UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE CIENCIAS BIOLÓGICAS

Máster en Zoología

Trabajo Fin de Máster

MOULT-BREEDING OVERLAP IN RELATION TO ENVIRONMENTAL CONDITIONS OF THE PIED FLYCATCHER WITHIN A CLIMATE CHANGE CONTEXT.

(Herraiz, 2018)

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Madrid, septiembre de 2024

Universidad Complutense de Madrid
Máster Universitario en Zoología

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SOLAPAMIENTO MUDA-CRÍA EN RELACIÓN A LAS CONDICIONES AMBIENTALES DEL PAPAMOSCAS CERROJILLO DENTRO DE UN CONTEXTO DE CAMBIO CLIMÁTICO.

- Trabajo Fin de Máster -

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Septiembre, 2024

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ANEXO I: DECLARACIÓN DE NO PLAGIO

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ABSTRACT

Reproduction and moult are two fundamental events in the life of birds, and both are characterized for being energetically expensive. The overlap of both processes can lead to a reduction in the reproductive success and/or survival of the bird. Furthermore, this trade-off between reproduction and moulting is of vital importance in long-distance migratory birds due to the limited time they have to carry out both processes before returning to their winter quarters, specially under a framework of global warming. In this work, we investigate the influence of different intrinsic factors (sex, age, laying date) and extrinsic factors (temperature and habitat) on the occurrence of moult-breeding overlap and moult onset using 19 years of data from a long-term monitoring population of pied flycatchers. Our results show that the occurrence of moult-breeding overlap has not increased during the study period, and neither has advanced the onset of moult. We found that both moult-breeding overlap and onset of moult were sex and age-dependent: males showed greater overlap and earlier moult onset than females, and first-year birds tend to overlap moult and breeding more than older birds. However, temperature during incubation did not show any association with neither the occurrence of moult-breeding overlap nor the onset of moult. Our findings suggest that sexual differences in the moult-breeding overlap may indicate differential fitness costs for males and females and that moult may be more sensitive to endogenous conditions.

Keywords: moult-breeding overlap, pied flycatcher (*Ficedula hypoleuca*), climate change, physiological trade-off, intrinsic and extrinsic factors, fitness, environmental conditions.

RESUMEN

La reproducción y la muda son dos eventos fundamentales en la vida de las aves, y ambos se caracterizan por ser energéticamente costosos. El solapamiento de ambos procesos puede llevar a una reducción del éxito reproductivo y/o supervivencia del ave. Además, este compromiso entre reproducción y muda es de vital importancia en aves migratorias de larga distancia debido al tiempo limitado que tienen para llevar a cabo ambos procesos antes de regresar a sus cuarteles de invierno, especialmente en un marco de calentamiento global. En este trabajo, investigamos la influencia de diferentes factores intrínsecos (sexo, edad, fecha de puesta) y extrínsecos (temperatura y hábitat) en la ocurrencia del solapamiento muda-reproducción y el inicio de la muda usando 19 años de datos de una población de seguimiento a largo plazo de papamoscas cerrojillos. Nuestros resultados muestran que la ocurrencia del solapamiento muda-reproducción no ha aumentado durante el período de estudio, como tampoco ha adelantado el inicio de la muda. Descubrimos que tanto la superposición entre muda y reproducción como el inicio de la muda dependían del sexo y la edad: los machos mostraron una mayor superposición y un inicio más temprano de la muda que las hembras, y las aves de primer año tienden a superponer la muda y la reproducción más que las aves mayores. Sin embargo, la temperatura durante la incubación no mostró ninguna asociación ni con la ocurrencia de la superposición entre muda y reproducción ni con el inicio de la muda. Nuestros hallazgos sugieren que las diferencias sexuales en la superposición entre muda y reproducción pueden indicar costos diferenciales de aptitud física para machos y hembras y que la muda puede ser más sensible a las condiciones endógenas.

Palabras Clave: superposición muda-cría, papamoscas cerrojillo (*Ficedula hipoleuca*), cambio climático, equilibrio fisiológico, factores intrínsecos y extrínsecos, aptitud física, condiciones ambientales.

