Hybridization and adaptive mate choice in flycatchers

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Hybridization in natural populations is strongly selected against when hybrid offspring have reduced fitness. Here we show that, paradoxically, pairing with another species may offer the best fitness return for an individual, despite reduced fitness of hybrid offspring. Two mechanisms reduce the costs to female collared flycatchers of pairing with male pied flycatchers. A large proportion of young are sired by conspecific male collared flycatchers through extra-pair copulations, and there is a bias in favour of male offspring (which, unlike females, are fertile) within hybrid pairs. In combination with temporal variation in breeding success, these cost-reducing mechanisms yield quantitative predictions about when female collared flycatchers should accept a male pied flycatcher as a mate; empirical data agree with these predictions. Apparent hybridization may thus represent adaptive mate choice under some circumstances.

Animal and plant hybrid zones are often seen as natural laboratories in which to study evolutionary processes associated with speciation[1]. One very important stage in the speciation process is the development of pre-mating isolating mechanisms between two divergent populations. Reduced fitness of hybrids is thought to select for mechanisms that reduce the likelihood of mating events between populations. Although low rates of hybridization may occur relatively frequently between closely related taxa in some groups[1–3], it is quite difficult to study the fitness consequences of hybrid matings in natural populations. Here we show in two closely related species of birds that, despite the presence of effective pre-mating isolating mechanisms, hybrid pairings occur at an unexpectedly high frequency. Dissection of the fitness consequences of different pairing and mating events shows, however, that hybrid pairing may be selectively favoured in a seasonally changing environment.

Rates and consequences of hybrid pairing

Pied and collared flycatchers (*Ficedula hypoleuca* and *F. albicollis* respectively) have two separate areas of sympatry (range overlap) within their European range, where hybridization occurs[4–7]. In the isolated population breeding on the Swedish island of Gotland, collared flycatchers are numerically dominant, comprising 95% of all breeding birds. In central Europe a hybrid zone runs east–west through the Czech republic[6], and in our study area there, collared flycatchers are again numerically dominant (85% of breeding birds). Pairing in both populations is species assortative (see Table 1 for a test of the null hypothesis of random mating: Swedish population: $\chi^2_4 = 943.1$, $P \ll 0.0001$; Czech population: $\chi^2_4 = 310.0$, $P \ll 0.0001$). Extensive life-history data collected over 20 years in the Swedish population allows the estimation of fitness of hybrids relative to the parental species (Table 2). F_1 hybrids have reduced fitness relative to the parental species, with greatly reduced fitness for F_1 hybrid females owing to almost complete sterility (Table 2). There is no recorded case where an F_1 hybrid female has recruited offspring to the breeding population, and all females for which hybrid status has been established using genetic markers (see below) have been infertile. Males have slightly reduced fertility (92% as assessed by egg hatchabiity) relative to males of the parental species (Table 2). Data from the Czech population suggest that hybrid

fitness is similarly, or even more strongly, reduced there[6]. Studies using genetic markers have confirmed that hybrid males are fertile (ref. 5 and B.C.S., unpublished work). Neither sex of hybrid shows any sign of reduced viability once they have reached breeding age. Male hybrids apparently suffer reduced fitness owing to lower recruitment of offspring relative to collared flycatchers, but not relative to pied flycatchers (Table 2). The low recruitment of offspring for pied flycatchers, and thus possibly for male hybrids too, may be due to reduced philopatry (return to natal site) of their young[8]. These data suggest that the relative reduction in fitness for a female that pairs with a heterospecific male will be substantial (up to 75% of fitness lost).

Given the rarity of pied flycatchers in both hybrid zones, it is not difficult to understand why a female pied flycatcher may sometimes be forced to pair with a male collared flycatcher: there may simply be no unpaired male pied flycatchers remaining[3,9]. Males of both species apparently show no mate discrimination[10]. However, it is much more difficult to understand why a female collared flycatcher (the numerically dominant species) should choose to pair with a male pied flycatcher, particularly since males of the two species differ strikingly in terms of plumage, song and calls, and given that

Table 1 Mating frequencies of flycatchers (number of pairing events observed)

