Articles

Early arrival is not associated with more extra-pair fertilizations in a long-distance migratory bird

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When assessing the benefits of early arrival date of migratory birds, a hidden and often ignored component of males' fitness is the higher chance of early-arriving birds to obtain extra-pair fertilizations. Here we investigated how extra-pair paternity might affect the relationship between male arrival date and number of fertilizations in a model study system, the European pied flycatcher *Ficedula hypoleuca*. For this purpose, we sampled and genotyped breeding pairs, unpaired males and offspring (including embryos from unhatched eggs when possible) of a Dutch pied flycatcher population. Detailed information on arrival date of males, egg laying date of their social mates and nest success was also recorded. Early-arriving males had early-laying females and males with early-laying females had a higher probability of siring extra-pair eggs and obtain more fertilizations. However, male arrival date alone did not correlate with the probability to gain extra-pair paternity and neither to the amount of fertilized eggs. Both early- and late-arriving males had a higher probability of losing paternity in their own nest compared to birds with an intermediate arrival date. Finally, late-arriving males were more likely to remain unpaired but, interestingly, a few of these birds obtained paternity via extra-pair copulations. Because earlier arrival date did not lead to more extra-pair fertilizations and because such relationship seems to be driven mainly by the female's laying date, we conclude that the contribution of extra-pair paternity to the overall fitness benefits of early male arrival date is relatively small.

Migratory birds need to time the different stages of their complex annual cycle to take advantage of the distinct favourable conditions in their wintering and breeding environments (Alerstam et al. 2003, Buehler and Piersma 2008). The timing of migration from the wintering to the breeding grounds has been a topic of particular interest to evolutionary biologists, as the arrival time on the breeding grounds is considered a trait with major fitness consequences (Møller 1994, Velmala et al. 2015, Visser et al. 2015). Both males and females may suffer the costs of arriving too early or too late. Because males usually arrive earlier and are more likely affected by the costs of early arrival than females, and also due to their conspicuousness upon arrival, they are easier study subjects and thus the vast majority of studies have focused on male arrival phenology (Møller et al. 2003, Møller 2004, Reudink et al. 2009, Canal et al. 2012a, Velmala et al. 2015). An excessively early arrival may mean facing very harsh early spring conditions en route or at breeding territories (Møller 1994, Brown and Brown 2000), but these risks have to be traded off against the potential fitness benefits of early male arrival. These include the claim of better territories (Alatalo et al. 1984, Slagsvold 1986, Potti and Montalvo 1991, Hasselquist 1998, Canal et al. 2012a), earlier breeding (Cooper et al. 2010, Canal et al. 2012a, Velmala et al. 2015), more opportunities to find a mate (Alatalo et al. 1984, Møller 1994, Lozano et al. 1996, Canal et al. 2012a) and/or attract additional ones (Alatalo et al. 1984, Reudink et al. 2009, Cooper et al. 2010, Canal et al. 2012a), and more chances of second breeding attempts in case of failure of the first nest (Cooper et al. 2010). Ultimately, this would lead to higher breeding success for early-arriving males.

Early-arriving males might also have further opportunities to achieve extra-pair mating (Langefors et al. 1998, Møller et al. 2003, Reudink et al. 2009, Cooper et al. 2010, Canal et al. 2011, 2012a, b). Extra-pair paternity (henceforth EPP) is an important fitness component that can increase reproductive success in males, as long as this exceeds paternity loss in their own nest (Webster et al. 1995). As the number of arriving females is limited and the number of competing males in a breeding site increases throughout the season, males that arrive early (and thus mate early) would have more time and chances to achieve extra-pair fertilizations.

