THE ROLE OF SOCIAL RELATIONSHIPS IN TERRITORIAL AND COLONIAL FEMALE PASSERINES

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Memoria presentada por la graduada Mireia Plaza Cusiné para optar al grado de Doctora en Ecología por la Universidad Autónoma de Madrid, dirigida por los Doctores Juan Moreno Klemming y Diego Gil Pérez del Museo Nacional de Ciencias Naturales.

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Thesis structure

This thesis is structured in eight main sections. In addition to a general ABSTRACT, first section includes a general INTRODUCTION, where the conceptual framework is established, the AIMS and HYPOTHESES and the general METHODS that have been used during the research are presented, including study species, study sites and general fieldwork, laboratory and social network analyses. The methodology is supplemented by the specific procedures used to develop each objective included in its relevant chapter. The following six sections include CHAPTERS in standard scientific article format. The last sections include the INTEGRATIVE DISCUSSION and CONCLUSIONS which complete the present PhD thesis.

A mi madre

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Esta tesis ha representado no sólo la oportunidad de emprender un camino dentro de la ciencia y formarme profesionalmente, sino también de crecer y descubrir aspectos de mí que no conocía, de encontrar personas maravillosas con las que he compartido momentos que llevaré conmigo toda la vida. Estar escribiendo estas líneas supone el cierre de lo que ha sido una de las mejores etapas de mi vida y por eso representa satisfacción y alegría, pero también emoción y algo de nostalgia al llegar al final del camino.

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RESUMEN

Explicar la diversidad de comportamientos, su origen y la influencia de unos sobre otros, es uno de los retos históricos de la biología y la principal fuente de investigación para la etología clásica y la ecología del comportamiento. El objetivo principal de esta tesis es aumentar el conocimiento sobre la influencia del entorno social en la reproducción, bajo una perspectiva evolutiva y mediante una gran carga experimental. Para ello se ha estudiado el efecto de distintos tipos de interacciones sociales entre individuos de un mismo o distinto sexo y sobre diferentes procesos reproductivos como son la incubación o el propio comportamiento copulatorio, enfatizando el papel de las hembras. Se han utilizado dos especies modelo con sistemas sociales y reproductivos diferentes en distintos ambientes, en cautividad y en libertad: una especie territorial, el papamoscas cerrojillo *Ficedula hypoleuca* (sólo en libertad)*,* y una especie gregaria, el gorrión común *Paser domesticus*. Ello permite ofrecer una visión más amplia sobre los paseriformes y sus métodos de estudio.

Dado que las cavidades de nidificación son un recurso escaso para las aves trogloditas, objeto de investigación en la presente tesis, hay una fuerte competencia sobre ellas por parte de las hembras durante el periodo reproductor, y su disponibilidad ejerce una gran presión selectiva durante la incubación. En el contexto del primer experimento (**Capítulo 1**), se observó que una reducción drástica de la mancha alar en hembras experimentales de papamoscas cerrojillo, producía valores más altos de vigilancia frente a una situación simulada de intrusión territorial por parte de otra hembra, disminuyendo así sus niveles de intensidad de incubación. A partir de estos resultados, podemos deducir que las señales alares transmitirían información sobre el estatus social de las hembras, mediando sus interacciones sociales en contextos de competencia intra-sexual, permitiéndoles así mantener altos niveles de incubación al reducir la necesidad de vigilar. Se definen las señales de las hembras como producto de la selección social ejercida sobre ellas durante la reproducción.

En especies con monogamia social, es frecuente la detección de casos de paternidad extra-pareja como producto de cópulas de la hembra con machos ajenos a

la misma. A pesar de existir numerosos estudios sobre los patrones de este fenómeno, no existe aún consenso sobre las razones de su gran variabilidad entre especies e individuos. Mediante el segundo experimento (**Capítulo 2**) también en el papamoscas cerrojillo, comprobamos que una reducción de la superficie alar en las hembras durante su periodo fértil, con la consiguiente presumible disminución de la capacidad de vuelo, inducía un aumento de los niveles de paternidad extra-pareja. Además, en un tercer experimento (**Capítulo 3**) con la misma especie, se observó que el aumento de peso en las hembras, causado por la reducción drástica del coste de construcción del nido, producía un aumento de los niveles de paternidad extra-pareja. Considerando que la capacidad de vuelo (positivamente relacionada con la superficie alar y negativamente con el peso corporal), está asociada a la posibilidad de escapar de cópulas no deseadas por parte de las hembras de especies territoriales, estos resultados sugieren que la paternidad extra-pareja es en ocasiones el resultado de un conflicto sexual que se resuelve en beneficio de los machos ajenos a la pareja.

El contexto social es un elemento clave durante el periodo reproductor, ya que los individuos establecen relaciones para alimentarse, defenderse y también para reproducirse. En el **Capítulo 4**, estudiamos la importancia relativa del entorno social en la paternidad extra-pareja del papamoscas cerrojillo, mediante el análisis de su repetibilidad individual entre temporadas reproductivas. Además analizamos la influencia de los rasgos individuales de machos y hembras territoriales como su coloración o medidas corporales, y componentes contextuales como la sincronía y la densidad poblacional, de forma conjunta sobre la paternidad extra-pareja. La ausencia de repetibilidad tanto en machos como en hembras para este rasgo, indicó que la variación en el contexto social en el que las interacciones sexuales tienen lugar, debe reducir el peso de la influencia de los rasgos individuales.

La personalidad ejerce una influencia sobre distintos comportamientos de los individuos provocando fluctuaciones en su eficacia biológica. Para que un rasgo de comportamiento sea considerado parte de la personalidad, debe ser consistente en un mismo individuo y distinto del que presentan otros individuos de la población. En el **Capítulo 5**, estudiamos la consistencia inter-individual en los rasgos de sociabilidad del gorrión común, medidos a partir de sus interacciones con los demás individuos dentro de la red social de su población, entre distintos contextos y a distintas escalas

temporales. La existencia de repetibilidad (consistencia intra-individual) en las variables de medidas de sociabilidad, permite afirmar que éstas pueden ser consideradas rasgos de personalidad. Finalmente, la relación encontrada en el **Capítulo 6** entre estas variables y la frecuencia de cópulas extra-pareja en las hembras, apoya la idea de que la sociabilidad puede ser una estrategia para afrontar determinadas condiciones del entorno en esta especie, pudiendo con ello aumentar su eficacia biológica.

La importancia relativa de las características individuales tanto de machos como de hembras por un lado, y la influencia de entorno social por el otro, deben ser considerados como factores clave en la determinación de los patrones reproductivos.

ABSTRACT

Explaining behavioural diversity and its origins is an historical challenge for biologists and the main focus of research for classical ethology and for behavioural ecology. The main objective of this thesis is to increase our knowledge about the influence of the social environment on reproduction, under an evolutionary perspective and through a largely experimental approach. This has been achieved by studying the effect of different types of social interactions between individuals of the same or opposite sex on different reproductive processes such as incubation or copulatory behaviour by emphasizing the role of females. We have used two model bird species with different social and reproductive systems, in captivity and in the wild: the pied flycatcher *Ficedula hypoleuca* (only studies in the wild), which is a territorial species, and the house sparrow, *Passer domesticus,* which is a gregarious species. This offers a broader perspective on passerines and on their study methodologies.

Since nesting cavities are a scarce resource for hole nesters, there is strong competition for them during the breeding season. Their availability thus exerts a strong selective pressure during the incubation period. In the context of our first experiment (**Chapter 1**), it was observed that a drastic reduction of the wing patch in pied flycatcher females of the experimental treatment, caused higher values of vigilance against a simulated territorial intrusion by another female, decreasing their levels of incubation. Thus, wing patches may transmit information on female social status, mediating their interactions in contexts of intra-sexual competition. This would allow them to maintain high levels of incubation attendance by reducing the need for surveillance. Thus, these female plumage signals would be the result of social selection during breeding.

In socially monogamous species, cases of extra-pair paternity (EPP) are frequent as the product of female copulations with other males rather than their mate. Although there are numerous studies on patterns of EPP, there is still no consensus on the reasons for its great variability between species and individuals. Through our second experiment (**Chapter 2**) in pied flycatchers we found that an experimental reduction in female wing area during the fertile period, which presumably caused a decrease in their flight ability, led to an increase in extra-pair paternity levels compared with control females. In addition, in a third experiment (**Chapter 3**) on the same species, it was observed that the increased mass of experimental females caused by an induced drastic reduction in nest building costs, produced an increase in EPP levels compared with control females. Considering that flight capacity (positively related to wing area and negatively associated with female body mass) is presumably linked to the capacity of females to avoid unwanted copulations by extra-pair males, our experimental results suggest that EPP can be the product of a sexual conflict which turns to the advantage of extra-pair males in some situations.

The social context is a key element during the reproductive period, since individuals establish relationships with conspecifics in order to feed, defend territories and mate. In **Chapter 4** of this thesis, we studied the relative importance of the social environment in EPP for pied flycatchers by analyzing its individual inter-year repeatability. In addition, we studied the influence on EPP of individual features of territorial males and females such as age, plumage coloration and morphology, as well as contextual variables like breeding density or synchrony. The absence of inter-year repeatability in both males and females for EPP pointed out that variation in the social context in which sexual interactions occur may reduce the influence of individual traits.

Personality affects different individual behaviours, which in turn have effects on fitness. For a trait to be considered part of personality, it must be consistent within individuals and differ among them. In **Chapter 5** of this thesis, we studied individual consistency in sociability traits of house sparrows. Sociability of individuals was measured through their interactions with others inside the social network of the population, in different contexts and at different time scales. The presence of individual repeatability (intra-individual consistency) in sociality measures makes them ideal personality traits. Finally, the relationship found in **Chapter 6** between sociality and the incidence of EPP in house sparrows, supports the idea that sociality can be considered part of a strategy for females to cope with their social environments and probably thereby increase their fitness.

The relative importance of individual characteristics of both males and females on the one hand, and the influence of the social context on the other, should be considered as key factors in determining the resulting behavioural reproductive patterns.

INTRODUCTION

Understanding mechanisms that mediate social interactions is a major challenge for evolutionary biologists. Social competition occurs in a rich variety of contexts given that animals compete over food resources, space, reproduction, mates, social status and even parental care (West-Eberhard, 1979). Although competition in these various situations affects different components of fitness, the social interactions themselves are often mediated by similar physical signals independently of the context. Darwin developed his second evolutionary theory (sexual selection) to explain the evolution of those traits that seemed to pose a particular challenge to natural selection by not clearly enhancing survival (Darwin, 1871). He defined sexual selection as ''*the advantage which certain individuals have over other individuals of the same sex and species solely in respect of reproduction*". Thus, he pointed out that there may be different types of selection operating in nature. However further studies cast doubt on the separation between sexual and natural selection, suggesting that the encompassing concept of social selection (West-Eberhard, 1979) has much to offer to the current debates about both sexual selection and reproductive competition especially in females (Lyon & Montgomerie, 2012). It was not until fairly recently that the social environment has started to be considered as a comprehensive framework for the study of mating activities in both sexes, leading to the concept of sexual selection as a form of social selection which should not be studied separately.

A myriad observations and experiments support nowadays the broad details of sexual selection, confirming two main processes driving it: mate choice and intra-sexual competition over mates, which are widespread in nature and help explain much about the evolution of individual traits (Shuster, 2009). However, both mechanisms have traditionally been more intensively studied in males as their ornaments and behaviours are usually more extreme. The traditional underappreciation of the role of sexual selection for female traits has been based on the consideration of females as mere choosers and not as active participants in competition for reproductive opportunities. This has led to an asymmetrical perspective which views sexual and social forces as placing greater selective pressure on male traits (Doutrelant et al., 2020). A sharpened focus on the selective pressures operating on females at the time of breeding should attempt to remedy this bias in the literature.

This PhD thesis represents therefore an approximation to an improved understanding of female behavioural strategies during reproduction in different social contexts, in both territorial and gregarious passerine birds. We have approached the subject through five different research objectives grouping the six chapters of the thesis.

1. REPRODUCTIVE STRATEGIES: EFFECTS OF FEMALE-FEMALE SOCIAL COMMUNICATION ON REPRODUCTION IN TERRITORIAL BIRDS

Animal signals were defined by Maynard-Smith and Harper (2000) as traits that change another organism's behaviour while benefiting the sender. They should have evolved for this function and obtain their effects through the evolved response of the receiver. They convey necessary information for survival or information that leads to an increased individual fitness of the sender, acting in this way as communication channels (Stegmann, 2009).

Birds use a complex signalling system including essentially two types of cues: acoustic and visual. Variation in the song repertoires (intensity, duration and tone) and plumage traits (size, color or shape) that birds exhibit may transmit different types of information to conspecifics (Schaedler et al., 2020). A large body of literature has examined the signalling function of avian plumage traits, that are widely accepted to be important in both sexual (Ian & Hartley, 1998) and nonsexual communication (Senar, 2006). Plumage signals, both based on pigments and feather structure, have been attributed to mediate territory defence and/or attract potential sexual partners and are included in more complex, multimodal displays. They potentially inform about the quality condition and/or social status of the sender.

Two main hypotheses have considered the meaning of female signals. Firstly the genetic correlation hypothesis is based on the assumption that male and female phenotypes cannot evolve completely independently due to the correlated selective responses in both sexes (Lande, 1987). In this case, female traits would be partially the result of evolutionary forces acting on male signals through the genetic correlation between sexes. However, more recent theories argue that female showiness may have an adaptive meaning (Amundsen, 2000), resulting from sexual and social selection through male mating preferences or intersexual competition (Doutrelant et al., 2020).

Communication between same-sex individuals (or intra-sexual communication) usually occurs in cooperative (Krams et al., 2010) or competitive contexts, operating over limited resources like food, nesting-sites or mates in reproductive contexts. This type of competition might be even more relevant in territorial solitary species whose need to retain the exclusivity of certain resources during the reproductive season has direct implications for their fitness (Deng et al., 2010). Thus, social selection in females may operate to secure necessary resources for breeding (Tobias et al., 2012), favouring intra-sexual competition (Gowaty, 1981). The expression in females of male phenotypic signals in non-sex role reversed species is increasingly found to be correlated with female dominance (Moreno et al., 2014). Thus, such characters may reflect competition for nesting sites especially in cavity nesters, since this type of nesting resource is particularly scarce and sought after. What is more, given intraspecific synchronicity in the reproductive season, this competition may operate more strongly between individuals of the same species, considering the limited availability of breeding resources in a specific time period.

This block encompasses **Chapter 1.**

2. MATING STRATEGIES: DRIVERS OF EXTRA-PAIR PATERNITY IN FEMALES OF TERRITORIAL BIRD SPECIES

Mating systems reflect the result of natural selection on mate choice, and ultimately on strategies for maximizing individual reproductive success (Andersson, 1994). They describe how males and females choose a mate and reproduce. Different mating strategies range from the pairing of a single male with a single female (monogamy) to the association of one male with multiple females (polygyny), or on the contrary, the aggregation of many males and one female in its different forms (polyandry) (Wittenberger, 1979).

Under strict monogamy (the most common strategy in birds but rare in other taxa), the number of mating events is constrained to one per individual, and differences between the sexes in the strength of sexual selection are small (Petrie & Kempenaers, 1998). However, copulations with individuals outside the social pair bond are very common in monogamous systems, resulting in a variable percentage of extra-pair paternity (EPP). During the last two decades this phenomenon has been intensely studied revealing that around 90% of monogamous bird species present cases of offspring sired out of the social pair-bond (Griffith et al., 2002). Clearly, EPP increases the number of mating events and thereby creates additional opportunities for male–male competition (Forstmeier et al., 2014). It has the potential to alter the strength of sexual selection experienced by males. However, the benefits of mixed paternity are less obvious in the case of females, and there are still controversies in the studies that try to address this issue (Forstmeier et al., 2011, Westneat & Stewart, 2003a).

Evidence of direct and indirect benefits for females has been found in several studies, increasing opportunities to enhance the quality of the offspring (Fossøy et al., 2008). This frames a female choosiness scenario in which it would be expected for females to select high-quality extra-pair sires. To test this hypothesis differences in several traits between the social mate and the extra-pair male or their respective progeny have been compared, including age (Moreno et al., 2010b, Girndt et al., 2018), body condition (Kempenaers et al., 2001, Charmantier et al., 2004, Moreno et al., 2013a), survival (Johnsen et al., 2000), immune response (Wilk et al., 2008), heterozygosis (Moreno et al., 2013a, Hsu, 2014) or the expression of plumage ornaments (Edme et al., 2016, Mitrus J., 2014). Some of these studies have found evidence of benefits accrued from mating with extra pair partners, but this effect is by no means widespread. Furthermore, it remains controversial if those signs are truthful indicative of genetic quality (Dean et al., 2010, Kokko, 1998). It has also been found that females are more likely to pair with highly heterozygous extra-pair males in some cases ((Mingju et al. (2017), but see Reid et al. (2015)). Another adaptive hypothesis argue that females ensure fertility through extra-pair behaviour (Griffith, 2007). However, male infertility is rather common, and mating with more than one male can cause polyspermia which can result in embryonic death (Hasson & Stone, 2009, Griffith, 2007). Finally other adaptive theories have been based on infanticide avoidance, arguing that females prevent aggression to their offspring by engaging with EPP males who could damage their progeny (Wolff & Macdonald, 2004), or that females try to obtain a stronger contribution from cooperative parental care through EPP behaviour (Kleindorfer et al., 2005). As results from these studies even in the same or closely related species are contradictory, there are yet no firm conclusions and it is unclear whether these patterns reflect causes or consequences of female promiscuity (Lifjeld et al., 2019, Fossøy et al., 2006).

On the other hand, the sexual conflict hypothesis (Trivers, 1972) derived from sexual selection operating on both sexes towards conflicting ends (Bateman, 1948), posits that males may drive the incidence of EPP through their tendency to seek copulations independent of female choice (Westneat and Stewart 2003; Arnqvist and Kirkpatrick 2005). Females in many cases may be selected to resist EPC attempts by extra-pair males but be unable to completely avoid them. Evidence for this hypothesis arises from observations showing that sexual forcing is not rare in birds (McKinney & Evarts, 1998, Brekke et al., 2013) and from evidence showing costs for females from a reduction in parental investment of the social male (Arnqvist & Kirkpatrick, 2005) or sexually transmitted diseases (Poiani & Colin, 2000). In addition, in some species females never initiate EPCs or even try to avoid them by escaping (Alatalo et al., 1987) and have low fertilization success (Birkhead et al., 2001). Also, more recent studies (Lifjeld et al., 2019) based on comprehensive multivariate analyses found that the effect of sexual selection disappears from the first variables affecting female promiscuity. The incidence of EPP in different populations may depend on the relative strength of selection for or against involvement of EPC in the two sexes. Evidence for sexual conflict in several studies argues against the generality of female benefits (Westneat & Stewart, 2003b, Low, 2005, Sigrunn, 2008, Arnqvist & Kirkpatrick, 2005).

This block encompasses **Chapters 2 and 3.**

3. MATING STRATEGIES: DRIVERS OF EXTRA-PAIR PATERNITY (EPP) AND SOCIAL CONTEXT IN TERRITORIAL BIRD SPECIES

Any behavioural trait is always product of the interaction of two main components: the innate or heritable, and the environmental. Models of sexual selection assume that mating preferences are heritable, and thus, repeatable for individuals across multiple mating episodes (Lande, 1981). Estimates of heritability are often difficult to obtain and several studies have instead examined the ‘repeatability’ of mate choice, or what is the same, its individual consistency (Boake, 1989). Repeatability combines both genetic and environmental components of variation in a trait, and sets the upper limit for heritability (Falconer, 1981). A high repeatability is not sufficient but necessary to ensure heritability for individual preferences, because it depends on the level of environmental variation and whether genetic variance is additive or non-additive. However, low repeatability implies that heritability is also low (Dohm, 2002). Thus, having a repeatability estimate is a first step towards examining the evolutionary potential of individual mate choice in sexual selection. As mentioned, extra-pair matings are widespread in avian species, and they are expected to impact on sexual selection (Whittingham & Dunn, 2005). Therefore, the study of individual consistency in EPP is an important contribution to the knowledge of its evolution.

In addition, individual reproductive behaviour is the consequence of interactions between several individuals in a society. Specifically, EPP is due to the associations between the social pair and extra-pair males (Canal et al., 2011), in a context where each sex has its own reproductive interests which respond to its own traits and social characteristics (Westneat & Stewart, 2003a, Forstmeier et al., 2014). Contextual characteristics and ecological constraints like reproductive synchrony and breeding density (O'Brien & Dawson, 2008, Hoi-Leitner et al., 1999) will also contribute to shape the resulting extra-pair behavioural pattern (Richardson & Burke, 2001). In summary, to ascertain the factors that shape variation in EPP, an integrative view that includes the social context is essential (Brouwer & Griffith, 2019). However, many studies of EPP disregard the social context or are unable to checks its effects.

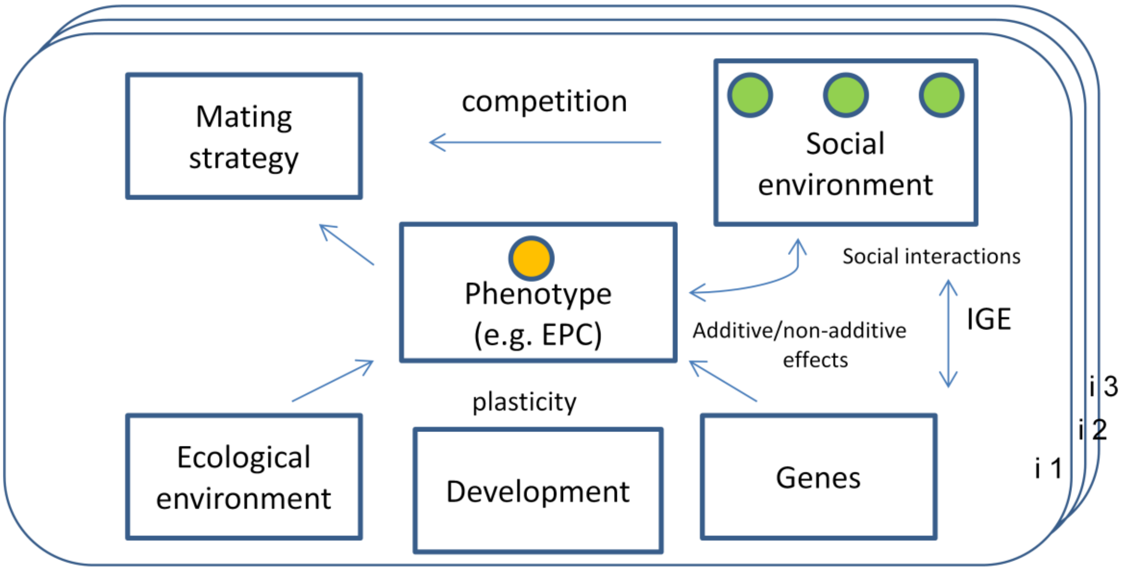

Fig. 1. Individual mating system diagram (i1-i3 represent different individuals in the population). Potential environmental and genetic effects on individual mating strategies are represented. An individual's phenotype (i.e. EPC, extra-pair copulation) results from direct genetic effects, early environmental variables determining their development, variability in ecological (physical) conditions and changes in the social context throughout their life. Also individuals can be affected via indirect genetic effects (IGEs) from their social environment (indicated by the green circles). They can respond to changes in the environment through plasticity. Note that individuals can modify their social environment and that the social environment can influence an individual's phenotype (e.g. tendency to mate outside the pair-bond; yellow circle) through its effect on the mating strategy. Thus the social and mating interactions among individuals in the group will determine the mating system. Modified from Maldonado-Chaparro et al. (2018)

This block encompasses **Chapter 4.**

4. SOCIAL STRATEGIES: SOCIAL NETWORK METRICS AS PERSONALITY TRAITS IN GREGARIOUS BIRD SPECIES

Behaviour has been shown to be flexible, allowing animals to respond to different situations in a changeable environment, and in particular, under different social conditions (Réale & Dingemanse, 2010). Consistent differences among individuals in their behavioural responses both across time and social contexts have been termed animal personality traits (Krause et al., 2015) and have been recorded in a wide range of animal taxa. These traits can also be used to characterize individuals as belonging to behavioural 'types'. This is useful for establishing that behaviour in one context can be used to predict behaviour in a different one, given that it is consistent in the same individual (Sih, 2004).

The first studies on personality were focused on the main axes of behavioural variation including boldness (risk taking), exploration or activity and aggression (Réale & Dingemanse, 2010). Also, in group-living animals, personality predicts leadership and social foraging strategies and the structure of those interactions (Leblond & Reebs, 2006). However, personality has mainly been studied by regarding social traits in dyadic relationships or on a hierarchical basis (i.e. individuals are more or less aggressive) in short-term observations. Thus, they capture only a small part of the overall social complexity and ignore the potential importance of indirect relationships beyond dyadic interactions observed between focal individuals. Recent research acknowledges that the role of personality in complex social dynamics and group level interactions is best characterized by analyzing social networks (Krause et al., 2015).

A social network reflects the structural characteristics of the relationships among a number of individuals (nodes) connected via social ties (edges), and that can be used to find sexual partners, engage in foraging and antipredator behaviour, and to develop and maintain cooperative relationships (Krause et al., 2015). Through the social network concept we can define the individual social phenotype, characterized by its node-based metrics that quantify the number and intensity of connections and the position of this individual with respect to interacting others. Social network analysis provides a novel array for describing the social fine structure of animal groups and populations (Maldonado-Chaparro et al., 2018).

The study of social connections in complex populations has experienced an increasing research interest in the last few years, with first studies on the analyses of animal social networks being published in the first decade after year 2000 (Krause et al., 2015, Croft et al., 2008). Moreover, there have been conceptual and empirical advances in the study of personalities (Dingemanse & Reale, 2005). After Krause et al. (2010) mentioned the power that social network analyses could offer to the field of animal personality, only a few studies have directly integrated the relation between both concepts (Kulahci et al., 2018, Jacoby et al., 2014) or at least have mentioned the idea (Aplin et al., 2015) that social node-based metrics can be considered as animal personality traits.

Personality features have been shown to influence individual fitness (Dingemanse et al., 2004, Réale & Dingemanse, 2010) through a wide range of other traits, like dispersal and migratory tendency (Chapman et al., 2011), response to environmental perturbation or predation (Sih et al., 2004), interspecific interactions and competition (Webster et al., 2009) or divergence in habitat use and resource polymorphism (Wilson & McLaughlin, 2007). And specifically it has been reported influencing reproductive success and sexual behaviour (Chen et al., 2018, Godin & Dugatkin, 1996). Thus, studying the connection between social networks (considered as personality traits) and reproductive behaviour, becomes a step forward in the study of animal personalities. For this reason, block 4 encompasses **Chapter 5** and represents a prerequisite for the next block.

5. MATING STRATEGIES: INFLUENCE OF SOCIALITY BY NETWORK METRICS ON FEMALE EXTRA-PAIR BEHAVIOUR IN GREGARIOUS SPECIES

Sexual selection is a major force in shaping the structure of animal societies and the different forms of individual behaviour, for example through its interaction with mating system and sex roles. Sex differences in the strength of sexual selection are the primary causes of the sex differences in behaviour and morphology found in many species (Andersson, 1994).

There is increasing evidence that animals choose, modify, and in part create their environment and thus, the selection they experience (Laland et al., 1999, Oh & Badyaev, 2010). Their behaviours allow individuals to establish preferred associations with habitats in which they are likely to experience high fitness. Behavioural habitat selection thus has important evolutionary consequences that promote divergent evolution when organisms are exposed to novel selective environments (Duckworth et al., 2004). In the same way, individuals discriminate among different social contexts. More specifically, by interacting preferentially with certain conspecific individuals they can effectively modify their social environment and hence, the selection they experience (Lewis, 2008). This agrees with the perception that the social environment is a critical determinant of fitness in animals (Brent, 2015), and that sociality may offer potential benefits to individuals (Brent, 2015, Grabowska-Zhang et al., 2011). More social individuals are more likely to obtain benefits such as access to location of resources or potential threats (Atton et al., 2014, Brent, 2015) by establishing cooperative relations and reducing aggressive interactions (Grabowska-Zhang et al., 2011). Thus, although a cost is paid in terms of competition and disease transmission (Corner et al., 2003, Silk et al., 2017), they may improve their fitness by obtaining essential information from others. During the breeding period, and especially for females, social associations are probably crucial to overcome this sensitive phase in which offspring survival becomes essential to ensure their fitness. For instance, infanticide by other group members has been found in some colonial breeders (Veiga, 2003) and it is possible that stable social associations may buffer females against this. Several studies have reported the effects of the social environment on reproductive performance (Krams et al., 2008, Beletsky & Orians, 1989).

Reproductive behaviour also includes extra-pair mating behaviour, and any promiscuous activity involves at least four parties: the social male, the social female, one extra-pair male and his social mate if the extra-pair male is paired (Westneat & Stewart, 2003a). Therefore the occurrence of EPP will result from the interaction between these individuals, and their social behaviour interactions must be of importance for the resulting outcome. Social associations previous to the breeding period may impact on female future mating decisions, and more specifically, they may affect the choices of partners, including the choice of extra-pair partners (Beck et al., 2020).

To sum up, if behavioural traits can explain individual variation in promiscuity (While et al., 2009, van Oers et al., 2008), resolving the connection between mating strategies and the social environment may allow us to discover how sociality interacts with the resulting mating decisions.

This block encompasses **Chapter 6.**

AIMS AND HYPOTHESIS

The main objective of this thesis is to explore how social processes affect reproduction in different social systems of passerine birds by focusing on the role of females. Our goal is to increase the understanding of reproductive strategies in different social contexts, by studying two model species such as the pied flycatcher (territorial breeder) and the house sparrow (colonial breeder) with different social systems. This thesis is framed within the scientific field of behavioural ecology and it is divided in six chapters with the following specific objectives:

- **Chapter I.** Explore the implications for reproduction of female-female social communication through plumage signals by studying the effect of female territory defence/vigilance on incubation intensity in a competitive context in pied flycatchers.

