

Article

Understanding Extra-Pair Mating Behaviour: A Case Study of Socially Monogamous European Pied Flycatcher (*Ficedula hypoleuca*) in Western Siberia

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Abstract: Extra-pair copulation (EPC) occurred in most socially monogamous bird species. The mechanisms leading to the frequent occurrence of extra-pair offspring (EPO, EPY) in socially monogamous couples, as well as the ‘function’ of EPC, are the subjects of strong debates and raise many unanswered questions. We studied the relationship between extra-pair paternity (EPP) and the different characteristics of males and females in the European pied flycatcher in Western Siberia (Russia). The analysis was based on the genotyping of 232 males, 250 females, 1485 nestlings (250 nests). The European pied flycatchers were predominantly socially and genetically monogamous, but about 20% of birds could be involved in EPP. Loss of paternity tended to be more frequent in one-year-old males. EPCs could be multiple: one individual may have up to three extra-pair partners. The EPP rate was independent of the breeding time. The extra-pair mates of an individual were mainly its near neighbours. The EPC status of an individual was unrelated to most of its morpho-physiological traits. The occurrence of EPP was almost twice as high in females nesting in good quality territories. The fitness of within-pair offspring, EPO, paternal half-sibs of EPO and maternal half-sibs of EPO did not differ statistically significantly. Assuming very low heritability of extra-pair mating, we argued that EPCs could be incidental side effects (by-product) of selection. We believe that the evolution and maintenance of extra-pair mating are the episelective processes in the case of the European pied flycatcher.

Keywords: European pied flycatcher; *Ficedula hypoleuca*; extra-pair mating behaviour; extra-pair copulations; extra-pair paternity; Western Siberia; episelective evolution



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1. Introduction

Monogamy is the most common system of social relationships in birds, which has been recorded for more than 90 percent of species [1]. Social monogamy is a pairing system in which one male and one female stay together and cooperate in breeding for at least one breeding cycle [2]. Long-term social bonds (offspring rearing, for example) between mates maintained for several years are generally called long-term mutual monogamy or true monogamy [1,3]. Sexual or genetic monogamy implies sexual exclusivity along with the social living arrangements between a male and a female, and it is quite rare among birds [4,5]. In the social relationships of birds, serial monogamy [6], facultative polygamy and extra-pair copulations (EPCs) have been frequently reported.

Social monogamy occurs as serial monogamy in many bird species. Serial monogamy implies that pair bonds are formed sequentially for only one reproductive attempt or breeding season [6]. The genetic consequences of serial monogamy for individuals living

for several years differs only little from polygamy: one male fertilizes the eggs of several females [7]. Additionally, species with serial monogamy can exhibit facultative polygamy. In its most general meaning, the facultative polygamy of serially monogamous species can be viewed as the realization of serial monogamy within the same reproductive season [7,8].

EPCs as another type of ‘mate infidelity’ has been observed in many bird species [5,9]. The term refers to copulations that occur outside the social bonds. Individuals involved in EPCs do not show any social interactions (except the copulations), making such contacts and their consequences in natural populations difficult to identify without special methods, e.g., DNA profiling. The widespread use of DNA profiling [10] in studies of the mating systems of natural bird populations has revealed that EPP occurs in more than 75% of surveyed socially monogamous bird species [4,5,9,11–13]. Thus, it is obvious that monogamous social living arrangements are very often associated with genetic polygamy.

Attempts to explain the broad spread of EPP in socially monogamous bird species are still ongoing [5,9]. An EPC can be costly for both males and females [14–17]. However, females as well as males, despite these risks, still engage in extra-pair copulations [11,18–23].

The most common explanation of the evolutionary origin and maintenance of promiscuity in socially monogamous bird species is based on the assumption that birds have a propensity for EPC because there should be benefits from the promiscuous mating behaviour that outweigh all its negative consequences. Several hypotheses have been put forward on this issue [5,9,11,12,14,24]. The advantage of extra-pair mating is thought to be obvious for males who may sire additional offspring without the cost of care, and thus increase the number of their descendants in the population (increase fecundity). Indeed, where the costs of infidelity are low and EPC avoidance in females is weak, EPC may result from direct selection among males generating a self-interest male tactic [25]. This view appears to be well supported by accumulated data [26].

Female birds cannot take advantage of increased fecundity (the number of eggs in a clutch) from EPC, however, in socially monogamous relationships, females may mate multiply because of indirect genetic (e.g., ‘good’ and ‘compatible genes’ hypothesis) ([11,19,27–31], but see [26,32]) and direct benefits. Direct benefits include, for example, a better chance of fertilization of eggs [33], nuptial gifts from several mates [34,35], but see [36], or getting extra help from extra-pair mates in caring for offspring at the nest [18,37,38]. By mating with extra-pair males, females may construct a social network, which also could provide benefits to them (vigilance, alarm calls, calling networks, predator mobbing, and so on) [24,39].

Other hypotheses, also based on adaptive selective mechanisms, consider the possibility of EPC evolution among females in socially monogamous systems as a side correlative effect of selection on some fitness-related traits with the pleiotropic gene effects on the female ‘inclination’ towards EPC. A female ‘propensity’ for EPC may evolve and persist because it is positively genetically correlated with a male or female reproductive fitness component, and, consequently, experiences positive cross-sex or within-sex indirect selection [40]. These refer to ‘intersexual’ and ‘intrasexual antagonistic pleiotropy’ mechanisms, respectively [14,41,42], but see [43].

In view of the difficulties in attempts to explain inter- and intraspecific EPP variation based on differences in contemporary ecological factors and benefits from the promiscuous mating behaviour, the alternative explanations of EPP were proposed. They are based on fundamental differences in reproductive biology among avian lineages rather than differences in contemporary ecology. They consider the cost of being involved with EPC rather than differences in the opportunities for engaging in such behaviours. Indeed, in species with high annual mortality rates, females that participate in EPCs will be less likely to suffer any subsequent payback from their social partner [11,44,45]. It was shown that for EPP, over 50% of the interspecific variation is due to differences among taxonomic families and orders. High EPP rates are associated with high rates of adult mortality and reduced paternal care [45,46].

The above list of hypotheses (most likely the list is not all-inclusive) alone tells eloquently that the mechanisms of evolutionary persistence of EPP remain unclear. This may

be because all proposed hypotheses have limited general applicability, and can differ even within a species or population or between years. Consequently, obtaining new data that can reveal the causes both facilitating and refuting various hypotheses about EPP persistence among monogamous species may help to understand the high degree of variation in social relationships.

The European pied flycatcher (*Ficedula hypoleuca*) is a small (~12 g) insectivorous long-distance migratory bird species that breeds in Europe and winters in Africa. In nature, the European pied flycatchers nest in tree cavities but readily accept nest-boxes, which has made it a model species for studying population ecology and evolutionary biology. Birds breed once a year. The female incubates an average of six to seven eggs. Male and female together feed 5–6 nestlings in Western Siberia [47]. European pied flycatchers are relatively short-lived birds. The average lifespan of an individual is about two years from hatching [48,49]. The maximum life span in most populations does not exceed 8 years. However, the European pied flycatcher has a large pool of non-breeding individuals [50–53]. Males and females may first begin breeding at one to five years of age [50,53].

The European pied flycatcher is predominantly socially monogamous during a breeding cycle with an annual change of partners (serial monogamy) and with facultative polygyny. The proportion of socially monogamous pairs is rarely less than 80–90% [49]. However, like in most passerine birds with serial monogamy, some individuals are involved in EPCs that result in EPPs (for the variety of social relationships that may exist within the species, see [8]). The proportion of broods containing nestlings sired by extra-pair males varied from 6.5% to 40% [8,54–65].

In most studies of EPP in the European pied flycatcher, attempts were made to find phenotypic traits of males that could serve as criteria for ‘extra-pair mate choice’ [55,58,59,66,67]. It has been shown that different traits can be correlated with the involvement of males in EPP such as male-specific plumage ornaments [58,67,68], body size, age [59,61,67], polygyny status [56], and timing of breeding [60,61,65]. The relationship between phenotypic traits of females and their involvement in EPC has been studied much less frequently [64,69,70]. Moreover, despite the relatively large number of studies carried out on the European pied flycatcher, the actual effects of EPC on individual fitness are still ambiguous [8,57,65,71]. Consequently, our understanding of the factors affecting variation in the EPP rate between populations or individuals of the European pied flycatcher, as well as mechanisms persisting EPP, is rather limited.

