Factor	d.f.	<i>F</i>	<i>P</i>
Laying date* (A)	1, 326	21.05	< 0.0001
Territory type† (B)	1, 326	0.180	0.671
Type of heterospecific pair‡ (C)	1, 326	< 0.001	0.991
A × B	1, 326	2.824	0.094
B × C	1, 326	0.079	0.779
A × C	1, 326	3.378	0.067
A × B × C§	1, 326	6.029	0.015
Territory [random effect]	248, 336		

*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 Fig 1.

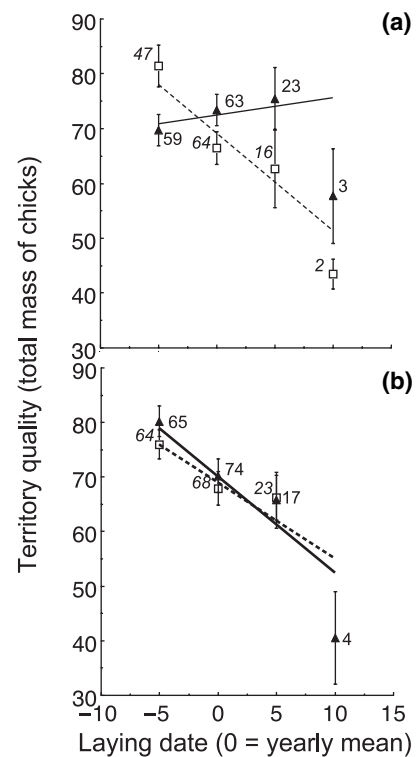


Fig. 1 Differences in the seasonal decline in territory quality (mass of broods of collared flycatchers reared in the box within 5 years of the focal pair) between nest-boxes that had been occupied by heterospecific pairs and those that had been used by control pairs of collared flycatchers. Panel a refers to heterospecific pairs with a female collared flycatcher, whereas panel b refers to heterospecific pairs with a female pied flycatcher. Each point refers to breeding attempts categorized according to their laying date, although lines refer to the fit of the mixed models to continuous data, with territory as a random factor. Sample sizes refer to the number of breeding attempts represented by each category of laying date. Open squares and the dashed line refer to territories that had been used by pairs of collared flycatchers, and filled triangles and the solid line refer to territories that had been occupied by heterospecific pairs. The interaction between laying date and territory type (heterospecific vs. collared) shown in Panel a is statistically significant (GLMM, $F_{1,150} = 8.96$, $P = 0.003$), whereas in Panel b no interaction exists (GLMM, $F_{1,180} = 0.30$, $P = 0.585$).

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 (Fig. 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 (Fig. 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.

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, $F_{2,61} = 6.399$, $P = 0.003$; see Fig. 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 2, Fig. 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 Bures (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 2). Additional MANOVAs for each species separately indicated that the significant interaction term in MANOVA 1 in Table 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

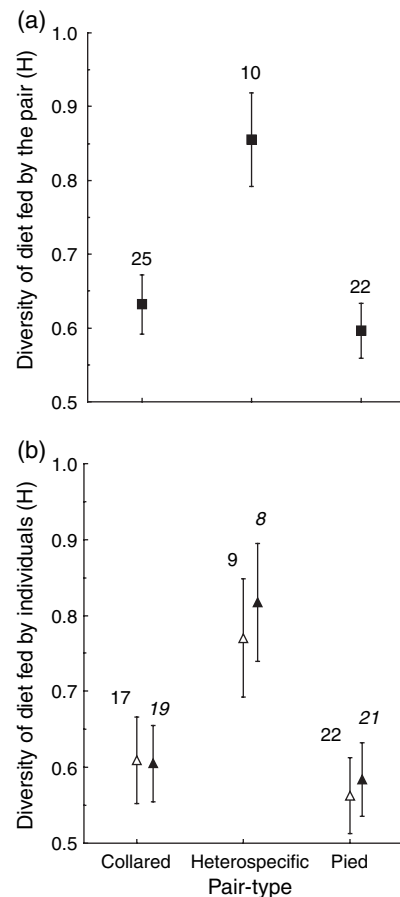


Fig. 2 Panel a shows the mean diversity of food brought to nests reared by collared flycatchers, heterospecific pairs, and pied flycatchers. Panel b shows the diversity of food delivered by each individual parent within each pair. The dietary diversity of one individual per pair was used to calculate 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 we 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.

(the two species feeding in identical habitat), no such interaction between the effect of species and laying date on diet was observed (Table 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 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: $t_{10} = -0.46$, $P = 0.657$;

Table 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.

Factor	d.f.	F	P
(1) Comparing pure pairs			
Species (A)	3,42	1.573	0.210
Laying date (B)*	3,42	0.954	0.424
A × B	3,42	3.134	0.035
(2) Comparing individuals in heterospecific pairs			
Species (A)	3,16	0.330	0.804
Laying date (B)*	3,16	2.450	0.102
A × B	3,16	0.168	0.916

*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.

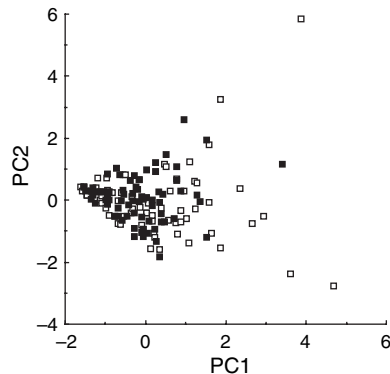


Fig. 3 Principle components analysis illustrating the high degree of overlap between the diets of collared and pied flycatchers. Collared flycatchers are shown with open squares, and pied flycatchers represented by black squares. All adults from heterospecific pairs and the swapped nests of pure pairs 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.

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 (Fig. 2). 'Pair' nested within 'pair-type' was a significant predictor

Table 3 Nested ANOVA showing factors important in determining an individual parent's dietary diversity.

Factor	d.f.	F	P
Pair [pair-type]	53, 90	1.818	0.033
Pair-type*	2, 90	9.062	< 0.001
Age-class†	1, 90	0.309	0.582

*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 Fig 3.

of dietary diversity (Table 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).

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).

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, $F_{1,47} = 0.49$, $P = 0.486$).

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 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.*, 2003), 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 (Parker, 1979; Parker & Partridge, 1998). 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 & 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 override the indirect costs of producing hybrid offspring. In such situations, direct benefits would be an important factor hindering reproductive isolation in sympatry. 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.

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