  We hypothesized that female plumage badges act as signals of social dominance, allowing them to communicate their status to other female individuals in a context of competition for nest cavities. Thus, more dominant females, as expressed through certain plumage signals (white wing patches), may enhance their reproductive performance by decreasing their territory defence/vigilance and increasing incubation intensity.

- **Chapter II.** Explore the role of female mobility on the propensity to experience EPCs (and thus on their EPP levels) by studying the effect on EPP of an experimental reduction of wing area during the fertile phase in pied flycatchers.

  We hypothesized that flight ability confers females the capacity of searching for attractive extra-pair sires or of avoiding the unwanted attention of extra-pair males during their fertile phase. We apply an experimental handicap that reduces flight efficiency (increases wing loading and creates air turbulence through the wing surface) to a group of females and compare EPP in their broods with that of control broods. Thus, if EPP is the product of adaptive mate choice, experimental females with a higher wing loading would suffer a reduced capacity to both evade their guarding mates and locate extra-pair sires, showing therefore reduced levels of EPP. However, if EPP is driven mainly by sexual conflict, we would expect the opposite pattern.

- **Chapter III.** Understand if changes in female body mass during the fertile phase impacts on their EPP levels. By performing an experimental manipulation on female pied flycatcher body mass and thereby on their wing loading, through a drastic reduction of nest building effort, to study the impact of this manipulation during the fertile phase on EPP levels compared with the control situation.

  We test the hypothesis that increases in female body mass during the fertile phase will lead to increased EPP levels through either improved condition (enhanced capacity to find preferred extra-pair mates) or reduced flight efficiency (reduced capacity to evade approaches by unwanted extra-pair males). The consequences for EPP of the normally maximal masses attained by females at this reproductive stage have not been considered previously in the literature.

- **Chapter IV.** Explore the relative importance of the social context for determining EPP as well as the relevance of male and female traits in explaining its incidence in pairs. First we analysed individual consistency in EPP through the study of its inter-year repeatability in male and female pied flycatchers. Secondly, we performed comprehensive multivariate analyses including both male and female traits and ecological and contextual factors.

  Our aim was to elucidate if EPP is individually repeatable between years in which the social context for breeding individuals may differ. Furthermore, we wished to explore the associations of EPP with individual traits included in other studies and the role of breeding synchrony and phenology discussed in the literature on EPP. We aimed at detecting the unmeasured effect of a varying social environment as a main driver of EPP patterns through the lack of individual inter-year repeatability.

- **Chapter V.** Elucidate if social characteristics measured by social network node-based metrics are individually consistent among different contexts and time scales, and thus, if they can be considered animal personality traits. We analysed repeatability of social node-based metrics in male and female house sparrows in a wild and in a captive population and after long and short term perturbations.

  We hypothesized that social node-based metrics are individually consistent across contexts and time scales and thus that they can be considered animal personality traits.

- **Chapter VI.** Explore the association between social characteristics measured by social network node-based metrics and the intensity of extra-pair mating behaviour. We analysed the relation between social node-based metrics and the proportion of extra-pair copulations (EPC). We also performed an experimental design manipulating the social structure of individuals to study the effect of this manipulation on EPC behaviour.

  We hypothesized that more social females would show an enhanced EPC proportion assuming that EPCs may confer social benefits.

GENERAL METHODS: STUDY SITES AND SPECIES

These general methods are focused on the study sites and species. A brief description of the field methods are included in each study site and of the molecular analyses applied for paternity assignments, as well as of the social network analyses that are common to several chapters. A more detailed description of the specific methods used will be found in each chapter. Specific field methods are described in Chapters 1, 2, 3, 4 and 5; Chapters 2, 3 and 4 detail specific paternity analyses methodology; Chapters 5 and 6 explain how to obtain data and apply social network analyses.

STUDY SITES

Results of this thesis have been obtained at five different study sites; three wild areas: Valsaín, Lozoya and Lundy island, and two captive sites: Silwood Park and Seewiessen.

The studies described in Chapters 1-4 were conducted during the spring seasons of the years 2015 to 2017 in Valsaín (and also in Lozoya in chapter 4). The study presented in Chapter 5 was conducted during the winter seasons of the years 2013 to 2016 in the Lundy Island, Silwood Park and Seewiessen areas, and the study described in Chapter 6 during the winter and spring seasons of 2018 in Silwood Park. Description of the areas has been grouped by similarity as follows:

1. VALSAÍN AND LOZOYA

Both areas are montane forests of Pyrenean oak, *Quercus pyrenaica*, located at 1200m a.s.l. in Valsaín (40˚ 54'N, 4˚ 01'W) and 1500m a.s.l. in Lozoya (40° 58'N, 3°48'W), Central Spain. Environmental conditions in both areas are continental and strongly correlated between each other given the distance between them (20 km) and the habitat similarity, although population connectivity is relatively low as ascertained through the scarce presence of recruits. There are scattered pines *Pinus sylvestris* among the oaks while the shrub layer consists mainly of *Cistus laurifolius* in Valsaín and *Crataegus monogyna* in Lozoya. Some seasonal watercourses run through both areas.

A total of 435 and 100 nest-boxes respectively were placed in Valsaín and Lozoya and their occupation has been checked since 1991 and 2001 respectively (see Sanz et al., 2003 and, Moreno et al., 2019 for a general description). Breeding activities have been followed routinely every year and laying and hatching dates and brood sizes at hatching and fledging have been determined. Nest-boxes were placed hanging from a branch attached to a metal hook (Lambrechts et al., 2010), and they were occupied mainly by pied flycatchers, blue tits *Cyanistes caeruleus*, great tits *Parus major*, nuthatches *Sitta europaea* and rock sparrows *Petronia petronia* (the last species only in Valsaín).

To characterize the study area each nest-box location was identified with a Global Positioning System (GPS) waypoint. All the nest-boxes were cleaned every year after the breeding season.

Study site in Valsaín (Segovia), Spain

2. SILWOOD PARK AND SEEWIESEN

Those areas involve captive populations of house sparrows kept at the Imperial College in Silwood Park (Ascot, UK) and at the Max Planck Institute for Ornithology (Seewiesen, Germany) respectively. Both populations consisted in originally wild-caught animals born in 2005 and 2006 and their offspring born in captivity, on which a series of long term studies have been conducted (Girndt et al., 2017). All individuals were individually marked with a unique combination of three colored pvc rings and a numbered metal ring to be easily identified by sight. In addition, each sparrow was provided with a subcutaneous passive integrated transponder which is read by a receiver device attached in different positions depending on the experiment.

All aviaries were provided with *ad libitum* food consisting of dry mealworms (larvae of *Tenebrio molitor*) or sunflower seeds, nesting material based on cotton, heather branches and textile components, and nesting sites (nest-boxes) to avoid resource competition between individuals (Girndt et al., 2018). The same person cleaned the installation and replaced food and materials once per day to avoid them to run empty. Each aviary was divided in same-sized sections where individuals where located depending on experiment requirements.

Study site in Silwood Park (Ascot) UK.

3. LUNDY ISLAND

This area is the largest island located in the Bristol Channel, 19 km off the coast of Devon (51°10’N, 4°40’W). The population is based around a small village and farm, which are situated in the south-east corner of the island and cover an area of approximately 1 km^2. The Lundy house sparrow colony fluctuates around 30-40 breeding pairs per year. This wild population of house sparrows has been monitored since 2000 as most of them breed in the nest-boxes fixed at the walls of the stone houses (Cleasby et al., 2011). Around 150 nest-boxes have been installed. Inter-specific competition for them is non-existent because no other bird species of a size that could utilize the nest-boxes breeds on Lundy (Davis & Jones, 2007). Levels of migration reach a maximum of three birds every four years, making it a nearly closed population suitable for longitudinal studies (Lattore et al., 2019). Life histories of individual birds and a full pedigree of the population are available. Birds were also individually marked with the same system of rings described above, as well as with a subcutaneous passive integrated transponder read by antennas attached to each nest-box on the island (Sánchez-Tójar et al., 2018). Environmental conditions respond to the Atlantic influence and are relatively mild and windy. The vegetation on the plateau is mainly dry heath *Erica arborea* and a mosaic of acidic grassland and stone hills.

Study site in Lundy island, UK

STUDY SPECIES

In order to be able to offer a broad point of view on how the social environment impacts on reproductive behaviour, and to generalize concerning mating patterns, this thesis has been conducted on two model species of passerine birds with very different social and mating systems: a territorial and a gregarious species, namely the pied flycatcher *Ficedula hypoleuca* and the house sparrow *Passer domesticus*.

1. PIED FLYCATCHER

The pied flycatcher is a small (12-13 g) migrant passerine bird which breeds in the Palearctic region. Its wintering areas are located in the tropical zones of West Africa (Lundberg & Alatalo, 1992). It remains in the Iberian Peninsula and other areas of the Western Palearctic during spring and summer as a breeder (Merino & Potti, 1997). Being a natural cavity nester, the species quickly adapts to nest-boxes, which constitutes an important advantage for behavioural studies that need to capture and manipulate individuals. This has made it one of the most intensively studied birds (Lundberg & Alatalo, 1992).

The first males start arriving at their breeding areas in the mountains of central Spain in the middle of April to select a territory and attract females, which start arriving approximately one week later. Their territory includes the nest cavity and its immediate surroundings, and given the short breeding season (less than 3 months), pairs are able to raise only one brood. They moult the whole plumage in July-August before migrating to Africa in September. After pair formation, nest-buiding is carried out mainly by females during periods of 4-11 days (Moreno et al., 2009b, Moreno et al., 2010a), although some males can contribute (Martínez-de la Puente et al., 2009). In our study areas nests are essentially made of bark of *Cistus laurifolius* and of pine *Pinus sylvestris,* dry oak leaves and dry grass (Moreno et al., 2009a).

Females incubate alone during around 13-16 days (Lundberg & Alatalo, 1992), while males feed the females during this period (Moreno et al., 2011). The mean clutch in our study areas has six eggs, with some clutches containing 5 or 7 eggs. Nestlings fledge at ages of 15-19 days (Moreno et al., 2019) and both parents contribute equally to feeding them. From laying of the first egg the total duration of the breeding period covers 35-40 days.

The subspecies present in the Iberian Peninsula is *Ficedula hypoleuca iberiae* (Curio, 1960). It shows a strong sexual dimorphism after the partial molt in winter, just before spring migration. The potential functions and characteristics of the conspicuous black and white contrasting plumage in males have aroused much curiosity (Sirkiä & Laaksonen, 2009). On the other hand, females show a brownish-greyish colouration with less contrasting white wing and forehead badges. Studies on female plumage in our populations have convincingly shown their important role in social and sexual selection as signals of quality (Moreno et al., 2014, Moreno et al., 2013b) and dominance (Morales et al., 2007, Moreno et al., 2019, Cantarero et al., 2016).

Although the pied flycatcher shows a reproductive system based on social monogamy and biparental care, a relatively high percentage of genetic polygamy (and some social polygamy as well (Alatalo et al., 1984)) has been always detected in molecular studies, ranging between 13 and 25% of extra-pair offspring (Moreno et al., 2013a) in the Iberian populations. All this makes the species suitable for carrying out studies on its reproductive behaviour and more specifically, on extra-pair behaviour.

Female (left) and male (right) adult pied flycatchers

2. HOUSE SPARROW

The house sparrow is a non-migratory passerine bird with multiple broods per season. It is a gregarious species that roosts communally and feeds in flocks. There is a huge variability in its habitat preferences in the wild, being adapted to a wide range of latitudes and different environmental conditions (Cramp & Perrins, 1993) and showing a wide distribution throughout Europe (Anderson 2006). It becomes easily adapted to anthropic activity and is often found in cities as a human commensal (Choudhary et al., 2019).

This colonial breeder uses natural cavities to reproduce and readily accepts nest-boxes as nesting sites (Reynolds et al., 2019). Its diet is based on vegetable elements, seeds and some invertebrates that provide protein to the growing nestlings during the rearing period (Peach et al., 2014). Once the pair is formed, both members construct a nest, although male contribution is frequently higher. In our wild study population, nests are mainly formed of heather twigs, dry grass and feathers. Both males and females incubate (11-19 days) and the mean clutch size is around five. The species shows biparental care and nestlings are able to fly in around 10-14 days (Salleh Hudin et al., 2017). Males defend their nests during the breeding period from conspecifics with aggressive displays and songs. Between one and four broods per season have been found in different populations during reproductive seasons that may cover the period from mid-April to July.

Its mass is 24-40 g depending on sex, age and condition, and it shows a marked sexual dimorphism (Summers-Smith, 1963). The main difference between sexes is the conspicuous black patch that males exhibit on the throat and chest, and the bib whose size has been shown to be a signal of social status (Nakagawa et al., 2007). Even though they breed with a single social partner, promiscuous behaviour has been regularly detected with an average of 17% of extra-pair offspring in broods (Hsu et al., 2017). Given the gregarious nature of house sparrows, the species shows a complex pattern of social and sexual relations including aggressive interactions, male pre-copulatory displays and female solicitations of copulations during the reproductive season (Girndt et al., 2018). This makes it a suitable species for studies on social and sexual network analyses and promiscuous behaviour.

Female (left) and male (right) adult house sparrows

PATERNITY ANALYSES

Parents and nestlings of pied flycatchers were genotyped in order to explore paternity. Type-it kits (QiaGen, Duren, Germany) were used to amplify approximately 5 ng of template DNA. Following published primer sequences described in Leder et al. (2008) we used ten pied flycatcher microsatellite loci for genotyping in two multiplex PCR reactions (Moreno et al., 2015). We amplified loci in two Multiplex PCR reactions (Table 1). In both sets the PCR program consisted in a denaturing step of 94°C during 2 min, then 30 cycles with 30 s at 94°C, 30 s at 55°C and 30 s at 72°C, and a final extension step of 2 min at 72°C. Fluorescently labeled PCR products were separated on a DNA analyser. Subsequently allele lengths were determined using Genemapper 4.0 software.

We then compared the genotypes of chicks with those of female and male nest owners by running a paternity analysis in CERVUS. We specified the identity of the mother for all chicks and the software assigned the genetic father from the sample of captured adult males (Westneat & Mays, 2005) if their genotypes were compatible for the assigned loci. We accepted a minimum number of 6 loci typed, a proportion of candidate parents sampled of 85%, using a 95% level of confidence, and allowing a 5%

proportion of mistyped loci. CERVUS assigned paternity to the male with the highest LOD (logarithm-of-odds) score (calculated by taking the natural log of the overall likelihood ratio; the likelihood ratio is the probability for the candidate parent to be the true parent divided by the probability for the candidate parent of not being the true parent). The combined non-exclusion probabilities of a second parent are specified in Chapters 2, 3 and 4. We accepted a candidate as the genetic father of a given nestling only when the difference between the LOD scores of the first and the second most probable fathers was statistically significant (Kalinowski et al., 2007). Those nestlings with two or more mismatched loci with respect to their social fathers were considered as extra-pair offspring. We also visually checked if those males assigned by CERVUS as fathers of extra-pair offspring matched the genotypes of the nestlings they were assigned to (Potti & Canal, 2011, 2010c).

Table 1. Microsatellite used in paternity analyses, including primer sequences, repeat motif of longest perfect repeat, size range (base pairs) and Multiplex PCR reaction (Mix).

Locus	Primer sequence (5'–3')	Repeat unit	Size (bp)	Mix
Fhy301	F-TTCCAGGTATATGTAACCAAAAC RGTTTATAGAAGCTGTGAATCCCTTAAT	$GTTT_{10}$	127-158	1
Fhy466	F-TCGGAATTGAAGACCTAGT R-GTTTGATGCCAAATAGTTATGAA	$GATA_{13}$	142-179	1
Fhy336	F-CTATGCAGTAGTCCTATTCAA R-GTTTCAAACAGATCCTAAGTAGAG	$GAAT_{12}$	127-204	1
Fhy370	F-CTCCAGGTAAGCAAACACAG R-GTTTCACCAACCTTCTTCATGTCA	$GAGT_{19}$	256-324	1
Fhy452	F-ATATCCAATAGCCGCAAACT R-GTTTGGATGCAGATTATTAGCCTT	$GATA_{13}$	308-341	1
Fhy328	F-CATGGTTTGAGGAGGTTGTT R-GTTTCAGGCTGATGCTGTTGTAAC	$GTTT_{11}$	134-182	2
Fhy223	F-TTCTCCTGGTCCTTAGCTTA R-GTTTCCATCTGCTTCTCTATCCC	$GGTA_{11}$	116-165	2
Fhy236	F-GGGTAGAGCCAAGATCCTG R-GTTTAGGTGCACACAGACATTGAGC	GT_{23}	154-200	2
Fhy304	F-GTTCCTCCTCTCATTAGTTC R-GTTTCTGTATCACTTGTAGCCATT	$GTTT_{15}$	222-263	2
Fhy407	F-AAAGTTAGCCTATGTCTACCAGA R-GTTTAGCTCTTCCCAGATTCTAAG	$GTTA_{15}$	196-241	2

SOCIAL NETWORK ANALYSES

Social network analyses are based on the construction of the social network model of a population. To do so we need to collect social data. With this purpose, we recorded individual interactions of captive house sparrows by using an automated radio frequency identification system (RFID) in a foraging context. Each individual was tagged with a passive integrated transponder (PIT), which provided a unique 10-digit code when scanned with a portable reader. Social associations were recorded inside a feeder fitted with two RFID antennas at the 'in' and 'out' sides of its entrances. The antennas were connected to a data-logging reader, that recorded the code of each individual, as well as the date and time of each event (Bonter & Bridge, 2011). We used the co-occurrence of two individuals inside the cage for longer than three seconds as a proxy for a social interaction (Maldonado-Chaparro et al., 2018).

We then constructed a social network per occasion (with weighted edgelists using the R package 'iGraph' v.1.2.4.1) and extracted three social node-based metrics: strength, betweenness and closeness (Csardi & Nepusz 2006). Strength is the number of direct connections of a focal individual with the others in the population, and it is weighted by the total number of interactions, so it represents the intensity of the sociability of an individual; betweenness is the number of shortest connections between a pair of individuals that 'pass through' the focal individual, and describes how well an individual connects different individuals of the network; and closeness is the length of the average shortest path between a focal individual and all the other individuals within the network, so it quantifies how central an individual is in the population (Csardi & Nepusz 2006).

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CHAPTER I

FEMALE INCUBATION ATTENDANCE AND NEST VIGILANCE REFLECT SOCIAL SIGNALING CAPACITY: A FIELD EXPERIMENT

This chapter reproduces entirely the manuscript:

Plaza M., Cantarero A., Cuervo J.J. & Moreno J. 2018. Female incubation attendance and nest vigilance reflect social signaling capacity: a field experiment. Behavioral Ecology and Sociobiology. 72:24

ABSTRACT

Due to the reduced conspicuousness of female signals, their evolution has traditionally been interpreted as a by-product of sexual or natural selection in males. Recent studies have argued that they may be the result of sexual or social selection acting on females. Here, we explored the role of the white wing patch during the incubation period in female-female competition contests in a migratory cavity-nesting songbird, the pied flycatcher *Ficedula hypoleuca*. At this stage, female investment is crucial for offspring survival, while competition among females for nest cavities is still operating. We experimentally performed an extreme reduction of signaling capacity by covering the wing patch with dark paint in a group of females and compared their incubation attendance and social interaction patterns (vigilance and aggression at the nest as defense variables) during simulated territorial intrusion tests with female decoys, with those of an unmanipulated group of females. Tests were performed both before and after the manipulation. We hypothesized that these patches constitute signals of social dominance in female-female interactions that allow them to maintain high levels of incubation attendance by reducing the need for vigilance. We found a marked decrease in incubation attendance in experimental females after manipulation, a change that was not found in the control group. Moreover, vigilance decreased in the control group after the manipulation, a change not detected in manipulated females. No effects on aggressive nest defense were found. Female wing ornamental traits would act as a badge of status in social interactions allowing more intense incubation and reduced vigilance. Implications of social interactions on incubation patterns should be incorporated in future studies

Keywords experimental patch manipulation, female ornamental traits, incubation behavior, nest vigilance, signaling status, social interaction.

INTRODUCTION

Animals can transfer information to conspecifics through morphological or behavioural traits, such as colored ornamental plumages or courtship displays (Maynard Smith & Harper, 2003). These signals may function to communicate phenotypic traits of the signaler like those involved in competitive capacity or reproductive efficiency. This type of communication has usually been studied from the male perspective, presumably due to the higher conspicuousness of male signalling traits, compared with those of females. Since Darwin (1871), the evolution of female ornamental features has been thought of as a by-product of sexual or natural selection in males through their genetic correlation (Lande, 1980). This conclusion has been supported by several studies (Muma & Weatherhead, 1989, Hill, 2006, Cuervo et al., 1996, Rohde et al., 1999, Dale et al., 2015). However some authors have argued that female signals may have an adaptive meaning (Amundsen, 2000, Jones & Hunter, 1993, Torres & Velando, 2005, López-Idiáquez et al., 2016). Two scenarios could lead to female functional signals. On the one hand, males may choose some traits in their mates, and females may compete for the attention of males (intersexual selection), so sexual selection could act on females in exactly the same way as it operates on males of many species (Clutton-Brock, 2009). On the other hand, because of their high energetic investment in gametes and parental care, females are expected to compete for limited resources during the breeding period (intrasexual competition), a form of social selection that is not sexual (Bleiweiss, 1997, Cain & Langmore, 2016), and conspicuous signals could be positively selected during these interactions by social selection (West-Eberhard, 1983, Rosvall, 2011, Soler & Moreno, 2012, Morales et al., 2014, Hegyi et al., 2008). Both possibilities are not exclusive, and vestigial trait expression in females resulting from genetic correlation with male traits, can be the starting point for further trait elaboration through sexual and social selection (Lyon & Montgomerie, 2012, Tobias et al., 2012, Dale et al., 2015). Thus, female sexual selection has recently been included under the wider concept of social selection (West-Eberhard, 1983, Lyon & Montgomerie, 2012, Tobias et al., 2012). In this context, it has been shown for instance that female song is widespread and ancestral in songbirds to communicate territorial defense or coordination of breeding activities (Odom et al., 2014, Langmore, 1998, Riebel et al., 2005, Webb et al., 2016). Again, there is recent evidence that in some species like the rock sparrow *Petronia petronia*, females collect specific materials to decorate their nests, to indicate their status to the rivals or predators (García-Navas et al., 2015).

Several scenarios have been proposed to explain the functionality of plumage badges of status (Senar et al., 2006, Maynard Smith & Harper, 2003, Rohwer & Ewald, 1981) as the expression of the concomitant costs of developing or bearing the signal (Senar et al., 2006). Theoretical studies suggested that only high quality individuals are able to sustain the cost of expressing an enhanced signal of status due to the behavioural cost of dominance interactions in a context of a high intrasexual encountering rate (Social Control Hypothesis) (Slotow et al., 1993, Ketterson, 1979) or of avoiding increased predation due to elevated conspicuousness (Differential Predation Hypothesis) (Fugle & Rothstein, 1987, Moreno Rueda, 2003). A physiological cost of badge production has also been proposed in relation with the cost of modified hormone levels or trade-offs with the immune response, that could be related to individual quality (Owens & Hartley, 1991, Poiani et al., 2000). In addition, information about social dominance obtained from the signals must be backed by corresponding dominant behaviour because potential opponents may be focused on both aspects simultaneously (Skeptical Receptor Hypothesis) (Rohwer, 1977, Maynard Smith et al., 1988, Caryl, 1982). From another point of view, some studies have proposed that social hierarchies may be the product of mixed evolutionary stable strategies, whereby individuals of different social dominance are able to coexist evolutionarily by allocating their resources differently, leading to general benefits from signalling social status (Maynard Smith, 1988).

In many species of birds the two sexes exhibit common plumage signals, although with more intense expression in males (del Hoyo et al., 1992), which makes it easier to investigate in this sex. Some plumage characteristics of females have been shown to be positively associated with individual rank and aggressive behaviour, for example the wing patch size of the collared flycatcher *Ficedula albicollis* (Hegyi et al., 2008), the ornamental facial patterns of the northern cardinal *Cardinalis cardinalis* (Jawor et al., 2004), the bib size in the social weaver *Philetairus socius* (Rat et al., 2015) and the colour and size of the crown patch in the golden-crowned sparrow *Zonotrichia atricapilla* (Chaine et al., 2011). Nevertheless, in some comparative studies the functionality of ornaments is very clear in one sex, but not in the other, as is the case of the throat patch in the Eurasian tree sparrow *Passer montanus* (Mónus et al., 2017) which is suggested to be a status signal in males but not in females, or the white flank spots in the diamond firetail *Stagonopleura guttata* (Crowhurst et al., 2012) and the breast patch of the rock sparrow *Petronia petronia* (Cantarero et al., 2017) that, on the contrary, seem to be reliable signals in females but not in males. Thus the same ornaments can have different functions in the two sexes.

Competition among female birds could operate in order to achieve access to preferred males (Petrie, 1983), to protect male parental investment (Slagsvold et al., 1992, Slagsvold & Lifjeld, 1994), or over resources necessary for breeding like territories or nest sites (Servedio et al., 2013, Stockley & Campbell, 2013), that directly influence the probability of reproducing successfully. It operates not only during territory acquisition, but also during the incubation period. This competition could be crucial in species with limited access to strictly necessary breeding resources like nest sites for cavity-nesting birds (Sandell & Smith, 1997). In this case, documented female defence behaviours against female intruders (Jawor et al., 2006, Goymann et al., 2008, Jawor & Ketterson, 2006) may have been favoured especially when nests can be taken over during the initial stages of reproduction (Rosvall, 2011). In this context, the female signalling function could be interacting with male behaviour giving a final result in terms of reproductive performance, so the influence of mutual ornamentation and male behaviour must be taken into account to correctly interpret the results of experimental manipulations of female signalling capacity (Kötél et al., 2016, Kraaijeveld et al., 2007).

Incubation is a crucial phase of avian reproduction, given the intense need for care of developing embryos and the impact of embryonic condition for subsequent nestling growth (Deeming & Ferguson, 1991). Thus, parental investment during this phase has important consequences for fitness (Reid et al., 2000). In many species like the pied flycatcher *Ficedula hypoleuca*, females incubate alone (Deeming, 2002), although males often feed them at this stage (Kötél et al., 2016, Cantarero et al., 2014, 2016b). This is why female behaviour during this period may become essential, as females must find an equilibrium between the time spent on feeding herself and attending the clutch in species with uniparental incubation, as predicted by the female nutrition hypothesis (von Haartman, 1958, Royama, 1966, Cantarero et al., 2016b). A factor not often considered when discussing the compromise between embryo thermal needs and the incubating parent's energy requirements, is the added demand to defend the territory, nest site or nest cavity against potential competitors. In some studies, aggressive competition between females has been detected (Moreno, 2015, Rosvall, 2011, Rosvall, 2008). Sometimes, these competitive interactions involve clutch destruction and replacement through egg burial and ejection during egg laying or incubation, and/or results in the loss of the reproductive season (Moreno, 2015). The intensity of competition between females during early-breeding phases may depend on the degree of limitation in the availability of resources crucial for breeding successfully, such as nest cavities or high quality mates or territories. Accordingly, there could be strong selection to advertise social dominance in the pied flycatcher during forays out of the nest while incubating (Morales et al., 2014, Moreno et al., 2013). Thus, social signalling at this stage could ensure adequate incubation attendance.

Here, we explored the role of a female plumage trait, the white wing patch, in female-female competition in a migratory cavity-nesting songbird, the pied flycatcher, during the incubation period. Both males and females exhibit a conspicuous white patch on the wing based on the white edges of tertials and secondary coverts and on white bands on some secondaries and primaries, male patches being larger and more conspicuous (Lundberg & Alatalo, 1992). These patches are exhibited by birds of both sexes in social interactions by repeatedly flicking the folded or partly folded wings (Curio, 1978). Sexual selection has been shown to act in favour of larger wing patch size in males (Sirkiä & Laaksonen, 2009, Sirkiä et al., 2010). Furthermore, females with larger wing patches breed earlier and have a higher hatching success (Morales et al., 2007). The extent of the wing patch in females is also positively linked to testosterone levels during incubation (Moreno et al., 2014, Cantarero et al., 2015, Cantarero et al., 2016a). Moreover, there is strong female intrasexual competition for nest cavities during the incubation stage (Moreno, 2015) as indicated by the intense aggression of territorial females towards female intruders during initial breeding stages (Moreno et al., 2016, Morales et al., 2014, Breiehagen & Slagsvold, 1988, Cantarero et al., 2015, Lifjeld & Slagsvold, 1989).

There is a large degree of variation in the extent of the white wing patch of females, ranging from highly conspicuous badges to barely noticeable feather edges (Moreno et al., 2014, Cantarero et al., 2016a). Given the positive links between the extent of female wing patches and testosterone, early breeding and hatching success, we hypothesized that these patches constitute signals of social dominance in female-female interactions that allow them to maintain high levels of incubation attendance by reducing the need for vigilance at this stage. To evaluate this hypothesis we experimentally performed an extreme reduction of female signalling capacity by covering the wing patch in an experimental group of females, and compared their incubation attendance and social interaction patterns during simulated territorial intrusion tests with female decoys with those exhibited by unmanipulated females. We predicted that the absence of the wing patch should be associated with an increase in territorial defence and a decrease in incubation attendance.

MATERIAL AND METHODS

General field methods

The study was carried out during the spring of 2015 in a montane forest of Pyrenean oak *Quercus pyrenaica*, located at 1200 m above sea level near the village of Valsaín, Central Spain (40˚54'N, 4˚01'W), where long-term studies on breeding pied flycatchers have been ongoing since 1991. In the area, 300 nest boxes (Lambrechts et al., 2010) are routinely checked during the pied flycatcher breeding season, lasting from the middle of April when the first males arrive, to the first days of July when the latest broods fledge. Nest boxes were checked every 4 days from April 15 to the finalization of nest construction, to detect the initiation and progress of nest building for every breeding pair. Afterwards, all the nest-boxes occupied by flycatchers were checked every 3 days to record laying date. As most females begin incubation on the laying of the penultimate egg (Ruiz-de-Castañeda et al., 2012) and the modal clutch size in the population is 6, we considered incubation to begin on the laying of the fifth egg (mean incubation period is 14 days). Hatching success was estimated as the percentage of hatched eggs in relation to clutch size. It was not possible to record data blind because our study involved focal animals in the field.