INTRODUCTION

Plumage fulfills a great variety of functions: 1) it is a protective and thermoregulatory barrier, 2) plays an important role in visual communication and camouflage and among all 3) it enables birds to fly (Jenni & Winkler, 2020). Environmental and biological factors such as sunlight, weather conditions, abrasion and the presence of parasites contribute to plumage degradation, which reduces its quality and compromises the activities of the individual (Barbosa *et al.*, 2002; Swaddle *et al.*, 1996; Weber *et al.*, 2005). All birds replace their plumage (or part of it) at least once a year during moult. Overall, moult is considered a costly process for two main reasons: 1) it requires substantial amounts of energy and special nutrients and 2) it causes a temporary decrease in the performance of plumage related functions (e.g. thermal insulation, flight) (Jenni & Winkler, 1994; Lindström *et al.*, 1993; Mcnamara & Houston, 2008; Newton, 2009). Given these costs, moult is expected to be temporally separated from other time-consuming and energetically demanding activities like reproduction. In temperate regions, passerines show a strict and regular organization of their annual cycles due to seasonality (Jenni & Winkler, 2020). That means that essential processes like breeding, moult and migration take place in certain time windows. In the case of European passerines individuals typically start to moult once reproduction is completed, right before the worsen of environmental conditions (e.g. onset of autumn-winter) or migration (Jenni & Winkler, 2020). However, the overlap between moult and breeding has been reported in both sedentary and migratory passerines species (Foster, 1975; Morales *et al.*, 2007; Payne, 1969; Solís *et al.*, 2021), a phenomena with negative consequences on breeding performance, survival and feather quality (Hemborg *et al.*, 1998; Hemborg *et al.*, 2001; Morales *et al.*, 2007).

The occurrence of moult-breeding overlap can be influenced by external (e.g., temperature, habitat) and internal factors (e.g. sex, age, body condition), as well as by their interactions (De La Hera *et al.*, 2010; Jenni & Winkler, 1994; Kiat & Izhaki, 2016; Siikamäki *et al.*, 1994). For example, within the same population, males are more prone to overlap breeding and moult than females (Hemborg, 1999; Hemborg *et al.*, 2001; Moreno *et al.*, 2001; Orell & Ojanen, 1980; Siikamäki *et al.*, 1994; Solís *et al.*, 2021), starting to moult during the incubation and egg-hatching periods. Furthermore, first-year birds also tend to overlap breeding and moult more frequently than adults (Hemborg *et al.*, 2001; Kiat & Izhaki, 2016; Siikamäki *et al.*, 1994; I. Solís *et al.*, 2021), presumably due to their low quality plumage or a later start of reproduction (Hemborg *et al.*, 2001). Lastly, breeding phenology may also affect the onset of moult, as individuals breeding late in the season or laying a second brood are more likely to overlap both annual events to complete moult before autumn or migration (Hemborg *et al.*, 2001; Svensson & Nilsson, 1997).

Environmental conditions can also affect the prevalence of moult-breeding overlap. Indeed, populations of the same species situated at different latitudes show differences in the frequency of moult-breeding overlap (Hemborg *et al.*, 1998, 2001), with northern populations showing a higher proportion of individuals overlapping breeding and moult. Climate change is changing environmental conditions (e.g., temperatures, precipitations), which may alter time constrains across annual cycles (Tomotani *et al.* 2018), and hence, the stages of the annual cycle of passerines (e.g. breeding, moult and migration). Shifts in the timing of these stages may impose fitness costs, especially if these changes are unequal across stages (Tomotani *et al.*, 2017, 2018). Rising temperatures due to climate change could reduce the breeding period and moulting in several bird species. This suggests that, with higher temperatures, reproduction is accelerated and the time available between the end of breeding and the beginning of moulting is reduced. Tomotani *et al.* (2017, 2018) further noted that these changes in life cycles could have significant consequences for the survival and adaptation of birds. In addition, the decrease in food supply and the poorer quality of the territory are other environmental factors that can alter these processes (Perrins, 1970; Verhulst, Balen, & Tinbergen, 1995; Wiggins, Pärt, & Gustafsson, 1994). Therefore, understanding the factors that cause the overlap of reproduction and moult, as well as the alterations caused by climate change in these components of the life cycle of migratory birds, is essential to understand whether species can to adapt to such rapid environmental changes.

The pied flycatcher (*Ficedula hypoleuca*; Pallas, 1764) is a long-distance migratory passerine. In Iberian populations, pied flycatchers arrive during April and May for reproduction. Once they complete breeding and moult, they migrate to their winter quarters in Africa during mid-August. In this study, we used 19 years of data from a longitudinal study of a pied flycatcher population to investigate the occurrence of moult-breeding overlap. Our aim is to examine the influence of intrinsic (e.g., sex, age and laying date) and extrinsic (e.g., temperature and habitat) factors that may influence moult-breeding overlap and its onset as well as to track possible changes in the incidence of moult-breeding overlap and timing of moult at the population level across the study period. The specific objectives are to: 1) analyze whether the proportion of individuals that overlap reproduction and moult have changed over the study period, 2) assess whether the annual timing of moult onset has varied during this period, 3) examine how intrinsic or extrinsic factors influence the probability of moult-breeding overlap and 4) the timing of moult onset.