Swedish birds

	Female of pair			
Male of pair	Collared	Hybrid	Pied	Total
Collared	5,567	15	84	5,666
Hybrid	110	1	8	119
Pied	72	3	172	247
Total	5,749	19	264	6,032

Czech birds

	Female of pair			
Male of pair	Collared	Hybrid	Pied	Total
Collared	601	19	15	635
Hybrid	18	0	5	23
Pied	12	6	69	87
Total	631	25	89	745

experimental studies have shown perfect discrimination by females in areas of sympatry[7]. Theoretical models of mate choice suggest that the criteria determining the acceptance of a potential mate should be relaxed if mate choice occurs under time constraints[11], and as with many birds breeding in temperate regions, flycatchers are selected to begin breeding as early as possible[12–15]. Female collared flycatchers pairing with male pied flycatchers breed later (2.6 days later in Sweden and 3.5 days in the Czech Republic) than females in pure collared pairs ($F_{1,5493} = 12.51$, $P = 0.0004$, and $F_{1,512} = 3.56$, $P = 0.06$ respectively, with a general linear model (GLM) controllng for year-associated variation). These observations suggest that females may choose to breed in mixed pairs because of time constraints on breeding, but it remains difficult to understand how time constraints could be so severe that females accept the substantial loss in fitness that seems to result from hybridization.

Conspecific extra-pair paternity

Long-term pedigree data from the Swedish population suggest, however, that the cost of mating with a male pied flycatcher may be reduced by extra-pair copulations with conspecific males. Of 28 offspring recruited from PF × CF (male pied flycatcher × female collared flycatcher) pairs, only 12 (43%) were identified as first-generation hybrids when first handled, the rest (57%) being identified as pure collared flycatchers (identification was made without any knowledge of the identity of an individual's parents). In contrast, 25 of 36 offspring (69%) recruiting from CF × PF pairs were identified as first-generation hybrids when first handled ($\chi_1^2 = 6.98$, $P < 0.01$). One interpretation of these data is that the offspring of females in PF × CF pairs are frequently sired by conspecific males. However, these data also suggest occasional errors in field identification, since 8/36 (22%) of offspring from CF × PF pairs were identified as pure collared flycatchers, which should not be possible given that intraspecific brood parasitism is rare or absent in this population[16]. Mis-identifications could arise in this fashion if alleles from collared flycatchers were dominant to those from pied flycatchers at loci controlling plumage patterns.

We tested whether rates of extra-pair paternity were elevated in PF × CF pairs by microsatellite genotyping families of different specific combinations from both the Swedish and Czech populations. A male's share of paternity in the brood depended on pair-type for both the Swedish population (Fig. 1a; GLM: $F_{3,41} = 12.66$, $P < 0.0001$) and the Czech population (Fig. 1a; GLM: $F_{3,45} = 5.47$, $P = 0.0027$). This effect was largely due to the higher rate of extra-pair paternity among PF × CF pairs, compared with the other three pair types (Fig. 1a). There was no difference between the two populations in either the pattern of paternity across different pair types (GLM: $F_{3,86} = 0.527$, $P = 0.67$) or the overall rate of paternity ($F_{1,86} = 0.811$, $P = 0.37$). The overall mean rate of extra-pair paternity in pure collared flycatcher families (14.5%) is similar to

a previous estimate from the Swedish population (15.5%; ref. 16), whereas the mean rate of extra-pair paternity in PF × CF pairs is approximately four times greater at 59% in the Swedish population, agreeing well with the figure of 57% suggested by the pedigree data.