Female patch manipulation

Nests were assigned randomly to either control or experimental treatments on the 6th day of incubation. On that day, all females were captured in the nest box during daytime incubation without the need of using a trap, as at this stage they are not easily frightened away from the nest. They were identified by their rings or ringed if necessary, and a digital photograph of the natural white wing patch was taken from above at a height of 10 cm from the animal by placing the wing in its natural folded position on a flat surface with a ruler besides for reference, and forming a 135º angle with the wing. All photographs were taken with the same camera, and during the morning hours so that lighting conditions were always similar. The same photographic technique has been used in previous studies (Moreno et al., 2014, Cantarero et al., 2016a). After that, the experimental treatment was applied: experimental females were painted on the wing patch with permanent nontoxic water-based brown paint markers (GALIAN COGASA, Murcia, Spain), obtained by mixing 60ml of green, 20ml of red and 4ml of yellow to achieve a colour similar to that exhibited by brown dorsal body feathers, and within the natural range of plumage colour (Fig. 1). This colour was not estimated by using a spectrophotometer because this would have prolonged the manipulation in the field and augmented stress. This kind of paint is commonly used by pigeon keepers and is suitable for birds because it quickly dyes the feathers but keeps barbule integrity intact (and thus flight and insulation capacity), in contrast to non-water based paints. A reduction (albeit extreme) of the natural patch was simulated in this way in experimental females, while control ones were swabbed with water on the same area of the wing, as it was impossible to find in the market a nontoxic water-based white paint that did not affect feather integrity. The paint used in the experimental group had exactly the same texture and consistency as water, thus expanding on feathers and covering the whole white patch as soon as it was applied. This prevented the establishment of other experimental groups through covering only part of the white wing patch as would have been ideal, or through increasing patch size. In the field, we have observed a wide patch size range, from females showing large badges to others with practically imperceptible ones (Fig. 2). We assume that females in the experimental group approached the signalling capacity of some females with minimal wing patches, as observed in our population. In total, we included 31 experimental females and 31 control females. Manipulation intensity was identical for both groups, and it took the same time (around 5 minutes for the whole procedure per individual), so the potential stress suffered by all animals was approximately the same. All females resumed incubation after being put back on the nest and no nest was deserted after manipulation.

Fig. 1 Adult pied flycatcher female with (right) and without (left) applied treatment

Male ornamental plumage measurements

All males were captured in their nest boxes with traps while feeding nestlings of 7–8 days old (nestlings fledge 16 – 17 days after hatching). They were identified by their rings or ringed if necessary, and digital photographs of the white forehead and wing patches were taken using exactly the same methodology described for females. Moreover the percentage of black feathers on back and head was recorded on a 0 – 100 scale with 10 % interval scores, as they exhibit a melanin-based dorsal coloration.

This scale is strongly correlated with the Drost scale used by other authors (Galván & Moreno, 2009).

Photo analysis

Digital photographs were analyzed with Adobe Photoshop CS5 v.11.0. To estimate surfaces, the distance of 1 mm on the ruler was related to number of pixels. A zoom of 400 % and a paintbrush of 17 pixels, with 100 % hardness and 25 % spacing were used to score white wing patch areas estimated in cm^2 (Sirkiä et al., 2015).

Simulated territorial intrusions tests and video recordings

Simulated territorial intrusions were staged by presenting a pied flycatcher female decoy on a flat surface hanging on a branch, at approximately 0.5 m away from the nest-box, during the whole duration of the test. This distance was chosen because female aggressiveness towards intruders decreases with increasing distance to the nest, as the maximum level of aggressive behaviour occurs at a distance of less than 1 m (Rätti, 2000). Thus, we tried to maximize the potential effect of our treatment. As decoys, we used 4 stuffed females that had been found naturally dead in the same population in previous years, and that had been preserved at -20 °C until preparation (Morales et al., 2014). Tests were conducted in an identical manner twice, firstly three days before the experimental manipulation (3^{rd} day of incubation) and again three days after the experimental manipulation (9^{th} day of incubation). We randomly selected one decoy for each nest in both tests, taking into account not to repeat any decoy in the same nest for the second test, following the methodology of other studies (e.g., Morales et al., 2014, Moreno et al., 2014, Vergara et al., 2007). Every nest box and its surrounding area including the decoy were filmed during 40 min (first tests: mean ± SE = 42.6 ± 5.5 min, n = 62; second tests: mean ± SE = 41.3 ± 5.5 min, n = 62) with digital video cameras placed 50 m away from the nest box tree, and covering a space of approximately 2 m around the nest box.

All films were recorded from 8:00 to 12:00 h, and we did not find significant relationships between behavioural variables and time of day ($P > 0.10$ in all tests). We ignored if females were inside the nest box when we started video recordings, and we decided not to force them to escape from the nest box, as we did not want to introduce an unwanted factor in the analyses (scared females compared with non-scared ones). Due to time constraints, we could not wait until females left the box unprompted before filming. Thus, some females detected the decoy after naturally flying out of the nest box, while others did when returning after having left the box without being prompted. We assume that the response to the decoy would be similar in both situations. However, this precluded us from using latency time as a response variable. No evidence of stress or unnatural behaviour like extremely long absence periods from the nest were observed after the visit.

Fig. 2 Adult pied flycatcher females with different sizes of patches, ranging from a minimum signal (right) to a large one (left).

Behavioural data analysis

Films were viewed with VLC Media Player software always by the same observer, and from them we extracted the following information about female defensive behaviour: appearance on film estimated as the percentage of time that the female is in the field of view with respect to the observation time (vigilance), percentage of time considered spent at less than 30 cm from the decoy with respect to the time that the female appears in the film (proximity), number of flights towards the decoy per time unit (min) and number of attacks or physical contacts with the decoy per min (attack rate). Following Rätti (2000), proximity is significantly associated with female attack rate, so it is considered an index of aggressive disposition. Because of technical problems, we failed to record the behaviour at some nests, in which cases behavioural response variables could not be estimated (one nest each for the first and second tests).

We also estimated three incubation variables: number of incubation sessions per hour (recalculated as the number of incubation sessions per 60 min with respect to actual observation time), mean duration of incubation sessions (incubation rhythm), and percentage of time spent by the female inside the nest-box (intensity of incubation or “nest attendance”) which includes the time allocated to incubating and turning the eggs (Cantarero et al., 2015).

When an intrusion trial was started, three different situations could be present, and the observation time used to obtain the variables for each of these situations was calculated as follows: a) the focal bird was present outside the nest-box in the image within the field of view, so all variables were calculated using the total film time; b) the focal bird was not present within the field of view at the start and it arrived in the course of the film, so variables were calculated using the total film time excluding the latency time (the time that it took for the bird to appear); c) the focal bird was inside the nest-box, so variables were calculated in reference to the film time after she left the nest-box. No female took more than 10 min to notice the presence of the decoy, so the minimum time used to calculate variables was 30 minutes.

Male behaviour during incubation was also analyzed and two variables were extracted: male incubation feeding behaviour (number of feedings to the female per hour) and male presence (defined as the percentage of film time that the male was present within the field of view).

Statistical analyses

All the analyses were performed using STATISTICA package, version 10.0 (StatSoft, Inc., Tulsa, Oklahoma, USA). Due to the strong association among defensive variables on one side, and among incubation variables on the other, but the weak links between the two groups of variables, we performed two different principal component analyses (PCA) for defensive and incubation groups of variables respectively. Both sets of data (1st and 2nd tests) were included in these analyses.

For female defence variables, two factors were extracted by a principal component analysis (PCA1) that together explained 81.1 % of behavioural variation. The criterion for factor extraction was an Eigen value >1 and we used Varimax rotation to maximize the normalization of the variables. Factor1 explained 52.8 % of variance and was strongly positively loaded for vigilance and proximity (correlations were 0.97 and 0.96 respectively). Factor2 explained 28.3 % and was positively loaded for attack rate and flights towards the decoy per hour (0.78 and 0.82 respectively). Thus, Factor1 represents an index of female vigilance and Factor2 provides an index of aggression.

For female incubation related variables, the PCA (PCA2) extracted only one factor that explained 59.8 % of behavioural variation, where mean incubation sessions and incubation intensity were negatively loaded (correlations were -0.91 and -0.71 respectively). Therefore, this factor represents an inverse index of incubation attendance. This inverse index was multiplied by -1 before analyses.

Given that defence and incubation PCA factors were normally distributed, we used two-way ANCOVAs with repeated measures in one factor to analyze potential differences between groups in relation to the test comparison for the vigilance and defence factors. Two covariates were included in analyses: the laying date, since previous work has shown that females breeding later in the season tend to have higher levels of testosterone, which is positively associated with nest defence behaviour (Cantarero et al., 2015), and the wing patch size before manipulation as it has been related to individual signalling capacity in previous studies (Cantarero et al., 2016a) and thereby could interact with the effect of the manipulation (Osorno et al., 2006). Tests of collinearity to explore the influence of covariates on the vigilance, aggression and incubation factors were not significant, so the effects of the independent variables can be generalized. Our main prediction is that for the treatment to be effective, there should be a significant interaction between treatment and the temporal (test) factor. Differences between first and second tests within each experimental treatment were checked a posteriori with paired t-tests. As the requirements for parametric statistics were not obtained for hatching success, a Mann–Whitney *U*-test was used for comparing this variable between groups.

Male ornamental features were also analyzed in order to avoid unintended differences in male behaviour between experimental and control groups. We used *T*-Student tests to analyze the extent of the white wing and forehead patches given the normal distribution of these ornamental variables, and a Mann–Whitney *U*-test for the percentage of dorsal black, as the normal distribution was not obtained for this variable.

To analyze the interaction between male and female behaviour during incubation we performed two-way ANOVAs with repeated measures in one factor to compare male incubation feeding and vigilance behaviour during incubation in order to investigate potential differences between experimental groups in male behaviour caused by the treatment applied to the females. We also analyzed the correlation between male behavioural variables and the female incubation factor in the second test to ascertain if male behaviour directly affects female incubation attendance.

RESULTS

Our patch manipulation treatment had an effect on female vigilance, as shown by a significant interaction between treatment and test (Table 1, Fig. 3). This effect is due to an absence of significant differences in vigilance between first and second tests for the experimental treatment (paired t-test: t = -9.97, P = 0.339), in relation with a decline in the control group (paired t-test: t = 2.051, P = 0.048) (Table 1, Fig. 3). A significant interaction was found also between laying date and test, due to an increase in vigilance scores with laying date only for the second test. No effect of treatment on female aggression was found (Table 1).

We also found a strong effect on female incubation attendance scores as shown by a significant interaction between treatment and the test factor (Table 1, Fig. 4). There was a marked decrease in incubation attendance for the experimental group between first and second tests (paired t-test: t = 2.749, P = 0.009), while there were no significant differences between tests for the control group (paired t-test: t = -1.166, P = 0.252) (Table 1, Fig. 4). A significant interaction was found between laying date and test, due to a decrease in incubation scores with date only for the second test.

There were no significant differences between control and experimental nests in hatching success (U = 483.5, P = 0.5).

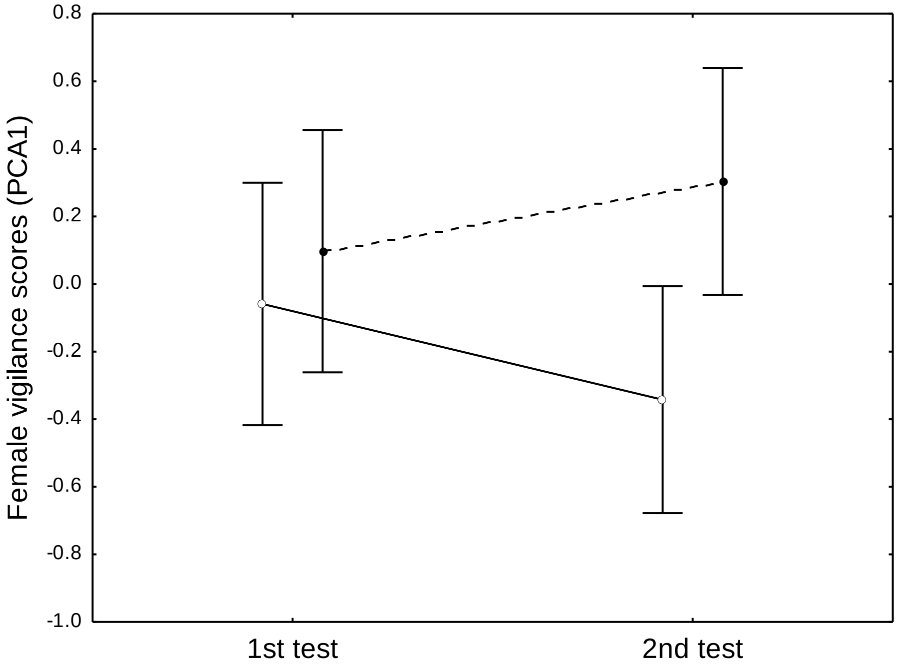

Fig. 3 Mean (±0.95 SE) vigilance behaviour scores (Factor1, PCA1) in “Experimental” and “Control” groups, 1st and 2nd test. Empty dots and continuous line represent the “Control group” while filled circles and dashed line represent the “Experimental group”.

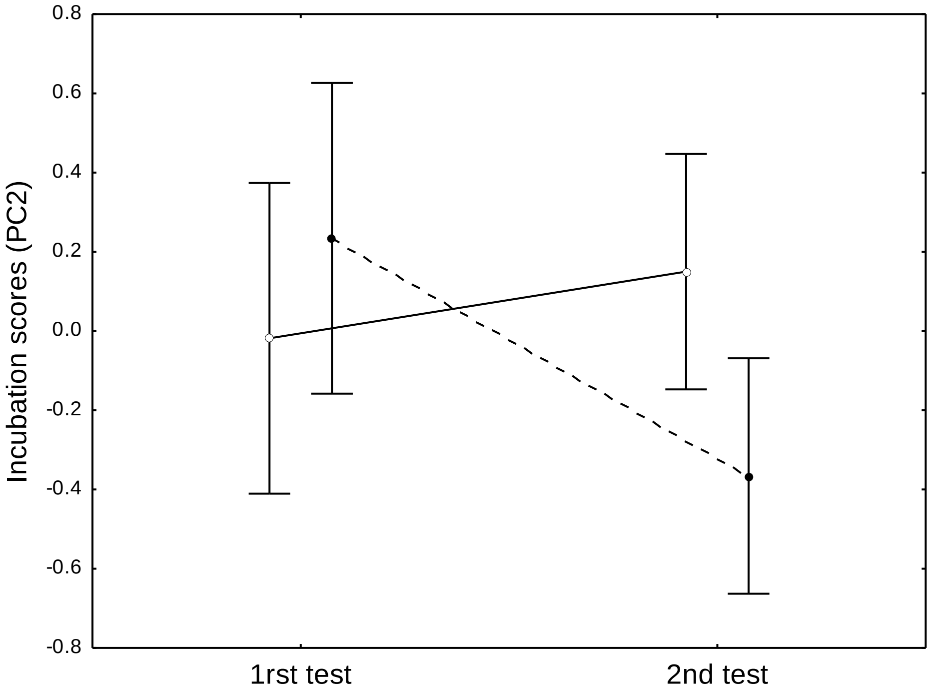

Fig. 4 Mean (±0.95 SE) incubation attendance scores (Factor1, PCA2) in “Experimental” and “Control” groups, 1st and 2nd test. Empty dots and continuous line represent the “Control group” while filled dots and dashed line represent the “Experimental group”.

Table 1 Two-way ANCOVAs with repeated measures in one factor for vigilance behaviour scores (Factor1, PCA1), aggression behaviour scores (Factor2, PCA1) and incubation attendance scores (Factor1, PCA2) in relation to experimental treatment ("Experimental" and "Control" groups), the test factor (before manipulation, 1st test, and after manipulation, 2nd test) and their interaction.

Response	**Predictor**	**Degrees of freedom**	***F***	***P***
Vigilance	Treatment	1	3.904	0.052
	Test	1	7.735	**0.007**
	Test*Treatment	1	4.176	**0.045**
Aggression Factor2(PCA1)	Treatment	1	0.695	0.407
	Test	1	1.883	0.175
	Test*Treatment	1	1.120	0.294
Incubation Factor1(PCA2)	Treatment	1	0.447	0.505
	Test	1	4.031	**0.049**
	Test*Treatment	1	8.699	**0.004**

We found a significant interaction between female treatment and male presence ($F_{1,31}$ = 4.05, P = 0.048), caused by a longer male presence in the experimental group in the second test. There was a negative, albeit no quite significant association, between female incubation and male presence (r = -0.245, P = 0.054), but we found no association between female vigilance and male presence (r = -0.04, P = 0.75). No effect of female treatment was found for male incubation feeding behaviour ($F_{1,31}$ = 0.05, P = 0.816). Furthermore there was no correlation between male incubation feeding behaviour and the female incubation factor in the second test (*r* = 0.021, *P* = 0.87).

There were no significant differences between experimental groups in the extent of the male white forehead patch (*t* = -0.01, *P* = 0.990), the male white wing patch (*t* = -0.65, *P* = 0.512) or male dorsal blackness (*U* = 449.5, *P* = 1.0).

DISCUSSION

Experimentally covered wing patches resulted in a marked decrease in incubation attendance and the absence of a decrease in female vigilance when an intrusion was simulated when compared with unmanipulated control females. No significant effects on aggressive nest defence or hatching success were detected. Male vigilance increased after the manipulation although it had no bearing on female incubation. In addition, females breeding later in the season showed significantly lower incubation attendance and higher vigilance scores after the experimental manipulation compared to females breeding earlier. No effect of the original white wing patch on treatment was detected.

The extent of white on female pied flycatcher wings, has been proposed as a signal of dominance through its association with testosterone levels during incubation (Moreno et al., 2014, Cantarero et al., 2015). Moreover, female vigilance towards an intruder is negatively associated with circulating levels of this hormone (Cantarero et al., 2015). Vigilance and aggression have been also related in other species to levels of progesterone (Weiss & Moore, 2004, Goymann et al., 2008) and corticosterone (van Duyse et al., 2004) or dehydroepiandrosterone and its conversion to other steroid hormones (Jawor et al., 2006), whose levels fluctuate throughout the reproductive period and interact with testosterone, leading to a joint behavioural expression. Thus, dominant females may enforce respect from competitors through signalling, thereby being able to reduce vigilance at the nest during incubation. The link between wing patch size and dominance in females is supported by the association of large patches with early breeding and improved hatching success in this species (Morales et al., 2007). Females with larger wing patches either arrive earlier at the breeding grounds or are more effective at securing a nest cavity, and they seem to be more efficient incubators as well (Morales et al., 2007). Here we show that late-breeding females without signalling capacity had to increase their vigilance more and incubate less intensively than early-breeding females, while no association between breeding date and female behaviour was observed prior to the manipulation. No replacement clutches have been detected in our population so they cannot be considered as a factor exerting differences in female's behaviour. These results support the increasing importance of the signal with decreasing female status as expressed by laying date.

The link between competitiveness and incubation efficiency could be mediated through plumage signals, if the latter are perceived by rival females as signals of resource holding capacity. We have proposed that females could ensure proper incubation attentiveness through signalling during their foraging forays out of the nest. Here we show that females being incapable of signalling with their wing patches incubate less intensively which could have detrimental effects on embryonic growth and development. Although we did not find an effect on hatching success, it has to be borne in mind that our disturbance by intruder simulation was brief and unique, and we don't know the real intruder pressure throughout incubation at the different nests. Presumably, only when exposed to intense intruder pressure, as in high-density populations, would reduce incubation intensity translate into heightened embryo mortality. However, more subtle effects on embryonic fitness due to reduced attendance cannot be ruled out (Hepp et al., 2015).

Incubation intensity would be affected by the need to frequently leave the nest to check on intruders and to try to evict them. This need would presumably decrease in the course of the incubation stage for two reasons: first, intruder pressure may decline with date given the seasonal decrease in reproductive prospects (Perrins 1970), and second, there may exist a territorial 'residency effect' (Davies 1978; Kemp and Wiklund 2004) whereby increased time since settlement leads to higher competitive capacity. These trends would explain why vigilance decreased from the first to the second test for control females. Females with experimentally concealed wing patches did not show this decline in vigilance from the first to the second test, possibly because they became more restless and insecure than control females. By leaving the nest more frequently and spending more time outside, they also reduced incubation attendance. Probably for the same reason, they did not attack intruders more than control females, as they would not be able to properly signal their status. An altered signalling capacity caused by experimental size modification of the female white forehead patch has been shown before in this species to cause a decrease in incubation attendance (Moreno et al., 2013).

Male ornamentation did not differ significantly between experimental treatments, so the quality or social status of mates can have hardly interfered with the experiment on females. Our results regarding male behaviour show no effect of the experiment on male incubation feeding frequency, but we found longer male presence caused by the absence of female signalling capacity. However, these changes do not clearly influence female incubation patterns, and what is more, it seems that those females whose males are more present at the nest-box may even incubate less. So that our results could be considered as conservative, pointing out that although the change in the female signals could potentially be perceived by males as a change in the quality of their mate (Kötél et al. 2016), female incubation can hardly have suffered because of loss of interest by mates as these increased instead of reducing their presence near the nest-box. Moreover, it seems that male presence can hardly explain the results of the experiment as it showed no significant association with female incubation attendance (the tendency if anything was negative) or female vigilance. Thus, we conclude that neither male quality nor male behaviour explain the results reported here regarding female incubation behaviour.

In the framework of our results, social selection might enforce a high pressure on female ornamental traits, and females, as well as males, would experience intense competition over ecological resources, which may select for traits that signal their competitive hability. This results, are consistent with those obtained in the collared flycatcher (Hegyi et al., 2008) during nest settlement, where the aggressiveness of females against simulated intruder female decoys was related to wing patch size. Similar results were found in studies of the function of status signals by recording female reactions towards taxidermy models or conspecifics in other species (Murphy et al., 2009a, Murphy et al., 2009b, Griggio et al., 2010, Midamegbe et al., 2011). The capacity to signal social dominance by females during nest box settlement and incubation through wing patches, may have associated physiological costs in terms of reduced antioxidant defences possibly mediated by social control. It has been observed in pied flycatchers that female oxidative status is associated with plumage badges, and that a reduced nest attendance may be the outcome of increased social interactions (López-Arrabé et al., 2014, Moreno et al., 2013). Thus, badges of status in female pied flycatchers may operate as badges of oxidative status as a consequence of female capacity to obtain resources necessary for breeding (Rosvall, 2011) in competitive circumstances.

This signalling capacity may also contribute to efficient incubation in the presence of significant intruder pressure in some populations. Clutch destruction and replacement is often observed in some populations, being accompanied in some cases by evidence of violent fights between females and destruction of eggs or hatchlings (Moreno, 2015). The importance of intrasexual dominance status on reproductive success due to aggression between females has been documented in various studies in different species (Jawor & Ketterson, 2006, Jawor et al., 2006, Goymann et al., 2008). Our findings suggest that female social requirements can influence incubation patterns, in association with other more commonly studied factors such as female food requirements and embryo thermal needs (Deeming, 2002, Deeming & Reynolds, 2015). The importance of female social interactions for incubation intensity has not been sufficiently stressed in recent reviews on incubation behaviour (Marasco & Spencer, 2015) and should be considered as an additional factor, modulating the evolution of incubation patterns.

Some studies have related incubation attendance to social or sexual signals but without experimentally manipulating the social context. In the closely related collared flycatcher it was found that, although the extent of the female white wing patch was negatively correlated with incubation intensity, the incubation behaviour of both partners was related to their own or to their partner's ornamentation (Kötél et al., 2016). So that plumage badges may act as potential signals of reproductive performance in both sexes. Similar results were found in plumage ornamental characteristics of other species like the european starling *Sturnus vulgaris* (Komdeur et al. 2005), the bar-throated apalis *Apalis thoracica* (van Dijk et al., 2015) or in some cardueline finches (Bókony & Liker, 2005), where negative associations between ornament expression and incubation duration were found. In contrast, plumage coloration in the great tit *Parus major* does not predict nest attentiveness of either parent, which is related mainly to environmental conditions (Matysioková & Remeš, 2010, 2011). However, all results of these studies were no experimentally related to female social competition.

In summary, information transmission patterns are complex, modulated by a combination of information sources and parental decisions, and highlight the role of signals under a complex picture that depends on a tangle of relationships, including hormonal interactions, life history traits and sex role components (Harris & Uller, 2009). Within this complex network, ornamental traits may be acting as quality signals. Female wing ornamental traits in our population should be therefore functional, and may act as a signal of dominance status in social interactions. This signal should not only be of importance during territory acquisition and mating but during incubation as well. Social selection in females should thus operate during a good part of the breeding cycle (Tobias et al., 2012). The implications of social status for incubation patterns should be considered in future studies.

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ETHICAL APROVAL

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Permissions for handling birds were provided by ‘Consejería de Medio Ambiente de Castilla y León’ (regional government with attributions on capture of wild birds). J. Donés, director of ‘Centro Montes de Valsaín’ allowed us to work in the study area. The study was ethically approved by the Ethical Committee of ‘Consejo Superior de Investigaciones Científicas’ (CSIC) and by the regional administration competent in matters related to animal protection in research according to Royal Decree 53/2013 (Dirección General de Producción Agropecuaria y Desarrollo Rural, Junta de Castilla y León).

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CHAPTER II

EXPERIMENTALLY FLIGHT-IMPAIRED FEMALES SHOW HIGHER LEVELS OF EXTRA-PAIR PATERNITY IN THE *PIED FLYCATCHER*

This chapter reproduces entirely the manuscript:

Plaza, M., Cantarero, A., Gil, D. & Moreno, J. 2019. Experimentally flight-impaired females show higher levels of extra-pair paternity in the pied flycatcher *Ficedula hypoleuca*. Biology Letters **15**: 20190360.

ABSTRACT

There is no consensus yet on the reasons why females engage in extra-pair copulations (EPC). In some species, females have been shown to accrue some indirect benefits, but these effects are not consistent across species and studies. The sexual conflict hypothesis posits that extra-pair paternity (EPP) is the result of strong selection for male pursuit of EPC without real benefits for females. In order to test this hypothesis, we experimentally reduced wing area (reversibly tying together some primary feathers), in a group of pied flycatcher females (*Ficedula hypoleuca*). The manipulation increases wing loading (body mass/wing area), which is negatively associated with flying ability, and thus with the capacity to escape from unwanted copulations. We compared the levels of EPP in this experimental group with those of a group of un-manipulated females. Experimental females almost doubled the proportion of extra-pair young (EPY) with respect to control females. In addition, more males sired EPY in experimental than in control broods containing EPY. These results suggest that in our study population, EPP could be partially a product of female capacity to avoid EPCs. We also discuss the alternative hypothesis that results might be due to an eventual reduction of female attractiveness.

Kewords extra-pair paternity, flying performance, mate choice, sexual conflict, female traits.

INTRODUCTION

Most socially monogamous birds show extra-pair paternity (EPP) (Petrie and Kempenaers 1998; Westneat and Stewart 2003) and, although 90% of them display bisexual parental care (Lack 1968), at least 70% also present cases of offspring sired by a male outside the pair bond (Griffith et al. 2002). However, even though EPP may constitute a strong driver of sexual selection, its incidence is poorly understood (Møller and Birkhead 1994; Brouwer et al. 2017).

The main adaptive explanations for female involvement in extra-pair copulations (EPC) propose that females may obtain indirect benefits (Griffith et al. 2002; Forstmeier et al. 2014) mainly by gaining “good genes”, enhancing heterozygosis or ensuring fertilization. Great effort has been made to test these hypotheses (Griffith et al. 2003; Krist and Munclinger 2011; Forstmeier et al. 2014; Boulton et al. 2018), but results are not clear even within the same or closely related species (Griffith et al. 2002; Westneat and Stewart 2003; Forstmeier et al. 2014). Contrary to this, the sexual conflict hypothesis argues that males may drive the incidence of EPP towards their benefit overriding female choice (Westneat and Stewart 2003; Arnqvist and Kirkpatrick 2005; Boulton et al. 2018). Costs for females derived from EPC have been mentioned in the literature (Alatalo et al. 1987; Poiani and Colin 2000; Birkhead et al. 2001; Arnqvist and Kirkpatrick 2005) while benefits have not always been found (Lifjeld 1997; Blomqvist et al. 2002; Moreno et al. 2010). For an extra-pair fertilization to be successful, it is required that an extra-pair male encounters a female and achieves copulation. In some species, females seek out such encounters, but in others males initiate them (Westneat 1992; Dickinson 1997; Kleven 2006). There is little information on the proportion of EPP obtained through female solicitation of EPCs, and male-initiated EPCs constitute the most commonly observed events in birds (Westneat and Stewart 2003). In some birds females never initiate or solicit EPCs but stay passive or try to escape from extra-pair males (Björklund and Westman 1983; Alatalo et al. 1987).

Under a female mate choice scenario, it is expected that older, more experienced or larger females should be more able to escape from their mate and more capable of selecting high-quality extra-pair sires (Bouwman 2005; Whittingham and Dunn 2010; Ramos et al. 2014). But if EPP is the consequence of sexual conflict (Westneat and Stewart 2003; Arnqvist and Kirkpatrick 2005), we expect it to depend on the social mate's mate-guarding capacity (Moreno et al. 2010), and on the female's ability to avoid unwanted extra-pair male encounters (Alatalo et al. 1987), which may vary with their size, age, social dominance (Plaza et al. 2018) or flight ability. A recent study (Moreno et al. 2015) found that older pied flycatcher (*Ficedula hypoleuca*) females, with longer wings, showed a lower occurrence of EPP, suggesting that traits related to capacity of avoiding unwanted males can decrease the incidence of EPP. Wing loading (body mass/wing area (Videler 2005)) has been theoretically and empirically negatively related to flight capacity at short distances (Pennycuick 1982; Kullberg et al. 2002) through the modification of the centre of gravity (Videler 2005), and in a sexual conflict scenario we should expect a positive relationship between female wing loading and EPP (Westneat and Stewart 2003).