When assessing fitness in wild species, often only the number of recruiting offspring from the social brood to the

breeding population is used (Perrins 1965, Visser and Lessells 2001), overlooking EPP. If there is a relationship between arrival date and the probability of obtaining fertilizations outside the social nest, considering only the recruits from the social nest might provide a misleading picture of the actual fitness benefits of early arrival (Albrecht et al. 2007). Correct fitness estimations are needed for an unbiased estimate of selection on arrival date. This is especially true as seasonal timing is currently shifting due to anthropogenic alterations (e.g. climate change), which calls for studies aiming to understand how quickly species can respond and adapt to rapid environmental changes (Parmesan and Yohe 2003, Visser 2008). A number of studies have described either advancement trends or unchanging migration dates (Both and Visser 2001, Lehikoinen et al. 2004, Gill et al. 2014) and to understand such (lack of) responses, there is also an increasing interest on the fitness consequences of arrival date in long distance migrants (Velmala et al. 2015, Visser et al. 2015).

EPP has been shown to be affected by many factors, including secondary sex features (Møller et al. 2003, Lehtonen et al. 2009, Canal et al. 2011), body size (Canal et al. 2011), age (Moreno et al. 2010, Canal et al. 2012b), polygyny (Lubjuhn et al. 2000), and timing of breeding (Canal et al. 2012a, b). However, to date, the number of studies that have investigated how male arrival date associates with paternity (gain or loss) is small (Table 1). Such studies can be divided in cases in which early males gained paternity by having a higher probability of obtaining EPP (Table 1; Langefors et al. 1998, Cooper et al. 2010) and cases in which early males had a lower probability of losing paternity in their own nest (Table 1; Møller et al. 2003, Cooper et al. 2010). In any case, both situations lead to clear fitness benefits for early-arriving males.

Due to the potential relevance of extra-pair fertilizations when accounting for the total fitness benefits of early arrivals, here we investigated the contribution of this 'hidden' fitness component to the selection for early arrival. To this end, we collected data on arrival and laying dates and we sampled and assigned paternity for eggs and chicks of a Dutch pied flycatcher population. We then explored how the probability of gaining or losing paternity is associated with arrival date, and tested whether the relationship between fertilizations and arrival date is significantly affected by EPP. Moreover, we not only focused on sampling the surviving chicks but attempted to sample any fertilized egg that was produced by our breeding population, gaining additional information on the fertilization attempts of the males in our population.

Methods

Study system and study area

European pied flycatchers *Ficedula hypoleuca* ([Pallas], 1764), Muscicapidae, are long-distance migrants that breed in Europe and winter in Africa. They breed in tree cavities but readily accept nest boxes, which makes the species suitable for field research. Pied flycatcher males are usually easy to distinguish from females by the presence of a wider white wing patch, a white forehead patch and the presence of black or grey plumage, while females are brown. In our study area in the Netherlands, however, some males have a female-like appearance, but they can be distinguished from females by their conspicuous singing behaviour. Voucher material of this population was deposited in the ornithology collection of the Naturalis Biodiversity Center (Leiden, the Netherlands) under the inventory numbers RMNH 592347, RMNH 592348 and RMNH 592349.

Our study was conducted between early April and late June, 2014, in the National Park de Hoge Veluwe (the Netherlands; 52°02′07″N, 5°51′32″E). Forested areas in the park are dominated by pedunculate oaks *Quercus robur*, northern red oaks *Quercus rubra*, Scots pines *Pinus sylvestris*, larches *Larix* spp. and birches *Betula* spp. We provide around 400 nest boxes year-round in an area of 171 ha which are occupied in spring by cavity-nesters such as pied flycatchers, great tits *Parus major*, blue tits *Cyanistes caeruleus*, nuthatches *Sitta europaea* and coal tits *Periparus ater*.

Field sampling and data collection

Arrival time was assessed by daily scoring newly arriving males in our study area from early April using a protocol very similar to the one described in Potti (1998), Visser et al. (2015) and Both et al. (2016). Birds choose a territory upon arrival and advertise their cavity or nest box to the females by singing continuously at or close to the potential nest site. Two or three trained observers walked independently pre-established routes covering the whole study area and visiting all boxes. Routes and direction of the routes were alternated daily among observers in order to prevent any potential bias among them. Detected birds were described in terms of plumage and aluminium/colour ring combinations. Male pied flycatchers display relatively large individual variation in plumage features which, combined with colour ring combinations, allows an initial recognition in the field without the need of capturing the birds. In our study site, plumage

Table 1. Review of studies that correlated arrival dates with paternity gain or loss in different bird species. The symbols show the direction of the pattern in relation to early-arriving birds.