A more relevant comparison, in terms of the fitness of the offspring produced, is of the proportion of young that are sired by a conspecific male (Fig. 1b). In all pure pairings ($N = 44$ CF × CF pairs and $N = 16$ PF × PF pairs), all offspring were sired by a male
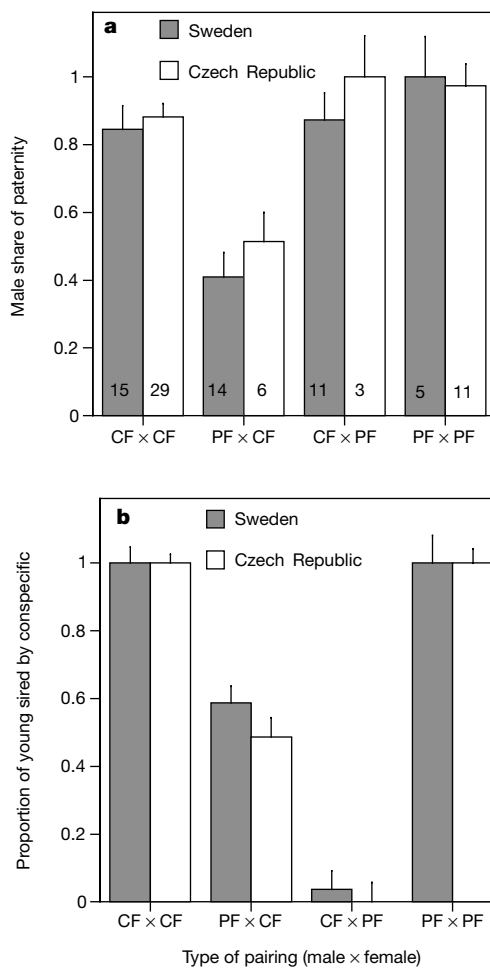

Figure 1 Male share of paternity in relation to pairing type in flycatcher families. **a**, Mean (+s.e.) share of paternity for male flycatchers in relation to pairing type in two separate hybrid zones. Numbers in bars give sample size (number of broods analysed). **b**, Mean (+s.e.) proportion of young sired by a male flycatcher conspecific with the female of the pair; sample sizes as for **a**.

Table 2 Fitness components for individual collared and pied flycatchers and their hybrids in the Swedish hybrid zone

Species	Sex	Fitness component (mean ± s.e.)									
		Hatchability	N	Fledging success	N	Lifetime fledged young	N	Reproductive attempts	N	Lifetime recruitment	N
CF	Male	0.946 ± 0.003†	1,700	0.744 ± 0.006†	2,375	6.26 ± 0.09†	2,259	1.65 ± 0.02†	3,264	0.67 ± 0.02†	2,316
	Female	0.884 ± 0.005†	2,266	0.615 ± 0.007†	3,157	5.21 ± 0.08†	2,978	1.59 ± 0.02†	4,192	0.54 ± 0.02†	3,008
Hybrid	Male	0.873 ± 0.049‡	29	0.664 ± 0.051†	47	5.28 ± 0.61†	47	1.58 ± 0.10†‡	79	0.33 ± 0.14‡	52
	Female	0.092 ± 0.047‡	31	0.151 ± 0.057‡	42	0.84 ± 0.31‡	38	1.70 ± 0.16†	43	0.00 ± 0.00‡	39
PF	Male	0.952 ± 0.010†	107	0.811 ± 0.023‡	141	6.70 ± 0.36†	140	1.42 ± 0.07‡	173	0.35 ± 0.08‡	148
	Female	0.867 ± 0.024†	141	0.720 ± 0.026§	200	5.44 ± 0.27†	199	1.20 ± 0.04§	262	0.27 ± 0.04§	212
Test statistic	Male	F = 4.67*		F = 4.94**		F = 2.04		F = 4.19*		F = 10.11***	
	Female	F = 144.8***		F = 41.04***		F = 20.37***		F = 29.34***		F = 15.15***	

Hatchability and fledging success are expressed as the proportion of eggs hatching, and young fledging, relative to the number of eggs laid in the clutch. Lifetime fledged young, N reproductive attempts, and lifetime recruitment are the total number of young fledged, number of times observed breeding, and the number of offspring recruited to the breeding population, respectively, over an individual's lifetime. All statistics refer to comparisons within sexes. Values with different superscript symbols differ significantly from each other (Tukey tests, $P < 0.05$).
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

conspecific with the female of the pair. In mixed pairs, there was a highly significant difference between PF × CF and CF × PF pairs ($F_{1,32} = 35.38$, $P < 0.0001$; $N = 20$ and $N = 14$ respectively). All extra-pair sired young in PF × CF pairs were sired by a male conspecific with the female, resulting in 56% of offspring from these pairings being non-hybrid. In only one case (involving two nestlings from a brood of five in the Swedish population) were extra-pair offspring from a CF × PF pair sired by a male conspecific with the female. Pedigree data from this population also suggest a low rate of conspecific extra-pair copulation in CF × PF pairs, because 3/36 recruited offspring (8.3%) were identified as pure pied flycatchers when first recaptured. We interpret the difference in the rate of conspecific extra-pair paternity between the two types of mixed pairs to be due to the great difference in the abundance of potential conspecific extra-pair sires.