In the present study we manipulated wing loading by reversibly reducing wing area in an experimental group of females, to investigate the effect of this manipulation on EPP in pied flycatchers, a model species in this context as it shows genetic polyandry (Björklund and Westman 1983; Alatalo et al. 1987; Gelter and Tegelström 1992; Ellegren et al. 1995; Rätti et al. 1995; Lifjeld et al. 1997). We had previous evidence of the effects of experimental modification of flying ability by reducing wing area in closely related species (Senar et al. 2002) which induces gaps in the wing and impairs flight performance (Kiat et al. 2016; Kiat 2017; Tomotani et al. 2018). Our prediction is that, if EPP is explained by an adaptive mate choice hypothesis, experimental females with a higher wing loading would suffer a reduced capacity to both evade their guarding mates and locate extra-pair sires, thus showing reduced levels of EPP. However, if EPP is driven mainly by sexual conflict, we would expect the opposite pattern (Björklund and Westman 1983).

MATERIAL AND METHODS

(a) Field methods

The study was conducted in 2017 in an oak *Quercus pyrenaica* forest in central Spain, where 300 nest-boxes have been installed (Lambrechts et al. 2010).

Nests were randomly assigned to either control or experimental treatments on the first day of construction (Moreno et al. 2009). On that day, females were captured by using a conventional nest-box trap (Cantarero et al. 2016). The trap was active for a maximum of 1 h to minimize disturbance. All females were identified by their rings or ringed if necessary and mass was recorded with a Pesola spring balance (accuracy 0.25 g). After that, experimental females were handicapped by taping primary remiges five to seven as described in Senar et al. (Senar et al. 2002). A rectangular notch was cut on each side of the 3 rachises and they were placed side by side. These remiges were tied all three together with a strip of tape within the notched area, thus creating two wing gaps (Fig.1). We also cut the notched area from the same feathers in control birds, but no strip was added. We took a digital photograph of the wing before and after applying the treatment in 8 experimental females. Pictures were analyzed to estimate surfaces (Plaza et al. 2018). Reduction in wing area was of 5.75±0.73%, which falls within the normal range for birds when molting (Hedenström 1998). On the 2nd day of incubation females were again captured in the nest-box during daytime. They were weighed and the tape in the experimental group was removed. Two females from the experimental treatment and two controls changed nest-box to restart breeding and they were removed from the experiment, so 24 control and 25 experimental nests were included in analyses.

There is previous evidence that only inseminations (and also extra-pair inseminations) occurring from day −2 (laying date=0) until the day the penultimate egg is laid (Von Haartman 1956; Alatalo et al. 1987; Chek 1993) result in fertilizations (Von Haartman 1956; Lifjeld et al. 1997).

All adults were captured during daytime while feeding nestlings of 7–8 days (nestlings fledge 16–19 days after hatching (hatching day=day 1). They were identified by their rings or ringed if necessary and again weighed. A sample of blood from the brachial vein (10–20µl) was taken and stored on Flinders Technology Associates reagent loaded cards (Whatman Bioscience, Florham Park, NJ, USA) until needed for paternity analyses. The following traits associated with EPP in a previous descriptive study of the same population (Moreno et al. 2015) were recorded: female age estimated from ring data, female wing length measured with a ruler and male dorsal blackness as percentage of black plumage on the mantle.

When nestlings were 13 days old they were ringed, and a small blood sample from the brachial vein was taken for paternity analyses. Carcasses and abandoned eggs found inside the nest-boxes were collected and frozen for paternity analyses through tissue extraction. Hatching failure affected 42 of 297 eggs in 24% of the nests. Of these, 15 did not show any trace of embryonic development suggesting that they were infertile.

Figure 1. Female pied flycatcher female showing the wing manipulation of the experimental group (primary remiges five to seven tied together).

(b) Genetic analyses

We collected samples from 49 families, all of them including the two social mates and their brood at 12 d of age (98 adults, 270 nestlings). DNA was obtained from blood samples using a standard extraction protocol that digests the cards where the blood is fixed and animal tissues from the carcasses and eggs. BioSprint Blood kits (QiaGen, Duren, Germany) were used to extract and purify genomic DNA and Type-it kits (QiaGen, Duren, Germany) to amplify it in the PCR.

For genotyping the samples we used 10 pied flycatcher microsatellite loci (Leder et al. 2008) and the PCR standard protocol described (Moreno et al. 2015). By running a paternity analysis in CERVUS 3.0.7 (Kalinowski et al. 2007) we determined parentage using the same published criteria (Kalinowski et al. 2007; Moreno et al. 2015) (see supplementary material).

(c) Statistical analyses

We explored possible differences between treatments in breeding variables by using *t*-tests for hatching date (normally distributed) and Mann–Whitney *U*-test for clutch size (not normal). We then compared changes in female body mass during the treatment period between groups to detect if differences in female flight capacity were caused by changes in wing area (unpaired *t*-test).

We analyzed two indicators of the intensity of extra-pair mating interactions, namely the incidence of EPP (presence/absence of EPY in the nest) and the proportion of EPY (number of extra-pair young divided by brood size) as dependent variables in two sets of Generalized Linear Models in R (v 3.5.3; R Foundation for Statistical Computing, Vienna, Austria) with binomial and quasi-binomial distributions to test the effects of our treatment on the dependent variables. We included as covariates the three variables that were significantly associated with EPP in a previous descriptive study in the same population (Moreno et al. 2015): female wing length, female age and male dorsal blackness. In the case of EPY proportion, we calculated the over-dispersion parameter (ĉ) in the full model and used this value to adjust the Akaike information criterion values (AICc), yielding quasi-AICc values corrected for over-dispersion (QAICc). We conducted the *dredge* automated model selection function (MuMIn package Barton, 2019) on all possible combinations of the 3 covariates. We report the conditional average models taking into account all models that differed in less than 4 units from the model with lowest QAIC.

We also compared the number of extra-pair mates in nests with EPP in the two treatments with a Mann-Whitney U-test. All values are presented with SE.

RESULTS

EPP was found in 22 out of 49 broods (44%) and affected 65 of 270 nestlings (24.1%). Nests with EPP contained an average of 2.95±0.41 EPY (range 1-6) and mostly involved one extra-pair male (66%). In six nests we found extra-pair sires that could not be identified.

No significant differences were found with respect to hatching date and clutch size between treatments (Table 1). Mass and mass changes between captures were not substantially different between groups (Table 1).

Our treatment caused a strong effect on the proportion of EPY, which was significantly higher in the experimental group (Table 2, Fig.2 and Table S4) and doubled the proportion of EPY with respect to the control group (effect size=58%). Treatment was included in 6 of the 8 most plausible models. The frequency of nests with EPP was 0.33±0.10 in the control group and 0.56±0.09 in the experimental group (effect size=41%). Treatment was conserved in 4 of the 8 most plausible models (delta AIC<4), with a near-significant effect in the average model including male dorsal blackness and female age and wing length (Table 2 and Table S3) although the null model had the lowest AIC.

Within nests with EPP, there were more extra-pair fathers per brood in the experimental (1.93±0.16) than in the control group (1.30±0.22) (Mann-Whitney U-test: Z=2.12, *P*=0.034).

Table 1. Average values (±SE) and results of Unpaired *t*-tests and Mann–Whitney *U*-test for relevant variables in the experimental and control groups.

	Control	**Experimental**	**Statistic**	***P***
Hatching date	61.87 ± 0.80	63.44 ± 0.79	*t* = 1.38	0.17
Clutch size	6.12 ± 0.12	5.96 ± 0.11	*U* = 256	0.37
Female mass I	13.48 ± 0.32	12.96 ± 0.30	*t* = -1.17	0.24
Female mass II	14.74 ± 0.17	14.49 ± 0.16	*t* = -1.00	0.32
Change in female mass	1.25 ± 0.34	1.47 ± 0.31	*t* = 0.46	0.64

Table 2. Average models calculated from the set of most plausible models for EPY proportion (number of EPY/brood size) (deltaQAIC<4) and EPP (occurrence/absence) (deltaAIC<4) as dependent variables with binomial distributions, and experimental treatment, female age, female wing length and male dorsal blackness as predictors (models in supplementary material). Estimates for the binomial model were transformed (antilogarithm) to convert them into odds-ratios.

	EPY				EPP			
	Estimate	Std. Error	Z value	*P*	Estimate	Std. Error	Z value	*P*
Treatment	1.49	0.38	3.74	<0.01	3.25	0.69	1.64	0.09
Female age	-0.03	0.17	0.18	0.85	1.36	0.33	0.91	0.36
Female wing	-0.52	0.19	2.61	<0.01	0.98	0.35	0.05	0.95
Male blackness	-0.01	0.01	1.19	0.23	0.98	0.02	0.88	0.37

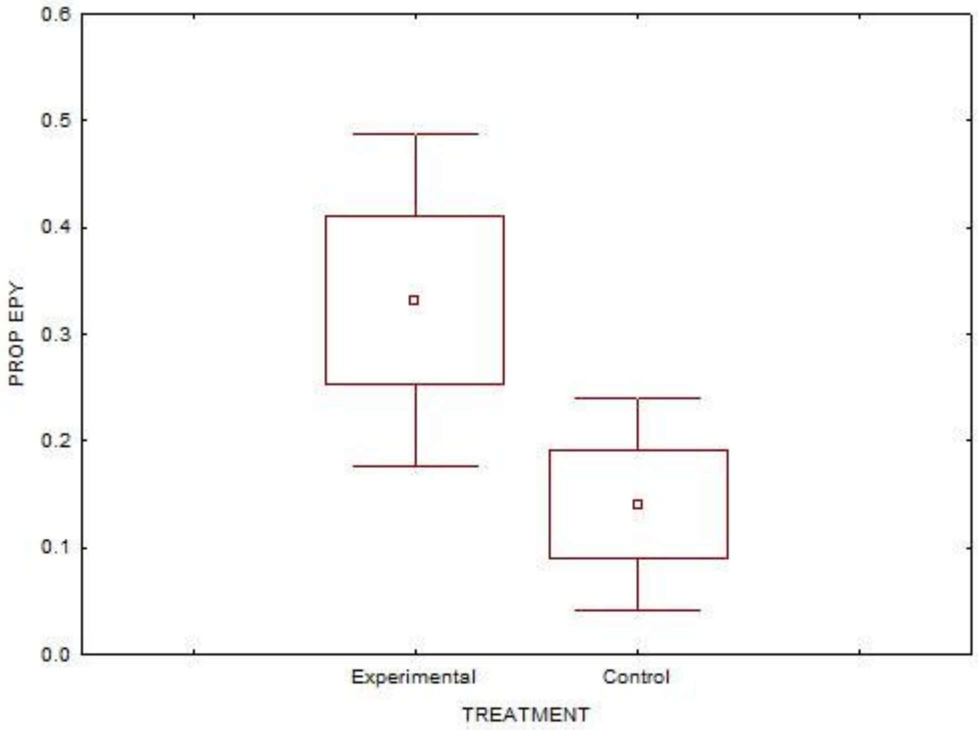

Figure 2. Proportion of EPY in "Experimental" (mean±SE, 0.33±0.06) and "Control" (0.14±0.06) treatments (Central points represent means, boxes SE and whiskers 95%CI).

DISCUSSION

We found that females with an impaired flight capacity caused by experimental reduction of wing area showed a large and significant increase in the proportion of EPY and a near-significant increase in the incidence of EPP, controlling for certain traits of the social mate or of the female involved. Experimental females effectively doubled the proportion of EPY in their broods with respect to controls. Moreover, a higher number of extra-pair mates fathered young in experimental than in control broods containing EPY. Brood EPP occurrence and percentage of nestlings affected are slightly higher than in other studies in the same population (28.8 and 13.1% in 2010, (Moreno et al. 2013a); 38.3 and 17.6% in 2011 (Moreno et al. 2015)).

The majority of the females from both groups increased their body mass during the experiment, although this increase was not significantly different between groups and it probably responds to a parental strategy to carry energetic reserves to lay high quality eggs. The absence of differences between treatments in mass variation suggests that any modification in female flight capacity was exclusively due to changes in wing area in the experimental group.

Our main results suggest that handicapped females were less able to escape unwanted copulations with extra-pair males. This increase in EPP frequency is compatible with a scenario in which the levels of EPP are influenced by male pursuit instead of female choice, and is backed up by previously published descriptive data from the same population (Moreno et al. 2015). This effect was robust after controlling for the possible influence of additional factors in EPP (Sirkiä and Laaksonen 2009; Moreno et al. 2013b). However, alternative explanations cannot be ruled out if we assume that the manipulation could lead to reduced female attractiveness. In this case, males paired to experimental females may experience: (1) reduced mate-guarding, thus allowing them to pursue EPCs; (2) reduced copulation rate or sperm transfer, leading to sperm-depletion (Pizzari et al. 2003). Both possibilities would lead females to show increased levels of EPP. However, we have no evidence that experimental females were less attractive to their males (Cantarero et al. 2014). Similarly, we could argue that handicapped females may seek EPC to secure feedings or protection from extra-pair partners, however there is no evidence that this happens in this species (Sonia Kleindorfer 2005).

Our results provide evidence in agreement with the idea that EPP may not be adaptive for females in some species being the result of strong selection in males (Arnqvist and Kirkpatrick 2005; Forstmeier et al. 2011). The occurrence of EPP is likely the result of behavioural and ecological issues (Westneat and Stewart 2003; Arnqvist and Kirkpatrick 2005; Moreno et al. 2015) in which both males and females interact. Since each of the players has its own reproductive interests depending on their own costs and benefits (Westneat and Stewart 2003; Arnqvist and Kirkpatrick 2005), our results suggest that in our study population, EPP is at least partially driven by extra-pair male pursuit and not female benefit.

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ETHICAL APROVAL

This study was approved by the Ethical Committee of CSIC and by regional authorities according to the Royal Decree 53/2013 to the projects CGL2013-48193-C3-3-P and CGL2017-83843-C2-1-P.

SUPPLEMENTARY MATERIAL

Genotyping

We used 10 pied flycatcher microsatellite loci for genotyping, following published primer sequences described in Leder et al. (2008). Two multiplex PCR reactions were designed as described before (Moreno et al., 2015), in which we amplified loci Fhy301, Fhy466, Fhy336, Fhy370 and Fhy452 in one reaction (set I) and Fhy328, Fhy223, Fhy236, Fhy304 and Fhy407 in the other (set II). We amplified approximately 5 ng of template DNA in the PCR. The program consisted in a denaturing step of 94°C during 2 min, then 30 cycles with 30 s at 94°C, 30 s at 55°C and 30 s at 72°C, finally an extension step of 2 min at 72°C. Conditions were the same for both multiplex sets. With 11, 14, 15, 20, 16, 16, 23, 11, 28 and 14 alleles respectively, all loci where polymorphic and a combined non-exclusion probability of second parent of 0.00000165 as calculated by CERVUS 3.0.7 (Kalinowski et al. 2007). Hardy Weinberg equilibrium tests after Bonferroni correction were not significant for four loci (Fhy336, Fhy466, Fhy452 and Fhy304), but in three of them (Fhy301, Fhy336 and Fhy407) the null allele frequency was higher than 0.05.

Paternity analysis

We determined genetic parentage by comparing the genotypes of chicks with those of female and male nest owners. If their genotypes were compatible for the loci typed, we considered that chicks were the offspring of the adults. We ran a paternity analysis in CERVUS by specifying the identity of the mother and allowing the software to assign the genetic father from the sample of adult males for all chicks. Those nestlings with two or more mismatched loci with respect to their social fathers were considered as extra-pair offspring (the mismatch never involved markers that deviated from Hardy–Weinberg equilibrium). In 56 cases Cervus assigned a non-social father inside the population male pool. Whereas in 9 cases it failed to assign a genetic father. Due to a single locus mismatch between the genotypes of the male and a chick could be due to mutation or genotyping mistakes, we considered these as extra-pair offspring indicating this decision to software. In the paternity analyses, we allowed a proportion of 5% mistyped loci and assumed that the proportion of candidate parents sampled was 85%, a level of confidence of 95%, with a minimum number of 6 loci typed. The software assigned paternity to the male with the highest LOD score (considering the likelihood ratio is the probability for the candidate parent to be the true parent divided by the probability for the candidate parent of not being the true parent, the LOD score is obtained by taking the natural log of the overall likelihood ratio). Only when the difference between the LOD scores of the first and the second most probable fathers was statistically significant, we accepted this as the genetic father of a given nestling. Nestlings with two or more mismatched loci with respect to their social fathers and considered as extra-pair offspring (56 cases in total) were assigned to non-social inside the population male pool. We could not assign a genetic father when the difference in LOD score between the first and the second most probable father was not significant (15 cases in total). However, we ensured if those males assigned by CERVUS as fathers of extra-pair offspring matched the genotypes of the nestlings they were assigned to by visually checking. Only one mismatch between females and offspring occurred in 9 cases, and in 12 cases with fathers.

Table S1. Ranked General Lineal Models (obtained with dredge and model.avg functions (MuMIn package Barton, 2019) with EPP (Occurrence vs. Absence of EPP) as dependent variable with binomial distribution and male dorsal blackness, female age and female wing length as factors. We report models that are within 4 units from the model with lowest AICc.

MODEL	df	AICc	delta	weight
Null	1	19.34	0.00	0.34
EPP~treatment	2	20.81	1.47	0.16
EPP~female age	2	21.33	1.99	0.13
EPP~male blackness	2	21.51	2.17	0.11
EPP~female wing	2	21.69	2.35	0.10
EPP~male blackness+treatment	3	22.90	3.56	0.06
EPP~female age+treatment	3	23.28	3.94	0.05
EPP~female wing+treatment	3	23.28	3.94	0.05

Table S2. Ranked General Lineal Models (obtained with dredge and model.avg functions (MuMIn package Barton, 2019) with EPY proportion as dependent variable with binomial distribution and male dorsal blackness, female age and female wing length as factors. QAIC values computed by calculating the over-dispersion parameter (ĉ) on the full model. We report models that are within 4 units from the model with lowest QAICc.

MODEL	df	QAICc	delta	weight
EPY~treatment+female wing	3	55.27	0.00	0.27
EPY~female wing+treatment	2	55.36	0.09	0.26
EPY~male blackness+female wing+treatment	3	57.33	2.05	0.10
EPY~male blackness +treatment+female wing	4	57.58	2.31	0.09
Null	1	57.67	2.39	0.08
EPY~female age+treatment	3	57.85	2.58	0.08
EPY~female age+female wing+treatment	4	57.88	2.60	0.07
EPY~female wing	2	58.86	3.58	0.05

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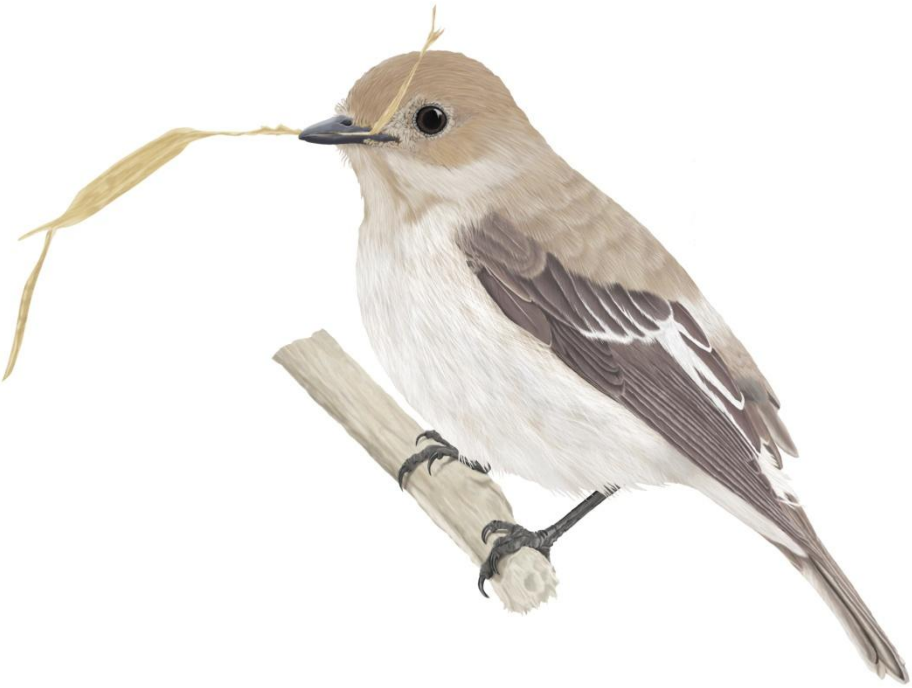
CHAPTER III

AN EXPERIMENTAL INCREASE IN FEMALE MASS DURING THE FERTILE PHASE LEADS TO HIGHER LEVELS OF EXTRA-PAIR PATERNITY IN PIED FLYCATCHERS *FICEDULA HYPOLEUCA*



This chapter reproduces entirely the manuscript:

Plaza, M., Cantarero, A. & Moreno, J. 2019. An experimental increase in female mass during the fertile phase leads to higher levels of extra-pair paternity in pied flycatchers *Ficedula hypoleuca*. Behavioral Ecology and Sociobiology **73**: 161.

ABSTRACT

Female mass in most altricial birds reaches its maximum during breeding at egg laying, which coincides temporally with the fertile phase when extra-pair paternity (EPP) is determined. Higher mass at laying may have two different effects on EPP intensity. On the one hand, it would lead to increased wing loading (body mass/wing area), which may impair flight efficiency and thereby reduce female’s capacity to resist unwanted extra-pair male approaches (sexual conflict hypothesis). On the other hand, it would enhance female condition, favouring her capacity to evade mate guarding and to search for extra-pair mates (female choice hypothesis). In both cases, higher female mass at laying may lead to enhanced EPP. To test this prediction, we reduced nest building effort by adding a completely constructed nest in an experimental group of female pied flycatchers (Ficedula hypoleuca). Our treatment caused an increase in mass and thereby wing loading and this was translated into a significantly higher EPP in the manipulated group compared with the control group as expected. There was also a significant negative relationship between EPP and laying date and the extent of the white wing patch, an index of female dominance. More body reserves at laying mean not only a higher potential fecundity but a higher level of EPP as well. This interaction had not previously received due attention but should be considered in future studies of avian breeding strategies.

Keywords Extra-pair paternity, wing loading, flight ability, nest building costs, female traits.

INTRODUCTION

Most female altricial birds show important changes in body mass in the course of the breeding cycle, increasing in mass before egg laying to a maximum just at laying, maintaining partly this high mass during incubation and losing it after hatching when feeding the chicks, thus returning to pre-breeding levels (Moreno 1989). This seasonal variation in female body mass has been interpreted as the result of a parental adaptive strategy and constitutes an important aspect of avian breeding biology. Firstly, a high body mass at laying would allow females to carry enough energetic reserves to lay high quality eggs, and then to keep a good condition when activity is reduced during incubation, when foraging is compromised. Later on, body mass would have to be reduced to enhance flying efficiency during nestling provisioning (Norberg 1981). Those changes in female body mass have been observed even in experiments where parents were supplementary fed (Moreno 1989; Sanz and Moreno 1995; Lothery et al. 2014). Changes in mass affect crucially female flight ability during the breeding cycle through the modification of wing loading (body mass/wing area) (Videler 2005), a trait that has been theoretically and empirically negatively related to flight capacity at short distances (Pennycuick 1982; Kullberg et al. 2002).

In the last two decades, increasingly accurate molecular tools have revealed that 90% of socially monogamous bird species show extra-pair paternity (EPP), resulting from mating outside the social pair-bond (Petrie and Kempenaers 1998; Westneat and Stewart 2003). Given its influence on fitness, EPP must be an important factor in sexual selection (Møller and Birkhead 1994; Griffith et al. 2002; Garamszegi and Møller 2004). However, although great effort has been made to test adaptive explanations behind extra-pair copulation (EPC) behaviour within and across species, there is yet no consensus on the key factors that are behind it (Griffith et al. 2003; Forstmeier et al. 2014; Boulton et al. 2018).

EPP results from the complex interaction between a female, an extra-pair male and the social mate, so the behaviour and traits of each of those parties is of importance for the resulting EPP patterns. Most adaptive explanations propose that females may obtain indirect benefits from EP behaviour (Møller and Birkhead 1994;

Forstmeier et al. 2014), e.g. through improving offspring viability trough the choice of more attractive extra-pair sires. Under this point of view, the outcome of EPP depends on the interplay of two factors. Firstly, on the social male's capacity to guard their mates and fight off male intruders, which is a function of his aggressiveness and dominance (Moreno et al. 2010b). And secondly, on the female's ability to evade mate guarding tactics (Alatalo et al. 1987), which may depend on her size, age (Bouwman and Komdeur 2005; Ramos et al. 2014), social dominance expressed through ornaments (Plaza et al. 2018) or flight ability (Stutchbury and Robertson 1987). In this respect, a high female body condition would favour the capacity of females to evade the attention of their mates and fly in search of extra-pair mates, roaming more easily through the breeding area.

In contrast with the above explanation, the sexual conflict hypothesis (Westneat and Stewart 2003; Arnqvist and Kirkpatrick 2005) derived from sexual selection, proposes that EPP results from a dynamic interplay in which both sexes strive towards conflicting ends. Under this scenario, strong selection in males to seek copulations independent of female choice would lead to higher incidence of EPP despite female costs to avoid EPCs (Arnqvist and Kirkpatrick 2005; Forstmeier et al. 2014). A consideration of female traits that relate to EPP may help us detect whether variation in female capacity to avoid EPCs explains EPP patterns. For instance, if EPCs are the result of male coercion (Westneat and Stewart 2003; Boulton et al. 2018), an increase in female mass would result in a higher wing loading which is translated into a reduced flight ability and a diminished capacity of the females to evade unwanted suitors. Indeed, such a negative relation between EPP and female flight ability has been found in some recent studies (Moreno et al. 2015; Plaza et al. 2019).

Bird nests have traditionally been considered as a simple receptacle for eggs and nestlings (Deeming 2013), while their functional characteristics in relation to avian reproduction have recently been taken into account (Cantarero et al. 2015b; Bailey et al. 2016). The costs of nest building have largely been documented (Hansell 2000) in terms of physiological stress for the builders (Morales et al. 2008; Moreno et al. 2008), their health and body condition (Tomás et al. 2006) or survival (Gill and Stutchbury 2005). The effort spent on this task may constrain reproductive behaviour during subsequent breeding phases, particularly so for the sex that is mainly involved in nest building. We have shown in a previous experiment that females whose nest construction costs are experimentally reduced, display improved body condition that results in a higher reproductive success (Moreno et al. 2010a). In many species, nest building precedes or overlaps in time with the fertile period and the time when reserves are accumulated in preparation for egg laying. Thus, we may expect that experimentally reducing or eliminating the cost of nest building may lead to an enhanced accumulation of reserves prior to laying (Moreno 1989), resulting in a higher condition but also in a higher wing loading during the fertile phase.

In the present study, we manipulated female body condition and wing loading, by drastically reducing female nest building effort in order to investigate the effect of this manipulation on EPP in pied flycatchers (*Ficedula hypoleuca*), a model species in studies of genetic polyandry e.g. (Ellegren et al. 1995). The manipulation involved adding a completely built nest to an experimental set of nest-boxes. In this species, nest building is conducted mainly (Gelter and Tegelström 1992; Martínez-de la Puente et al. 2009) or exclusively (Curio 1959) by the female. Our previous evidence shows that this modification of nest building effort exclusively increases female body condition (Moreno et al. 2010a), whereas a food supplementation experiment would have also affected males (Moreno et al. 1999). We test the hypothesis that increases in female body mass at this sensitive period will lead to increased EPP levels through enhanced condition or reduced flight efficiency. To take into account female quality and dominance we included laying date and the extent of a female social plumage signal as independent variables, as well as a plumage signal of the social mate's dominance.

MATERIAL AND METHODS

General field methods

This study was conducted during the spring of 2016 in a deciduous forest of Pyrenean oak *Quercus pyrenaica*, at 1200 m.a.s.l. near Valsaín, central Spain (40˚54'N, 4˚01'W). A total of 450 nest-boxes have been installed in this area since 1991, leading to a series of long term studies of pied flycatchers breeding in them (the bottom area of the nest-box was 175 cm^2 and the distance from the bottom to the entrance hole was 12.5 cm, Lambrechts et al. (2010)). The breeding season of this species lasts from the middle of April when the first birds arrive from migration, to the beginning of July when all chicks have fledged. We clean all nest-boxes every year after breeding is over. Daily checking was done from April 15 to detect the initiation and progress of nest building until the end. Afterwards, all occupied nest-boxes were checked every 2-3 days to record laying date (Julian calendar), clutch size, hatching date and brood size. The modal clutch size in the population is 6, and most females begin incubation on the laying of the penultimate egg (Ruiz-de-Castañeda et al. 2012) so we considered incubation to begin on the laying of the fifth egg (mean incubation period is 14 days).

Nest manipulation

The average reported time spent in nest building by flycatchers is 4 to 11 days (Curio 1959; Lundberg and Alatalo 1992; Moreno et al. 2008). Although intra-pair copulations have been reported 9 days before the laying of the first egg (Von Haartman 1956), experiments by Lifjeld et al. (1997) showed that only inseminations occurring from day −2 before the laying of the first egg until the day the penultimate egg is laid result in fertilizations. This short fertilisation window coincides in time with most observed copulations, which are confined to this relatively short period immediately before the start of egg laying (Von Haartman 1956; Alatalo et al. 1987; Chek et al. 1993). In the year in which this study was conducted (2016), a cold spell in May at the time of nest building led to delays in laying (the average time between the end of nest construction and laying date was 11±SE 0.57 days). This is in contrast with the typical pattern in which only a few days elapse between nest completion and laying (Moreno et al. 2010a). Thus, nest building did not overlap the period when females were fertile, so the effects of the experiment in terms of changes in EPP cannot be due to behavioural changes occurring during nest building. There was no association between the length of the interval from finished nest building to start of laying and EPP (Spearman´s rank correlation: $r_{57}=0.15$, $P=0.23$). This suggests that the degree of overlap between nest building activities and the fertile phase did not affect the results of our experiment.