Species	Percentage of broods with EPP	EPP gain	Paternity loss	Fertilized eggs/offspring	Authors, year of publication
Sedge warbler *Acrocephalus schoenobaenus*	23%	+			Langefors et al. 1998
Barn swallow *Hirundo rustica*	32.4%		−		Møller et al. 2003
American redstart *Setophaga ruticilla*	43%		−	+	Reudink et al. 2009
Eastern kingbird *Tyrannus tyrannus*	58–70%	+			Cooper et al. 2010
Pied flycatcher *Ficedula hypoleuca*	19.8%	0 (only via female lay date)	+ (quadratic relation)	0	Present study

coloration varied from female-brown or light grey to almost entirely black (Drost 1936). The forehead white patch also varied in size, from absent to a large patch covering most of the forehead, and also in shape from two distinct dots to a rectangular-shaped patch. We associated singing males to the closest nest-box in the vicinity. During chick rearing phase (see below), those males were caught and described again in terms of plumage characteristics and ring combinations. This allowed us to link the original identifications to an individual ring number and assign individual arrival dates. Any bird still singing by the 1 May (around one week after the first egg was found) was considered a 'bachelor' and was captured to be identified and blood sampled. Some of those bachelors became breeding birds later in the season.

Upon arrival of females and start of nest building, nests were checked daily to assess the date of first egg laid, clutch size, date of egg hatching and number of hatched chicks. As part of an experiment running in parallel, some eggs were also marked and swapped (see also the assignment of paternity section). Both adults were then captured when their chicks were seven days old with a spring door trap or a wire door trap installed in the nest box entrance. These breeding birds were identified, measured and blood sampled (10 μl) by brachial venipuncture for paternity analysis. When 12 d old, chicks were also measured and a 10 μl blood sample was taken for paternity analysis. Blood samples were stored in 1 ml of Cell lysis Buffer (Qiagen, Venlo, the Netherlands). Any unhatched egg or dead chick found before blood sampling were collected and stored at –20°C. Tissue samples from unhatched embryos, dead adults (all found dead inside nest-boxes as a consequence of agonistic interactions with other flycatchers, great tits or blue tits) and dead chicks were taken for DNA analysis. In the few cases in which blood samples were missing but feather samples were available, DNA was isolated from the proximal tip of the feather (i.e. calamus).

Genotyping

FavorPrep 96-well Genomic DNA kit (Favorgen Biotech, Ping-Tung, Taiwan) was used to isolate the DNA from blood and tissues, following the manufacturer's protocol and using 200 μl of blood-Cell lysis buffer mixture. In the case of tissue samples, around 25 mg were processed like proposed in the FavorGen protocol with 2 h of lysis incubation. 0. PCR was performed by using five microsatellite DNA loci: Fhyu336, Fhyu234, Fhyu304, Fhyu453, Fhyu448 (Leder et al. 2008). Separation of the PCR fragments took place using an ABI 3130 Genetic Analyser (Thermo Fisher Scientific, Waltham, USA). The capillary electrophorese results of the ABI were analysed with the software GeneMapper 5.0 (Thermo Fisher Scientific, Waltham, USA) that determined the sizes of the amplification products.

Assignment of paternity

The paternity of offspring was analysed with Cervus ver. 3.0.7. (Field Genetics, London, UK; Kalinowski et al. 2007) (see also Supplementary material Appendix 1 Table A1). Cervus calculates a likelihood ratio for each father–offspring pair using genotype data from young and adults as candidate parents. It assigns paternity according to the difference in the likelihood ratio scores between the most likely and second-most-likely fathers. Five microsatellite loci were used to determine genotype and assign parentage with one mismatch allowed. Confidence was set at 95% level. Based on that information, we determined whether a male sired an extra-pair young or not, whether it lost paternity in its own nest or not and, whether it was able to father any egg at all or not; finally, we determined how many eggs and 12 days-old chicks each male fathered in total. The probability of assignment was 0.99 and mismatches between known pairs of parent and offspring were also re-visualised for accuracy (see Supplementary material Appendix 1 for more details).