MATERIALS AND METHODS

Study system

The pied flycatcher is an insectivorous passerine bird that shows sexual dimorphism both in plumage coloration and slightly in size (Lundberg & Alatalo, 1992; Potti & Merino, 1996). After spring migration from Africa, the species breeds in temperate forests of Eurasia, both deciduous and coniferous, producing a single brood per season and establishing a small territory around a nest hole (Lundberg & Alatalo, 1992). Males arrive at breeding areas before females, search for a suitable nesting site, defend their possession and try to attract a female (Canal *et al.*, 2012).

Data have been collected from a pied flycatcher population breeding in nest boxes, near La Hiruela in central Spain (41°04′N, 3°27′W–40°40′N, 4°80′W) that has been monitored since 1984. The study area consists of two ecologically distinct habitats separated by only 1.1 km: a mature deciduous forest (9.3 ha) dominated by Pyrenean oak (*Quercus pyrenaica*) and a coniferous plantation (4.8 ha) dominated by Scots pine (*Pinus sylvestris*). Birds can move between habitats without being hindered by physical barriers; however, the presence of rocky outcrops and riparian vegetation that separates these habitats is unsuitable for pied flycatcher breeding. In 1984 and 1988, respectively, nest boxes were placed in the oak forest (n = 156) and pine forest (n = 81). Since then, the number and location of nest boxes has not changed, being 20 ± 9.2 m (mean ± standard error) apart.

Field procedures

Nest boxes were regularly inspected every three days during the breeding season, from mid-April to early July to determine occupancy (Figure 1). After occupation, nest boxes were checked daily to determine laying date, clutch size (typically 5-6 eggs), hatching date, and number of nestlings. On day 13 after hatching, all nestlings were marked with numbered metal rings and tarsus length was measured to the nearest ±0.05 mm. On the other hand, breeding adults were captured while incubating (in the case of females) or feeding chicks between days 8 and 10 after hatching (in the case of males and females) using a nest box trap (Friedman *et al.*, 2008). All individuals were uniquely identified with metal and colored rings.

Many individuals are born in the next boxes; thus, their age is known (50% approx). Unringed birds were aged based on plumage criteria (1 year or older; Karlssoln *et al.*, 1986). Standard morphological measurements were taken on each individual, including tarsus length (± 0.01 mm), wing length (± 0.5 mm), forehead patch size (± 0.01 mm) and body mass (± 0.1 g). For each individual, we recorded whether it had begun the moult process (individuals with or more primary feathers absent or with new feathers growing) or not. We also scored each primary feather from each wing according to its stage of growth: old feather = 0, the feather has been dropped and the new feather s =1, new feather fully developed with no trace of sheath = 5 or from 1 to 4 depending on its length relative to its full length (Jenni & Winkler, 1994). Once all primary feathers were scored, we sum all the scores to obtain a moult score for each wing. Pied flycatchers take between 45-50 days to complete post-nuptial moult (Ginn & Melville, 1983) and assuming a linear increase of moult scores of the primaries, moult speed in flycatchers is approximately 1 point/day (Hemborg, 1999). Due to asymmetry in moult, we selected the wing with higher score to estimate moult onset, assuming the linear increase of 1 score point/day mentioned above.

Figure 1. Inside of nest box with female pied flycatcher and chicks. Image created by the author.

Environmental variables

Meteorological data used in this study were obtained from the AEMET station of Colmenar Viejo (40°39′N, 3°45′W; https://opendata.aemet.es/centrodedescargas/productosAEMET), as it is the only nearby station (within 50 km) that has a complete record for the entire study period. The average temperatures and precipitation recorded did not differ from the data available for the Buitrago de Lozoya meteorological station (41°00′N, 3°36′W), the closest to the study area, but with an incomplete temporal record. During the years in which both stations operated simultaneously, temperature and precipitation indices showed a high correlation, supporting the validity of the long-term climate data used in our analyses (see Le Vaillant *et al.*, 2021) for more details).

Initially, we considered the average, minimum and maximum temperatures and precipitation in different periods for each year: April, May, June and spring, as well as those calculated for each brood during the incubation period (from the laying of the last egg to hatching) and the nestling stage (from the date of hatching to day 13 of nestling age) of each brood. After checking the correlations between all climatic variables (Table S1), we decided to use the average temperatures during the incubation period for three reasons: 1) this index is correlated with those of other periods (Table S1), 2) considering the temperature during the incubation period is the most biologically appropriate approximation given the focal variable and our field protocols. On the one hand, the incubation period is the period immediately preceding the moult, which occurs during the nestling stage (no moulting individual has ever been found during the incubation period; D. David observation based on sporadic captures and captures in the framework of experiments). On the other hand, as our sampling protocol involves capturing adults when their chicks are 8 days old, considering environmental conditions during the nestling phase would imply using future conditions (those occurring between 9-13 days of chick age) to account for events that have already taken place (onset of moult before 8 days of age). 3) These indices are calculated specifically for each brood (unlike, for example, monthly indices), thus providing a more accurate representation of the conditions faced by chicks in each nest box.