A further test of whether the rate of heterospecific fertilization differs between the two classes of mixed pairs can be made using F_1 hybrid individuals and a polymerase chain reaction (PCR)-based test which detects the presence/absence of a 32 base pair (bp) mitochondrial DNA indel, which is a fixed difference between the two species[17], as this allows identification of the maternal species of the hybrid. If extra-pair copulations are more frequent in PF × CF pairs, then F_1 hybrids with collared flycatcher mothers should be under-represented relative to the proportion of PF × CF pairs comprising mixed pairings. We screened 31 adult F_1 hybrids from the two populations combined (individuals not included in the paternity analysis above), for which hybrid status was confirmed by microsatellite genotyping, and found that 9/31 (29%) had collared flycatcher mtDNA. This is almost significantly fewer than expected based on the proportions of mixed pairs of the two types in the two populations (99 CF × PF and 84 PF × CF pairs: $\chi_1^2 = 3.55$, $P = 0.059$), but close to the proportion expected (28%) if the rate of conspecific extra-pair mating is taken into account (goodness-of-fit $\chi_1^2 = 0.03$, $P = 0.87$).

The elevated rates of extra-pair paternity in PF × CF broods need not represent elevated rates of extra-pair copulation, because sperm from male collared flycatchers may have an advantage owing to conspecific sperm precedence[18,19]. Further work is required to determine whether female collared flycatchers in mixed pairs pursue an active strategy to increase the rate of extra-pair copulation dependent on the species of the male mate. Irrespective of the behavioural mechanism, the result is that female collared flycatchers in mixed pairs produce a high proportion of non-hybrid offspring, meaning that the costs of heterospecific pairing are lower than they appear at first sight. Female collared flycatchers would be able to reduce the costs of hybridization even further by completely cuckolding their heterospecific mates, yet we found only 3/20 such families. Male collared flycatchers seem to adjust their rate of parental care in response to their perceived share of paternity[20,21], and it is possible that the share of paternity that male pied flycatchers gain, on average, reflects a minimum level at which a male's parental assistance will still be guaranteed. The reproductive success of unassisted female flycatchers is substantially reduced relative to those whose offspring receive paternal care[22].

Sex ratio bias

The long-term data from Sweden also suggest that there may be a male bias in the sex ratio of recruits from mixed pairs, compared to pure collared pairs, because 18/28 recruits (64%) from PF × CF pairs were male, compared to 1,218/2,528 (48%) from CF × CF pairs: ($\chi_1^2 = 2.892$, $P = 0.089$). We tested whether the same bias was present at the nestling stage by using genetic markers to identify the sex of nestlings from PF × CF and CF × CF broods. Combining data for the two populations, we found that the mean brood sex ratio in PF × CF pairs was higher than in pure collared pairs (Fig. 2a; GLM: $F_{1,169} = 5.24$, $P = 0.023$), and corresponded quite well to that suggested by pedigree data. The biased sex ratio in mixed pairs

might be explained by increased mortality of females (the heterogametic sex) at any time after fertilization, in accordance with Haldane's rule[23]. Surprisingly, however, long-term data from the Swedish population give no indication that either the proportion of eggs hatching, or the proportion of eggs fledging, are lower for PF × CF pairs than CF × CF pairs; in fact the reverse is true for fledging success (Fig. 2b). Both would be expected to be lowered in mixed pairs relative to pure pairs, if heterogametic female hybrids suffered higher mortality. Hence, if the male-biased sex ratio is the result of differential mortality of male and female embryos it must occur very early in development.

An alternative hypothesis is that the sex ratio bias occurs because females in PF × CF pairs adjust the sex ratio of eggs they ovulate in favour of males. Recent evidence suggests that female birds may have some degree of control over their offspring sex ratio[24–27]. Selection for such control would be strong and consistent when one sex of offspring had a reproductive value of zero, although conditional sex allocation of this precision may seem biologically implausible. Whether the sex ratio bias is caused by biased mortality or biased production, the result is that females in mixed pairs invest less parental effort, and hence fewer resources, in female offspring with lower reproductive value. This represents a second means by which the costs of heterospecific pairing are lower than they appear at first sight.