This investigation was carried out as part of an ongoing long-term population study initiated in 2001 [8,53,69,72]. To determine parenthood and EPP status, we collected 1967 blood samples from 250 bird families in 2005 and genotyped them by microsatellite analysis [8]. We compared the traits of within-pair and extra-pair mates in both males and females. We attempted to find the phenotypic traits associated with the involvement of birds in EPCs. The recruitment rate of fledglings of the European pied flycatcher in the Western Siberian population is one of the highest among all populations of this species [53]. Therefore, we could directly estimate whether there are differences in the survival and the total fitness of the fledgling from different EPP backgrounds. Using these data, we tried to identify the most likely mechanisms of EPC evolution (ultimate explanation) in a given bird species and to identify the most likely environmental factors (proximate explanation) determining the involvement of individuals in EPCs.

2. Materials and Methods

2.1. Study System

This study was part of an ongoing long-term study of the European pied flycatcher population in Western Siberia in the Tomsk region. This region is now considered to be the eastern boundary of the species distribution range. We used nest-boxes ($n = 381$ in 2005) to attract these birds for breeding. The nest-box area of the study site was established in 2001. It is located 13 km southwards of Tomsk ($56^{\circ}21' \text{ N } 84^{\circ}56' \text{ E}$) in a mixed forest which consists of aspen (*Populus tremula*), birches (*Betula spec.*), spruce (*Picea abies*), Siberian fir

(*Abies sibirica*) and pine (*Pinus sylvestris*). The study site consists of four subplots—two 5 ha and two 10 ha areas equipped with nest-boxes (Figure 1). To build the map of the study area, we first GPS-referenced the coordinates of the corner nest-boxes of each subplot, and then, using the triangulation method, we determined the position of other nest boxes in the map. The map was created in Inkscape. The coordinates were recorded in pixels, which were back converted to meters for the calculations.

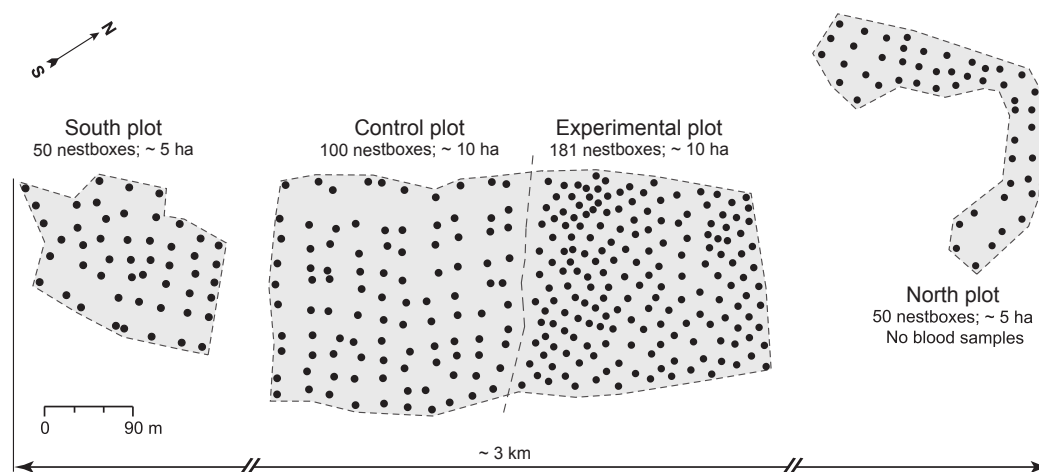


Figure 1. A simplified map of the study area. The map shows all the plots, their areas, and the number of nest-boxes at each site.

The distance between the most remote nest-boxes in the whole study site was approximately 3 km (Figure 1). On average, the interval between nest-boxes is about 30 m on the South, Control and North plots and about 20 m on the Experimental plot. The nest-box density was ~10 nest-boxes/ha for South, Control and North plots and 18.1 nest-boxes/ha for the Experimental plot (Figure 1). In 2005, the actual breeding density of European pied flycatchers was 8.2–9.1 pairs/ha (South, Control and North plots) and 11.5 pairs/ha (Experimental plot).

The European pied flycatcher is the only mass breeder at our sites. Other bird species (great tit, coal tit, Eurasian nuthatch, common redstart) occasionally nest in the nest boxes (1–3 pairs) and not every year.

During the breeding season, we checked each nest-box once every five days to record the laying date of the first egg, clutch size, brood size and the number of successfully fledged nestlings. To trap birds, we used small traps mounted inside the nest-box. We trapped females during the incubation period with active (spring-loaded) flap traps, which, when triggered by birds, close the entrance to the nest box from the inside. We trapped parents feeding nestlings with passive (springless) traps, which only allow birds to enter the nest box, but block their exit. We captured almost all females two times during the breeding season. For the first time, each female was captured on day 7 to 9 of clutch incubation, and for the second time, each female was captured when feeding 9–11-day-old nestlings. Males were captured only when feeding 9–11-day-old nestlings. When a bird was caught, we attached an aluminium ring to the bird's leg to enable individual identification. We did not use colour rings made of plastic for the birds' banding. No nest failed after the capture of adults. We measured weight, wing and tail length, tarsus length, fat index, post-breeding moult stage, and primary score index each time a European pied flycatcher was captured. For each caught male, we also recorded forehead spot size and the colour type of breeding plumage on Drost's colour scale [73]. The scale describes the degree of melanization of the upper-body feathers in the male breeding plumage and overall plumage brightness. Males of type 1 are the most conspicuous because of the deep black colouration of the body top and purely white colouration of the body bottom. Males of type 7 are most cryptically coloured because of the brown body top and dirty brownish-white body bottom.

The colouration of breeding plumage of males of type 7 is practically identical to that of females.

Immigrant flycatchers (all adults without rings) to the study population were trapped and ringed as described above and released; they can be identified individually throughout a lifetime. Flycatchers, born in the study area, were ringed and monitored from birth through all breeding attempts until they disappeared (when they most likely died).

Additional information about the monitoring scheme of the population, the bird treatment, as well as a description of the research area can be found in our earlier publications [8,74].

2.2. Paternity Analysis

To determine paternity, blood samples were taken from each bird. Both males and females were blood-sampled when feeding 9–11-day-old nestlings. Nestlings were blood-sampled on day 10–12 after hatching. We collected blood samples from all caught birds breeding in the three subplots in 2005 (South, Control and Experimental plots). This year, all nests in which females started incubation were successful, i.e., they survived until the time of blood sampling and the nestlings fledged. Therefore, blood samples were taken from all females. In 13 nests, we did not manage to detect/catch males. Some males could not be caught using our trapping methods, because they became extremely cautious (they stopped feeding their nestlings if they saw a trap inside the nest box). Some males were absent near the nests (males may have been predated, and it could be bigamous males who do not assist one of their females to feed nestlings). Therefore, we sampled 232 males, 250 females, 1485 nestlings (250 nests; 1967 blood samples). DNA fingerprinting was carried out using eight microsatellite loci, which were amplified by two multiplex-PCRs and analysed by capillary electrophoresis. For the analysis, we used FHU1/PTC2, FHU2/PTC3 [75]; FHU3, FHU5 [76]; FHY336, FHY403, FHY427, FHY452 [77] microsatellite loci. The degree of genetic relationships was determined by CERVUS 3.0 [78]. All work related to DNA genotyping of individuals and paternity analysis was completed at the Institute of Pharmacy and Molecular Biotechnology of Heidelberg University in Germany. Further details of genotyping by microsatellite analyses were described in the publication of Grinkov et al. [8].

For the present study, we assumed that the number of offspring sired by an extra-pair male is a good phenotypic measure of the extra-pair mating behaviour. Direct registration of copulation behaviour of all birds breeding in the study area was impossible in our case. In general, a linear dependence between the observed rate of EPCs and EPP was not demonstrated across an analysed species [79,80]. However, it seems that in the case of the European pied flycatcher there is a covariance between the observed rate of the EPP and the true rate of EPCs [74]. Nonetheless, we are unable to assume that females and males without EPY have not had EPCs at all. However, the assumption that EPP correlates positively with the degree of EPC behaviour across females and males within the population of the European pied flycatcher looks reliable and parsimonious [26,74].