Statistical analysis

Statistical analyses were performed using R-4.2.0 (R Core Team 2022).

To analyse whether the frequency of individuals that overlap moult and reproduction has changed during the study period, we ran a generalized linear model (GLM) with a Beta distribution (*glmmTMB* package; Brooks *et al.*, 2023). The response variable was the proportion of individuals overlapping moult and reproduction (a proportion). The predictor variables were sex (1: male vs. 2: female) and year (continuous).

To assess whether the average onset of the post-nuptial moult has varied over the study period, we used the *moult* package (Erni *et al.*, 2013)based on two analyses: (a) using presence/absence data (moulting/non-moulting individuals), we calculated the average annual moult onset date with a “probit model”, a generalized linear model (“glm” function) with a binomial distribution and a probit link function (Erni *et al.*, 2013). For this purpose, the scores of the variable moult response were transformed into a binary variable with categories "have not started moulting" (0) and "have started moulting" (1). The date of day 8 of chick hatching (continuous) was used as a predictor variable, and these analyses were also performed for each year and each sex. (b) Using the average start date of moult, a regression line was made with the function "lm" by which the start of moult of the population was obtained by year. We used the start of moult as a response variable and sex and year as explanatory variables.

To study the factors that influence the overlap between moult and breeding, we fitted a GLMM model with a binomial distribution (*glmmTMB* package). The response variable was moulting while breeding (0: “no” vs. 1: “yes”), the predictor variables were sex, laying date, habitat (2 level factor: oak vs. pine forest) and the interaction between individual age (2 level factor: 1-year-old (young) vs. older (adults)) and mean temperature during the incubation period, while individual identity and year were included as random factors. The interaction between sex and incubation temperature was not used in the minimal model because it was not significant in the saturated model.

To analyse the influence of extrinsic and intrinsic factors on the onset of the post-nuptial moult at the individual level, we fitted a linear mixed model (*lmer* package; Bates *et al*., 2023). The response variable was the estimated moult date, the predictor variables were habitat, sex, estimated age, laying date and incubation temperature, and individual identity and year were used as random factors.

The fit of all models was checked by visual examination of residuals and using the “DHARMa” (Hartig, 2017) and “performance” (Lüdecke *et al*, 2021) packages, and we ruled out collinearity (all VIFs < 3, (Zuur *et al*., 2010) using the “car” package (Fox & Weisberg, 2011). Statistical analyses were performed using R-4.2.0 (R Core Team 2022).

RESULTS

The proportion of individuals overlapping breeding and moulting has not varied over the study period in the population (Table 1). However, this proportion differed between sexes as females overlap moult and breeding less frequently compared to males (Table 1 and Figure 1).

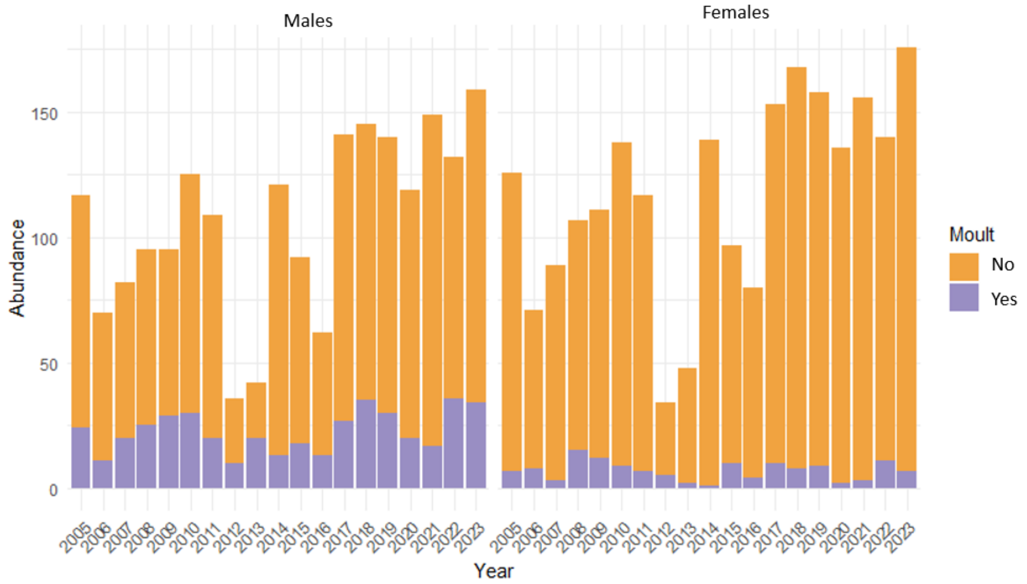

Figure 2 This figure represents the proportion of individuals, males and females separately, in the population that do or do not overlap moult and breed during the study period.