We randomly assigned nests to either control or experimental treatments on the first building day, which was detected by the presence of a few nest material pieces placed in a circle (*Cistus laurifolius* bark strips and oak leaves). We discarded nests if they were more advanced than this early stage. In total 36 control nests and 23 experimental nests were included in the experiment. A full description of nest material composition for pied flycatchers in our study area is provided in Moreno et al. (2009). The manipulation consisted in placing a completed flycatcher nest inside the nest-box on the day when the treatment was assigned to the experimental group. Control nests on the contrary were not manipulated until they were naturally completed and simply exchanged for other completed flycatcher nests. In this way, we made sure that all active nests (where eggs were laid) had experienced the same level of human disturbance, with the difference that in the experimental group female building costs were greatly reduced with respect to the control group. Nest completion was determined by the same observer following the standard criteria of the presence of a rounded compact nest cup (Moreno et al. 2010a). All added (experimental) or exchanged (control) nests were obtained from freshly completed Pied flycatcher nests that we had previously found abandoned in the study area before hatching of nestlings in previous reproductive seasons, since when they had been frozen at -20ºC until use. We weighed all of them once defrosted and shortly before their usage, as well as all the substituted nests in the control group. No differences in mass between introduced (21.80±SE1.63 g) and substituted (24.20±SE1.45 g) nests were found ($F_{1,57} = 1.27$, $P = 0.48$). As in both groups females added some material after the manipulation, all nests were also weighed after laying so the amount of material collected by females was known for both groups (difference in mass between the supplemented nests and the final ones). Accordingly, the average total amount of material collected by control and experimental females was 24.72±SE1.50 and 5.46±SE1.88 g respectively, showing that control females provided almost five times as much material as experimental females, with the difference being significant between the two treatments ($F_{1,57} = 63.5$, $P < 0.01$). After manipulation no nest desertion was detected.

Capture and sampling

All females were captured on day 7 of incubation in order to weigh them after laying (capturing them sooner may lead to desertion), by simply blocking the nest-box entrance and catching them during daytime. Later in the season, all adults were captured in their nest-boxes while feeding nestlings of 7–8 days (nestlings fledge 16–19 days after hatching) by using a conventional nest-box trap set at the entrance of the nest-box (Cantarero et al. 2016b). The trap was active for a maximum of 1 h to minimize disturbance to adult birds and nestlings, and it was removed earlier if both adults were trapped before that time. No individual remained more than 5 minutes inside the nest-box after the trap closed. All birds were identified by their rings or ringed if necessary and mass was recorded with a Pesola spring balance (accuracy 0.25 g). Females were aged by their rings, and for the ones that were not ringed we assigned the age of 2 years (typical age at which females are recruited to the breeding population in our studies). We also measured wing length with a stopped ruler to the nearest mm. As a measure of female plumage ornaments, a digital photograph of the white wing patch was taken from above at a height of 10 cm from the animal by placing the wing in its natural folded position on a flat surface with a ruler besides for reference, and forming a roughly 135° angle with the wing. The same photographic technique has been used in previous studies (Moreno et al. 2014; Cantarero et al. 2016a). All digital photos were later analyzed with Adobe Photoshop CS5 v.11.0. to estimate surfaces with the reference to the ruler. A zoom of 400 % and a paintbrush of 17 pixels, with 100 % hardness and 25 % spacing were used to estimate white wing patch areas estimated in cm^2 (Sirkiä et al. 2015). The percentage of male dorsal blackness was estimated by scoring black feathers in the head and mantle at 10 point intervals from 5 (0-10%) to 95 (90-100%) (Canal et al. 2011). A small sample of blood from the brachial vein (10–20 µl) was taken and stored on Flinders Technology Associates reagent loaded cards (Whatman Bioscience, Florham Park, NJ, USA) until needed for the paternity analyses. All captures were performed between 8 and 10 a.m. in the morning.

We ringed all chicks when they were 13 days old (hatching day = day 1), and we similarly collected a small blood sample from the brachial vein for paternity analyses. All carcasses and abandoned eggs found inside the nest-boxes during regular checks were collected and frozen on the same day for later paternity analyses through tissue extraction. Hatching failure affected 20 of 348 eggs in 33% of the nests (N = 20). However, 13 eggs did not show any trace of embryonic development suggesting that they were infertile (this can easily be visually detected by examining the egg in contrast to the light). Moreover, 10 chicks (of two different nests) were predated so we left those nests out of the experiment.

Genotyping

We obtained samples from 59 families, including the two social mates and their whole brood at 12 d of age (112 adults, 325 nestlings). DNA was obtained from blood samples using a standard extraction protocol that digests the cards where the blood was fixed and animal tissues from the carcasses and eggs. We used BioSprint Blood kits (QiaGen, Duren, Germany) to extract and purify genomic DNA from the blood samples and Type-it kits (QiaGen, Duren, Germany) to amplify approximately 5 ng of template DNA in the PCR.

We used 10 pied flycatcher microsatellite loci for genotyping, following published primer sequences described in Leder et al. (2008). Two multiplex PCR reactions were designed as described before (Moreno et al. 2015), in which we amplified loci Fhy301, Fhy466, Fhy336, Fhy370 and Fhy452 in one reaction (set I) and Fhy328, Fhy223, Fhy236, Fhy304 and Fhy407 in the other (set II). The PCR program consisted in a denaturing step of 94°C during 2 min, then 30 cycles with 30 s at 94°C, 30 s at 55°C and 30 s at 72°C, finally an extension step of 2 min at 72°C. Conditions were the same for both multiplex sets. With 13, 14, 18, 17, 15, 25, 17, 29, 10 and 15 alleles respectively, all loci where polymorphic and a combined non-exclusion probability of second parent of 0.00000114 as calculated by CERVUS 3.0.7 (Kalinowski et al. 2007). Three loci (Fhy336, Fhy236 and Fhy452) significantly deviated from Hardy Weinberg equilibrium after Bonferroni correction, but in only one locus (Fhy452) CERVUS estimated a null allele frequency that was higher than 0.05.

Paternity analysis

We determined genetic parentage by comparing the genotypes of chicks with those of female and male nest owners. We considered that chicks were the offspring of the adults if their genotypes were compatible for the loci typed. To confirm this, we ran a paternity analysis using CERVUS (v 3.0.7. Field Genetics), specifying for all chicks the identity of the mother and allowing the software to assign the genetic father from the whole sample of adult males. In the paternity analyses, we used a level of confidence of 95%, we allowed a proportion of 5% mistyped loci and assumed that the proportion of candidate parents sampled was 85%, with a minimum number of 6 loci typed. CERVUS assigned paternity to the male with the highest LOD score (obtained by taking the natural log of the overall likelihood ratio; the likelihood ratio is the probability for the candidate parent to be the true parent divided by the probability for the candidate parent of not being the true parent). We accepted this as the genetic father of a given nestling only when the difference between the LOD scores of the first and the second most probable fathers was statistically significant (Kalinowski et al. 2007). We considered as extra-pair offspring those nestlings (82 in total) with two or more mismatched loci with respect to their social fathers by CERVUS (the mismatch never involving markers that deviated from Hardy–Weinberg equilibrium). From all these nestlings, 46 cases were assigned to a male which was not included in the population male pool (most probably a non-territorial floater). However, when the difference in LOD score between the first and the second most probable father was not significant, we did not assign a genetic father (36 cases in total). We also visually checked if those males assigned by CERVUS as fathers of extra-pair offspring matched the genotypes of the nestlings they were assigned to. We took a conservative rule, and considered as a father–offspring pair in 9 out of the 46 cases of extra-pair chicks assigned by the program, since these mismatched the social male in only one locus.

We considered that a single locus mismatch between the genotypes of the male and a chick could be due to mutation or genotyping mistakes, and for this reason we overruled the CERVUS decision of considering these as extra-pair offspring. One mismatch between females and offspring occurred in 14 cases (8 cases in the control group and 6 in the experimental group), and in 16 cases with fathers (7 cases in the control group and 9 in the experimental group).

Statistical analyses

We first investigated possible differences between groups in breeding variables (hatching date and clutch size) and relevant female and male traits which could influence the effect of our treatment. When they were not normally distributed we performed Mann–Whitney *U*-tests.

We then examined the effect of our treatment on female wing loading by performing an unpaired *T*-test as it was normally distributed. Following Moreno et al. (2015) we extracted an index of wing loading (g/dm^2) by dividing female body mass by the square of wing length (n = 47). This index was validated with direct measurements of wing areas in the field in a pilot study conducted on birds not included in the experiment. In this study conducted in 2017, wing area was estimated from photographs (n = 41) of the contour of flattened wings against a sheet of paper with a ruler for reference as described above. The correlation of the two measures shows that our index was an acceptable proxy for wing loading (Spearman's rank correlation: $r_{71} = 0.77$; $P<0.001$). As we wanted to examine potential changes in wing loading caused by the effect of our treatment on female body mass, we first checked for differences in female wing length and then also in female mass. To that end, we performed two unpaired *T*-tests as both variables were normally distributed. As these mentioned traits are related to age, we also examined differences between groups in female age by performing a Mann–Whitney *U*-test, due to its lack of normality. All analyses mentioned were done with the STATISTICA package, v 10.0 (StatSoft, Inc., Tulsa, Oklahoma, USA).

The incidence of EPP was analyzed in two ways. On the one hand, as a binary response (occurrence vs. absence of EPP) within nests by a univariate generalized linear model using the GENMOD procedure in SAS v9.4 (StatSoft, Inc., Tulsa, Oklahoma, USA), with a binomial distribution, to test the effect of our treatment on EPP occurrence. Three additional potentially relevant independent variables were also taken into account, trying not to add unnecessary complexity to the analyses. First, we included laying date since extra-pair behaviour could be influenced by the availability of reproductive individuals, which varies throughout the season as reproductive pairs are established. Second, we took into account the extent of the area of the female white wing patch (since we have previously shown that it is a predictor of individual social signalling capacity (Plaza et al. 2018) and territorial defence behaviour, through testosterone levels (Cantarero et al. 2016a). Finally we also included the social male dorsal blackness as a measure of his dominance which is positively related to the mate guarding effect, and significantly negatively related to EPP in previous published studies (Moreno et al. 2015). We also examined potential differences in those female and male characteristics between groups. On the other hand, we conducted a similar analysis using a different univariate generalized lineal model following the same procedure, but using instead the proportion of EPY (number of extra-pair young divided by brood size with “event/trial” syntax) as a measure of extra-pair paternity. All values are presented with standard error.

RESULTS

EPP occurred in 21 out of 59 broods (35.59%) and affected 82 of 325 nestlings (25.23%). We found non-identified extra-pair sires in five nests. The number of EPY in nests with EPP ranged from 1 to 7 nestlings, being on average 3.90±0.42 EPY, which represents 67.76±0.40% of the broods on average.

The two experimental groups were similar in hatching date and clutch size (Table 1, both $P > 0.40$). We did not find differences in female wing length and age between experimental and control groups (Table 1). However, we did find significant differences in female mass (Table 1), which was higher in the experimental than in the control group. Also the experiment was successful in inducing differences in female wing loading during incubation between treatments due to higher values in the experimental group with respect to the control one (Table 1).

Table 1. Average (±SE) values for breeding variables and female and male measurements in the experimental and control groups and results of Mann–Whitney *U*-test and Unpaired *t*-test analyses (day 1 = April 1) of Pied flycatcher (N=59).

	Control	**Experimental**	**Statistic**	***P***
Hatching date	67.91 ± 0.40	67.52 ± 0.50	$U = 20$	0.62
Clutch size	5.91 ± 0.09	5.86 ± 0.11	$U = 207$	0.40
Female wing length (mm)	77.38 ± 0.32	76.86 ± 0.40	$t = 0.99$	0.32
Female age	2.72 ± 0.23	3.00 ± 0.31	$U = 371$	0.50
Female wing patch size	1.35 ± 0.04	1.41 ± 0.07	$t = -0.61$	0.53
Male dorsal blackness	0.86 ± 1.31	0.87 ± 1.93	$U = 356$	0.76
Female mass (g)	14.14 ± 0.13	14.74 ± 0.16	$t = -2.79$	0.007
Female wing loading (g/dm^2)	0.23 ± 0.002	0.24 ± 0.003	$t = -3.07$	0.03

Our manipulation also caused an effect on the occurrence of EPP, which was significantly higher in the experimental group (Table 2, Fig. 1). The final model also included significant negative associations between EPP occurrence and the extent of the female white wing patch (Table 2; mean wing patch area for nests with EPP and without EPP were 1.28±0.07 and 1.42±0.05 cm^2 respectively; $t = 1.41$; $P = 0.16$), and between EPP and laying date (Table 2; mean laying date for nests with EPP and without EPP were 49.10±0.46 and 49.92±0.33 respectively, day 1=April 1 ; $t = 1.45$; $P = 0.15$).

We also found a significant effect of our treatment on the proportion of EPY (Table 2) which was higher in the experimental than in the control group (means for each group were 0.31±0.07 and 0.15±0.05, effect size was 67% following Nakagawa and Cuthill (2007). We also found significant negative relationships between the proportion of EPY and both the extent of female white wing patch and laying date (Table 2), although the associations on their own were not significant (white wing patch Spearman's rank correlation: $r_{57}= -0.19$, $P > 0.05$; laying date Spearman's rank correlation: r_{57}= -0.22, $P > 0.05$) (Table 2). There were no differences between groups in the extent of the female white wing patch and male dorsal blackness (Table 1).

Table 2. Results of GLM models with EPP (absence/presence), proportion of EPY (number EPY / number total young) as dependent variables and experimental treatment, laying date, female white wing patch and male dorsal blackness as predictors of Pied flycatcher (N=59).

	Treatment (control)			**Laying date**			**Female wing patch**			**Male dorsal blackness**		
	Estimate	**χ^2**	***P***	**Estimate**	**χ^2**	***P***	**Estimate**	**χ^2**	***P***	**Estimate**	**χ^2**	***P***
EPP	1.61	5.94	0.01	0.36	5.28	0.02	2.33	4.91	0.02	0.00	0.03	0.86
EPY	-1.36	5.43	0.01	-0.62	19.75	<0.05	-2.25	6.50	0.01	0.01	0.19	0.66

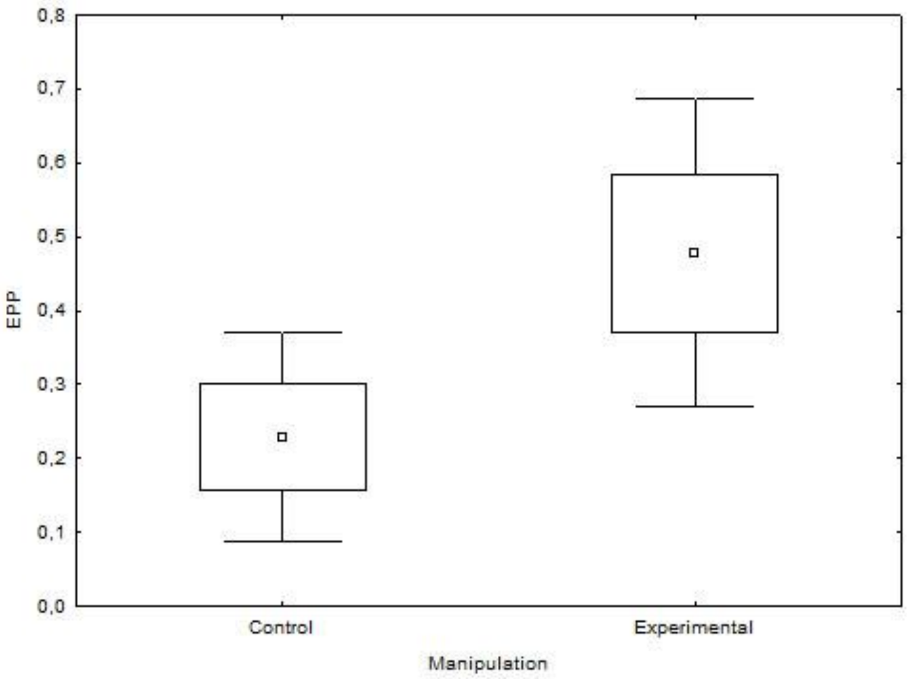


Figure 1. Proportion of pied flycatcher nests with EPP in the “Experimental” and “Control” groups (central points represent mean values, boxes represent Standard Errors and whiskers represent 95% confidence intervals, N=59).

DISCUSSION

The experimental reduction of nest building effort resulted in a significant increase in female body mass and wing loading and a subsequent increase in the occurrence of EPP and EPY in the experimental group. We also found that the probability of a nest containing EPP and the proportion of EPY were negatively related to the extent of the female white wing patch and laying date.

In the present study, control females took an average of 3 days to build the nest and collected almost 24 g of nest material, which is similar to values reported in other studies of populations breeding in central Spain (Moreno et al. 2008; Moreno et al. 2010a). Females constructed their nests at a rate of 6 g/day. These high rates may imply important energy costs as indicated by associations of building rate with female physiological costs in this species (Moreno et al. 2008), causing a significant effect on female body mass and therefore on wing loading, as we detected when comparing this variable between groups. Predation on adult females has been found to be high during nest building and egg laying, caused by vulnerability when collecting nest materials due to the increased female mass during this stage (Slagsvold and Dale 1996). However there was no predation in our study population in either of the experimental groups as deduced from the absence of cases of early nest abandonment.

Our experimental results showed that females of the experimental group displayed a higher condition and wing loading as well as higher EPP levels. These results are in accordance with a previous study reported by (Plaza et al. 2019), in which handicapped females with a diminished flying ability caused by an increased wing loading, also displayed higher EPP levels. Wing loading has previously been negatively correlated with flying capacity and a reduced manoeuvring ability (van den Hout et al. 2010; Salewski et al. 2014). In our treatment we found a higher body mass (translated into higher wing loading) in the experimental group during the incubation period (soon after our nest manipulation treatment was applied). Assuming that mass at incubation reflects mass during nest building, these results suggest that females that did not have to build a complete nest before laying could dedicate more time to feed themselves and increase their reserves to better provision their eggs with resources. Moreno et al. (2010a) found that a reduction in nest building effort was translated into increased offspring fitness.

One interpretation of our results would support the role of sexual conflict in the evolution of EPP. This interpretation would explain the patterns as caused by experimental females being less able to escape from unwanted copulations with extra pair males, thereby increasing their EPP rate (Plaza et al. 2019). This is in agreement with a scenario in which the levels of EPP would be influenced by male coercion instead of female choice (Björklund and Westman 1983) and it is consistent with the results found in a non-experimental study by Moreno et al. (2015), where a positive association between wing loading and EPP was reported. In contrast, an adaptive mate choice explanation would support the interpretation that improved body condition in experimental females led to increases in female condition and extra time, allowing them to seek out EPC by spending more time in extra-territorial forays and evading their social mate's guarding. None of these two options can be discarded. An alternative explanation would predict a potentially enhanced experimental female attractiveness due to the improved body condition translated into a higher capacity to lay a large number of high quality eggs (increased fecundity and fitness perception). In this case, males paired to experimental females would increase mate-guarding and copulation rate (Pilastro et al. 2002; Griggio et al. 2003; Griggio et al. 2005) leading to lower levels of EPP. We can now rule out this hypothesis as our results do not support it.

During the fertile period of the female, the social male would face a compromise between mate guarding and searching for potential EPCs. We consider that this compromise would not be affected by our treatment, as female fertility (Lifjeld et al. 1997b) could be easily perceived by the male through female behavioral signals (e.g. solicitations) rather than from the state of nest completion. Some individual characteristics expressing phenotypic quality may influence a male's ability or willingness to perform mate guarding. However, male dorsal blackness as an index of social dominance did not affect EPP. Furthermore, the extent of the male wing patch as another potential male social signal showed no association with EPP (Spearman's rank correlation: $r_{57} = -0.06$, $P = 0.63$).

Previous studies in pied flycatchers did not detect differences between extra pair and within-pair males in age, size or ornamentation (Moreno et al. 2010b) and there is no evidence of indirect benefits for extra-pair offspring in terms of good genes, as measured by microsatellite heterozygosity or body condition (Lifjeld et al. 1997a;

Moreno et al. 2013). Although there is evidence of good-gene effects in other species accrued thorough EPP (e.g. Kempenaers et al. 1992; Blomqvist et al. 2002), the picture is not so clear and recent analyses of the evolution of infidelity in monogamous passerines suggest that EPP is not adaptive for females in some species and that it may be the result of strong selection in males (Arnqvist and Kirkpatrick 2005; Forstmeier et al. 2011). However, there might be benefits for female extra-pair behaviour that researchers just have not investigated or thought of yet (Mennerat et al. 2018).

The extent of white on female pied flycatcher wings has been proposed as a signal of dominance through its association with testosterone levels (Moreno et al. 2014; Cantarero et al. 2015a). Moreover, female vigilance and dominance behaviours are positively associated with the size of this patch (Plaza et al. 2018). Thus, dominant females with larger patches may enforce their dominant status through signalling, being more able to resist unwanted males and thereby negatively interacting with EPP occurrence. This result supports previous evidence in the same population regarding female age (Moreno et al. 2015). That old and dominant females (more experienced) exhibit lower EPP values contradicts the presumption that EPP is the result of adaptive female choice as precisely these females would be in a better position to select extra-pair sires and resist mate guarding by their social mates.

Values found in brood EPP occurrence are similar to others in the same population and slightly higher in the percentage of nestlings affected (22.4 and 7.5% in 2003, Moreno et al. 2010a; 28.8 and 13.1% in 2010, Moreno et al. 2013; 38.3 and 17.6% in 2015, Moreno et al. 2015). They are also similar to those found in another Iberian population studied by (Canal et al. 2011) (39 and 20% respectively), and to the medium EPP rate in socially monogamous passerines which is above 25%. The importance of breeding synchrony and density on the interspecific variation in EPP has previously been reported (Stutchbury 1995; Griffith et al. 2002). It is assumed that temporal availability of reproductively active individuals may differ across the breeding season. In our highly synchronous breeding population (Griffith et al. 2002; Moreno et al. 2013), density of males not yet involved in parental duties may markedly decline throughout the season. As a consequence, the pressure of males seeking EPC may decrease, resulting in the negative relation between laying date and the incidence of

EPP. Previous studies in the same population showed no relation (Moreno et al. 2015) or a negative relation (Moreno et al. 2013) between EPY and laying date. In fact, Canal et al. (2012) described for the same species a decrease in EPP values during the days before the laying date, followed by an increase during egg laying and incubation, and no EPC occurring after those periods, suggesting that the demands of paternal care decreased the availability of males for EPCs. This pattern is in accordance with the general negative relation we found.

To conclude, we have found that females with a higher body mass during the fertile period display higher EPP levels. The evolution of mass change strategies in breeding altricial birds (Moreno 1989) has thus implications for EPP patterns. More body reserves at laying mean not only a higher potential fecundity but a higher level of EPP as well. This interaction had not previously received due attention but should be considered in future studies of avian breeding strategies. If female condition at laying denotes a high EPP for their partners, the possible negative consequences of a good breeding condition for females in terms of reduced mate incubation feeding (Cantarero et al. 2014) or help with nestling provisioning would merit further studies (Arnqvist and Kirkpatrick 2005). We also found that females with signals of higher social dominance show lower EPP values. Those results underline the role of female social traits in the evolution of avian EPP.

ETHICAL APROVAL

We were legally authorized to capture and handle pied flycatchers by Consejería de Medio Ambiente de Castilla y León (competent regional authority, protocol number EP/SG/706/2016, according to Royal Decree 53/2013), and by J. Donés, director of “Centro Montes de Valsaín”, to work in the study area. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The experiments comply with current Spanish laws, and grant holder and field researchers were officially licensed for animal manipulation following current EU regulations on animal manipulation (authorization types C and D with reference numbers CAP-T-0123-15 and CAP-T-0121-15). The study was ethically approved by the Ethical Committee of the ‘Consejo Superior de Investigaciones Científicas’ (CSIC).

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CHAPTER IV

EXTRA-PAIR PATERNITY IS NOT REPEATABLE BETWEEN YEARS IN PIED FLYCATCHERS: A ROLE FOR SOCIAL CONTEXT

This chapter reproduces entirely the manuscript:

Plaza M., Cantarero A., Gil D. & Moreno, J. (under review) Extra-pair paternity is not repeatable between years in pied flycatchers: a role for social context. Journal of Evolutionary Biology.

ABSTRACT

Patterns of variation in extra-pair paternity (EPP) have been studied in depth, but there is a surprising lack of strong evidence about the factors that explain their sources of variation. EPP is the consequence of the interactions between a social pair and extra-pair males during the female's fertile phase in a specific social context and ecological environment. Accordingly, the relative importance of individual characteristics on the one hand and of their immediate social environment on the other, may affect the repeatability of EPP and thereby its capacity to evolve. In the present study, we report an analysis of long-term data on EPP in two natural populations of pied flycatchers *Ficedula hypoleuca*. Firstly, we analyzed the repeatability of this trait in male and female individuals based on data from several breeding seasons. Secondly, we studied the relation between EPP and individual male and female traits and context variables like breeding synchrony and population density. Our results showed no repeatability for EPP in either sex. In addition, we found a positive association with laying date and stronger associations of EPP with male plumage and morphological traits than with female characteristics. The absence of individual repeatability suggests that variation in the social context in which interactions lead to EPP patterns may reduce the role of individual traits. The poor or absent knowledge of the social environment in many field studies of EPP, may explain the contradictory results on different or in the same species even including the same population in different years.

Key words: Extra-pair paternity, heritability, passerines, repeatability, sexual selection, social context

INTRODUCTION

In the last decades, hundreds of studies have provided evidence for mating outside the pair bond in socially monogamous avian species (reviewed in Brouwer & Griffith, 2019). This type of sexual polygamy has been reported in around 76% of birds (Griffith et al., 2002, Brouwer & Griffith, 2019), resulting in extensive levels of variation in extra-pair paternity (EPP) among species, populations and individuals. The study of the drivers that explain this variation in EPP has become crucial for the understanding on the evolution of mating systems (Brouwer & Griffith, 2019), and multiple hypotheses have been developed around it, ranging from stochastic demography to mating strategy theories (Forstmeier et al., 2014).

Extra-pair paternity is the consequence of the interactions between the social pair and extra-pair males during the female's fertile phase (Canal et al., 2011). In this context, each sex would have their own reproductive conveniences depending on their costs and benefits (Westneat & Stewart, 2003, Forstmeier et al., 2014). The interests of both pair members may be coincident in reducing EPP to the minimum possible in the existent social context. Alternatively, females may be interested in obtaining fertilizations from extra-pair males to the detriment of their mates' reproductive interests (Mingju et al., 2017, Arnqvist & Kirkpatrick, 2005). In the first case, the existence and variation of EPP should be related to the social pressure effected by extra-pair males and to the capacities of the pair male to counteract it through effective mate-guarding and mate defense (Petrie & Kempenaers, 1998). However, the female's evasion and aggressive capacities may also reduce the risk of EPP in this context (2019b, Plaza et al., 2019a). In the second scenario, variation in EPP may be related to the female's capacity to avoid mate guarding and effectively locate alternative mates in relation to the capacities of the social partner to impede such activities (Moreno et al., 2010). The social pressure by extra-pair males is more important in the first context in combination with the social male's defense capacity. The female's ability to avoid mate guarding and the discrepancy in sexually selected traits between the social partner and other potential mates appear paramount in the second scenario. In both cases we would expect significant individual repeatability in EPP between different reproductive occasions. However, a variable social environment may override individually based capacities in the first context, while changes in the degree of variation in male trait expression between seasons may affect female choosiness in the second (Wang et al., 2020, Lindstedt et al., 2007). While traits related to male aggressiveness such as age, size and signals of social dominance may explain some variation in EPP in the first scenario (Canal et al., 2011), traits associated with female capacity to override mate guarding like age, size (Moreno et al., 2015) and signals of social dominance (Plaza et al., 2018) may be more important in the second.

Only a few studies have reported information on individual repeatability in EPP for the two sexes between reproductive seasons (Dietrich et al., 2004, Reid et al., 2011). However repeatability in EPP provides basic information to understand the evolution of this trait, since repeatability sets an upper limit to heritability (Dohm, 2002, Falconer, 1981). Repeatability between first and second broods has been interpreted as a female trait in a mate choice scenario, ranging from high (Whittingham et al., 2006) to moderate (Schroeder et al., 2016) or absent (Dixon et al., 1994). Some studies relate these changes to between-year differences in factors that influence EPP like the boundary conditions (Dietrich et al., 2004). However, the repeatability of EPP between first and second broods of the same pair in the same season confuses individual traits with pair traits and does not adequately cover interseasonal differences. To ascertain the importance of individual traits for EPP it is necessary to estimate repeatability of individuals across different pair contexts as caused by mate changes between seasons. A high repeatability may be related to the importance of individual capacities to avoid (males) or affect (females) EPP, making this trait potentially heritable. On the other hand, a low repeatability may indicate the overriding influence of the immediate social context for EPP, making adaptations to control its expression inefficient. In this scenario, EPP will be the result of variable social circumstances and unlikely to evolve. However, a low repeatability of EPP may also be related to low repeatabilitiy of the individual traits affecting the incidence of EPP. Thus, this relationship should be tested to ascertain the impact of the social context on EPP (Weatherhead, 1999).

Context-dependent, spatio-temporal and ecological variables like timing of breeding, population density or reproductive synchrony, which have been traditionally proposed as determinants of EPP rates, are not under the control of individuals (Johnsen & Lifjeld, 2003, Canal et al., 2011). Population density and breeding synchrony can influence the encounter rate between mates and extra-pair mates (Richardson & Burke, 2001). Thereby, high breeding densities have been predicted to increase opportunities for extra-pair copulations, and consequently enhance the occurrence of EPP in songbird species (Charmantier & Perret, 2004, Gelter & Tegelström, 1992). However, while comparative studies within species report a positive relationship between reproductive density and EPP (Westneat & Sherman, 1997), a negative or inexistent association has been shown across species (Møller & Ninni, 1998, Mingju et al., 2017, Kasumovic et al., 2009). Timing of breeding has been proposed to influence EPP levels (Canal et al., 2011), tending to increase late in the season. This pattern could emerge if males maximize mate guarding during their mate's fertile period (Evans et al., 2008), while searching for extra-pair copulations when females are incubating (Birkhead & Biggins, 1987).