2.3. Overlapping in Fertility Periods

The physiological readiness of females and the sexual activity and maturity of males primarily determine the chances that the EPC could lead to an EPY. We calculated fertility periods of females in this population to estimate the proportion of individuals in which it overlaps. Females of the European pied flycatcher can store sperm for 7–9 days [81]. Copulations can occur 9 days before the first egg is laid [66,82,83], but it seems that the earliest mating that can lead to fertilization was two days before the first egg is laid [81,83]. The extent and degree of sperm storage by females of the European pied flycatcher has not been determined in detail [81]. Males of the European pied flycatcher are physiologically ready for mating and fertilization from the arrival to the breeding area and until the hatching of the nestlings [84]. Therefore, we define a fertility period as following: the fertility period starts 6 days before the laying of the first egg and finishes when the last egg has been laid (a similar estimate was used, for example, in [56,85]). Obviously, among

males, the period in which they can inseminate eggs is longer, and they can visit females in different stages of egg-laying.

2.4. Nest Site Quality

To estimate the quality of the breeding territory, we used the number of recruits in the local population, which were produced in each nest-box from 2001 to 2009 (four years earlier and later than the year of parenthood determination). All territories were divided into four groups. In the 'best' nest site group, the number of recruited fledglings varied from 6 to 13 individuals, in the 'good' nest site group—from 3 to 5 individuals, in the 'bad' nest site group—from 1 to 2 individuals, and finally, in the 'worst' areas, no recruits were obtained for 9 years.

The boundaries were stated so that there were at least 30 nests in the categories (in fact, the number of nests in the smallest group is 31). This number of nests gives a number close to the recommended [4] number of offspring for EPP estimation. The European pied flycatchers have an average of 6 nestlings per brood, yielding about 180 nestlings in 30 broods (200 recommended in the publication; the smallest group in our analysis contains 195 nestlings).

2.5. Classification of Individuals

We used the following classification of individuals for the statistical processing of data. We divided all males into three groups:

M_{EPY-0} —Males that did not gain EPP, and did not lose paternity in their own nest, EPY-neutral, mostly monogamous in our opinion (but see [8]).

M_{EPY+} —Males that gained EPP, but could have also lost paternity in their own nest, EPY-positive males.

M_{EPY-} —Males that lost paternity in their own nest, but did not gain EPP elsewhere, EPY-negative males.

We again want to point out here that females of some EPY-positive males were involved in EPC, i.e., among EPY-positive males, there were also lost-paternity males [8]. We did not distinguish this group of males separately because of the small sample size. They were all part of the EPY-positive group of males.

We also divided females into three groups:

F_{EPY-0} —Females not obtained EPP and mated with EPY-neutral males, EPY-neutral, mostly monogamous females.

F_{EPY+} —Females obtained EPP, EPY-positive females.

F_{EPY-} —Females breeding with EPY-positive males.

We divided the nestlings according to their genetic background into four groups:

WPO—Within-pair offspring, the genetic descendants of the EPY-neutral males and EPY-neutral females.

Mat HSib of EPO—maternal (within-pair) half-siblings of extra-pair offspring.

EPO—Extra-pair offspring.

Pat HSib of EPO—Paternal (within-pair) half-siblings of extra-pair offspring.

Mat HSib of EPO and EPO are the genetic offspring of EPY-positive females, they are in this female's broods (nests). Mat HSib of EPO nestlings were sired by within-pair males of the EPY-positive females (M_{EPY-}). EPO nestlings were sired by extra-pair males (M_{EPY+}). Thus, Mat HSib of EPO and EPO nestlings were reared by EPY-positive females and their within-pair males (M_{EPY-}). Mat HSib of EPO and EPO nestlings are thus maternal half-siblings: they have a common mother but different genetic and social fathers. Pat HSib of EPO nestlings are genetic descendants of EPY-positive males, and these males have reared them together with their within-pair females (F_{EPY-}). EPO and Pat HSib of EPO nestlings are therefore paternal half-siblings. For reference, we list all types of social and genetic relationships between offspring and parents in Table S1 in the Supplementary Materials.

Sometimes we had to group individuals by other criteria for statistical calculations. Such instances are indicated and described in the legends of figures and tables or the text.

2.6. Fitness Estimates

We traced the survival and the reproduction of the nestlings of the 2005 cohort until 2012, until the last individual of the cohort was recorded. These data allowed us to calculate both the survival rate and the overall fitness of the nestlings in connection with their genetic origin and social parents. No experimental manipulations that could affect the fitness of birds were performed during this time.

Here, we define recruitment rate not as per capita recruitment probability, but instead as the number or proportion of individuals, i.e., what number or proportion of individuals of the 2005 cohort survived and returned to the population. We calculated apparent recruits, corrected recruits and local survival probability for different groups of nestlings (see above). Apparent recruits are the simple sum of returned individuals to the study area from nestlings ringed in 2005 during all subsequent years (e.g., if one individual returned in three different years, it was only counted as one). We calculated the corrected recruits using our earlier approach, which is based on the assumption of a 50% average annual individual mortality rate [50,51,53]. The corrected recruits make the adjustment for the fact that some recruits may have been missed (not bred) and, therefore, some may have died and may not have been captured in the following years. A more precise measure of the local survival probability, taking into account all possible inter-annual variation in mortality, we calculated based on the Cormack–Jolly–Seber (CJS) models [86–89] in the MARK program [90], using RMark [91] as R interface for the MARK.

The calculation of corrected recruits is much easier than the local survival probability with MARK. The two estimates are comparable (see results). The assumptions for the computing of corrected recruits are simple. The mean survival of individuals of different groups does not differ after the first year of life (50% as written above). The encounter probability is 0 before the first breeding for all individuals. In general, for most individuals, these assumptions are met, although not always.

We used lifetime reproductive success (LRS) and the individual intrinsic rate of increase (λ) calculated for a specimen (per capita) as an estimate of total fitness of fledglings [92]. The individual intrinsic rates of increase were calculated as the dominant eigenvalue of an age-structured population projection matrix constructed from life-history data, collected for an individual [93]. Both estimates were calculated from the number of fledglings and the number of recruits produced by an individual during a lifetime. These data were used to compare all groups of nestlings.

2.7. Statistical Analysis

We estimated the statistical significance of differences between the proportions (for example, proportions of apparent recruits and corrected recruits in all types of nestlings) using χ^2 -test (prop.test() function in R). Inferences on whether there were statistically significant differences in local survival probability between nestlings groups were drawn based on Akaike's Information Criterion corrected for the effective sample size, AIC_c , and the normalized Akaike weights, ω_i , calculated for all CJS models (involving or not involving the variation in local survival probability between different nestlings groups and in different breeding years of pied flycatchers). We used the open-source R software environment for statistical computing and graphics (version 3.5.0) [94] under an integrated development environment for R-RStudio (RStudio Desktop version 1.1.447) [95] to analyse data.

In particular, to test statistical significance differences in the central tendency between the two groups, we used the Mann–Whitney U test. To estimate linear or monotonic relationships between variables, we used Spearman's correlation. Both between groups tests of the central tendency and correlations were used to describe associations between various variables pointed in previous parts of the Materials and Methods section. The use of either test in each case was determined by the type of the variable (numerical, nominal,

ordinal, etc.) under treatment. We used the false discovery rate method (“fdr”) to correct p -values. We used spatstat package [96] in R to calculate distances.

3. Results

Paternity analysis revealed that 21.2% (53) of studied broods had EPY [8]. Therefore, 53 females definitely took part in EPCs. The number of extra-pair mates in EPY-positive females could be 1 (84.9%, $N = 45$), 2 (13.2%, $N = 7$) and 3 (1.9%, $N = 1$). Thus, the number of sexual partners in EPY-positive females varied from 2 to 4 including within-pair social males. Paternity analysis assigned 40 males as extra-pair sires. EPY-positive males successfully sire EPY in 1 extra-pair female in 85.0% cases ($N = 34$), in 2 extra-pair females in 12.5% cases ($N = 5$) and in 3 extra-pair females in 2.5% cases ($N = 1$). The number of sexual partners in EPY-positive males also varied from 2 to 4 including within-pair social females. We found that 8 females of EPY-positive males in turn also copulated with extra-pair males [8], i.e., there were 8 males that participated in EPCs whose social mates reciprocally cuckolded them.