Table 1. Results of a generalized linear mixed model analysing the proportion of overlapping breeding and moult in relation to year and sex.

	Estimate	SE	z	P
Intercept	44.085	±28.064		
Year	-0.022	±0.014	-1.564	0.118
Sex (female)	-1.406	±0.172	-8.184	**<0.001**

For each fixed effect, the estimate, standard error (SE), z value (z) and p value (P) are given. Parameters that have shown a 95% significance level are highlighted in bold.

The onset of moult has not varied over the years in the population (Table 2). However, as with the probability of overlapping, there are differences between sexes, with females showing a positive trend and males the opposite. In other words, it has been observed that males start moulting earlier and females later (Table 2 and Figure 2).

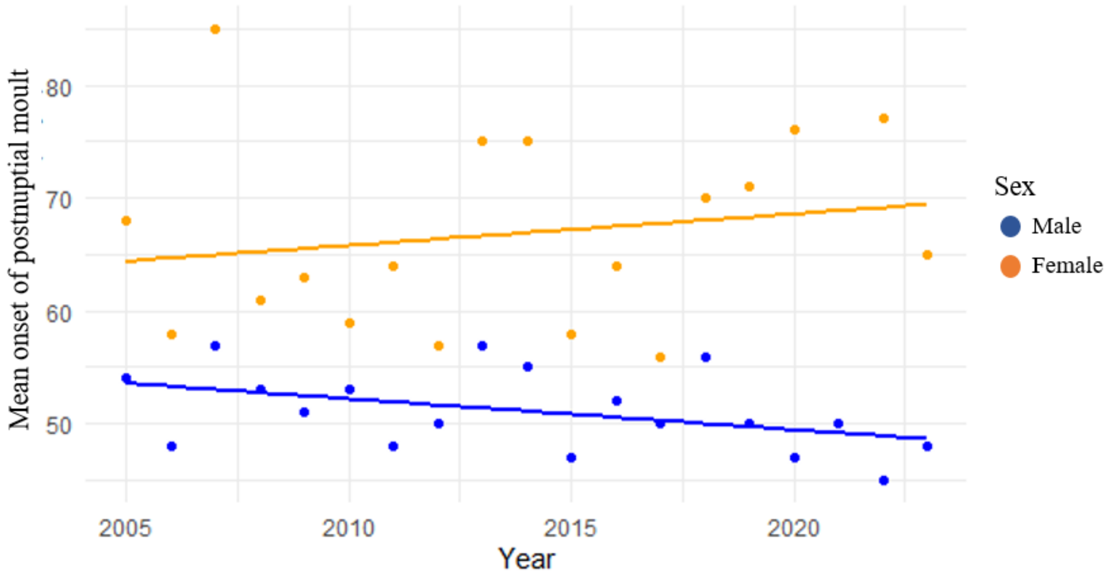

Figure 3. Means onset of moult in males (blue) and females (orange), over the study period. The y-axis indicates the day on which the post-nuptial moult starts relative to 1 May, which is day 1

Table 2. Results of the linear model analysing the moult initiation date in relation to year and sex.

	Estimate	SE	t	P
Intercept	72.490	±392.984		
Year	-0.011	±0.195	-0.054	0.957
Sex (female)	15.668	±2.119	7.396	**<0.001**

For each fixed effects, the estimate, standard error (SE), t value (t) and p value (P) are given. Parameters that have shown a 95% significance level are highlighted in bold.

The probability of overlapping moult and reproduction was influenced by all the variables considered. Specifically, the probability of overlapping moult and breeding was positively related to the laying date (Figures 3B and 3C) and was higher in the oak than in the pine forest (Table 3) and for males compared to females (Figure 3B). In addition, the interaction between age and incubation temperature affected the probability of moulting while breeding (Table 3 and Figure 3C). The probability of overlap increased in adults as incubation temperature increased, while in juveniles such probability remained relatively stable over the range of incubation temperatures (Figure 3A).