Comparisons of the individual traits of the two sexes related to levels of EPP are also scarce in the literature, as studies have usually focused on just one of them (Griffith et al., 2002). Moreover, the traits of the social male are frequently linked to relative attractiveness in relation to other coexisting males (Forstmeier et al., 2014, Bonderud et al., 2018) rather than to its capacity to repel and attack intruding males. Similarly, female traits are usually linked to the capacity to evade mate guarding (Bouwman & Komdeur, 2005) rather than to avoid contact with intruding males or repel their approaches (Moreno et al., 2015, Plaza et al., 2019a). Analyzing traits of both pair members at the same time in the context of EPP, allows a comparison of the relevance of the two sexes in order to explain the huge interpopulation and interspecific variation found in the literature (Ramos et al., 2014, Wang et al., 2020).

Individual traits potentially relevant in the context of EPP are age and experience (Moreno et al., 2010, Girndt et al., 2018, 2015), body size (Bonderud et al., 2018) and signals of social dominance like specific plumage ornaments (Mitrus et al., 2014, Edme et al., 2016). If male characteristics related to dominance override female traits when explaining EPP (Moreno et al., 2010), a possible interpretation is that the ability of the male partner to reduce the incidence of EPP through active mate defense may override any tendency of the female partner to avoid mate guarding and seek extra-pair partners (Alatalo et al., 1987, Griffith et al., 2002). The alternative scenario in which females are able to seek partners at will would predict a stronger influence of traits of the female partner on the resulting EPP.

The pied flycatcher *Ficedula hypoleuca* has been a model species for the investigation of EPP even before the advent of molecular techniques (see early studies like Alatalo et al. (1987)). The advantage of studying such a species in this context is that so much is known about the potential traits explaining EPP. The effects of breeding phenology and synchrony or population density (Rätti et al., 1995, Björklund & Westman, 1983, Canal et al., 2011), and of male traits involved in mate guarding (Alatalo et al., 1987), territorial defense (Lehtonen et al., 2009), and sexual attractiveness (Edme et al., 2016, Mitrus et al., 2014) have been explored in numerous studies on the same a closely related species, with some of them also including female traits (Moreno et al., 2015, Plaza et al., 2019a, Rätti et al., 1995). However, to our knowledge, there is no previous study on this model species including individual repeatability and exploring how the traits of the two sexes explain the incidence of EPP. Here we present a comprehensive study by using long-term data on EPP in two populations of pied flycatchers. The aim is to explore the relative importance of social context for determining EPP as well as the relevance of male and female traits in explaining its incidence in pairs. We included laying date and synchrony as social context information, and individual age, body size, the extent of the white forehead and wing patches and the extent of male black dorsal plumage as individual characteristics.

MATERIAL AND METHODS

General field methods

The study was carried out in two different areas in central Spain where long-term monitoring of pied flycatchers has been conducted. The two study sites in Lozoya (40°58′N, 3°48′W, 1400–1500m a.s.l.) and Valsaín (40°54′N, 4°01′W, 1100–1200m a.s.l.) are montane deciduous forests of Pyrenean oak *Quercus pyrenaica* where 100 and 435 nest-boxes respectively have been placed and their occupation has been checked since 2001 in Lozoya and 1991 in Valsaín. Environmental conditions in both areas are strongly correlated given the distance between them (20 km straight) and the habitat similarity, although population connectivity is relatively low as ascertained through the presence of recruits. The study was performed during the breeding seasons of 2015-2017 in Valsaín and 2016-2017 in Lozoya.

The breeding period of the species lasts from the middle of April when the first males arrive from migration, to the beginning of July when most chicks have fledged. Regular checking from April 15 to the end of breeding period is done to detect the presence and settlement of every flycatcher breeding pair and to record laying dates (day 1 = April 1), hatching dates, clutch sizes and reproductive performance (breeding success). We clean all nest-boxes every year after breeding is over.

As this study involves some areas and years where different experiments took place (2019a; Moreno unplished data, Plaza et al., 2018), only control nests were included in the analyses, excluding the possibility that experimental treatments may affect the results. The total number of nests along the breeding season was of 70, 111 and 68 in Valsaín in 2015, 2016 and 2017 respectively, while it was 41 and 40 in Lozoya in 2016 and 2017. The study areas cover 65 ha in Valsaín and 84.18 ha in Lozoya. Thus, the breeding density of pied flycatchers in Valsaín (1.49 pairs/ha) was higher than in Lozoya (0.48 pairs/ha), and nest-box density was also higher in the first area (4.61 nest-boxes/ha) in comparison with the second area (1.18 nest-boxes/ha). What is more, we have evidence from previous years that competition for nest-boxes is therefore stronger in Lozoya (Cantarero et al., 2015).

Capture and sampling

All adults were captured in their nest boxes while feeding 7–8 days nestlings (fledging takes place 16–19 days after hatching) during daytime, by using a conventional nest-box trap set at the entrance (Cantarero et al., 2016). The trap was removed once both adults were captured and for a maximum of 1h. All birds were ringed or identified by their rings. Body mass was recorded with a Pesola spring balance (accuracy 0.25 g) and wing length was measured with a stopped ruler to the nearest mm. A digital photograph of the white wing and forehead patch was taken from above at a distance of 10 cm from the animal by placing the wing in its extended position on a flat surface with a ruler aligned beside the wing for reference. All photographs were taken during the morning hours with the same digital camera and following the method described in previous studies (Moreno et al., 2014, Plaza et al., 2018). Surfaces of the white wing area were estimated with Adobe Photoshop CS5 v.11.0. following Sirkiä et al. (2015). Repeatability of wing and forehead patches was estimated from different photos of the same bird after the same capture event (N=25) and was extremely high in both cases (wing patch 0.96 SE=0.02 p<0.01, forehead patch 0.98 SE=0.04 p<0.01). We measured the percentage of black feathers with values from 0 (0–10%) to 9 (90–100%) in the mantle and head of males as “blackness”. We were able to establish the exact age of individuals only if they had been ringed as nestlings (126 females and 122 males in the total sample included in this study). Age for unringed birds was estimated assuming they were 2 years old when captured for the first time as breeders, as 48% of recruits in the population are recruited at this age (Moreno et al., 2015).

A small sample of blood from the brachial vein (10–20 µl) was taken and stored on Flinders Technology Associates reagent loaded cards (Whatman Bioscience, Florham Park, NJ, USA) until needed for paternity analyses.

We ringed all chicks when they were 13 days old (hatching day = day 1), and also a small blood sample from the brachial vein was taken for paternity analyses. All carcasses and abandoned eggs found during regular checks inside the nest-boxes were collected and frozen on the same day for later paternity analyses through tissue extraction. Hatching failure affected 105 of 1130 eggs in 18% of the nests. However, 45 eggs did not show any trace of embryonic development suggesting that they were infertile.

Genotyping

We obtained samples from 189 families. All of them included the two social mates and their broods (178 males, 181 females and 1002 nestlings). We used BioSprint Blood kits (QiaGen, Duren, Germany) to extract and purify the genomic DNA from the cards where the blood was fixed and from the carcasses and eggs tissues. Type-it kits

(QiaGen, Duren, Germany) were used to amplify approximately 5 ng of template DNA in the multiplex PCR reactions.

Following published primer sequences described in Leder et al. (2008) we used 10 pied flycatcher microsatellite loci for genotyping in two multiplex PCR reactions described in Moreno et al. (2015). We amplified loci Fhy301, Fhy466, Fhy336, Fhy370 and Fhy452 in one reaction (set I) and Fhy328, Fhy223, Fhy236, Fhy304 and Fhy407 in the other (set II). In both sets the PCR program consisted in a denaturing step of 94°C during 2 min, then 30 cycles with 30 s at 94°C, 30 s at 55°C and 30 s at 72°C, and a final extension step of 2 min at 72°C. All loci were polymorphic (Kalinowski et al., 2007). We used CERVUS (v 3.0.7. Field Genetics) software, to calculate the following genetic statistics: the number of different alleles in each set of data (Table S1 of the Electronic Supplementary Material (ESM); the loci that deviated from Hardy-Weinberg equilibrium and the ones with a null allele frequency higher than 0.05 (both shown in Table S2).

Paternity analysis

For each year and area we determined genetic parentage by comparing the genotypes of chicks with those of female and male nest owners by running a paternity analysis in CERVUS. We specified the identity of the mother for all chicks and the software assigned the genetic father from the sample of captured adult males (Westneat & Mays, 2005). We considered that chicks were the offspring of the adults if their genotypes were compatible for the assigned loci. We accepted a minimum number of 6 loci typed, a proportion of candidate parents sampled of 85%, using a 95% level of confidence, and allowing a 5% proportion of mistyped loci. CERVUS assigned paternity to the male with the highest LOD (logarithm-of-odds) score (calculated by taking the natural log of the overall likelihood ratio; the likelihood ratio is the probability for the candidate parent to be the true parent divided by the probability for the candidate parent of not being the true parent). The combined non-exclusion probabilities of second parent calculated by CERVUS are shown in Table S2. We accepted a candidate as the genetic father of a given nestling only when the difference between the LOD scores of the first and the second most probable fathers was statistically significant

(Kalinowski et al., 2007). Those nestlings with two or more mismatched loci with respect to their social fathers (the mismatch never involving markers that deviated from Hardy–Weinberg equilibrium) were considered as extra-pair offspring. All these nestlings (177 cases in total) were assigned to a non-social male inside the population male pool. However, we did not assign a genetic father when the difference in LOD score between the first and the second most probable father was not significant (66 cases in total). We also visually checked if those males assigned by CERVUS as fathers of extra-pair offspring matched the genotypes of the nestlings they were assigned to (Potti & Canal, 2011, 2010). We took a conservative rule and only accepted as father–offspring pairs 25 out of the 66 cases of extra-pair chicks assigned by the program, rejecting these mismatches of the social father that only involved one locus. We considered that a single mutation or genotyping mistake can produce a single locus mismatch between two genotypes, and for this reason we overruled the CERVUS decision of considering these as extra-pair offspring (Moreno et al., 2015, 2010). Mismatches between females and offspring occurred in 36 cases.

Statistical analyses

We compared EPY (number of extra-pair young divided by brood size, EPY from now on) between years to study the repeatability (individual consistency) of this trait in both males and females. We used the repeated measures of 68 individuals from the total sampled population (38 males and 30 females). Repeatability is the proportion of phenotypic variation that can be attributed to between subject variation (Nakagawa & Schielzeth, 2010). By using the rptR package (v0.9.22, Stoffel 2019) we performed Generalized linear mixed models with binomial distributions, “Proportion” as data type and the logit link function. This analysis uses EPY as dependent variable, and the individual (male/female) as random factor. We ran 1000 iterations of the model by repeatedly generating data from the distribution defined by the estimated parameters, and then obtaining the CI from this simulated distribution (Nakagawa & Schielzeth, 2010). We also explored repeatability of the tested variables for EPP in the previous analyses. All estimate values are presented with standard errors.

We then analyzed the incidence of EPP (presence/absence of EPY in the nest, EPP from now on) and the proportion of EPY as dependent variables in two sets of Generalized Lineal Models with binomial and quasi-binomial distributions respectively, to assess their relationship with male and female traits. These included wing length, wing and forehead patch size, age for both sexes and male dorsal blackness, as well as breeding success (proportion of fledged young = N fledged young/N hatched young) and laying date. In addition, we considered the degree of synchrony of each nest, and the study area as factor given the differences between areas in density. We calculated a synchrony index for each nest based on the proportion of fertile females on the laying date of the first egg at the focal nest (Kempenaers, 1993). The female's fertile period was defined as day -2 to day +5 (mean clutch size for pied flycatchers is 6 (Lifjeld et al., 1997)).

We estimated the VIF (Variance Inflation Factor) to explore possible multicollinearity among independent variables. However, none of the predictors included in the models showed collinearity (all VIF < 2.21). We z-standardized all variables before analyses to allow comparisons across estimates. In the case of EPY, we calculated the over-dispersion parameter ($\hat{c}$) in the full model and we used this value to adjust the corrected Akaike information criterion (AICc), yielding quasi-AICc values corrected for over-dispersion (QAICc). We applied the *dredge* model selection function (MuMIn package Barton, 2019) that uses all possible combinations of the covariates to obtain the conditional average model taking into account all models that differed in less than 4 units from the model with lowest QAIC.

All analyses were performed by using R software (v 3.5.3; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

We found EPP in 71 out of 189 broods (37.56%) affecting 243 of 1002 nestlings (24.25%). In 23 nests, non-identified extra-pair sires were detected. In nests with EPP, the number of EPY ranged from 1 to 7, being on average (SE) 3.42 (0.22) EPY. Detailed descriptive results in each population and year are shown in Table 1.

Table 1. Proportion of nests with EPP (%EPN = number EPN / number Total nests), Proportion of extra pair young (%EPY = number EPY / brood size per nest), proportion of extra-pair young with non-identified father (%EPY no father = number EPY with non-identified father / number EPY per nest), mean number of EPY in nests with EPP (EPY / nest = number EPY / brood size) and rank (minimum-maximum number of EPY per nest and total number of nests (N) in each area and year of the study.

	Valsaín					**Lozoya**				
	%EPN	**%EPY**	**%EPY no father**	**EPY/ nest**	**N**	**%EPN**	**%EPY**	**%EPY no father**	**EPY/ nest**	**N**
2015	0.38±0.06	0.24±0.04	0.29±0.08	3.21±0.38 (1-5)	63	-	-	-	-	-
2016	0.24±0.06	0.16± 0.05	0.20±0.13	4.0±2.26 (1-7)	41	0.50±0.09	0.37±0.08	0.41±0.11	4.21±0.54 (1-7)	28
2017	0.33±0.08	0.17±0.05	0.53±0.21	2.70±0.47 (1-6)	30	0.33±0.08	0.29±0.05	0.10±0.01	3.35±0.53 (1-7)	27

Between-year repeatability analyses for EPY showed extremely low and nonsignificant levels for females and males (Table 2). Individual traits showed in all cases higher repeatability than for EPY which suggests that the low individual consistency in EPY is not due to high lability of individual traits across years.

Table 2. Estimated (between years) repeatability (R) for the proportion of extra-pair young (%EPY) and morphological male and female traits. Stardard error (SE) and confidence intervals (CI) are shown.

Trait	**R**	**SE**	**CI**	***P***
Male %EPY	0.00	0.04	0, 0.13	0.50
Female %EPY	0.06	0.09	0, 0.301	0.48
Male laying date	0.11	0.09	0, 0.309	0.16
Female laying date	0.65	0.07	0.50, 0.76	0.48
Male wing patch	0.61	0.11	0.30, 0.75	<0.01
Female wing patch	0.39	0.16	0.00, 0.67	<0.01
Male dorsal blackness	0.14	0.14	0, 0.46	0.18
Male wing length	0.49	0.12	0.22, 0.69	<0.01
Female wing length	0.21	0.16	0, 0.55	<0.01

Final average models yielded more effects of male and female traits for EPY than for EPP. However, the sign of the estimates was invariably in the same direction using either measurement, which suggests that EPY is a more sensitive measure of extra-pair paternity than EPP, while measuring the same phenomenon.

The final average model showed that later broods were more likely to contain EPY than early ones (Table 3). Several male traits also explained EPY: older males, dorsally blacker males and those with smaller wing patches had a higher EPY in their broods (Table 3). We also found some female predictors: EPY increased with increasing female age, but decreased with wing length. In addition, there were higher levels of EPY and EPP in the high density area (0.33±0.04 and 0.50±0.06 respectively) than in the low density area (0.20±0.03 and 0.32±0.04 respectively) (Table 3). Female forehead patch size and the Synchrony Index were not included in the resulting average models and male forehead patch was not retained either in the final model with EPP as dependent variable.

Table 3. Average models calculated from the set of most plausible models for EPP (occurrence/absence) (ΔAIC<4), and EPY proportion (number of EPY/brood size) (ΔQAIC<4), as dependent variables with binomial distributions and laying date and reproductive success (% of fledged young = N fledged young*100/N hatched young) and female and male wing length, wing and forehead patch size, age and male dorsal blackness as predictors. Estimates for the binomial model were transformed (antilogarithm) to convert them into odds-ratios.

	EPY				**EPP**			
	Estimate	Std. Error	Z value	*P*	Estimate	Std. Error	Z value	*P*
Study area	-1.44	0.25	5.73	**<0.01**	-0.90	0.45	1.96	**0.04**
Laying date	0.08	0.01	4.46	**<0.01**	0.06	0.03	1.87	0.06
Female age	0.18	0.07	2.61	**0.01**	0.19	0.14	1.32	0.18
Female wing length	-0.10	0.04	2.26	**0.02**	0.01	0.09	0.15	0.87
Female wing patch	-0.09	0.09	0.95	0.34	-0.21	0.19	1.09	0.27
Male dorsal blackness	0.04	0.00	4.46	**<0.01**	0.02	0.01	1.37	0.16
Male age	0.22	0.06	23.69	**<0.01**	0.13	0.12	1.09	0.27
Male wing patch	-0.25	0.05	4.88	**<0.01**	-010	0.10	1.01	0.31
Male wing length	-0.04	0.04	0.94	0.34	-0.05	0.09	0.54	0.58
Male forehead patch	-0.59	0.82	0.71	0.47	-	-	-	-
Reproductive success	-0.00	0.00	0.43	0.66	-0.00	0.00	0.40	0.68

DISCUSSION

In this long-term study of the incidence of EPP in two pied flycatcher populations we have found no individual consistency in both its incidence and extent for both sexes. Repeatability was much lower than for individual traits related to EPP. The strongest determinant of EPY was laying date, with late broods showing higher levels. Furthermore, we have found marked associations of EPY with male traits related to social dominance and sexual attractiveness like age, male dorsal blackness and extent of the white wing patch, although the trends show opposite directions. Finally, older and smaller females showed higher levels of EPY in their broods. Although the low density area showed higher levels of EPP and EPY, the lack of further replication makes this result provisional. Competition caused by the availability of nest-sites, may enforce a high influence on EPP by increasing the number of social interactions at the start of the breeding season. This effect might be higher than the one caused by the raw number of individual density.

The low repeatability of EPY indicates that the varying social context between years rather than individually consistent traits like size and plumage signals explain the incidence of EPP in broods in our pied flycatcher populations. The fact that age in both sexes shows increasing trends with EPP supports that social context is crucial (Wang et al., 2020). The effect of individual age on EPP might be relative to the effect of the age of surrounding competitors, so the strength of this variable supports that individual strategies to control EPP can have different outcomes depending on the social environment (Ramos et al., 2014). The low repeatability also relates to the impossibility of EPP evolving in a zero-sum game where successful and unsuccessful individuals compete for the same pool of reproductive outcomes. EPP would not be an individual trait but the result of the interaction of individuals in a varying and fluid social context affecting behaviours operating during a brief period of time each season.

Given that plumage traits in the populations studied show marked phenotypic plasticity and are affected by prior conditions in both the breeding and wintering ranges (Moreno et al., 2019), it is not surprising to find that such traits explain part of the variation in EPP despite the low repeatability in EPP itself. Blacker males apparently are less able to control EPP in their broods despite evidence indicating that they are socially dominant and sexually attractive (Slagsvold & Lifjeld, 1988, Sætre et al., 1997, Galván & Moreno, 2009). This is in contrast with the trend of males with larger wing dominance signals attaining lower levels of EPP in their broods. The first result contradicts evidence in the same and nearby populations obtained in other years, namely that blacker males show lower levels of EPP (Moreno et al., 2015). This inter-year variation in trends again supports that EPP depends on social context. In a highly competitive social context, blacker males may suffer more from their aggressive tendencies and guard their mates less efficiently through involvement in frequent aggressive interactions. This highlights the importance of controlling for immediate social context in EPP studies.

Females are expected to be interested in improving their social mate choice through seeking EPP with more attractive extra-pair mates (Griffith et al., 2002, Forstmeier et al., 2014). Older females could be more able to avoid intense mate guarding by their partners and thus attain a higher EPP in their broods. This result found here contradicts that obtained for the same area (Valsaín) in a previous study (Moreno et al., 2015). Again, the varying trends between years and studies strengthens the idea that the relationships found are more related to a fluid and changeable social context that to the traits of the individuals involved (Maldonado-Chaparro et al., 2018). In some social conditions but not in others, older females may have a higher probability of improving their reproductive success through extra-pair mate choice leading to opposite trends of EPP with age in different studies of the same populations (Moreno et al., 2015). Regarding the negative association of EPP with female wing length, larger females may be able to escape from harassing extra-pair males and thus reduce EPP in their broods as shown here, supporting another previous study in Valsaín (Plaza et al., 2019a, Moreno et al., 2015).

Finally, the relevance of male traits for explaining the incidence of EPP appears stronger than female traits. This suggests that the defensive capacity of males relative to their social competitors is more important than those of their partners to either attract foraying extra-pair males and avoid mate guarding or alternatively to escape from male intruders (Ramos et al., 2014). Our study also contributes to recent investigations suggesting that no single factor can explain EPP patterns, and that interactive effects of multivariate analyses per individual are needed (Brouwer & Griffith, 2019). A hierarchical explanation for variation in the occurrence of extra-pair fertilizations was postulated by Griffith et al. (2002), considering fundamental life history and socio-ecological parameters as well as individual characteristics. What is more, the occurrence of EPP might likely be the result of behavioural and ecological interactions (Moreno et al., 2015, Arnqvist & Kirkpatrick, 2005, Westneat & Stewart, 2003). In a recent review, Maldonado-Chaparro et al. (2018) proposed that EPP should be studied as a meta-trait that cannot be understood without the analyses of all its integrative variables.

In summary, dependence of EPP on the social context of each pair makes this a nonrepeatable trait, which cannot evolve independently in individual members of populations. Fluctuations of the social context make also results from different studies of the same population non-repeatable as found here. The crucial determinants of EPP in each nest are presumably the outcome of interactions of the two members of the pair with their neighbours and floaters during a short window of time each season. Until this social context can be adequately studied and its elements quantified, results of EPP studies will continue to be unrepeatable even in the same population.

ETHICAL NOTE

We were legally authorized to capture and handle pied flycatchers by Consejería de Medio Ambiente de Castilla y León (Valsaín) and by Comunidad de Madrid (Lozoya) (competent regional authorities, protocol number EP/SG/706/2016, according to Royal Decree 53/2013), and by J. Donés, director of “Centro Montes de Valsaín”, to work in the Valsaín study area. The experiments comply with current Spanish laws, and grant holder and field researchers were officially licensed for animal manipulation following current EU regulations on animal manipulation (authorization types C and B). The study was ethically approved by the Ethical Committee of the Spanish ‘Consejo Superior de Investigaciones Científicas’ (CSIC).

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M.P. conducted field and lab work, analyzed data and wrote the manuscript, A.C. conducted field work and revised the manuscript, A. M. designed and supervised the lab work, E.S.-D. participated in field work and revised the manuscript, D.G. designed the study and revised the manuscript and J.M. designed the study, conducted field work and revised the manuscript. All authors approved the final version of the manuscript and agree to be held accountable for the content therein.

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CHAPTER V

REPEATABLE SOCIAL NETWORK NODE-BASED METRICS ACROSS POPULATIONS AND CONTEXTS IN A PASSERINE

This chapter reproduces entirely the manuscript:

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ABSTRACT

Behavioral traits are considered animal personality traits when individuals differ consistently in their expression across time and across context. Here, we test this idea on three metrics derived from social interaction networks (strength, betweenness, closeness). Using experimental data from house sparrows in captive populations, and observational data from house sparrows in a wild population, we show that all three metrics consistently exhibit repeatability across both study populations and two methods of recording interactions. The highest repeatability values were estimated in male-only captive groups, while repeatabilities estimated in single-sex networks subsetted from mixed-sex groups showed no sex-specificity. We also show that changes in social group composition led to a decrease in repeatability for up to six months. This work provides substantial and generalizable support for the notion that social network node-based metrics can be considered animal personalities. Our work suggests that social network traits may be heritable and thus could be selected for.

Keywords: animal personality, repeatability, passerine, social network, social traits

INTRODUCTION

Behavior is considered to be flexible over time and context, allowing animals to respond to variation in the environment, and in particular, in social conditions (Réale & Dingemanse, 2010, Koolhaas et al., 2010, Wilson et al., 1998). Where individuals differ consistently in their behavioral phenotype over both time and context, such as differing social or physical environments (Dall et al., 2004, Montiglio et al., 2013, Sih, 2004), individual differences are termed animal personality (Réale *et al.* 2007) and have attracted extensive research (Wolf *et al.* 2007; van Oers & Mueller 2010, Wolf & Weissing 2012) .

Previous studies on animal personality have primarily focused on the shy-bold continuum, which mainly quantifies behaviors based on an individual's willingness to take risks (Dingemanse *et al.* 2004; Dingemanse & Réale 2004; Smith & Blumstein 2007; Abbey-Lee & Dingemanse 2019). This research has shown that these personality types have a heritable component (Dochtermann *et al*. 2019; van Oers *et al.* 2004; Montiglio *et al.* 2013; Winney *et al.* 2018). Different animal personality types have associated advantages and disadvantages, such as predator avoidance, access to social information, disease transmission and survival probability (Wilson *et al.* 2013; Abbey-Lee & Dingemanse 2019; Moiron *et al.* 2019). Presumably, adaptive personality is based on life-history trade-offs, implying that selection favors the evolution of personalities depending on future fitness expectations (Nicholaus et al., 2012, but see Moiron et al., 2020). Less attention has been dedicated to the study of animal personality in the context of sociability (Bell et al., 2009), defined as the tendency of individuals to interact with other conspecifics (Dingemanse & Réale 2004; Krause, James & Croft 2010). More social individuals are likely to obtain essential information from others that will benefit their fitness, such as potential threats or the location of resources (Bergmüller & Taborsky 2010; Brent 2015; St Clair *et al.* 2015). Contrary, less social individuals – i.e. those that take part in fewer interactions – may obtain benefits by avoiding costs of being social, such as conflict, competition and disease transmission (Corner, Pfeiffer & Morris 2003; Atton *et al.* 2014; Silk *et al.* 2017).

The social environment, namely the group of individuals that interact with a focal individual, is crucial in shaping personality because social factors and interactions can both affect and be influenced by other individuals, creating feedback loops (Krause *et al.* 2010; Bergmüller & Taborsky 2010). Thus, these traits may also be governed by indirect effects, originating from individuals other than the individual in which the trait is measured (e.g. Schroeder et al. 2019). Yet, even in the presence of indirect effects, we expect direct effects to play a larger role. Until now, data supporting the basic hypothesis that social interactions are repeatable, potentially heritable animal personality traits, are still limited. The inherent complexity of social interactions requires the integration of social network methodology (Krause et al., 2010).

Social interactions can be characterized through social network analysis – where associations between individuals are quantified depending on with how many other individuals they interact with and how often they interact with them (Krause *et al.* 2015). The social network is defined as the structural characteristics of the individual identities (termed nodes) interacting via social connections (termed edges). These connections can be social associations, antagonistic, reproductive, or foraging relationships (Krause et al., 2015). From such networks we can calculate node-based social metrics, and as such, the concept of animal personality cannot be directly applied. This is because a social interaction is the result of at least two individuals, so the outcome depends on the combination of their social phenotypes. This complexity can be rather impactful the more individuals are involved in an interaction, with the possibility of creating feedback loops and resulting in complex social phenotypes.

Yet, node-based metrics could function as proxies for the latent, underlying personality traits such as extraversion, or sociability. Indeed, it has been shown that individuals differ consistently in their node-based metrics in wild populations across long time scales in a variety of species like great tits *Parus major* (Aplin *et al.* 2015), wild ring-tailed lemurs *Lemur catta* (Kulahci, Ghazanfar & Rubenstein 2018), wild eastern water dragons *Intellagama leseurii* (Strickland & Frere 2018), wild vervet monkeys *Chlorocebus pygerthrus* (Blaszczyk 2018) and yellow-bellied marmots *Marmota flaviventris* (Blumstein *et al*. 2012). Those differences are also present on shorter time scales in wild guppies *Poecilia reticulata* (Krause *et al.* 2017). What is more, this consistent between-individual variation has been shown to be maintained in different habitats including field and laboratory in vampire bats *Desmodus rotundus*

(Ripperger et al., 2019), or across social environments in captive sharks like the small-spotted catshark *Scyliorhinus canicula* (Jacoby *et al.* 2014), the lemon shark *Negaprion brevirostris* (Finger *et al.* 2018), and in captive forked fungus beetles like *Bolitotherus cornutus* (Formica *et al.* 2017) and wild Emei music frogs *Babina daunchina* (Deng & Cui 2019). Yet, it remains unclear, and if so, in which circumstances, those patterns in behaviors measured in captivity can be translated into the wild within the same species (Bell et al., 2009, Osborn & Briffa 2017, Herborn et al., 2010).

Social behavior is the result of selection pressures, and may well be sex specific, for instance, males may be selected to compete for mates, while females compete for other resources (Sterck et al., 1997). Therefore, sex differences are expected in social behaviors, making it essential to investigate if those sex differences affect their repeatability patterns (Wolf & Weissing 2010, Nakagawa et al., 2007). Indeed, in some species like the eastern water dragon *Intellegama leseurii*, sex-specific effects have been found, reporting repeatability in males but not in females (Strickland & Frere 2018), however studies in other species did not show such sex differences in social traits (Kulahci *et al.* 2018).

Here we hypothesize that node-based social network metrics describing social interactions can be considered animal personality traits. To test this hypothesis we test for its assumption, that personality traits show repeatability across both time and context. We test whether social network traits in house sparrows *Passer domesticus* (hereafter sparrows) show consistent between-individual differences across different timespans and social environments. We used a wild population and two captive populations, and tested repeatability across a short and a longer time span, and also while changing the social group composition. Furthermore, we tested for sex-specificity in repeatability. If social network metrics are indeed repeatable, this allows for the possibility of these traits being heritable, as repeatability combines both genetic and environmental components of variation in a trait, setting the upper limit for heritability (Boake 1989; Falconer 1981 but see Dohm 2002). Thus, if there was statistically significant repeatability, this would indicate that these traits could respond to selection.