We were able to reveal fathers for 65% ($N = 67$) of all EPY ($N = 103$) among the sampled males. The remaining 35% ($N = 36$) of EPYs for which genetic fathers were not identified are most likely the offspring of males that we were unable to detect or capture and the offspring of unmated non-breeding males [65].

3.1. Breeding Density and Breeding Time

Our study area consisted of several plots, differing in the number and density of nest-boxes. The density of nest-boxes ranged between 10 and 18.1 boxes per ha (Figure 1). In 2005, the actual breeding density of the European pied flycatcher was 8.8 pairs/ha, 9.1 pairs/ha and 11.5 pairs/ha on South, Control and Experimental plots, respectively. The corresponding EPP occurrence was 13.6%, 25.3% and 20.9%. We were not able to detect statistically significant differences in the level of EPP between plots ($\chi^2 = 2.42$, $df = 2$, $p = 0.298$). The breeding density was on average very high, and the relatively small differences between subplots did not affect EPP rates (but see [8]).

In the Siberian European pied flycatcher population, breeding was rather synchronous (Figure 2). When the first egg was laid in the first nest-box, already 27 sexually receptive females (>10% of all females) were present in the population. When 50% of the clutches contained at least the first egg, about 70% of the females were in a fertile period. Even when the last female in the population started to lay eggs, about a dozen fertile females were still available (Figure 2). Thus, males of the Siberian population had the opportunity at any given time to find a sufficiently large number of females for EPC. Moreover, because the fertile period of males lasts from the time of arrival until the eggs hatch [84], the latest nesting males had the potential to mate with all females in the population. The extended fertility period of both males and females is most likely the reason why we were not able to detect signs of an uneven distribution of EPP within the nesting period. The proportion of nests with EPY was not correlated with the breeding time (Figure 3a).

The number of nests with EPY in each particular nesting period was directly proportional to the corresponding total number of nests (Figure 3b). Thus, the proportion of males and females, copulating with extra-pair mates, was more or less stable throughout the breeding season.

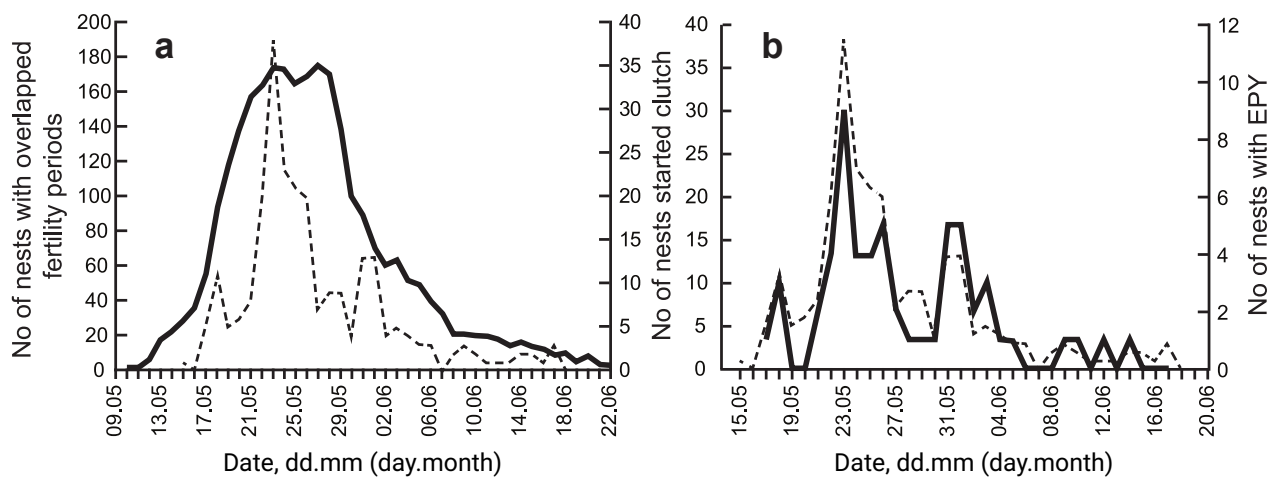


Figure 2. Clutch start dates, overlapping fertility periods (a) and number of nests with EPY (b). The dashed line on parts (a,b) is the number of nests that a clutch is started on the relevant date; the solid line on (a) is the number of nests whose female fertility periods overlap on the relevant date; the solid line on (b) is the number of nests with EPY on the respective date.

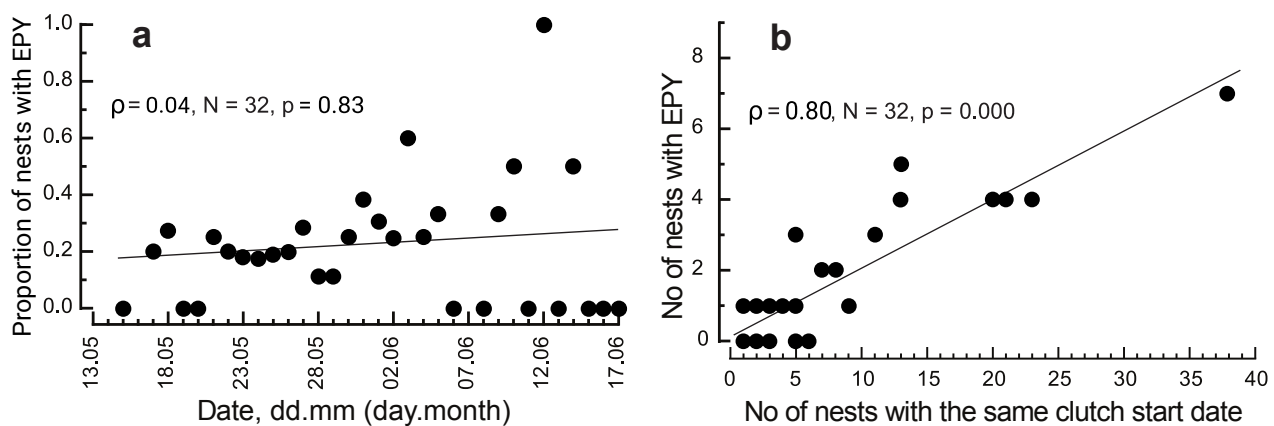


Figure 3. The link between the proportion of nests with EPY and the clutch start date (a) and between the number of nests with EPY and the total number of nests that a clutch has started on the respective date (b). The value of Spearman's correlation (ρ), sample size (N) and significance level (p) are given. Linear regression (solid line) is shown.

3.2. Distance between Extra-Pair Mates

Males and females copulated mainly with their near neighbours as extra-pair mates: the nests of extra-pair partners were spaced between 17.4 to 495.3 m apart (Figure 4). The distance between all nests (the set of distances from a nest-box to all others) varied from 12.1 to 1891.4 m at the plots where blood samples were taken. The mean distance between all nests was 594.14 m ($SD = 578.1$, $N = 31,125$), the median was 297.9 m (Figure 4). The average distance between the nests of extra-pair mates was 149.1 m ($SD = 115.3$, $N = 46$). In more than 90% of cases, nests of extra-pair mates were located between ~270–300 m from each other. No EPC could be detected between males and females nesting on different study plots, nor between partners nesting at the same site but which were more than 500 m apart (Figure 4). The distributions in distances of all nests and distances of nests of extra-pair mates differed significantly (Mann–Whitney U test $W = 1,123,036$, $p = 2.449 \times 10^{-11}$). A pronounced decrease in the likelihood of EPC between individuals, depending on the distance between their nests, had also been demonstrated in Spanish populations of the European pied flycatchers [61].