Fitness equivalence of mixed pairing

Our data suggest that, for a female collared flycatcher, the cost of pairing with a male pied flycatcher may be reduced by two mechanisms. In contrast, the rarity of suitable conspecific mates, even as extra-pair copulation partners, means that female pied flycatchers, the rarer species, pay almost full costs of hybridization. We tested the fitness costs of heterospecific mate choice directly by assessing the number of offspring and grand-offspring recruited to the breeding population from different pairings in the Swedish population. The mean number of recruited offspring was independent
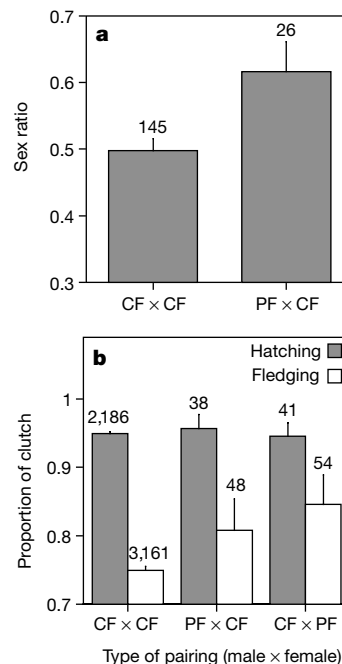

Figure 2 Effect of pairing type on sex ratio and viability of brood. **a**, Sex ratio within broods of flycatchers in relation to pairing type. Numbers above bars give sample sizes. **b**, Hatching and fledging success (mean + s.e. proportion of eggs hatched) in relation to pairing type. Numbers above bars give the number of breeding attempts in each category. Hatching success was independent of pairing type ($F_{2,3263} = 0.04$, $P = 0.92$), while there was a weak effect of pairing type on fledging success ($F_{2,3263} = 3.20$, $P = 0.04$).

of the type of pairing (Fig. 3a), suggesting that hybrid offspring do not suffer any reduction in viability compared with pure-bred offspring. However, if the cost of hybridizing is due to the production of sterile offspring, this cost should be seen in terms of the number of grand-offspring produced, which corresponds better to the reproductive value of offspring. We found a highly significant effect of pairing type on the number of grand-offspring produced (Fig. 3b). Female pied flycatchers mated to male collared flycatchers produced significantly fewer grand-offspring per breeding attempt than did either pure collared pairs or female collared flycatchers mated to male pied flycatchers; the latter two did not differ significantly.

The data on paternity and sex ratio within mixed species and pure families, together with the life-history data (Table 2) allow the calculation of the expected relative fitness of the three types of pairings (pure collared; male pied × female collared; and male collared × female pied). Assuming total sterility among female hybrids, we can calculate the expected fitness of the three types of pairings in two ways. First, discounting male fitness that is lost due only to reduced fertility suggests that the fitness of the two mixed pairings relative to a pure collared pairing will be in the ratio: CF × CF : PF × CF : CF × PF = 1.00 : 0.82 : 0.47. This calculation allows for the possibility that the reduced recruitment success of hybrid males is due to their offspring's showing greater dispersal. Alternatively, assuming that the recruitment data in Table 2 are representative of the relative recruitment success of male collared flycatchers and hybrids, and discounting the fitness of hybrid

males accordingly, yields a predicted ratio of CF × CF : PF × CF : CF × PF = 1.00 : 0.72 : 0.27. Both sets of predictions lie within the 95% confidence intervals of the observed fitness of the three pair types (Fig. 3b), although the prediction based on the full life-history data (that is, prediction 2) shows a closer agreement with the data.