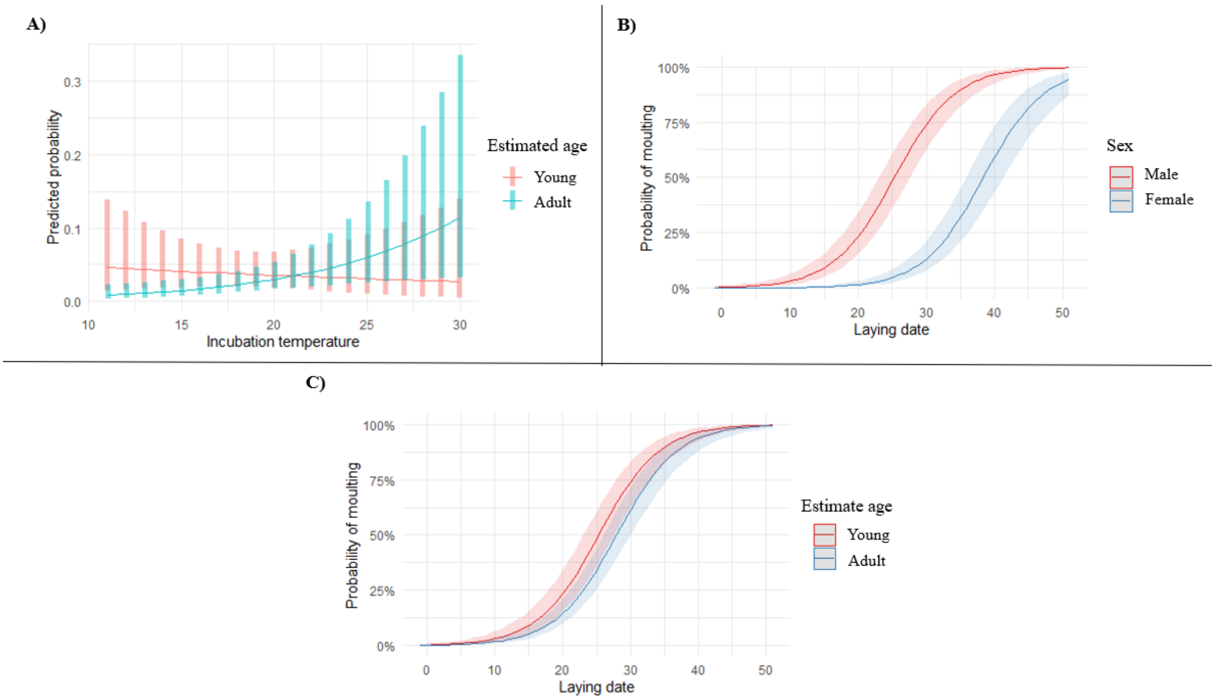

Figure 4. A) Predicted probability of overlap between moult and brood resulting from the interaction between incubation temperature and age (1-year-old (young) vs. older (adults)), B) the probability of overlap in relation to laying date and sex, C) the probability of overlap in relation to laying date and age.

Table 3. Results of the generalized linear mixed model analysing intrinsic (sex, laying date and age) and extrinsic factors (incubation temperature and habitat) that may influence the probability of moult-breeding overlap (N = 3990).

Fixed effects	Estimate	SE	z	Odds ratio (CI 95%)	P
Intercept	-5.152	±1.319			
Sex (female)	-2.925	±0.241	-12.131	**0.05 (0.03-0.09)**	**<0.001**
Laying date	0.224	±0.019	11.607	**1.25 (1.20-1.30)**	**<0.001**
Age (adult)	-3.622	±1.344	-2.695	**0.03 (0.00-0.37)**	**0.007**
Incubation temperature	-0.029	±0.072	-0.397	0.97 (0.84-1.12)	0.692
Habitat (pine forest)	-0.524	±0.161	-3.244	**0.59 (0.43-0.81)**	**0.001**
Age*Incubationtemperature	0.172	±0.070	2.446	**1.18 (1.03-1.36)**	**0.014**
Random effects	Variance	SD			
Ring	0.384	±0.619			
Year	1.429	±1.196			

For each fixed effect, the estimate, standard error (SE), z value (z), odds ratio and p value (P) are given. For each random effect, variance and standard deviation (SD) are shown. Parameters that have shown a 95% significance level are highlighted in bold.

The initiation date of the post-nuptial moulting was unrelated to the incubation temperature and the age of individuals (Table 4). However, the moult onset date was positively related to the laying date (Figure 4) and was later in the pine than the oak forest (Table 4) and females than males (Figure 4).