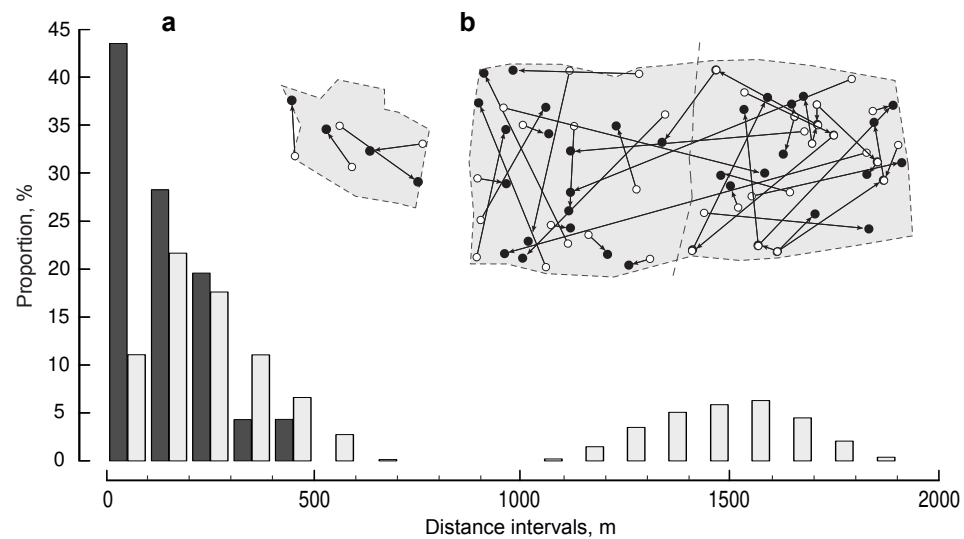


Figure 4. Occurrences of distances between nests of extra-pair mates (a) and the location of nests of extra-pair mates (b). In (a), the black bars indicate the distribution of distances between the nests of extra-pair mates, and the light bars depict the distribution of all possible distances between all nests. (b) Nests of extra-pair males (open circles) and extra-pair females (filled circles). The arrows mark the distance from the nest of the extra-pair male to the respective nest of the extra-pair female. Space between the plots is reduced. For the actual location of the areas, see Figure 1.

We found that the distance between nests of extra-pair mates was not associated with the probability of paternity loss in EPY-positive males (recall that 20% of them were EPY-negative males) (Mann–Whitney U test $W = 154$, $p = 0.40$), meaning that all EPY-positive males could lose paternity regardless of how far from their nest they found extra-pair females for EPC.

The distance between the nests of the extra-pair mates is positively correlated with the clutch start date of within-pair females of EPY-positive males (Figure 5a).

This is probably due to a smaller number of sexually receptive females at the end of the breeding period. A large distance between nesting sites of extra-pair mates would be the consequence.

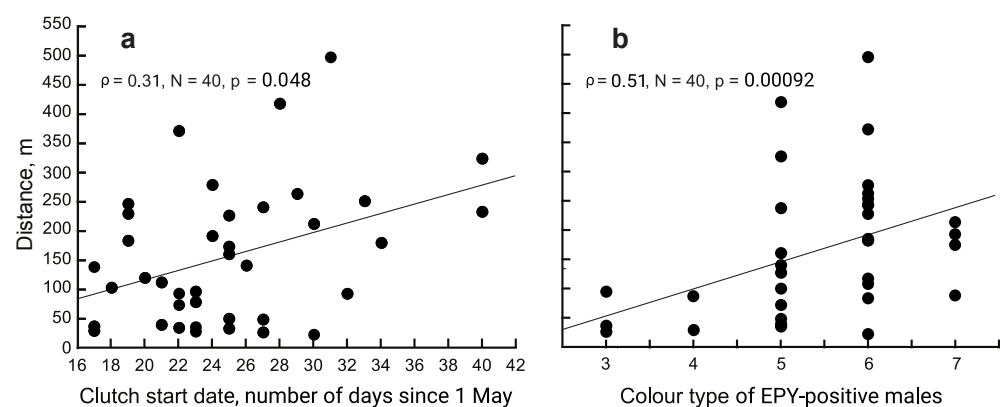


Figure 5. The distance between the nests of extra-pair mates depending on breeding timing of males (estimated as the clutch start date of within-pair female) (a) and the Drost's colour type of the male breeding plumage (b). The order of the categories of this scale denotes the degree of melanization of the upper-body feathers in the male breeding plumage and overall plumage brightness. Males of type 1 are the most conspicuous because of the deep black colouration of the body top and purely white colouration of the body bottom. Males of type 7 are most cryptically coloured because of the brown body top and dirty brownish-white body bottom. The colouration of breeding plumage of males of type 7 is practically identical to that of females. The value of Spearman's correlation coefficient (ρ), sample size (N) and significance level (p) are given. Linear regression (solid line) is shown.

3.3. Age of Birds

Among females with EPY (F_{EPY+}), the proportion of individuals older than one year was higher (58%, 31 out of 53 females) than among females without EPY (F_{EPY-} and F_{EPY0}) (49%, 97 out of 197). These differences are not statistically significant ($\chi^2 = 1.1$, $df = 1$, $p = 0.3$). EPY-positive males were older than EPY-negative males in overall terms (Figure 6).

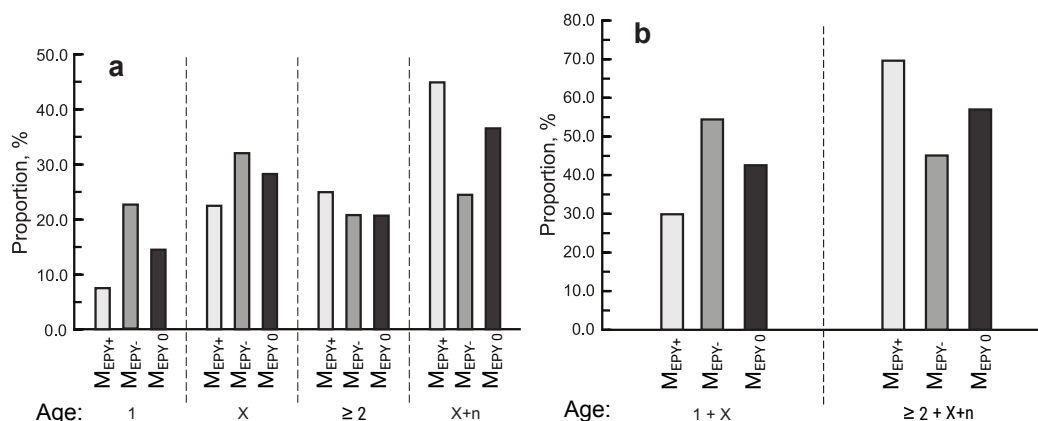


Figure 6. The age distribution (%) in males that mated with extra-pair females (M_{EPY+}), those who lost their paternity (M_{EPY-}) and monogamous males (M_{EPY0}). Bars painted in different shades of grey denote groups of males for which the proportions of age classes were calculated. Age groups are labelled as yearlings (1), males aged 2 years and older (≥ 2), males of uncertain age first caught in the study area (X), and males recaptured in the study area (X + n, where n = 1, 2, 3, etc.) One-year-old and older individuals (age 1 and ≥ 2) were born in the research area and were ringed as nestlings in previous reproductive seasons. Males of unknown age (X and X + n) were ringed as adult birds. (a) The age ratio of all four age groups. (b) The age ratio in the pooled samples.

Among males, whose age was accurately determined according to the ringing data (age 1, 2, 3, etc.), the proportion of one-year-old males (yearlings) was about two times higher than the proportion of older birds aged 2 years and older in the EPY-negative fathers in comparison with EPY-positive ones (52.6% (10 out of 19) and 23.1% (3 out of 13), respectively). Additionally, among males of unknown origin (age X, X + 1, X + 2, etc.), the proportion of newly caught birds (age class X) was higher than the fraction of recaptured individuals (age class X + n) in the EPY-negative fathers in comparison with EPY-positive males (57.7% (15 out of 26) and 33.3% (9 out of 27), respectively) (Figure 6). In a pooled sample, in which the yearlings were combined with the newly caught males and the older specimens with re-caught ones, respectively, the proportion of younger males (yearlings plus X) was higher among the EPY-negative males in comparison to EPY-positive males (55.6% (25 out of 45) and 30% (12 out of 40), respectively) (Figure 6). Only this difference is statistically significant ($\chi^2 = 4.63$, $df = 1$, $p = 0.031$). The EPY-neutral males have a proportion of young individuals that are intermediate between EPY-negative and EPY-positive males. Pairwise comparison of the proportion of yearlings among males of different EPP status using correction for multiple testing revealed no statistically significant differences (please see Table S6 in the Supplementary Materials).