Not all complete families were caught, so, for the nests in which only one of the parents was caught, the genetics of the offspring was used to determine the other parent. This reconstructed genotype was then matched with all other genotypes that we generated to either identify the parent among the captured birds or infer that the parent was another, not captured, bird. If the father could not be identified (which was the case for only 5 out of 208 birds), it was still possible to determine if the brood had any extra-pair eggs. When an unknown male had fathered an egg, it was counted as a paternity lost for the social male, but no paternity gain for another.

The data collection for extra-pair-paternity analysis was conducted in the same year in which a field experiment was also carried out. Purposes and results of this experiment will be presented elsewhere (Tomotani et al. 2016, unpubl.). This experiment had one potential implication for our analysis: when females laid a seventh egg, these were often stored or put under non-experimental birds and as a consequence they were significantly more likely to be lost, therefore not assigned to any male, than eggs number one to six (Fisher's exact test, $p < 0.001$). However, out of those eggs which were assigned, there was no significant difference of being extra-pair between eggs one to six and egg seven (Fisher's exact test, $p = 0.15$). Therefore for the purpose of extra-pair paternity analysis, eggs were treated in the same way, independent of their lay order (see Supplementary material Appendix 1 Table A2 for more details).

Unassigned eggs (not genotyped and thus without known paternity) needed to be considered differently depending on the analysis. When we analysed whether a father gained or lost the paternity of any extra-pair egg (see below in the data analysis part), any unassigned eggs had to be excluded. When we calculated the number of eggs a given male fathered, we took into account the probability of losing paternity of an egg in our population (9% based on all own and extra-pair eggs laid in 2014). Eggs not previously assigned to a male, were then given to the social father with a 91% of probability of being his own. All the males in the population received an extra paternity value which consisted of a 9% fraction of all eggs not assigned (72 eggs) divided by all males of the population (109 males). Four of the eggs that we genotyped did not match with any male of the population (within or extra-pair), they were also divided by all males of the population and added to the total number of fathered eggs as a fixed value. Therefore, for the final number of fathered/fertilised eggs we considered: own genetically-identified social eggs + identified eggs fathered in other nests (including polygyny and extra-pair) + 0.91 × (social

eggs not assigned) $+ 0.09 \times$ (all eggs not assigned/all males in the population) + eggs with no match in the population/ all males in the population.

Data analysis

Analyses were performed in R ver. 3.2.1 (18 June 2015; R Core Team). We used generalized linear models in R (logit-link and Binomial error-distribution) to test if the probabilities to gain extra-pair paternity or lose paternity could be explained by the arrival date of the male (as linear and quadratic term) or the laying date of the female. We performed backwards model selection, dropping non-significant terms in each step. Because the inclusion of the laying date of the female forced the model to only include males that had a nest (and no bachelors), we also tested in simple regression analysis the probability to gain paternity in relation to the arrival date of all males, in relation to the arrival date of only males that had a female and in relation to the female laying date. We also tested whether the probability of fertilizing an egg at all was related to arrival date of the male or laying date of the female, however, it was not possible to include both terms in the same multiple regression analysis since males with known arrival date that failed to fertilize eggs were all bachelors, so we only tested these probabilities in separate simple regressions (arrival date of the male including bachelors or laying date of the female). Using multiple linear regressions we tested whether or not the arrival date of the male correlated with the laying date of their female and whether or not the number of fathered eggs and chicks was related to arrival date of the male or the laying date of the female. For this analysis we only included data from males that had a female (no bachelors) and tested both fathered eggs with EPP or only the social eggs. Finally, to shed some light on the pattern of fertile female availability, we calculated for each male the difference between the laying date of his social female and the laying date of his extra-pair female. The difference was then correlated with the arrival date of the male and the laying date of its social female. For polygynous males, we only considered the laying dates of the primary brood as secondary broods could have a higher incidence of paternity loss due to differences in behaviour of the male (Canal et al. 2011). Moreover, the incidence of polygyny was very low in our dataset (only three males).