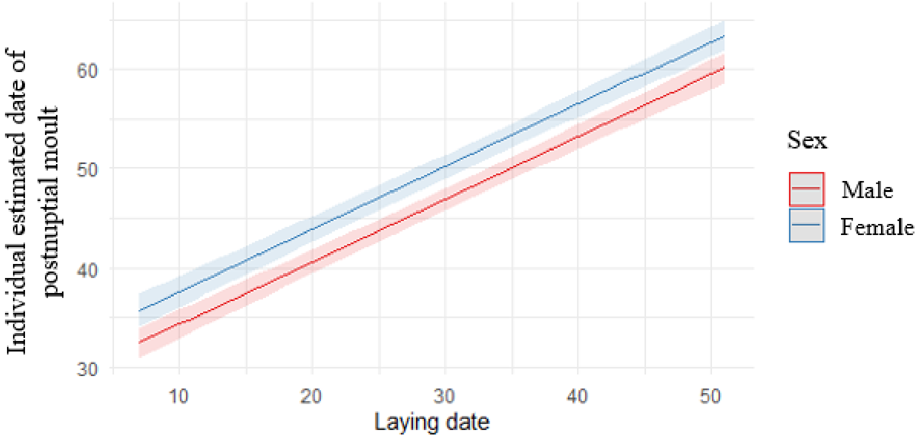

Figure 5. Estimated initiation date of the post-nuptial moult in relation to laying date and sex. The y-axis indicates the day on which the moult starts relative to 1 May, which is day 1.

Table 4. Results of a general linear mixed model analysing intrinsic (sex, laying date and age) and extrinsic factors (temperature incubation and habitat) that may influence the onset of moulting (N = 545).

Fixed effects	Estimate	SE	t	P
Intercept	26.466	±1.592		
Habitat (pine forest)	0.997	±0.323	3.083	**0.002**
Sex (female)	3.281	±0.377	8.697	**<0.001**
Incubation temperature	0.080	±0.097	0.828	0.408
Laying date	0.630	±0.024	25.797	**<0.001**
Age (adult)	0.218	±0.334	0.654	0.513
Random effects	Variance	SD		
Ring	1.979	±1.407		
Year	4.122	±2.030		
Residual	9.692	±3.113		

For each fixed effect, the estimate, standard error (SE), t value (t) and p value (P) are given. For each random effect, variance and standard deviation (SD) are shown. Parameters that have shown a 95% significance level are highlighted in bold.

DISCUSSION

Using a dataset of 19 breeding seasons, we have investigated the role of multiple extrinsic and intrinsic factors on the timing of moult and the probability of overlapping moult and breeding in a population of pied flycatchers. Overall, the date of moult onset and the probability of overlapping moult and breeding have not changed over the study period but were related to sex, age, laying date and habitat or the temperature faced by individuals immediately before moult. However, we did not observe any temporal trend neither in the number of individuals overlapping moult and reproduction nor a shift in the timing of moult.

In our population, males were more likely to be found overlapping moult and breeding and they started to moult earlier than females, which is consistent with other studies in the same and other species (Hemborg, 1999; Hemborg *et al.*, 2001; Moreno *et al.*, 2001; Siikamäki *et al.*, 1994; Solís *et al.*, 2021). Several explanations have been proposed to explain this. It is suggested that differential fitness costs may have shaped this sexual difference. It is likely that an overall lower and more inconsistent investment in breeding (Queller, 1997) may allow males to devote energy in moult and reproduction at the same time. It has been suggested that a higher incidence of extra-pair paternity may influence the willingness of a male to invest in the brood (Siikamäki et al., 1994). As a result, this may have forced females to delay moult to ensure the survival of their offspring. However, there are other authors who claim that females that overlap breeding and moulting can reduce reproductive effort, even going so far as to remove eggs from the nest, to increase the probability of survival by devoting greater effort to moulting (Hemborg et al., 2001; Lobato et al., 2006; Morales et al., 2007). In addition, other authors suggest that gonadal activity may delay moult in females (King, 1973; Miller, 1961), and thus, be more constrained than males in this regard.

Warming of temperatures may serve as a surrogate for the progression of the breeding season. As the breeding season advances, temperatures get hotter, day lengthens and environmental conditions such as food abundance and food quality decline. This may trigger moult overlap in breeding individuals, since time for completing the moult before migration shortens. Interestingly, only older individuals showed a higher probability of overlapping with increasing temperatures whereas young birds did not. One possibility could be that older individuals are capable of tracking changes in environmental cues due to their experience. In addition, as shown in other studies, we also found that first-year birds tend to overlap more frequently than older ones. One possibility is that yearlings are forced to moult earlier in the season due to a higher wear of their primary feathers (Siikamäki *et al.*, 1994). Moreover, it has been suggested that first-year individuals may prioritize moult by reducing reproductive effort and maximize their fitness through enhanced survival (Morales *et al.*, 2007; Solís *et al.*, 2021). However, robust studies (i.e. accounting for recapture probability) about the relationship between moult-breeding overlap and survival are sorely needed.