3.4. Morphological Characters

In our work, we evaluated the association of wing, tail, tarsus and beak length, beak height, body mass, fat and cloacal protuberance index (males), post-breeding moult stage, primary score index, Drost's colour type (males), and forehead spot size (males) with EPP status. We could find no differences in most morphological traits between EPY-positive males versus EPY-negative fathers (Table S6 in the Supplementary Materials). EPY-positive males tended to have a longer beak. Similarly, we were not able to identify morphological differences between EPY-neutral males and the two other groups of males that differed

in their EPP status (Table S6 in the Supplementary Materials). The distances between the nest-boxes of extra-pair mates were positively correlated to the colouration of the breeding plumage of EPY-positive males (Figure 5b): males with a blacker breeding plumage were more likely to copulate with extra-pair females in the nearer vicinity to their nests than were males with browner breeding plumage. Two explanations are possible: black top body males are more attractive than brown males ([97,98]; but see [99,100]). Alternatively, this may be due to the greater aggressiveness of resident males towards blacker males [101,102], which in turn may restrict their movement across the territory, unlike browner males. The remaining morphological traits of EPY-positive males (wing, tail, tarsus and beak length, beak height, body mass, fat and cloacal protuberance index, and forehead spot size) were not related to the distance between the nests of the extra-pair mates (Table S6 in the Supplementary Materials).

The females with EPYs (F_{EPY+}) tended to have shorter wing length and tail length in comparison with EPY-neutral females (F_{EPY0}), although these differences are not statistically significant (Table S6 in the Supplementary Materials). Body mass, fat index, tarsus length, post-breeding moult stage, and primary score index were not differ statistically significant among EPY-neutral, EPY-positive, and EPY-negative females (Table S6 in the Supplementary Materials).

3.5. Territory (Nest Site) Quality

We calculated the proportion of EPY-positive mothers in ‘best’, ‘good’, ‘bad’ and ‘worse’ nest sites (Figure 7).

Females who were sampled in nest-boxes with higher recruitment rates showed an about twice higher EPP rate (Figure 7a). Comparison of the proportions of EPY-positive females among nests of different quality did not reveal the statistically significant differences ($\chi^2 = 3.34$, $df = 3$, $p = 0.34$).

The proportion of the EPY-positive males did not differ statistically significantly across different quality groups of their nesting sites (Figure 7b) (Table S6 in the Supplementary Materials). The overall variability in the number of recruits per nesting site is very high, and the linear dependence between the number of recruits and EPP estimates is weakly positive and not statistically significant (Spearman’s correlation ρ : $\rho = 0.07$, $\rho = 0.07$, $\rho = 0.04$ for the presence/absence of the EPO in a brood, the EPO number in females and the EPO number in males, respectively; Table S6 in the Supplementary Materials).

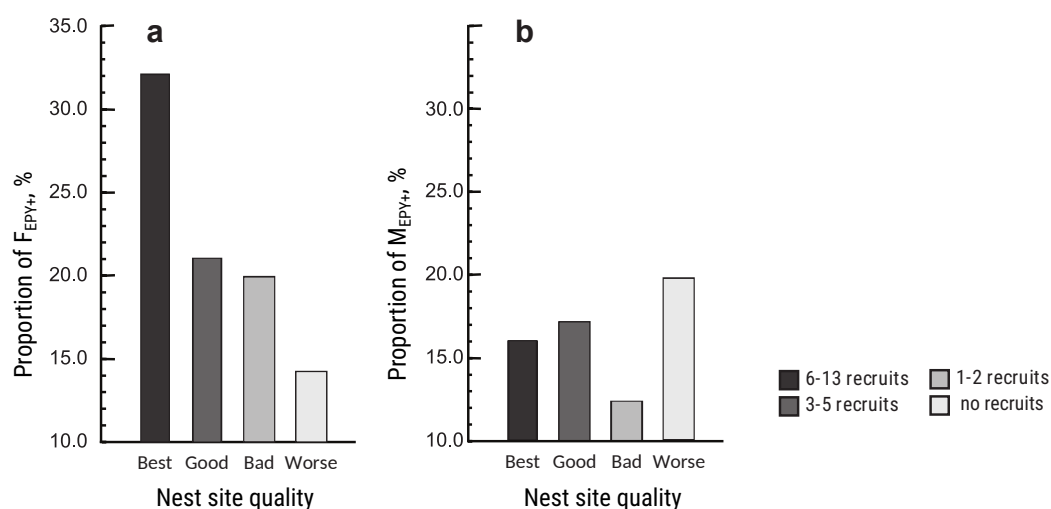


Figure 7. The proportion of females (a) and males (b) with EPY (F_{EPY+} and M_{EPY+} , respectively) among individuals nesting in territories of different quality. Territory quality was determined by the number of recruits born in the nest-box.

3.6. Fecundity of Adults, Fledglings Fitness

In our work, we cannot directly calculate and describe the variation in the total fitness of adults depending on their involvement in EPC. This requires long-term studies with annual genetic control of all descendants from all individuals. Using our material, we can estimate the effect of EPC on one of the components of fitness, namely, current fecundity. EPC can certainly increase the number of genetic offspring of male participants by simultaneously reducing the number of genetic offspring among EPY-negative males. The mean number of offspring of EPY-positive males, EPY-negative males and EPY-neutral was 7.0 ($N = 40$, $SD = 1.89$), 4.2 ($N = 45$, $SD = 1.64$) and 6.0 ($N = 165$, $SD = 1.39$), respectively. Pairwise comparisons using the Mann–Whitney U test showed statistically significant differences in all combinations with a significance level of $p = 0.00167$ (highest corrected p -value, Table S6 in the Supplementary Materials).

The mean number of descendants in EPY-positive, EPY-negative, and EPY-neutral females was roughly the same (6.1, $N = 53$, $SD = 1.1$; 5.7, $N = 32$, $SD = 1.2$; 6.0, $N = 165$, $SD = 1.4$, respectively; there are no statistically significant difference, Table S6 in the Supplementary Materials). The ratios of the number of fledglings to the number of eggs in EPY-positive, EPY-negative, and EPY-neutral females were 0.93 ($N = 53$, $SD = 0.10$), 0.88 ($N = 32$, $SD = 0.12$), and 0.91 ($N = 165$, $SD = 0.15$), respectively. This index incorporates two main causes of eggs' failure in females, i.e., fertilisation failure and embryo death. Here, we used it as the rough estimation of average female fertility. The clutch-to-brood ratio of EPY-negative females tended to be lower than that of EPY-positive and EPY-neutral females (the differences were not statistically significant, Table S6 in the Supplementary Materials). The mean clutch-to-brood ratio (average fertility) of EPY-neutral and EPY-positive females was nearly identical, as argued earlier [32].

The number of recruits and the recruitment rate of the fledglings related to the differences in genetic and social parents in the Western Siberian population of the European Pied Flycatcher are shown in Table 1. We compared the apparent and corrected recruitment rates of different types of offspring in all possible pairwise combinations (Table 1). We found that the differences in apparent recruitment rates of offspring were not statistically significant (Table S6 in the Supplementary Materials). The corrected recruitment of maternal (within-pair) half-siblings of EPO is significantly different from WPO, EPO and paternal (within-pair) half-siblings of EPO (χ^2 test; $p = 0.0009$, $p = 0.02$ and $p = 0.0009$, respectively; corrected p -values; Table S6 in the Supplementary Materials). A comparison of the models with the different type of variation of the local survival probability in the MARK program (Table S2 in the Supplementary Materials) revealed that the most parsimonious model best fitted the data was one in which the differences in the local survival probability between the types of offspring were not simulated (i.e., the local survival probability of all bird types was assumed to be the same). Thus, the survival of offspring of EPY-positive females from within-pair males (EPY-negative males) is at least as good (if not the best) as the survival of their half-sibs from extra-pair males (EPY-positive males) and offspring of EPY-positive males from their within-pair females (EPY-negative females).

The total fitness of the fledglings related to the differences in genetic and social parents in the Western Siberian population of the European pied flycatcher is presented in Table 2. A comparison of the mean intrinsic rate of increase and LRS values calculated from the number of fledglings and recruits between the different fledglings types in all possible pair combinations (Table 2) did not reveal statistically significant differences according to corrected p -values (Table S6 in the Supplementary Materials). However, in general, the fitness of EPY-positive females and EPY-negative males offspring was nearly twice that of all other offspring groups, although these differences were not statistically significant.