METHODS

Social interactions – general

The study took place in three different populations: a wild population located in Lundy island (UK), where all birds could freely interact with each other, and two captive populations located in Seewiesen (GER) and Silwood Park (UK), in carefully designed experimental social groups. Details of each population are explained below. In the captive population, the initial aviary occupation was chosen randomly (as detailed below), either unisex, or mixed sex. All mixed sex aviaries were assembled to a 50/50 sex ratio.

All social interactions were measured as follows in all populations: as interactions between two sparrows at a bird feeder. The feeder used for recording interaction data was always placed in the same location over the study period and consisted of a bowl of 15.5 cm in diameter, filled *ad libitum* with dry mealworms, wild bird food, or sunflower seeds, all of which are preferred food for sparrows. If two birds were present at the feeder at the same time, they were considered to be interacting. These interactions were measured using two methods (Table 1):

(1) We used transcriptions of video recordings of individually colour-ringed birds for the Lundy sparrows and the Seewiesen sparrows. Here, we noted the following dyadic interactions: pecks and fights (which are physical agonistic), threats (which are not physical agonistic), and when two birds were present at the feeder at the same time (i.e one bird is present, another lands and then one leaves). A digital video camera placed around 150 cm away from the feeder, recorded all interactions happening approximately 1 m around it, which aided colour ring reading. Video transcription was conducted by AST, DM, and MSF and it was constrained by observer hours. Video segments to be transcribed were selected randomly, using only segments with more than one individual present per frame.

(2) In the Silwood experiments, we equipped all sparrows with PIT (Passive-integrated transponder, Schroeder et al. 2011), which were read by PIT antennas flanking a caged-in feeding area (30x50 cm, 25cm high) containing the same type of feeder and food described above. Two entrances allowed sparrows to access the

feeding area at their leisure and to leave when they did not choose to interact with a newly arriving individual. Each entrance had two sequential antennas connected to a data logger that recorded the 10-digit unique number provided by the PIT, allowing us to determine the direction of a sparrow entering or leaving the feeding area. We considered an interaction to be dyadic when two individuals remained together in the feeding area for more than 3s using coexistence as a proxy of interaction (Farine, 2015). We did not separate agonistic and coexistence interactions in video recordings specifically so that we caould compare the wild and captive populations. Thus, video data collection including both types of interactions matched as closely as possible that collected with PITs. PIT antennas were registering 24 hours daily and we used all interaction data collected by them. However, we only had two PIT antennas available, and therefore the recordings took place in rotation around the aviaries.

The wild sparrows of Lundy

The sparrows population on Lundy island can be considered a closed population because Lundy Island is 19 km from the closest mainland, so migration is approximatly zero (Schroeder *et al.* 2015). Due to the isolated nature of the population, we were and are able to catch, visually identify (most birds on Lundy Island are ringed with a unique color ring combination) and automatically record all birds present on the island (Schroeder *et al.* 2011; Sánchez-Tójar *et al.* 2017). Doing so throughout the year allowed us to acquire comparably precise knowledge of which bird is present at any time, and to estimate and confirm no catching bias (Simons *et al.* 2015).

We performed ten discrete sampling occasions between the years 2013 and 2016: four during the non-breeding season (between mid-November and mid-February) and six during the breeding season (between mid-March and mid-July). For each occasion, we transcribed 2 days of video footage from, on average, 5h of interactions per day (range: 2.4-13h per day, total: 99.3 h). We placed the feeder close to the most frequently used roosting, breeding and feeding spots in the population. With these data we created ten social networks, one per sampling occasion, with weighted edgelists using the R package 'iGraph' v.1.2.4.1 (Csardi & Nepusz 2006). We then reduced the interaction dataset to interactions between same-sex individuals only to maximize comparability to data of the captive sparrows (see below). For the sex-specific analyses, we split these into two datasets, one for females, and one for males. For more detailed information about sampling and time points of each individual see Sánchez-Tójar *et al.* (2018).

The captive sparrows of Seewiesen

We collected data from 96 male sparrows held at the Max Planck Institute for Ornithology in Seewiesen, Germany, between 17th October 2014 and 15th December 2014. Most of the sparrows tested were captively bred progeny of wild sparrows caught in 2005 and 2006 (details in Laucht et al. 2010, and Girndt *et al.* 2018 for more information on animal husbandry). A total of 96 males was separated into four aviaries, corresponding to 24 males per aviary. One male died early in the experiment and was excluded from the analyses. Here, we transcribed 3h of video of interactions per aviary once per week across ten weeks, i.e. ten sampling occasions. From this dataset, we created 40 networks (i.e. one per aviary and sampling occasion) with weighted edgelists, as we did for the video data from the wild. The aviary social environments (which individuals were in which group) were otherwise kept stable throughout, and thus, the social environment of each aviary did not change across the ten sampling occasions.

The captive sparrows of Silwood

This population was held at Imperial College London in Silwood Park, Ascot, UK. The first Silwood dataset was collected over a period of ten weeks, between 27th December 2017 and 5th March 2018 in four aviaries with mixed-sex sparrow aviaries. Here we used PIT technology to record interactions (see above). Thus, the Silwood dataset can serve as a validation for the different method to record interactions. From the Silwood experiment, we collected data from four aviaries and across five sampling occasions, hence, here we constructed 20 social networks. The captive population sizes ranged between eight and 31 sparrows, with an average of 17 sparrows per aviary.

We then performed the following experiments to explore if node-based social network metrics were repeatable across different time scales and social contexts.

Across social environments – long term experiment

We then randomly re-distributed the Silwood sparrows after 5th March 2018 and split up the social groups into eight mixed sex groups (populations) to generate new social environments. The sparrows could breed in these groups during the summer of 2018. In the following winter, between 13th November and 31st December 2018, we re-assessed social interactions in these eight groups. This means the birds had seven months to adjust to their new social groups. We used data from the previous winter (as described above) as the first sampling event to estimate repeatability across social environments in the long term.

Across social environments – short term experiment

Then, sparrows were randomly re-distributed again, and sampled for a third occasion starting on 5th March 2019, to test for a short-term change in social environment. We only created seven populations to keep population sizes equivalent, due to natural mortality (Simons *et al.* 2019). In April and May we collected interaction data from these seven aviaries. We calculated the repeatability of these data using data from the winter 2018 occasion (see above), to test for short-term repeatability after the social environment was broken up. We created 15 networks, one for each aviary (2018: 8 aviaries, 2019: 7 aviaries) and occasion combination.

From each aviary we obtained one dataset and we considered this as one population.

Statistical analysis

We extracted the node-based estimates for strength, betweenness, and closeness from each social network (Csardi & Nepusz 2006). Strength describes the total number of interactions an individual engages in, regardless of who the other individuals are. Strength is weighted by the total number of interactions at the sampling occasion. Betweenness describes the number of shortest connections (combinations of connected edges) between a pair of individuals that ‘pass through’ the focal individual, thus describing how well an individual connects different parts of the network. Closeness describes how many connections are needed to link every other individual from a focal individual, thus it quantifies how central an individual is (Csardi & Nepusz 2006). Before all analyses, we log-transformed all metrics to approach normality, and then z-standardized them by network to allow comparing node-based metrics between social networks (Schielzeth, 2010).

We then estimated the repeatability – the proportion of variance explained by between-individual differences over the total phenotypic variance, in all three populations for all three metrics. We did this by running linear mixed-effects models, one for each metric for each dataset, with the individual identity of a bird as a random effect on the intercept. We then extracted the random effects estimated variance and calculated repeatability (Nakagawa & Schielzeth 2010).

In the analysis of association data, no individual is independent from the other individuals interacting with them. Hence, the network structure by itself might – by chance – produce a background level of repeatability given the struture of the data, which needs to be considered the null hypothesis for statistical testing instead of a repeatability of zero as normally done when using independent data (Bejder et al. 1998, James et al. 2009). To account for this possibility we ran 1000 iterations of the observed data for every model. In each iteration, we permuted the identity of the individuals (nodes) interacting with each other, and the number of times an association was observed (weights), while keeping the total number of associations, individuals, and the distribution of the weights constant. We constrained randomizations so that only individuals within the same group could be swapped. On each of these iterations, we conducted the same repeatability analyses as described above for the observed data. We consider the observed repeatabilities as statistically significant when their 95% credible intervals (hereafter 95CI) did not include the mean of the respective permuted repeatabilities.

We used the R package ‘MCMCglmm’ v.2.25 (Hadfield 2010) in the R environment v.3.5.2 (CRAN R Team 2018) for the statistical analyses. The Bayesian estimation allowed us to compute 95% credible intervals (hereafter 95CrI) for the variance components. We used the default priors. The auto-correlation for chains was <0.1 in all cases and the convergence of Markov-chains was checked visually.

RESULTS

For the wild Lundy sparrows, we collected 13085 dyadic interactions, including 471 individual birds, of which 49 birds had been seen only once, 28 birds had been seen twice, 68 birds had been seen between three and 10 times and 95 birds had been observed between 11 and 29 times, and 231 birds had been seen 30 times or more. Of these, 74 individuals had been seen 100 times or more.

For the Seewiesen sparrows, we collected 4432 dyadic interactions between 95 sparrows. One individual was only seen interacting once, 22 individuals were seen interacting between 2 and 29 times or less, and 73 individuals were recorded interacting 30 times or more. Of these, 36 individuals were observed interacting 100 times or more often. For the Silwood sparrows testing for between-individual differences across time, we collected 3775 dyadic interactions between 95 individuals. Of these, 80 birds were observed interacting 30 times or more. The dataset of the Silwood sparrows testing for long-term changes included 4244 dyadic interactions of 150 individuals. Of these, 86 individuals were recorded having 30 interactions or more. The Silwood dataset testing for short-term changes included 3826 dyadic interactions of 105 individuals, of which 33 were observed 30 times and more often.

Table 1. Descriptions of the four datasets, and the observed repeatabilities and their 95CIs of strength, betweenness, and closeness, of house sparrows interacting at a feeder. Note that the estimates for males in Seewiesen were quantified from a male-only group, while the sex-specific estimates for Lundy were quantified from a social network constrained to same-sex interactions only, even though birds did interact with both sexes on Lundy.

Population		Lundy (wild)			Seewiesen (captive)	Silwood (captive)	Silwood (captive)	Silwood (captive)
Sampling events		10			40	20	13	15
Sex		Mixed	Female	Male	Male	Mixed	Mixed	Mixed
Social environment between sampling events		Changing naturally through birth and death			Constant		Experimentally manipulated	
Habituation period		na			na	na	6 months	4 weeks
Sample size	Birds	471	218	249	95	95	150	105
	Interactions	13085	3151	3727	4432	3775	4244	3826
Repeatability	Strength	0.25	0.21	0.23	0.65	0.45	0.25	0.02
95CI		0.15 – 0.36	0.04 – 0.30	0.07 – 0.39	0.56 – 0.73	0.33 – 0.56	0.00 – 0.57	0.00 – 0.18
	Betweenness	0.28	0.23	0.20	0.53	0.24	0.03	0.01
		0.17 – 0.39	0.04 – 0.40	0.01 – 0.37	0.44 – 0.63	0.12 – 0.36	0.00 – 0.20	0.00 – 0.16
	Closeness	0.22	0.27	0.11	0.39	0.21	0.10	0.00
		0.12 – 0.32	0.10 – 0.42	0.01 – 0.27	0.29 – 0.50	0.10 – 0.33	0.00 – 0.53	0.00 – 0.05

In the wild population, and in both captive experiments across time where the social environment was not changed, the repeatabilities were statistically significant (Fig. 1), and ranged between 0.10 and 0.65 (Table 1). Repeatabilities were highest in the male-only captive set-up (Fig. 1). Interestingly, the repeatabilities only including single-sex interactions from the wild population were similar to those from mixed-sex groups, and their 95CI overlapped with the mean permuted repeatability (except for female closeness, Fig. 1A). In captivity, repeatabilities in the mixed-sex groups were lower than in male-only groups but did not differ much from mixed-sex repeatabilities in the wild (Table 1, Fig. 1A, C).

When sparrows had six months to habituate to a new social environment, strength and closeness still showed non-zero repeatability, whereas betweenness showed no repeatability (Table 1). After the social environment was changed, the 95CI of observed betweenness overlapped with the 95CI of the permutations (Figure 1C, D). Note that for the short-term measurements, we did not record enough interactions to achieve a high statistical power. However, the short-term repeatabilities differed from those calculated from the long-term habituation experiment (Fig 1D). When sparrows had less than a month to habituate to a new social environment, repeatability was practically non-detectable (Fig 1D).

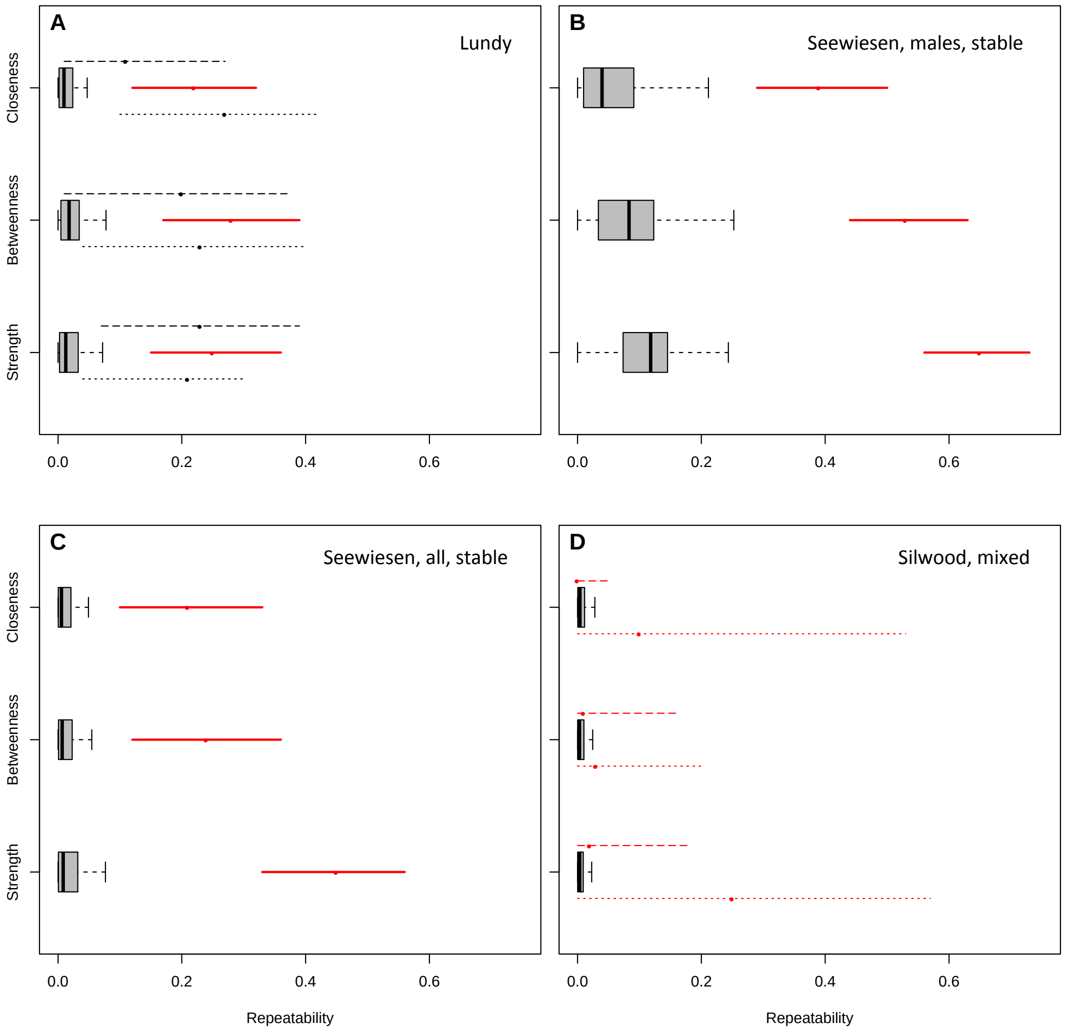

Fig. 1. Repeatabilities (red filled circles) and 95CI (lines) of individual social network variables in comparison to results from permutations (boxplots, whiskers: 95CI of permutation results, black filled circles indicate the mean). **A**: Lundy island sparrows across time. Red = all, black dotted lines = female-female interactions only, black dashed line = male-male interactions only. **B:** Seewiesen male sparrows across time. **C:** Silwood sparrows across time. **D:** Silwood sparrows after the social environment was changed. Red dashed lines: short-term habituation, red dotted lines: long-term habituation.

DISCUSSION

Our results show that sparrows' node-based metrics from social networks show repeatability across time and social environments. This fact was true for birds in the wild, and generally also in captivity, and in mixed- and single-sex groups. Thus, these results suggest that social network node-based metrics can be considered as animal personality traits. Our results thus corroborate previous findings in passerines showing that there are consistent between-individual differences in social traits across years (Aplin *et al.* 2015). We have also shown that video recordings and automated assays are adequate methods to collect individual interactions and to characterize the sociality of individuals.

Interestingly, we showed that between-individual differences across social contexts are dependent on the time frame studied, which suggests that populations need a minimum period of time to recover their initial social structure after a change in their composition. This is to be expected, as our concept of sociability, especially in the context of animal personality, may depend on a certain amount of familiarity between individuals. However, the concept of individual familiarity has to our best knowledge, not frequently been tested outside of family group structures (Lattore *et al.* 2019). These results suggest the importance of considering habituation time for any social experiments, and it may be that the time needed to habituate is longer than commonly expected, e.g. the habitutation period used in the literature is usually of 5 days (Boorgart et al., 2014; Nomano et al., 2014). Two conclusions can be drawn from these results. First, the habituation process potentially implies a mechanism of plasticity, which has the potential to direct future evolution by determining which trait values are exposed to selection; resulting in the final behavior we observe (West-Eberhard, 2003). In addition, personality traits have been shown to be heritable (Brent et al., 2013; Drent et al., 2003; Lea et al., 2010), thus if social components can be used as personality, it means they could be exposed to evolve via natural selection.

Recently, it has been suggested that male sociability may show higher repeatability than female sociability due to the different drivers of social behavior between sexes (Strickland & Frere 2018). This may affect the selection and maintenance of sex-specific behaviors and social aptitudes. The extent to which consistent variation is selected for, may be dependent on differences in selection pressures experienced by each sex as different mating strategies may impact their social interactions (Strickland & Frere 2018). Our data might be interpreted as supporting this notion, but with an important caveat: the social environment in which interactions are measured seems important. When we measured interactions in male-only environments, repeatabilities were surprisingly high. However, when we only used data of male-male interactions from mixed-sex environments, repeatability was nearly zero. Females did tend to have lower and bordering non-statistically significant repeatabilities in the wild compared to captivity values, suggesting a biological effect. The lower repeatability in females may overall reflect social plasticity. Thus it would be more benefitial for females to be socially adaptive in response to the dynamics of the changeable social environment, while the maintenance of specific behaviors in males might act towards their benefit when interacting with other males only (Nakagawa et al., 2007, but see Schroeder et al., 2016). It may also be possible that male-male competition increases in male-only environments, and in this case competition-related aptitudes might reach higher importance and should therefore be maintained, enhancing social repeatability values. Hence, future research exploring the extent of social plasticity, for example by differentiating between same-sex groups, and same-sex networks, is required.

Finally, while strength measures the number and weight of connections an individual has with others, the variables betweenness and closeness are less straightforward to interpret. This is because both measures not only take actions of the focal individual into account, but those connected with it and how well connected some of those are to others again. Some of this can certainly be governed by the focal individual, by choosing with whom to connect. For instance, certain individuals may be more likely to connect to other well connected individuals, while others may avoid this, and such behavior may well be what we picked up in our analysis. Although strength depends on the behaviours of others, as if an individual would refuse to associate with another it would affect their strengths. This effect is even stronger in betweenness and closeness given the nature of these variables. Yet, undoubtedly, the variables will be influenced paritally by indirect effects originating from interacting individuals (Brent, 2015; Fisher & McAdam, 2017). Quantifying these indirect effects is challenging, interesting and important.

To conclude, our comprehensive analysis across time and context, both in the wild and in captivity, using video analysis and automated data collection, suggests that, individual variation in social network node-based metrics in sparrows was consistent through time and context, suggesting that social network metrics can be considered animal personalities.

Author contributions

JSchroeder and TB conceived the ideas and designed the study; MP, TC, AFC, AG, GH, DAM, MSF, JSomerville, AST designed and performed the experiments; all authors contributed to data collection; JSchroeder, AFC and AST analyzed the data; all authors drafted the manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

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CHAPTER VI

FEMALES WITH MORE SOCIAL INTERACTIONS SHOW HIGHER LEVELS OF EXTRA-PAIR COPULATIONS PROPORTION IN THE HOUSE SPARROW

ABSTRACT

A large body of literature has revealed that most socially monogamous bird species show extra-pair mating behaviour. Even though, the reasons why females engage in extra-pair copulations (EPC) have not yet been clarified. Promiscuous behaviour results from the interactions between each individual and its social environment, which is characterized by the behaviour of all individuals in the population. Here, we test the hypothesis that three female node-based metrics derived from social association networks between individuals (strength, betweenness, closeness), will be positively associated with the frequency of EPCs in the house sparrow. We also performed an experimental design by manipulating the social structure of the population to study its effect in the EPCs. Our results showed marked significant positive associations between EPC proportion and strength, negative associations with betweenness, and no relation with closeness. Our experimental design also demonstrated that females with highest closeness values showed higher proportion of EPCs. This suggests that sociality is the strategy most females use to cope with their environments during the breeding period, allowing them to increase their fitness through promiscuous behaviour. Social metrics of individual centrality, provide different information from individuals and may enforce different effects on their reproductive behaviour: while strength represents the summary of direct interactions of a focal individual with the others in the population, closeness and betweenness are indicators of the specific position of an individual in the social network. Including the social context when studying mating behaviour (and specially extra-pair behaviour) is needed in future studies.

INTRODUCTION

Extra-pair paternity (EPP) is a widespread phenomenon (Petrie and Kempenaers 1998) that results from mating outside the social pair-bond in monogamous species. Although in the last three decades it has been intensely studied in birds (Westneat and Stewart 2003; Forstmeier et al. 2014), the reasons for the wide variation in the frequency of EPP across and between species, are not completely understood (Johnsen and Lifjeld 2003; Cohas and Allainé 2009; Wan et al. 2013). Nonetheless, great effort has been made to determine possible criteria explaining extra-pair mate choice (Griffith et al. 2002; Forstmeier et al. 2014), and the costs involved for females in engaging in extra-pair copulations (Hsu et al. 2014; Hsu et al. 2015). Adaptive explanations are based on studies that find evidence for that females obtain direct and indirect benefits from EPP. Those advantages include enhance the quality of their offspring, as they can gain good genes (Johnsen et al. 2000; Edme et al. 2016), avoid inbreeding (Arct et al. (2015) but see Hsu et al. (2015)), evade male infanticide (Wolff and Macdonald 2004) or ensure fertility (Griffith 2007; Hasson and Stone 2009).

However, evidence for sexual conflict argues against the generality of female benefits (Westneat and Stewart 2003; Arnqvist and Kirkpatrick 2005; Low 2005; Sigrunn 2008). Based on that drivers of selection must be different in each sex, males may drive the incidence of EPP through their tendency to seek copulations independent of female choice (Westneat and Stewart 2003; Arnqvist and Kirkpatrick 2005). And females may be selected to resist extra-pair copulation (EPC) attempts by extra-pair males (Plaza et al. 2019b; Plaza et al. 2019c) but be unable to completely avoid them (Poiani and Colin 2000; Arnqvist and Kirkpatrick 2005). Thus, the incidence of EPP in different populations may depend on the relative strength of selection for or against involvement of EPC in the two sexes. In summary, results from studies even in the same or closely related species are contradictory, and there are yet no firm conclusions on the reasons for female taking part in this behavior (Forstmeier et al. 2014).

An EPC involves at least four parties: the social male, the social female, one extra-pair male and his social female (Westneat and Stewart 2003a; Canal et al. 2012).

Therefore the occurrence of EPP will result from the interaction between these individuals, and their social behavior must be of importance for the resulting outcome. Additionally, the availability of individuals to mate with, the propensity and willingness of those individuals to also engage in EPC, and the ability of an individual to resist engaging in copulations (Girndt et al. 2018), determine the social environment, influencing the mating system and thus the EPCs frequency of the population (Maldonado-Chaparro et al. 2018). However and specifically, the consequences of consistent individual differences in social behavior (e.g. (Kulahci et al. 2018; Plaza et al. 2019a) for the evolution of social and mating strategies, have rarely been considered (Patrick et al. 2012). Thus, if behavioral traits can explain individual variation in promiscuity (van Oers et al. 2008; While et al. 2009), resolving the connection between mating strategies and social environment might allow us to discover how sociality takes part in the resulting mating decisions.

Although there are not many studies on this topic, several reviews have pointed out the importance of taking into account the social networks function in the behavioral ecology systems (Wey et al. 2008; Croft et al. 2009; Krause et al. 2009). The social network is the structural characteristics of a number of individuals connected via social ties, including finding sexual partners, engaging in foraging and antipredator behavior, and developing and maintaining cooperative relationships (Krause et al. 2015). A social network model includes a set of nodes that represent individuals connected through edges, that are the interactions between them. This drives towards the concept of the individual social phenotype, defined through its node-based metrics that quantify the number and intensity of connections and the position of this individual with respect to the others in the same network. The social phenotype is expected to vary among individuals in the population and to be consistent within them in different time and context scales (Plaza et al. 2019a).

The opportunity hypothesis suggests that social network dynamics are related to reproductive ones, and that the time an individual spends with others in social activities like feeding or communicating is positively correlated with the intensity of its mating behavior (Krause et al. 2015). Given this, it was hypothesised that individuals that have more social contacts and spend more time with others, are more likely to engage in mating activities, and EPP activities in particular (Maldonado-Chaparro et al. 2018).

In the present study we investigate female involvement in EPCs to increase our knowledge on the drivers of EPP patterns and their variability. We test the opportunity hypothesis in female house sparrows *Passer domesticus* of a captive population, a model species in this context as it shows genetic polygamy but social monogamy and biparental care (Girndt et al. 2018). To test this hypothesis we analyzed the associations between three social node-based metrics and the EPC proportion. We also performed an experimental design manipulating the social structure of individuals to study the effect of this manipulation in the EPC behavior. Our prediction was that female extra-pair mating will be positively associated with the number and intensity of social interactions inside a population network.

MATERIAL AND METHODS

Study population and breeding set-up

This study was conducted in a captive population of house sparrows *Passer domesticus,* kept at the Imperial College in Silwood Park, Ascot, UK, during 2017 and 2018. The population consisted in originally wild-caught animals born in 2005 and 2006 and their offspring born in captivity, that were looked after as described in Girndt et al. (2017), and a series of long term studies have been conducted with them.

The animals were allocated to mixed sex groups per aviaries (Table 2). In a previous study in the same population we demonstrated that the individual social network traits strength, betweenness and closeness are consistent across time and context, as they are repeatable (Plaza et al. 2019b). All aviaries were provided with *ad libitum* food consisting in dry mealworms or sunflower seeds, nesting material based on heather, cotton or wool and nesting sites (nest-boxes) to avoid resources competition between animals, allowing equal opportunities for them to reproduce (Girndt et al. 2019). The same person replaced food and materials once per day to avoid them to run empty.

Social data collection

To obtain social interaction data, we recorded individual interactions by using an automated radio frequency identification system (RFID) in a foraging context. Each individual was tagged with a passive integrated transponder (PIT), which provided a unique 10-digit code when scanned with a portable reader. Social associations were recorded inside a feeder fitted with two RFID antennas at the 'in' and 'out' sides of its entrances. The antennas were connected to a data-logging reader, that recorded the code of each individual, as well as the date and time of each event (Bonter and Bridge 2011).

All interactions were recorded from 27th December 2017 to 5th March 2018 (N = 280 hours), occurring between 07:00 – 17:00. A decoy RFID apparatus was used for a month before starting recordings to allow the animals to acclimatize to the presence of the equipment in each aviary (Plaza et al. 2020). Recordings for each aviary were taken in rotation (the non-decoy apparatus was placed five times in each aviary), as we were limited to one feeding station. We used the co-occurrence of two individuals inside the cage for longer than three seconds as a proxy for a social interaction (Maldonado-Chaparro et al. 2018).

Social network metrics

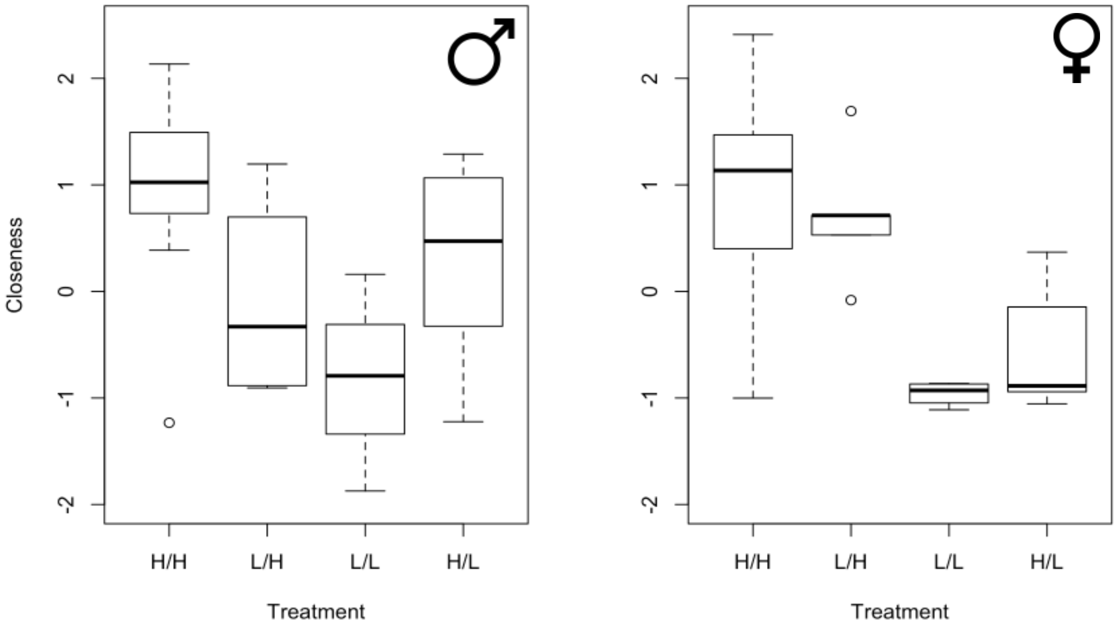
From the recorded interactions, we constructed a social network per aviary and occasion (with weighted edgelists using the R package 'iGraph' v.1.2.4.1) and extracted three social node-based metrics from this network: strength, betweenness and closeness (Csardi & Nepusz 2006). Strength is the number of direct connections of a focal individual with the others in the population, and it is weighted by the total number of interactions, thus, it represents the intensity of the sociability of an individual; betweenness is the number of shortest connections between a pair of individuals that 'pass through' the focal individual, and describes how well an individual connects different individuals of the network; and closeness is the length of the average shortest path between a focal individual and all the other individuals within the network, so it quantifies how central an individual is in the population (Csardi & Nepusz 2006).