The calculated average loss in fitness for a female collared flycatcher pairing with a male pied flycatcher, as opposed to a male collared flycatcher (18–28%), is still apparently substantial despite the cost-reducing mechanisms. However, this comparison ignores the fact that mean laying date in PF × CF pairings is 2.6 days later than in pure CF pairings. In the Swedish collared flycatcher population there is strong selection for breeding as early as possible[12,13], with the standardized selection gradient on laying date being $\beta = -0.343 \pm 0.035$ s.e. ($P < 0.0001$; s.d. of laying date is 4.5 days). A difference in mean laying date of 2.6 days should result in a loss of relative fitness of about 20%. However, the two pair-types differ with the respect to the seasonal decline in reproductive success (Fig. 4a), which explains why, despite breeding later, PF × CF pairs fledge a greater proportion of eggs than do pure collared pairs (Fig. 2b). Consequently, the overall reproductive success per breeding attempt does not differ between PF × CF pairs and pure collared pairs, despite the fact that the latter breed earlier. It is not clear why the relationship between reproductive success and laying date should differ in this manner, although one possibility is that males of the two species defend territories in habits with different seasonal declines in food availability[6]. Further studies are needed to address this question.

Combining the seasonal effect with the reduction in mean offspring reproductive value due to production of hybrid offspring allows us to predict the point at which pairing with a heterospecific male yields the same, or greater, fitness return as pairing with a conspecific male (Fig. 4a). This occurs when hybrid pairing occurs between 3 and 8 days later than the population mean, which will closely approximate the mean breeding date of pure collared pairs, depending on the degree to which reproductive value of hybrid male offspring is discounted relative to pure collareds (see Fig. 3b). Hence, after this point, female collared flycatchers that pair with a heterospecific rather than with a conspecific male should enjoy higher fitness. The relationship between the probability that a female collared flycatcher pairs with a male pied flycatcher and her relative laying date matches this prediction; the probability is at a maximum at +9 days relative to the mean breeding date and greater than that predicted from random mating between +0 and +17 days after the mean breeding date (Fig. 4b). We note that what we have calculated here (Fig. 4a) is the point at which conspecific and heterospecific pairings are of equal value given a simultaneous choice between the two. In fact, the problem for a female collared flycatcher is more likely to be that of choosing whether to accept a male pied flycatcher now, or to reject him and continue searching for a male collared. Thus, the loss in fitness due to late conspecific pairing is likely to be greater than that above.
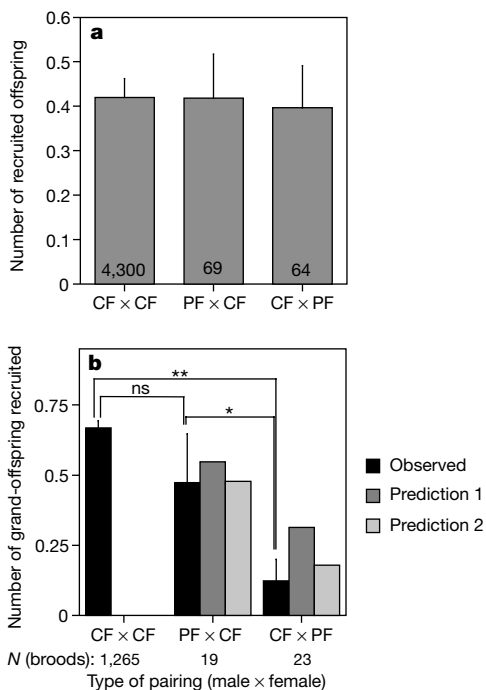
Discussion

Our findings show that hybrid pairing may sometimes represent adaptive mate choice, because the cost-reducing mechanisms that we identify compensate for the fitness that would otherwise be lost owing to producing unfit hybrids. This has implications for the study of speciation: hybrid zone dynamics may be more complex than previously recognized. Accordingly, predictions from current models of speciation, such as reinforcement[28], or sympatric speciation by sexual selection[29] may be too simple to capture the full diversity of possible interactions in hybrid zones. Our findings also have implications for understanding the evolution of extra-pair copulation behaviour in birds. Documented benefits for conspecific extra-pair copulation in birds are generally small[30–32], and are likely to represent an increase in fitness of only a few per cent[33], as with any