Table 1. The number of recruits and the recruitment rate of the fledglings related to the differences in genetic and social parents in the Western Siberian population of the European pied flycatcher.

Social and Genetic Mother	Sire	Social Father	Type	N	Recruited and Started Breeding in					Apparent Recruits		Corrected Recruits		ϕ [CI]
					2006	2007	2008	2009	2010	N	%	N	% [CI]	
F_{EPY-0}	M_{EPY-0}	M_{EPY-0}	WPO	981	30	28	–	1	1	60	6.1%	110	11.2% [9.2–13.2]	10.3% [7.4–14.1]
F_{EPY+}	M_{EPY-}	M_{EPY-}	Mat HSib of EPO	189	12	4	1	2	–	19	10.1%	40	21.2% [15.4–27.0]	14.6% [9.9–21.1]
F_{EPY+}	M_{EPY+}	M_{EPY-}	EPO	103	7	1	–	–	–	8	7.8%	9	8.7% [3.3–14.1]	10.6% [5.6–18.3]
F_{EPY-}	M_{EPY+}	M_{EPY+}	Pat HSib of EPO	212	5	4	1	–	–	10	4.7%	17	8.0% [4.3–11.7]	9.8% [6.1–15.2]

ϕ is the local survival probability from birth to the next breeding season as calculated in the MARK program, CI in square brackets denotes the lower and upper limits of the 95% confidence interval. F_{EPY-0} —EPY-neutral females, F_{EPY+} —EPY-positive females, F_{EPY-} —EPY-negative females, M_{EPY-0} —EPY-neutral males, M_{EPY+} —EPY-positive males, M_{EPY-} —EPY-negative males, WPO—within-pair offspring, Mat HSib of EPO—maternal (within-pair) half-siblings of extra-pair offspring, EPO—extra-pair offspring, Pat HSib of EPO—paternal (within-pair) half-siblings of extra-pair offspring (for details, see Classification of individuals section in Methods).

Table 2. The total fitness of the fledglings related to the differences in genetic and social parents in the Western Siberian population of the European pied flycatcher.

Social and Genetic Mother	Sire	Social Father	Type	N	$\bar{\lambda}_{Nfl}$ [CI]	$\bar{\lambda}_{Nrec}$ [CI]	$\bar{LRS}_{Nfl}$ [CI]	$\bar{LRS}_{Nrec}$ [CI]
F_{EPY-0}	M_{EPY-0}	M_{EPY-0}	WPO	981	0.15 [0–2.42]	0.02 [0–0.50]	0.28 [0–3.50]	0.02 [0–0.50]
F_{EPY+}	M_{EPY-}	M_{EPY-}	Mat HSib of EPO	189	0.27 [0–3.55]	0.04 [0–0.71]	0.53 [0–6.30]	0.04 [0–0.50]
F_{EPY+}	M_{EPY+}	M_{EPY-}	EPO	103	0.24 [0–3.47]	0.01 [0–0.00]	0.31 [0–3.95]	0.01 [0–0.00]
F_{EPY-}	M_{EPY+}	M_{EPY+}	Pat HSib of EPO	212	0.12 [0–2.14]	0.02 [0–0.36]	0.27 [0–3.36]	0.02 [0–0.36]

The per capita mean intrinsic rate of increase calculated using the number of fledglings and the number of recruits is written as $\bar{\lambda}_{Nfl}$ and $\bar{\lambda}_{Nrec}$, respectively. The per capita mean number of the lifetime fledglings and recruits produced by an individual is written as $\bar{LRS}_{Nfl}$ and $\bar{LRS}_{Nrec}$, respectively. CI in the square brackets denotes the location of 0.025 and 0.975 quantiles. F_{EPY-0} —EPY-neutral females, F_{EPY+} —EPY-positive females, F_{EPY-} —EPY-negative females, M_{EPY-0} —EPY-neutral males, M_{EPY+} —EPY-positive males, M_{EPY-} —EPY-negative males, WPO—within-pair offspring, Mat HSib of EPO—maternal (within-pair) half-siblings of extra-pair offspring, EPO—extra-pair offspring, Pat HSib of EPO—paternal (within-pair) half-siblings of extra-pair offspring (for details, see Classification of individuals section in Methods).

It is worth noting here, that our data on the fledglings fitness of different origin confirm that observations of a higher offspring fitness among broods containing EPY compared with those with only WPY do not provide conclusive evidence for indirect benefits of EPC behaviour among females because both a high offspring fitness and a high rate of EPY could be the result of a maternal and/or environmental effects [32].

4. Discussion

We carried out an observational analytical study of the EPP phenomenon in a Western Siberian population of the European pied flycatcher, attempting to find and quantify a relationship or association between registered variables (mainly individual phenotypical traits) and the involvement of a bird in extra-pair copulations. The revealed pattern of association between variables was rather complex.

In general, most of the associations between variables studied are weak. The statistical significance of most of the associations, with few exceptions, crosses only the uppermost threshold of significance level (0.05). The very revealing of statistically significant associations is very much dependent on the statistical analysis methods. This suggests that the data are poorly structured by the EPP status of individuals. Pure stochasticity seems to play a very important role in determining the involvement of individuals in EPC. Likely, many of the association of traits of individuals with their EPP status in the case of the European pied flycatcher researches are determined by the data structure per se, e.g., being data-specific.

We found that up to one-fifth of all birds can be involved in the EPP, which is just within the inter-population variability of the EPP rate described in this species so far (6 to 40%, see Introduction). Some of the relationships detected can be interpreted as a “function” of the EPCs, connecting this behaviour of an individual to its fitness and, hence, can be considered as support for selective scenarios persisting EPP (see Introduction). EPP tended

to be age-dependent, current fecundity was larger in EPY-positive males, offspring-to-eggs ratio in EPY-positive females (vs. EPY-negative ones) tended to be higher, distance to a nest of extra-pair females in EPY-positive males with bright and conspicuous breeding plumage was shorter. Many other relationships are more difficult to link to the presence of selective mechanisms. EPC was multiple, EPP rate was independent of breeding time, extra-pair mates were mainly near neighbours, association between most morpho-physiological traits and the EPC status of an individual was almost absent, the occurrence of EPP tended to be higher in good-quality territories, the average fertility of EPY-neutral and EPY-positive females were equal, and, finally, fitness between types of fledglings were roughly similar.

It is a challenging task to provide a coherent and logical explanation for our results based on all those hypotheses that assume the involvement of natural selection (in any of its forms) in the persistence of EPP in natural populations (most of such hypotheses were described in the Introduction). The difficulty is that many of the results we have obtained are contradictory and can be interpreted in different ways. Our results can be considered in favour or against a particular hypothesis about the function or evolution of EPC. To interpret the results within an adaptationist framework, we need knowledge about the genetic basis of EPC. However, the proportion of phenotypic variation in the number of EPOs and, apparently, in the involvement of individuals in EPC, due to genetic factors is very low among the European pied flycatcher [74]. This effectively means that selection can neither maintain EPP, nor change the mean EPP level among European pied flycatcher populations [74].

Therefore, when combined with earlier findings [8,74], the results of this study allow us to conclude that the extra-pair mating behaviour of the European pied flycatcher is an unpredictable side effect of selection on other phenotypic traits of an individual. Direct selection for fecundity, fertility and parental care performance is enough to produce EPCs as a by-product [8]. Here, our opinion is in general consistent with the repeated view that EPCs could be incidental side effects of behaviours (i.e., offensive male adaptations to gain sexual access to any females, resistance adaptations among females and defensive adaptations among males) that have evolved and are maintained primarily by direct benefits [32,103–105]. We believe that EPC evolved in the same way as all other non-monogamous forms of socially genetic relationships [8]. A very clear example of an incidental side effect of behaviour evolution is interspecific feeding [8,106,107], which no doubt does not arise evolutionarily by means of natural selection. The way in which a trait arises as an unpredictable by-product of selection for other properties of the phenotype, rather than as a correlative effect of it, is called episelective evolution [108,109]. Currently, within the field of ecological evolutionary developmental biology (Eco-Evo-Devo), this mechanism is considered as one of the main ways of evolution of novelties [110–112].