Late breeding individuals, irrespectively of their sex and age, show a higher likelihood of moult-breeding overlap, which is consistent with other studies (Hemborg *et al.*, 2001; Solís *et al.*, 2021; Svensson & Nilsson, 1997). As breeding season progresses, the available time to complete moult reduces. In migratory species like the pied flycatcher, moult schedule is even more constrained than in sedentary species because they must leave the breeding grounds and reach their winter quarters in West Africa. Thus, some degree of overlap between breeding and moult may be inevitable for late individuals as it may allow them to complete moult before migration commences. One unexplored yet interesting question is whether early and late overlapping individuals differ in the quality of their moulted plumage (Helm & Gwinner, 2006) as well as in the possible fitness consequences of superimposing these two processes at different sections of the breeding season.

The results indicate that individuals are more likely to overlap moult with breeding in the oak forest (although the size of the effect was small compared with other variables), which unlike the pine forest is the original habitat of the pied flycatcher in this locality (Potti & Montalvo, 1990). We do not have a clear explanation for this result. It could be reflecting differences in the age-structure or life-history traits between populations as well as differences in local environmental conditions which may require further study.

Our temporal trend analysis did not reveal an increase in the number of birds overlapping moult and breeding, which does not coincide with Moreno (2004) and Tomotani *et al.* (2017), who suggested that the number of individuals that overlap moult and reproduction would increase over time due to the delay in laying dates and the maintenance of the moult date. In our population, the start date of moult has not varied significantly in 19 years as shown by our analysis and laying dates have remained stable for the last 30 years (Le Vaillant *et al.*, 2021), which may suggest that the intervals for the annual cycle stages in our population have remained stable. However, a further study is needed to confirm it. Our results contrast with those from another population of pied flycatchers, in which male pied flycatchers showed a rapid advance of the timing of moult unrelated to temperature (Tomotani *et al.*, 2017). One possibility is that the onset of moult is determined earlier in the season as suggested by Tomotani *et al*. (2017). However, we do not possess information from that period. Another possibility is that the onset of moult is less susceptible to modulation by environmental factors in comparison with other annual cycle stages like arrival dates, being governed by endogenous factors (Gwinner, 1996; Tomotani *et al*., 2017). Indeed, our results show that individual onset of moult was not determined by incubation temperature, which may add support to the endogenous hypothesis. Lastly, it should be noted that pied flycatchers from Iberian populations conduct a shorter route migration than those belonging to northern populations.

One caveat of this study is that we had to discard data spanning from 1984 to 2004 due to the no availability of reliable moult data from those years. Therefore, it would be interesting to continue the research with a greater number of years of study to see if there really is no variation or if we have not seen it due to a lack of data.

CONCLUSION

In conclusion, we have shown that the occurrence of moult-breeding overlap is highly influenced by sex, age and laying dates. Specifically, males, first-year and late breeding individuals showed more likelihood of overlapping moult and reproduction. Despite the current global warming scenario, we did not detect an increase/advance in the proportion of overlapping individuals or the onset of moult in the last 19 years, suggesting that the onset of moult may be governed by other factors different that temperature during incubation. Further studies should investigate the consequences of moult-breeding overlap on fitness measurements such as breeding performance and survival.

ACKNOWLEDGMENTS

First of all, I would like to thank all the people and institutions that have contributed to the completion of this work. I would like to thank my tutors Iraida Redondo and David Canal, employees of the Museo Nacional de Ciencias Naturales (MNCN) and the Consejo Superior de Investigaciones Científicas (CSIC), for all their efforts during the completion of this work. They have been a great support to me both professionally and personally, encouraging me every day and answering my incessant emails. On the other hand, I would like to highlight the participation of my partner every day since I started the master's degree, my emotional support. Finally, I would like to thank the Universidad Complutense de Madrid (UCM), the Museo Nacional de Ciencias Naturales and the Consejo Superior de Investigaciones Científicas for their invaluable collaboration and for providing me with the resources and support necessary to carry out this study, and the Agencia Estatal de Meteorología (AEMET) for its contribution by providing essential data for the development of this study.

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ANNEXES

Table S1. Pearson correlations between the mean temperatures (A) and precipitations (B) variables considered for this study. Correlations significant for 95% confidence intervals are highlighted (*) in the cells below the diagonal.

A) Temperatures	Incubation	Nestling stage	April	May	June	Spring
Incubation	1	0.39	-0.21	0.43	0.47	0.29
Nestling stage	*	1	-0.19	0.34	0.52	0.28
April	*	*	1	0.12	0.06	0.58
May	*	*	*	1	0.76	0.83
June	*	*	*	*	1	0.79
Spring	*	*	*	*	*	1
B) Precipitations	Incubation	Nestling stage	April	May	June	Spring
Incubation	1	0.39	0.20	0.66	0.60	0.61
Nestling stage	*	1	0.26	0.40	0.57	0.44
April	*	*	1	0.53	-0.13	0.74
May	*	*	*	1	0.57	0.94
June	*	*	*	*	1	0.51
Spring	*	*	*	*	*	1