Figure 3 Numbers of recruited offspring and grand-offspring for each pairing type. **a**, Mean (± s.e.) reproductive success in relation to pairing type. There is no effect of pairing on reproductive success ($F_{2,4387} = 0.04$, $P = 0.96$). **b**, Number of grand-offspring recruited in relation to pairing type (Kruskal–Wallis test, $H_2 = 9.95$, $P = 0.007$), corresponding to the mean relative reproductive value of offspring produced from the three types of pairings. Values are means (+s.e.) for each brood. Pairwise comparisons between the means of each group are shown. Predicted mean reproductive values are shown for two scenarios based on the life-history data in Table 2, and the paternity and sex ratio data in Fig. 1b and Fig. 2a. Prediction 1 assumes that the reduction in fitness of hybrid males is only due to lower fertility, while prediction 2 assumes that the reduction is equivalent to the reduced recruitment success, relative to male collared flycatchers, in Table 2.
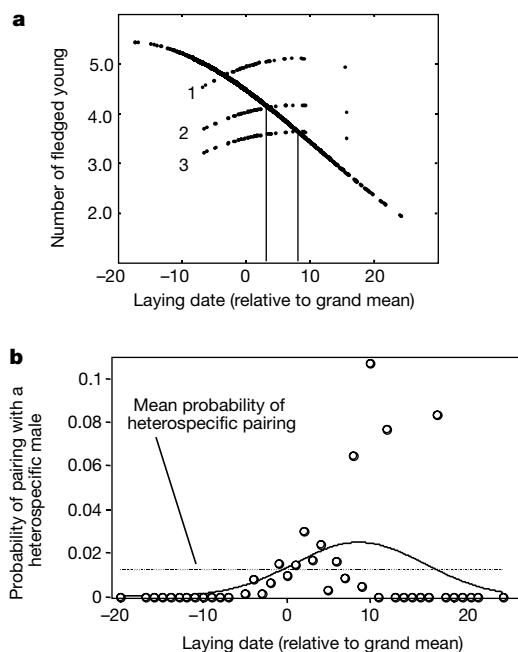
Figure 4 Breeding time, reproductive success, and mating decisions in flycatchers.
a, Relationship between breeding time (laying date) and number of young fledged from the breeding attempt for pure collared flycatchers and for male pied female collared flycatcher mixed pairs. The lines show the fitted relationships from a general linear model with Poisson error structure, and with terms for laying date ($P < 0.0001$) and laying date^2 ($P = 0.009$). The relationships differ between the two pair types ($F_{1,3144} = 4.55$, $P = 0.03$). For mixed pairs, three curves are shown: (1) that corresponding to the number of young fledged, ignoring the reduction in mean offspring reproductive value due to production of hybrid offspring; (2) that representing reproductive value discounted by 18% owing to reduced fertility of male and female hybrid offspring (corresponding to prediction 1 in Fig. 3b); (3) that representing reproductive value discounted by 28% owing to reduced recruitment of hybrids (prediction 2 in Fig. 3b). The point at which the curve for the mixed pairs intersects that for the pure pairs is the relative breeding date at which the fitness consequences of accepting a heterospecific mate are equivalent to that of accepting a conspecific mate. **b**, Probability of pairing with a male pied flycatcher in relation to relative breeding date for female collared flycatchers. The points show the proportion of female collared flycatchers laying on a particular date that were paired with a male pied flycatcher. The continuous curve shows the fitted relationship from a logistic regression with date ($P < 0.0001$) and date^2 ($P = 0.015$); the dotted line shows the mean probability (0.0125) of mixed pairing for the entire data.

form of indirect selection on mating preferences[34]. In contrast, the benefits from conspecific extra-pair copulation for a female in a mixed pair are large, as they may represent the difference between fertile and sterile offspring. Thus, even if mixed-species pairings are relatively infrequent, selection for extra-pair copulation behaviour may be sufficient to drive this behaviour to fixation. Female birds often prefer extra-pair copulation partners with exaggerated secondary sexual characters[16,30,35], and these characters often show the greatest divergence in pairs of sympatric closely related species[36]. Selection for conspecific extra-pair copulation in sympatry might thus have been an important first step in the evolution of extra-pair copulation behaviour.