Results

In total, 555 eggs were produced by the sampled breeding population. We could assign 87% (481) of those to a male (see Supplementary material Appendix 1 Table A3 for more details). The unassigned eggs were either broken, non-viable, predated, discarded by the female, had poor quality DNA or, if hatched, the chick had died and was not in the nest anymore by the time we could sample or collect it. 9% of the assigned eggs were fertilized by an extra-pair male; however, the proportion of clutches containing an extra-pair egg was close to 20% (Table 1). Out of 109 males analysed in total, their spring arrival dates and laying date of their social females were obtained for two overlapping (but not

the same) sets of 87 birds, with 72 males having both arrival and laying date known.

The probability of losing paternity showed a quadratic relation with the arrival date of the male, with both early and late-arriving birds showing a higher probability to have an egg fertilized in their own social nest by other males than males arriving on intermediate dates (quadratic term: $\chi^2_{1,n=72} = 5.27$, p = 0.02, estimate ($\pm$ SE) = 0.03 ($\pm$ 0.01) (Fig. 1a).

Late-arriving males were significantly more likely to not father any egg throughout the season than early-arriving males ($\chi^2_{1,n=87} = 6.90$, p = 0.01, estimate ($\pm$ SE) = –0.19 ($\pm$ 0.08); Fig. 2). The majority of those males were bachelors (no social nest), although they were captured performing song displays near nest-boxes. A few bachelor birds were able to obtain paternity via extra-pair copulations (Fig. 2).

Early-arriving males did not have a significantly different probability of gaining EPP compared to late-arriving males. Arrival date of the male was not significant when considered as the only term in a simple regression and also not when analysed in conjunction with the laying date of the female (Supplementary material Appendix 1 Table A4). The pattern of the simple regression was the same regardless of the inclusion of bachelor males or not. However the probability to gain EPP significantly declined with the laying date of a male's social female ($\chi^2_{1,n=87} = 4.39$, p = 0.04, estimate ($\pm$ SE) = –0.07 ($\pm$ 0.04); Fig. 1c). Also, early-arriving males had earlier laying females than late-arriving males ($F_{1,70} = 16.37$, p < 0.01, estimate ($\pm$ SE) = 0.81 ($\pm$ 0.2); Fig. 1b).

Male arrival date either alone or in conjunction with egg-laying date of his female did not explain the total number of eggs that the male was able to father, independently of the EPP (Supplementary material Appendix 1 Table A5). Laying date of a female was significantly related to the total number of eggs fertilized by her social mate throughout the season, also independently on whether EPP was taken into account or not (with EPP $F_{1,85} = 14.75$, p < 0.01, estimate ($\pm$ SE) = –0.09 ($\pm$ 0.02); only EPP lost $F_{1,85} = 13.69$, p < 0.01, estimate ($\pm$ SE) = –0.07 ($\pm$ 0.02); only social eggs $F_{1,85} = 9.71$, p < 0.01, estimate ($\pm$ SE) = –0.05 ($\pm$ 0.02); Fig. 1d), since clutch size normally declines over the season. As expected, the number of fathered eggs with EPP was significantly correlated to the number of social fathered eggs (without taking EPP into account) ($F_{1,85} = 47.87$, p < 0.01, estimate ($\pm$ SE) = 0.84 ($\pm$ 0.12)).

Males with early-laying females first fathered eggs in their own social nest and then eggs in other nests, reflecting fertile female availability. With the progress of the season, though, the pattern reversed, with males paired with late-laying females first fathering eggs in another nests and then eggs in their own nest ($F_{1,13} = 17.18$, p < 0.01, estimate ($\pm$ S) = –1.36 ($\pm$ 0.32); Fig. 3). This pattern was not observed in relation to the arrival date of the males (Supplementary material Appendix 1 Table A5).