It appears that the extra-pair mating behaviour of the European pied flycatcher is, at present, a highly variable phenotypic trait or a plastic character. The involvement of birds in extra-pair mating seems to be almost entirely determined by phenotypic conditions of the individual, their hormonal status, behaviour and environmental factors [8,74]. The participation of each bird in EPCs seems to be a little predictable and depends entirely on the ecological environment in which the bird finds itself at a given point in its life cycle. Phenotypic trait values with low ontogenetic and environmental reproducibility (repeatability) and with a minuscule genetic component in overall phenotypic variability could be arbitrarily associated with fitness: weakly or strongly, negatively or positively. The effects of such traits on fitness may thus be population-specific. In this regard, comparative studies of inter-population variability in EPPs of the same species may be very important for understanding the population-specific ecological and behavioural mechanisms behind the involvement of birds in EPCs.

EPCs can only occur when there is physical contact between individuals. Consequently, all factors that may influence the probability of contact between males and females have the potential to alter the occurrence of EPO in broods of social pairs. Population density, operational sex ratios and environmental heterogeneity seem to be the most important

among them [3,113,114]. Low nesting density should correlate with a low EPP rate merely because the probability of meeting an extra-pair mate decreases sharply as the distance between them increases (European pied flycatchers engage in the extra-pair copulations virtually without going beyond their home range). In this study, the variation in breeding density at forest plots was insufficient to affect the EPP incidence on these sites. However, the larger differences in the overall structural characteristics of the environment between the urban and forest research areas strongly influence the occurrence of EPP in the European pied flycatcher in Western Siberia [8].

The general relationship of nesting density to the occurrence of EPP in populations may, however, be more complex. Among European pied flycatchers, it is known that males who have not formed pairs can participate in EPCs along with breeding territorial males [65]. We could not reveal genetic fathers for 35% ($N = 36$) of the EPYs. Certainly, non-breeding non-territorial males could be the genetic fathers of some of these nestlings. Consequently, in the case of very low nesting densities, it is the non-breeding males that may sire a substantial proportion of EPY, and it is likely that the occurrence of EPP in such case will be determined by the number of non-breeders, their fertilisation performance and territorial linkages. While the movements of nesting birds are highly spatially restricted as they are tethered to the nest site, non-breeding males have no such restrictions and are likely capable of exploring much larger areas.

Further, knowledge of the sex role in EPC initiation is crucial to the understanding which ecological interactions and behavioural patterns affect EPP [23,115]. Mating attempts can be initiated by a male [12,28,36] or female [18,19,33]. The male-initiated mating does not necessarily always have to be allowed or solicited by the female to result in successful copulations, i.e., in fertilization: EPP can be a consequence of sexual coercion [116] and forced extra-pair copulations [22]. Moreover, because resisting the male-initiated mating attempts can be costly to females [116], female ‘cooperation’ with a male could reduce the net costs of the encounter resulting in inconvenience polyandry [12,117]. European pied flycatcher EPC could occur, as argued here, as an unexpected side effect of the reproductive performance [8]. The male at the peak of the reproductive state in the corresponding hormonal status will copulate anyway with a within-pair or an extra-pair mate, and he could achieve his aim through sexual coercion and forced extra-pair copulations [8,23,64,116]. On the other hand, since the fertilization of an egg is the most important physiological need for a female in a reproductive state, the female could ‘search’ for extra-pair mates if her social partner is somehow absent ‘soliciting’ the EPC [83,118,119]. Thus, the presence of EPO in bird nests does not automatically imply the existence of a ‘propensity’ for EPCs of any gender.

Apparently, EPCs among birds are most commonly male-initiated, which proves that a female-initiated pursuit of extra-pair copulation is rare [12]. In the case of the European pied flycatcher, EPCs are mostly initiated by males as suggested by previous observations [118,120]. Through territorial intrusion tests and the measurement of excreted corticosterone metabolites, it has been demonstrated that the likelihood of paternity loss is associated with reduced aggressiveness and increased stress among males of the European pied flycatcher. It has been inferred that paternity is lost mostly among males who cannot effectively guard their within-pair females and territory against the male intruders sexually harassing females [59]. On the other hand, a negative correlation between the proportion of EPY in the brood and the age and wing length of the female lead to the conclusion that first-year and shorter-wing females are less likely to evade sexual abuse attempts by extra-pair males [64]. Our findings are generally consistent with those of the study. Among Spanish European pied flycatchers, gaps were temporarily created in the wings of females, resulting in a reduction of their wing area. Consequently, experimentally flight-impaired females had a higher proportion of EPYs, they were more likely to have EPYs and the number of extra-pair mates was also higher [70]. Thus, EPCs among European pied flycatchers are mostly the result of sexual abuse and are mainly driven by the extra-pair male pursuit of

females, capable of overruling female avoidance and mate defence from within the pair males [64,70].

Likely, this mechanism is also responsible for the fact that the incidence of EPP tends to be higher among females nesting in the best territories. We do not know the characteristics of the microhabitats that bring local nesting birds to the production of more recruits. However, if this is due to a fragmented distribution of insects, then rich food patches simply attract birds. This has resulted in such sites being visited more frequently by more specimens and, as a by-product, resident females of these microhabitats may be more often sexually abused by extra-pair males.

5. Conclusions

There are two ways of looking at biological phenomenon: ultimate (evolutionary explanations) and proximate (ecological and functional explanations). Ideally, a thorough explanation of biological phenomena must include both, although neither of them is mutually exclusive. Ecological events can always be considered profitably within an evolutionary framework and vice versa. However, these explanations are still different and, for example, not all ecological causes of a phenomenon can be mechanisms of its evolution without explicit assessment of the key parameters characterising that phenomenon. The key parameters that need to be documented to understand the evolution of extra-pair copulation behaviour are the magnitude of genetic correlations, the intensity of selection on mating biases, additive genetic variation in mating biases, preferred male traits and net fitness, as well as repeatable differences in these across ecological contexts [105]. Without an explicit assessment of these key parameters, all evidence that a given ecological process is an evolutionary factor for extra-pair mating behaviour would be incomplete. In our work, we used heritability as the main criterion discriminating hypotheses about the evolution and maintenance of EPCs [74]. We argue that the evolution and maintenance of extra-pair mating seem to be the episelective process [109,110]. Extra-pair mating behaviour among the European pied flycatcher is a plastic trait and can be determined by a whole set of proximate causes, and it appears that these causes may be different in particular populations of the species.

There also appears to be a hierarchy among the proximate factors that determine the extent to which birds are involved in extra-pair mating. Structural environmental characteristics that influence the probability of physical contact between individuals is one such top-level factor [8]. Sexual abuse seems to be a low-level behavioural proximate cause of the involvement of specimens in extra-pair copulations of the European pied flycatcher [70].

However, many details remain ambiguous or unstudied. For instance, the role of unmated non-breeding males in shaping EPP levels in populations is poorly investigated. Theoretically, in some circumstances, these individuals may contribute significantly to the proportion of broods containing EPY. The relationship between male–male interactions and the likelihood of EPCs is also not known in any detail. We have sometimes observed unsuccessful mating attempts of females with a within-pair male in nature, where the extra-pair male simply physically pushed aside the within-pair male and mated with the extra-pair female. The female did not change her inviting mating posture. In this situation, there was no sexual abuse from the extra-pair male to the extra-pair female. It simply was the case that the within-pair male failed to protect his interests at the critical moment when his social female needed to fertilize her eggs. Studies of these relationships between individuals and others like them are purely ecological and ethological in nature, but they do not lose their significance in the understanding of the extra-pair mating behaviour. This ecological research and observations as well as individual-based behavioural studies which combine a detailed description of behaviour with the genetic control of paternity will, therefore, be particularly beneficial in the future.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d14040283/s1>, Table S1: The list of all types of social and genetic relationships between offspring and parents, Table S2: The set of models investigated in the MARK program, Table S3: EPP, breeding and phenotypic trait data, Table S4: Encounter histories of offspring in connection with their origin, Table S5: Fitness of offspring, Table S6: The list of *p*-values generated during the analysis.

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Institutional Review Board Statement: Our work conforms to the legal requirements and guidelines in the Russian Federation as well as to international ethical standards. All our treatments and samplings have been intravital and have not required prolonged treatment and handling of birds. The species from our study is not included in the ‘Threatened’ category of the IUCN Red List of Threatened Species. The Bioethics Commission of Lomonosov Moscow State University provided full approval for this research (Protocol No 89-o of 22 March 2018).

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