Methods

Life-history data

Data for the Swedish population, on the island of Gotland (57° 10′ N, 18° 20′ E) were collected as part of a continuous population monitoring study (1980 to present), of flycatchers breeding in artificial nest-boxes. Data on mating frequencies for the Czech population (Dlouhá Loucka: 49° 50′ N, 17° 15′ E) were collected from 1985 to the present. Laying date, clutch size, and number of young hatching and fledging were recorded by frequent visits to nest-boxes, although data on number of young hatching were not recorded for all breeding attempts. Adults were assigned to breeding attempts on the basis

of their capture (using internal nest-box traps) when feeding young. All adults were ringed with individually numbered aluminium rings on first capture, and all nestlings were ringed before fledging. Reproductive success (number of recruited offspring) was determined from recapture data in subsequent year, and lifetime reproductive success defined as the summed number of recruits over an individual's lifespan. In analyses of long-term data from the Swedish population we excluded all breeding attempts that had been subject to experimental manipulations (such as brood or clutch size manipulation), where these manipulations occurred before the relevant variable was measured. Therefore, the lifetime fitness measures are underestimates, but because experimental treatment was random with respect to species identity, comparisons of their relative sizes are unaffected by this restriction. In addition, for calculating lifetime fledgling production and lifetime reproductive attempts, we excluded all birds that were still alive in 1999, and for calculating lifetime reproductive success all birds that were still alive in 1998 or later, because some offspring do not recruit to the breeding population for two years. The expected relative fitness of the three types of pairing (pure collared; male pied × female collared; and male collared × female pied) was calculated as:

$$1 - (P_f \cdot P_{hs} \cdot (1 - W_f)) - (P_m \cdot P_{hs} \cdot (1 - W_m))$$

where P_f and P_m are the proportion of female and male offspring respectively, and P_{hs} is the proportion of offspring sired by heterospecific males. W_f and W_m are the fitnesses of hybrid females and males respectively, relative to a collared flycatcher offspring of that sex (relative fitness defined as unity). Flycatchers were identified on first capture as either pied, collared or F_1 hybrid based on the size and distribution of white plumage patches (males), the pattern of white on the nape feathers and the plumage tone of the upperparts (females) and on wing length and species-specific calls[37]. Identification of recruited nestlings was made without knowledge of their parents.

Molecular genetic analyses

Paternity was determined based upon allele-sharing at four polymorphic microsatellite markers: *FhU1*, *FhU2*, *FhU3* and *FhU4*. PCR conditions were as described[38,39]. The power of these markers to exclude conspecific paternity is not particularly high (approximately 0.96 in both collared flycatchers and pied flycatchers), which suggests that we may have overlooked a few cases of conspecific extra-pair paternity in our analyses of pure (CF × CF and PF × PF) pairs. However, of more relevance to our analyses is the fact that marked species-specific differentiation at these markers[39] greatly increases their power to detect cases of conspecific extra-pair paternity in mixed pairs, and heterospecific extra-pair paternity in pure pairs. The sex of nestling flycatchers was determined by PCR amplification of the pair of sex-linked *CHD1* genes, using either SSCP (single-stranded conformation polymorphism) analysis[24] or amplification using primers P2 and P8 (ref. 40) followed by silver-staining on 6% polyacrylamide gels. The maternal species identity of F_1 hybrids was determined by amplification of a stretch of mtDNA containing a 32-bp indel, as described[17]. Molecular species identification of both extra-pair sires and F_1 hybrids was possible using microsatellites because all four loci used for parentage analysis show some degree of species differentiation in allele size distributions, and in three cases there was little allele-sharing between the two species[39]. An assignment test[41] based on genotypes at these four loci correctly classified all of 108 adult pied flycatchers and 381 adult collared flycatchers to species. F_1 hybrids were classed as such if they were heterozygous for species-specific alleles at the three loci with largest R_{ST} values[39] (R_{ST} is a measure of genetic differentiation at microsatellite loci).

Statistical methods

Data were analysed using general linear modelling techniques[42] with binomial errors and logit link for proportional data (sex ratio, proportion of nestlings sired by male, hatching and fledging success), with scale correction for over-dispersed data. Count data (number of fledged offspring) were analysed with Poisson errors and a natural logarithm link, again with scale correction for over-dispersion. Changes in deviance were tested against the F-distribution, as recommended when data are overdispersed[42]. Our data contain repeated observation of some males and females; we chose to treat each pairing event as an independent data point since less than 1% of pairings involved the same pair of individuals in different years, and because the within-individual repeatability of life-history traits such as numbers of young fledged or recruited is very low[13,43].

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