Discussion

Our study supports the well-known pattern that early arrival date leads to higher breeding success by increasing the chances of finding a mate and/or promoting early breeding,
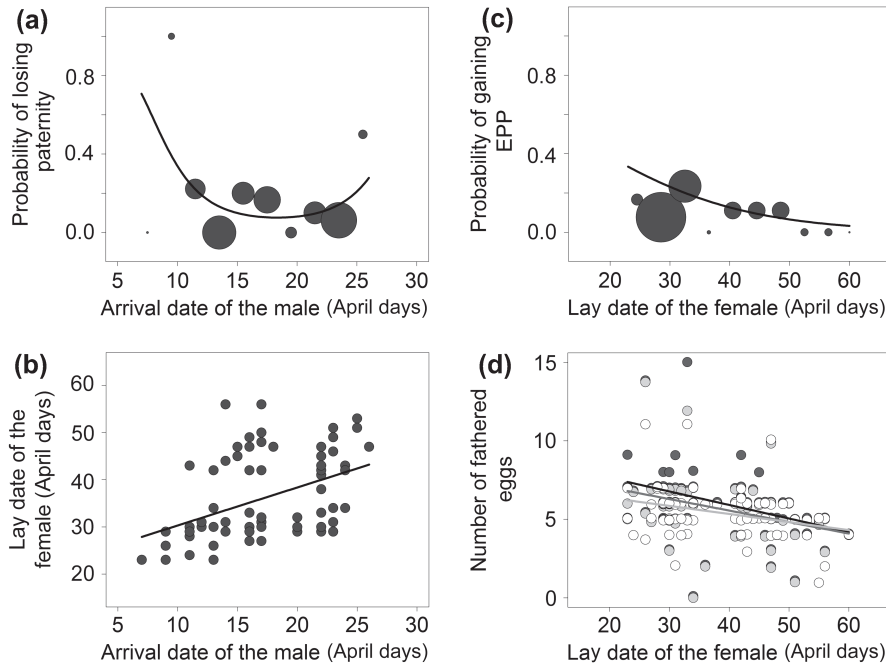
Figure 1. Correlation and model predictions of (a) the probability that a male loses paternity in his social nest in relation to his arrival date (given in April days: number of days since 31 March). Points correspond to the average probability of losing paternity of all males that arrived in each of the 2 day-bins starting from April day 7 (e.g. between days 7 and 8, 9 and 10, 11 and 12, etc.). The size of the symbols indicate sample size (largest = 15, smallest = 1). (b) The laying date of the female and the arrival date of the male (both given in April days). (c) The probability of a male to obtain extra-pair paternity in relation to the laying date of his social female (points correspond to the average probability of losing paternity of all males that had females laying in each of the 4 day-bins starting from April day 23; point sizes reflect sample sizes: largest = 26, smallest = 1). (d) The number of eggs males fathered and the laying date of his social female. From top to bottom: Dark gray circles represent the number of fathered eggs taking extra-pair eggs gained into account; the black line is the prediction of this model. Gray circles represent the number of fathered eggs without taking extra-pair eggs gained into account; the medium gray line is the prediction of this model. White circles represent the number of social eggs, the light gray line is the prediction of this model.

which is associated with larger clutch sizes. However, we did not find evidence supporting the hypotheses that early arrival date is associated with more EPP or less loss of paternity in the own social nest (Table 1). We observed a non-linear relationship between arrival date and paternity loss and a significant relationship between female laying date and the probability to gain EPP. This last result is fully consistent with observations from pied flycatcher populations breeding in the south-western edge of the distributional range of the species (Canal et al. 2012a, b).
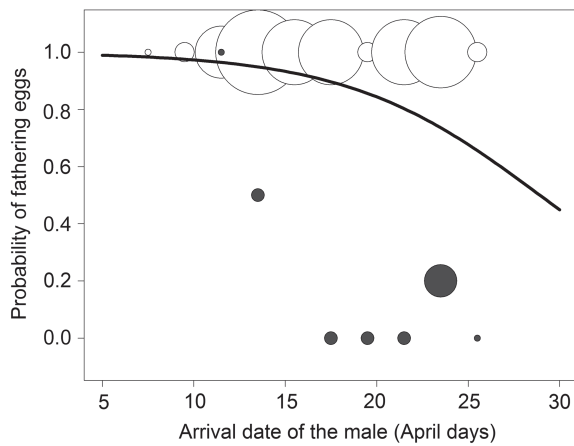

Figure 2. Probability of fathering any egg in relation to the arrival date of the male. Points correspond to the average probability of losing paternity of all males that arrived in each of the 2 day-bins starting from April day 7, size of the symbols indicate sample size (largest = 13, smallest = 1). Open symbols represent males with at least one social nest. Closed symbols represent males without a social nest (bachelor males).
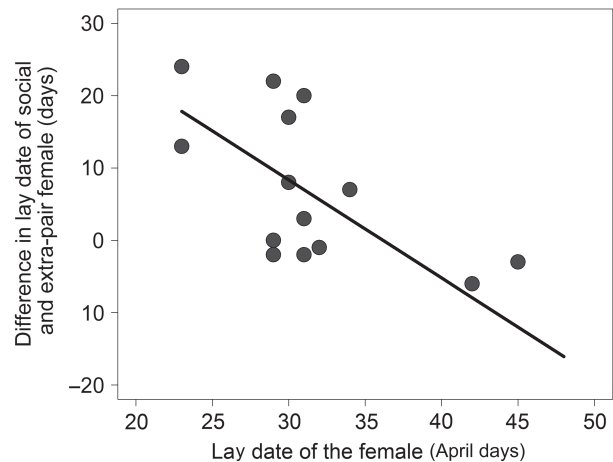

Figure 3. Difference in days between the laying date of social and extra-pair female (positive values: male first obtained paternity in social nest and then in extra-pair nest, negative values: male first obtained paternity in extra-pair nest and then in social nest) in relation to the laying date of social female.

Our results suggest that a gain in EPP for an early-arriving male would occur indirectly via the laying date of his social female and not exclusively due to his own arrival date. The lack of significant association between the probability of EPP and the arrival date of the male could be explained by the very large variation in the relationship between arrival and laying dates. Despite this relationship being significantly positive there is much residual variance (Supplementary material Appendix 1 Fig. A1), as has been also described previously for our study site (de la Hera et al. 2013) and for other geographically close pied flycatcher populations (Both et al. 2016). Arrival dates of males, although repeatable across years, are not necessarily related to their breeding date in different years (Both et al. 2016).

If EPP attempts are male initiated, breeding synchrony among individuals of the population can affect the relationship between laying date and EPP (Canal et al. 2012b). Thus, more synchronous breeding is associated with reduced rates of EPP, and this could in turn increase the difference between early and late breeders (Canal et al. 2012b). In the year that our data were collected (see Supplementary material Appendix 1 Fig. A1 for more details), the breeding synchrony was below the average (although still similar to other populations, such as those studied by Canal et al. 2012b). According to this, it could be argued that our lack of association between arrival date and EPP might be a year-specific phenomenon, caused by the low breeding synchrony in 2014. However, the explanation of Canal et al. (2012b) might also not apply here because it requires that arrival and laying dates are highly correlated or that such correlation became stronger in years with high breeding synchrony. Since this was not the case in our population (Supplementary material Appendix 1 Fig. A1c), we do not think the relationship between arrival date and EPP changes significantly under different degrees of breeding synchrony, but a more grounded statement would require the repetition of this data collection in several years with distinct breeding synchronies.

In our results, when EPP is considered in relation to laying date, males that mated early (i.e. early laying dates of their social female) obtained extra-pair fertilizations shortly after their social female initiated a clutch, while males that obtained a social female relatively late in the season had first fertilized an extra-pair female shortly before fertilizing their social female or even before obtaining a social nest (i.e. while still a 'bachelor'). This result partially supports the findings from Canal et al. (2012b) and suggests that the middle of the breeding season is the period when more fertile females are available and more chances exist of being cuckolded (Fig. 1c). Interestingly, if a similar comparison is done with arrival dates, the pattern changes: early and late-arriving males had a higher probability of losing paternity in their own nests than intermediate males (Fig. 1a), again suggesting that egg laying date is a better predictor of EPP than arrival date.

We also acknowledge that other factors could play a role in explaining the observed variation in EPP. Thus, it is also possible that our observed variation in EPP paternity probability could be explained by female choice of other male traits, such as body condition or some secondary sexual characters (Møller et al. 2003, Reudink et al. 2009) that are known to contribute to paternity gain or loss in pied flycatchers (Sirkiä and Laaksonen 2009, Moreno et al. 2010, Canal

et al. 2011). Moreover, female traits could also be important in explaining variation in EPP (Moreno et al. 2015). For our population we had data on males' blackness and front patch size that are signs of attractiveness in other populations (Sirkiä and Laaksonen 2009). Thus we tested whether those were related to EPP (Supplementary material Appendix 1 Table A6). However, we did not find any relationship between these secondary sexual traits and male arrival date. Our findings do not match what is reported for other populations supporting that female preference on male trait may vary between populations of pied flycatchers (Galván and Moreno 2009) and this relationship between EPP and male traits is not always straightforward as shown in Moreno et al. (2010, 2013).

It is important to take the sampling moment into account when evaluating the differences between the present study and others that evaluated the relationship between arrival date and EPP (Table 1). Here, we looked at the egg stage to define fertilization probability in relation to arrival time instead of using only samples of the surviving chicks. Although the final fitness measure is not affected by evaluating eggs instead of chicks, our method would have the potential to reveal details, such as a different survival probability of genetic and extra-pair offspring (Sardell et al. 2011), or explain the differences in paternity loss in relation to other studies (Table 1).

Another factor that could contribute to the dissimilarity of our results when compared to earlier studies is the relatively low proportion of extra-pair broods in our population (19.8%, Table 1), which would reduce the power of the tests associating arrival date and paternity. Our rate of extra-pair broods is consistent with the values obtained in other pied flycatcher populations: 10.4 to 18.9% in Germany (Lubjuhn et al. 2000); 15% (Lifjeld et al. 1991) or 16% (Slagsvold et al. 2001) in Norway; 13% (Lehtonen et al. 2009) or 22% (Rätti et al. 1995) in Finland; 22.4% (Moreno et al. 2010) or 28.8% (Moreno et al. 2013) in Spain. However, Canal et al. (2012b) and Moreno et al. (2015) reported higher rates of nests involved in EPP (between 33 and 40%) in Spanish populations. We supposedly had a higher than normal rate for our population in comparison to previous years due to the high breeding asynchrony (Canal et al. 2012b) and indeed a much lower proportion was reported in a previous year for our population (< 10%, de la Hera et al. 2013). Thus, for the majority of the populations, rates of EPP are generally low and fitness benefits of EPP are not always that clear (Slagsvold et al. 2001, Moreno et al. 2013).

We also attempted to sample as many bachelor males as possible, which allowed the observation that late arriving males have a higher probability of not obtaining paternity at all (similar results were obtained by Potti and Montalvo 1991). However, since bachelors comprise a different category of males and could behave very differently from breeding birds, their inclusion in the paternity gain analysis could offer another explanation on why our results were not consistent with previous studies. However, even when we excluded the bachelors, the results remained the same (Table 1), and thus the observed pattern is not related to a different behaviour of bachelor males. A very interesting outcome of the inclusion of bachelor birds in the analysis is the fact that they seem to be able to achieve some paternity.

Some studies report that the identity of a few extra-pair fathers remained unknown, even when a high proportion of the breeding birds are identified (Canal et al. 2011). These missing individuals could either be birds breeding outside of the study area or even floaters. Here we provide evidence that it is indeed possible for a male pied flycatcher to obtain paternity without a social nest.

In conclusion, our results suggest that the component of the male pied flycatcher phenology that affects the variation in EPP is the laying date of its female. Although arrival date is significantly related to laying date, the arrival date effect on EPP will largely depend on how strong the relationship is between arrival and laying dates in a given year or population. Thus, considering all fitness components that affect selection of arrival date (clutch size, probability to obtain a female, etc.), the final contribution of EPP will probably be fairly small. Therefore, we suggest that in this long-distance migrant, the estimation of fitness of arrival date should not be largely affected when information on EPP is not available.

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