Experimental design

We used the social network traits measured in the winter 2017-18 to assign birds to two categories of either high and low closeness. This trait was chosen as it indicates how central, or well linked, an individual is. We hypothesised that many short links to many other individuals might be something that is a prerequisite for many interactions, and also sexual interactions. We chose birds that had in their network either the 40% smallest closeness values per sex (in the following: group 'high' because they are highly social) or the 40% top values per sex ('low').

During the breeding season of 2018 we redistributed these birds to four aviaries. On the 1st of March we allocated 10 males and 10 females to each treatment group, such that either males and females had a similar trait, or opposite trait, in the same aviary (male-female combinations: high/high, high/low, low/high, low/low). This distribution let to some overlap between the high and low group in terms of their 2017 score – this is because their score was relative to others in their then aviary and sex group (Fig. 1).

Fig. 1. Experimental design of breeding in 2018. Closeness was scored in captive house sparrows in winter 2017 and scaled based on their treatment group and sex. The treatment group refers to the category of males/females.



Extra-pair behaviour data collection

We performed daily observations from 1rst May to 22nd July 2017 and 2018, which represents the beginning and the main breeding season of house sparrows. All observations were conducted between 8:00 a.m. and 11:30 a.m., as it has previously been reported to be the time of the day when the highest frequency of sexual encounters occur (Girndt et al. 2018). Birds were individually marked with a unique combination of three colored pvc rings, and a numbered metal ring, to be easily identified by sight. All recordings were carried out exclusively by two observers that registered the individuals involved in all copulations by using a Zeiss Victory 10 × 42 mm binocular. Both observers were blind to the age and pairing status of individuals when recording copulation behaviour. We divided each aviary into three same-sized sections so that they could be observed with an unobstructed view. Each aviary section was observed separately for 10 minutes resulting in a total observation time of two hours and a half per day in 2017 and three hours and a half per day in 2018.

Pair-bonds and nest-box owners were identified by simple repeated observation of the birds in each nest box, attending and building nests, defending them or incubating the eggs inside. As house sparrows commonly form pair-bonds after a male has got a nest site, the repeated presence of a male and a female at the nest is a strong indication of their pair-bond.

Statistical analysis

We analyzed an indicator of the intensity of extra-pair mating interactions, namely the proportion of extra-pair copulations in relation to the total number of copulations of an individual female (EPC). This variable was used as dependent variable in three sets of Generalized Linear Mixed Models with quasi-binomial distribution, to test the effect of the three social variables (strength, betweenness and closeness) on the female copulation behavior. We included age of the female as covariate because it has previously been reported to influence extra-pair behavior (Moreno et al. 2015), and the aviary where the animals were located in because it represents our unit (network).

To solve the problem of the non-independence characteristics inherent to the social data, we ran 1000 iterations of the observed data for every model. In each iteration, we permuted the identity of the individuals (nodes) interacting with each other, and the number of times an association was observed (weights), while keeping the total number of associations, individuals, and the distribution of the weights the same. On each of these iterations, we conducted the same analyses as described above for the observed data. We constrained randomizations so that only individuals within the same group could be swapped. We considered the observed values as significant when the 95% confidence intervals (CI) did include the mean of the respective permuted value for the regression model coefficient.

We then ran a Generalized Linear Mixed Model using the same dependent variable described above, and the aviary (each group with the combination of closeness category) as independent variable and the age of individuals as a covariate, to study potential differences in the proportion of EPC between treatment groups.

Before all analyses, we log-transformed and standardized all social network metrics (Schielzeth 2010). We reduced total datasets to those only for females with which we conducted all statistics. We also performed same analyses but using the total number of copulations instead of EPC as dependent variable, to test for variation in general sexual activity instead of promiscuous activity driving the pattern. All the analyses were performed using lm4 package (Bates et al. 2015) in R v.3.5.2 (CRAN R Team 2018) software.

RESULTS

EPC occurred in 167 out of 433 observed copulations (38.56%) with 66 females and 60 males involved in them. The number of EPC ranged from 1 to 10 per female, and 1 to 9 per male, being on average 2.20± 0.20SE and 2.21±0.10SE respectively (Table 1).

Table 1. Number of male, female, total individuals, social interactions, copulations and extra-pair copulations for 2017 and 2018 years in the different aviaries.

Year	Aviary	N females	N males	N social interactions	N copulations	N EPC
2017	1	12	6	620	97	25
	2	17	16	1687	-	-
	3	12	12	897	-	-
	4	7	11	572	147	44
	TOTAL	48	45	3776	244	69
2018	1	10	10	-	9	4
	2	10	10	-	42	26
	3	10	10	-	31	17
	4	10	10	-	12	5
	5	10	10	-	22	13
	6	10	10	-	63	25
	7	10	10	-	10	8
	TOTAL	70	70	-	189	98

We found a significant positive relationship between female proportion of EPC and strength (531.31±77.88SE) but a negative relationship with betweenness (0.01±0.00SE), and no significant relation was found between the dependent variable and closeness (19.32±1.93SE) (Table 2, Fig. 2). We found no significant relationship between total number of female copulations and the three social node-based metrics.

Table 2. results of Generalized Linear Models with quasi-binomial distribution for females, using the EPC (Number of extra-pair copulations / number of total copulations) as dependent variable and three social metrics (strength, betweenness and closeness) as predictors and aviary and as random factor and female age as covariate.

MODEL	Parameter	Estimate	Uncertainty	Z	Probability
EPC proportion ~ Strength + female age + Aviary	*Fixed effects*	*B*	*SE*		*P*
	Intercept	-0.72	0.52	-1.39	0.16
	Strength	0.57	0.24	2.33	**0.01***
	Age	0.14	0.08	1.70	0.08
	Random effects	*Variance*			
	Aviary	0.19			
EPC proportion ~ Betweenness + female age + Aviary	*Fixed effects*	*B*	*SE*		*P*
	Intercept	-0.46	0.54	-0.84	0.39
	Betweenness	-1.06	0.35	-2.98	**<0.01***
	Age	0.07	0.08	0.86	0.38
	Random effects	*Variance*			
	Aviary	0.12			
EPC proportion ~ Closeness + female age + Aviary	*Fixed effects*	*B*	*SE*		*P*
	Intercept	-0.82	0.53	-1.53	0.12
	Closeness	-0.21	0.20	-1.02	0.30
	Age	0.18	0.08	2.23	0.02
	Random effects	*Variance*			
	Aviary	0.21			

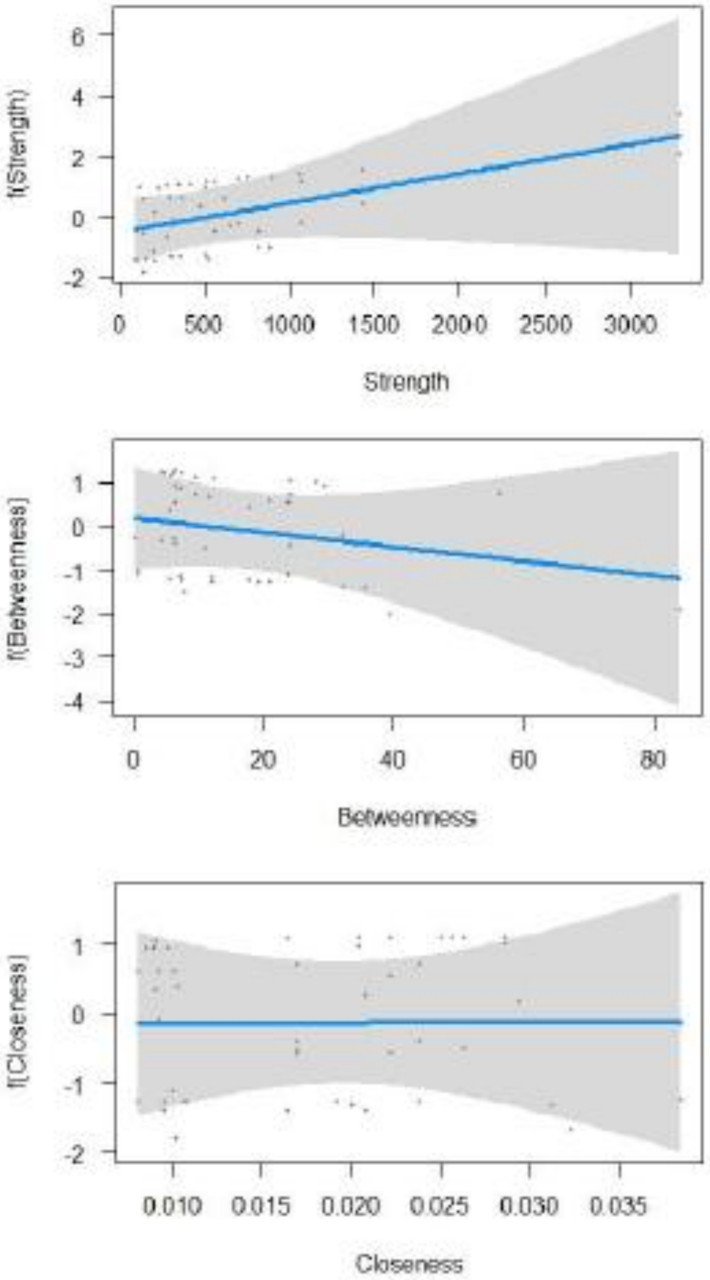


Fig. 2. Conditional plot of Generalized Linear Models with quasi-binomial distribution for females, using the EPC as dependent variable and three social metrics (strength, betweenness and closeness) as predictors and aviary as random factor and female age as covariate. It expresses the relation between partial residuals of EPC (f(variable)) and each dependent variable using the "Visreg" package 2.5-1 (Breheny, 2019). Blue lines describes the prediction values and the grey band describes the confidence intervals.

Our experimental design showed significant differences for the proportion of EPCs between groups. This effect was due to higher values in the high/high treatment compared to the low/low treatment (Z = 2.32; *P* = 0.02, Table 3; Fig. 3). No significant differences were found between groups in the total number of female copulations.

Table 3. Experimental distribution of individuals classified by their social closeness category. Mean values of EPC (±Standard Error) and results of a Generalized Linear Model with quasi-binomial distribution for females, using the EPC (Number of extra-pair copulations / number of total copulations) as dependent variable and the experimental group (Aviary) as predictor and female age as covariate. Aviary 2 is included in the intercept.

Parameter	**EPC proportion**	**Estimate**	**Uncertainty (SE)**	**Z**	**Probability *P***
(Intercept) Low/High	0.54±0.15	0.02	0.62	0.03	0.96
High/High	0.75±0.22	-1.69	0.88	-1.91	**0.05***
High/Low	0.70±0.20	-0.27	0.84	-0.32	0.74
Low/Low	0.51±0.16	-0.33	0.62	-0.53	0.59
Female age	-	0.28	0.16	1.68	0.11

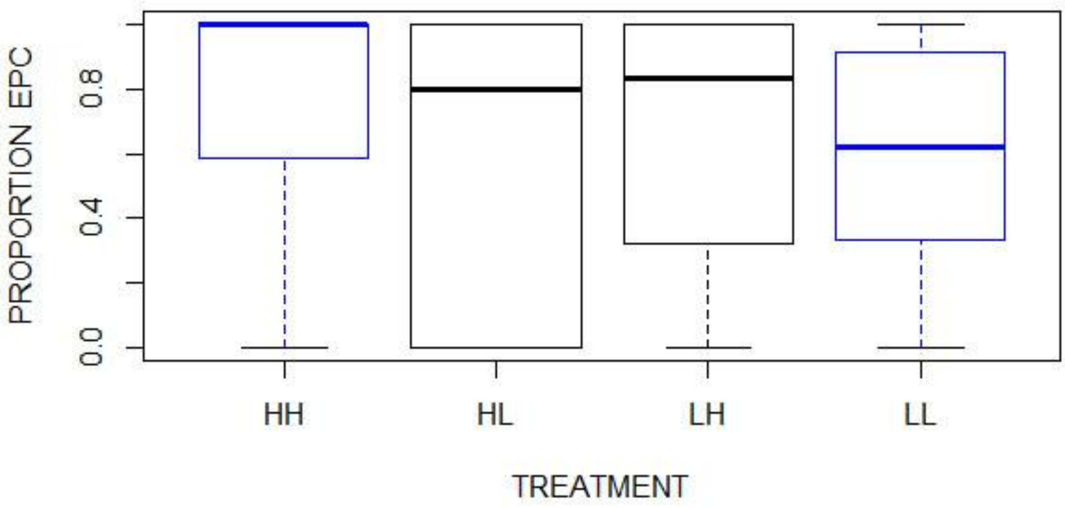


Fig. 3. Proportion of EPC in the four experimental aviaries. Central bars represent the mean value, boxes represent Standard Error and whiskers represent 95% confidence intervals.

DISCUSSION

Our results showed marked significant positive associations between EPC proportion and strength, negative associations with betweenness, and no relation with closeness. Also our experimental design presented meaningful differences between treatments, where females with highest closeness values showed also higher proportion of EPCs.

It has been described that more social individuals (those which take part in more interactions), are more likely to obtain benefits such as access to location of resources or potential threats (Atton et al. 2014; Brent 2015), by establishing cooperative relations and reducing aggressive interactions (Grabowska-Zhang et al. 2011). Thus, even a cost is paid on competition and disease transmission (Corner et al. 2003; Silk et al. 2017), they may improve their fitness by obtaining essential information from others. Also, specifically during the breeding period, social associations may be crucial for females to overcome this sensible phase in which offspring survival becomes essential to ensure individual fitness. Several studies reported the effects of social environment on reproductive performance (Beletsky and Orians 1989; Krams et al. 2008) and sexual selection (Oh and Badyaev 2010); In particular individuals can manipulate their social environment to get access to the best quality partners (McDonald 2007). In our study, social interactions were measured during the winter, out of the breeding seasons when EPCs occurred. Few studies have investigated how social associations during the non-breeding period, impact on future mating behavior (Choudhury and Black 1994; Firth and Sheldon 2016). Specifically Beck et al. (2020) reported how individuals that forage more together in winter, are also more likely to be extra-pair partners in the next season. Indeed, individuals tend to breed closer to familiar birds, as this offers potential benefits. Breeding near prior social associates may also potentially result in increased matings and specifically extra-pair matings, as extra-pair partners use to be in close territories (Canal et al. 2012; Kaiser et al. 2017; Mennerat et al. 2018) and prior social associations may increase attractiveness (Choudhury and Black 1994). Several studies on extra-pair paternity described some ecological or behavioral benefits that extra-pair males can assign to females like courtship feeding, providing resources or direct extra parental care in feeding their young (Wolf 1975; Kempenaers and Dhondt 1993; Kleindorfer et al.

2005). Also, extra-pair males might be more prone to do so if their social bonds are yet established with females offering a potential ecological advantage (Møller 1987).

Our results must be interpreted in the same line: strength has a key relation with sociability as it represents an overall total tendency to interact with other individuals. However, closeness and betweenness have plainly been described as measures that count paths between pairs of individuals (Krause et al. 2015). An individual could be very central in the network with a high betweenness despite interacting with very few other individuals, or have high betweenness with many interactions. Besides, closeness summarizes direct and indirect connections through all individuals in the population and the focal subject, so an individual can directly interact with other few or many individuals independently of its level of closeness. These two variables therefore capture sociability in a different way that strength does, becoming indicators of a different kind of sociability closer to the concept of individual social position. Moreover, betweenness and closeness measures appear more strongly dependent on the interactions of partners, as the number of connections they have in the network will exert a strong effect on the measure. Consequently potential differences in the relation between each social variable and other behavioral traits are expected to be found. Our results pointed out that more sociable females, those which have more interactions with other individuals in the population (showing higher strength), tended to be more promiscuous. On the contrary, the "connectivity" of a concrete female individual (measured by betweenness), was negatively related with its extra-pair reproductive behavior, while its "position" in the network (measured by closeness) showed no relation with its extra-pair reproductive behavior.

Additionally our treatment showed that more sociable females interacting with more sociable males (measured by closeness), had an increased EPC proportion in comparison with the less sociable group for both sexes. Those differences became significant when we looked at the extreme of sociality combinations between groups (H-H and L-L). In our experimental design we mixed individuals with the same or different social category. Combined effects of their social phenotype can affect the resulting individual EPC behavior by enhancing it. It has previously been hypothesized that the individual level of EPC could vary in response to the social environment that it experiences (Maldonado-Chaparro et al. 2018). This can explain why we did not find a relation between closeness and the proportion of EPC in the general analyses, but its effects appear to be magnified in the experimental design.

One interesting point is that our results are based on differences in EPC proportion, but when we looked at the analyses including the total number of copulations (which is an indicator of the total sexual activity), we did not find significant tendencies. This allows us to ensure that the effects of our results are not due to a direct relationship between sociability and general sexual activity. For example it could have been suggested that more sociable females can be more prone to display a general enhanced sexual activity, which was not the case.

These results point out an integrative concept of population network. Social network analyses arise from individual interactions (bottom-up), but also selects for individual behavioral strategies (top-down) (McDonald et al. 2013). This idea is also suitable for sexual interactions and also extra-pair interactions, if the overall relations in the population can be interpreted as a sexual network. It leads towards a wider concept of social network by including sexual relations. Mating behavior should be considered as a social network of sexual interactions: a new insight in sexual selection. Both types of interactions are closely related and must be analyzed under an integrative point of view (McDonald et al. 2013; Krause et al. 2015).

To conclude, our results support the idea that sociality is a strategy most females use to cope with their environments during breeding, allowing them to increase their fitness by enhancing proportion of EPC. Also, our results point out that different social metrics used as measures of individual centrality, provide different information about individuals which future studies must account for. Including the social environment when studying mating behavior (and especially extra-pair behavior) is needed.

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INTEGRATIVE DISCUSSION

The research described in the present thesis and its results explore how the social environment, approached from different points of view, impacts on reproductive processes in female passerine birds of both a territorial and a colonial species. The social context of reproduction for females includes not only their social partner, but also potential alternative mates and female rivals. Competition for reproductive opportunities with other females and conflicts of interest with extra-pair mates, may crucially affect female fitness driving the evolution of social signals and modulating promiscuous behaviour. This thesis aims at unraveling the impact of social interactions on female reproductive adaptations.

Competition over resources necessary for reproduction may drive the behaviour of individuals during the breeding season. Nest cavities constitute a scarce resource for obligate hole-nesting birds that limits the availability of breeding opportunities, leading to strong competition over them (Ingold, 1994, Li & Martin, 1991). This competition is expected to constitute an important selective force for the evolution of female social behaviour (Stockley & Campbell, 2013) that may favour the expression of social status through plumage signals. Few studies have investigated the effect of this competition on female breeding activities (Jawor et al., 2006, Jawor & Breitwisch, 2006).

Chapter I shows that female plumage signals can be considered badges of status in territorial social contexts. In accordance with this assumption, it has been shown that levels of female vigilance against intruders decrease with the size of their white wing patches during the crucial incubation phase (Deeming, 2002). Females with larger patches may experience a reduced threat imposed by female intruders, and consequently ignore rather than attack them, thereby enhancing allocation of time for reproductive activities such as incubation attentiveness (Cantarero et al., 2015). We show also that females with larger wing patches arrive earlier at the breeding sites, thereby securing a nest cavity. This enforces the link between wing patch size and fitness, in the same way as previous work has shown that there is a positive association between the expression of female signals of dominance and improved hatching success (Morales et al., 2007). Female breeding activities are thus not only the product of their physical condition but crucially also of their social status as expressed through the extent of plumage signals.

In addition to the described female-female competitive interactions during the reproductive season, females of socially monogamous species may be involved in mating interactions not only with their social partner, but also with other males in the population. Forced copulation has been reported in a wide variety of animal species, including birds (Thornhill & Palmer, 2000), and it is generally characterized by male force and female resistance. However, female active resistance may be weak in cases where males gain sexual access through threat of force and females passively accept because of high costs of resistance (Palmer, 1989). However, it has been alternatively proposed that females may seek matings with extra-pair males in search of fitness benefits through improved offspring genetic quality. To avoid approaches by unwanted extra-pair males or to search for matings with preferred extra-pair males, flight capacity during the fertile phase may be crucial. We would predict that impaired flight capacity should favour extra-pair matings in the first scenario and reduce their chances in the second. The results of the experiment described in **Chapter II** confirm that females with a diminished flight capacity during the fertile period show increased EPP levels in their broods in accordance with the first scenario. Accordingly, patterns of EPP in pied flycatchers are not consistent with the predictions of female choice, and are more readily explained as a result of extra-pair males pursuing their own fitness interests, thus supporting the sexual conflict hypothesis. These results are in accordance to those previously found in the same population showing that longer-winged females show reduced EPP levels in their broods (Moreno et al., 2015). In addition, no benefits for females in terms of larger or more heterozygous extra-pair offspring have been detected in the same population (Moreno et al., 2013).

Continuing with the study of the influence of female traits on EPP, **Chapter III** demonstrates that females with increased body mass during the fertile period as a consequence of avoiding the cost of nest construction, display higher EPP levels in their broods. This highlights the impact of adaptive body mass changes during reproduction in females (Moreno & Carlson, 1989, Moreno, 1989) on paternity of their offspring. Female birds usually attain their highest levels of body mass during the fertile phase when reserves needed for egg formation are being accumulated (Moreno et al., 2010a). However, mass has implications for wing loading and thus for mobility. The interaction between female reserve adjustments during the fertile phase and paternity has not been previously addressed, but as shown in the previous chapter female flight capacity may have important implications for EPP rate. An enhanced body condition can lead to an increased female capacity to seek EPCs which would support a female adaptive mate choice scenario. However, an increased female body mass can also diminish flying ability leading to females being less able to avoid unwanted copulations, supporting sexual conflict. In addition, the negative relationship found between EPP and the extent of the female white wing patch supports that dominant females show lower levels of EPP (see Chapter I for the social impact of the white wing patch). Altogether and given that there is no previous evidence for female benefits derived from promiscuous behaviour in our population (Moreno et al., 2010b, Lifjeld et al., 1997, Moreno et al., 2013a), results from this chapter support those presented in Chapter II in favour of a non-adaptive explanation of EPP for females in our population. In the social context of reproduction, both individually based capacities and the social environment may affect the resulting mating behaviour to a variable degree.

Most studies focus on the implications of individual male or female traits for the resulting EPP patterns in avian populations, while the importance of a variable social context is seldom taken into account. The literature presents a huge variation in trends and patterns concerning EPP without any major consensus about the sources of this variation. Studies of the same population in different years may lead to different results concerning the individual traits explaining the incidence of EPP. This lack of repeatability of results between years in the same population or between different populations of the same species may be based on an inadequate knowledge of variation in the social contexts in which mating behaviour occurs. For individual traits to explain EPP, we should expect a significant repeatability of individual EPP between years. **Chapter IV** shows that EPP is not repeatable between years in either males or females in the pied flycatcher. Given that the EPP phenotype of an individual depends on both genetic and environmental components (Whittingham et al., 2006), these results point out that the effects of variation in the social environment are presumably the main factor affecting EPP patterns in our study populations. In addition, some male plumage traits were found to explain part of EPP variation in multivariate analyses while female plumage traits were less important. However, the results concerning male traits did not agree with those from previous studies of EPP in one of our study populations, indicating that it is difficult to find a consistent inter-year pattern when changes in the social context are unknown. The conclusion is that aspects like density of neighbouring territories, presence and number of floating males in the population and their traits which are seldom included in analyses and not frequently studied, may explain a good part of inter-population and inter-year differences in EPP patterns of territorial birds in the literature.

For being able to estimate the direct effects of the social environment on reproductive behaviour in gregarious species, a prior analysis of the sources of variation in social relationships is needed. **Chapter V** describes how the social phenotype of house sparrows measured through node-based metrics like strength, closeness and betweeness (Krause et al., 2010) differs consistently between individuals in both sexes, and how it is individually consistent across the different times (long term and short term) and context (in the wild and in captivity) studied scales. Although the highest repeatability values were estimated in male-only captive groups, suggesting that male-male competition increases in male-only environments, single-sex networks subsets from mixed-sex groups showed no sexual differences in the consistency of social network node-based metrics. We also show that this consistence remains in both captive and wild environments, even after a disturbance that creates a new group social composition leading to a decrease in repeatability for up to six months. Given that social interactions are repeatable, this allows us to suggest that they can be considered as animal personality traits (Réale & Dingemanse, 2010), providing a reference for a first estimate of heritability and suggesting that they can be selected for.

The latter assumption allows us to investigate how individual female social phenotypes affect reproductive behaviour, given personality impacts on mating systems, by determining the propensity, willingness and ability of individuals to interact with others (Maldonado-Chaparro et al., 2018). The importance of social relationships for female mating decisions is shown in **Chapter VI,** which describes how females with more social interactions (measured by strength) prior to reproduction display a more intense extra-pair mating behaviour (measured through the proportion of EPCs). Additionally our treatment showed that more sociable females interacting with more sociable males (measured by closeness), show an increased EPC proportion. This could be explained if more sociable females are more likely to obtain reproductive fitness benefits (Atton et al., 2014, Brent, 2015). However, although females occasionally solicit EPCs (Whitekiller et al., 2000), they are also targets for attempts at forced extra-pair copulations. Communal displays involving a single female and several males have usually been reported during the fertile phase of the species (Møller, 1987, Summers-Smith, 1954). During communal displays females react aggressively to non-mate males suggesting copulation resistance. Given this, our results could also be interpreted as more sociable females using EPCs as a strategy to avoid aggression by extra-pair males in accordance with results in Chapters II and III in territorial species.

Social network analyses arise from individual interactions (bottom-up), but also selects for individual behavioural strategies (top-down) (McDonald et al., 2013). This framework is also suitable for interpreting sexual interactions including extra-pair interactions, if the overall relations in the population can be interpreted as a sexual network. It leads towards a wider concept of social networks by including sexual relations, so mating behaviour is considered as a social network of sexual interactions, leading to a new insight in sexual selection. Both sexual and social interactions are closely related and must be analyzed under an integrative point of view (McDonald et al., 2013, Krause et al., 2015). In addition, no association was found between EPCs and closeness or even a negative association was found with betweenness. While strength represents the summary of direct interactions of a focal individual with others in the population, closeness and betweenness are indicators of the specific position of an individual in the social network (Krause et al., 2015). These results point out that different social metrics used as measures of individual centrality, provide different information from individuals, and that they may enforce different effects on reproductive behaviour. Thus, future studies should take into account these divergences.

CONCLUSIONES GENERALES

- Los rasgos ornamentales de las hembras son funcionales y actúan como señales de estatus social, mediando las interacciones entre ellas durante la competencia por las cavidades de nidificación en contextos reproductivos, en el papamoscas cerrojillo. Esta suposición implica que las señales sociales en hembras son el producto de la selección sexual y social. Puesto que su tamaño está positivamente relacionado con el comportamiento de incubación, las hembras deben ser seleccionadas para aquellos rasgos que señalizan su habilidad competitiva e incrementan así el éxito reproductor.
- La paternidad extra-pareja en especies territoriales, debe ser el resultado de un conflicto social en el cual, la fuerza selectiva de los machos por copular con hembras fuera de su pareja social, es mayor que la de las hembras en evitar estas cópulas, o alternativamente de la capacidad de las hembras por escoger machos de mayor calidad fuera de la pareja. Las hembras de papamoscas cerrojillo experimentalmente mermadas en su capacidad de vuelo durante la fase fértil, presentan mayores niveles de paternidad extra-pareja en comparación con hembras control.
- Mayores reservas corporales durante la puesta, suponen una mayor fecundidad potencial, pero conllevan también mayores niveles de paternidad extra-pareja. Ello nos permite deducir que la masa corporal afecta a la actividad de las hembras, ya sea a través de una mejora en la condición corporal para la búsqueda activa de machos extra-pareja o al contrario, a través de una reducción en la capacidad de vuelo y de su capacidad para evitar el acoso de otros machos. Una reducción del esfuerzo de construcción del nido, produciendo un aumento de la masa corporal durante la fase fértil en hembras de papamoscas cerrojillo, está asociada a un incremento de los niveles de paternidad extra-pareja en comparación con hembras control, y puede ser atribuido a ambos escenarios adaptativos. La evolución de las estrategias de

cambio de masa corporal en las hembras con desarrollo altricial, tiene implicaciones en los patrones de paternidad extra-pareja.

- La dependencia del fenómeno de la paternidad extra-pareja por el contexto social debe convertir a este rasgo en no repetible. En dos poblaciones de papamoscas cerrojillo, la extra-paternidad no fue individualmente repetible entre años en ninguno de los dos sexos. La variación en el contexto social en el que las cópulas extra-pareja tienen lugar en distintos individuos entre y a lo largo de los años, puede potencialmente explicar la ausencia de repetibilidad individual. Ello tiene importantes repercusiones en futuras investigaciones ya que las fluctuaciones del contexto social hacen que los resultados de diferentes estudios en una misma población no sean repetibles.
- Los fenotipos sociales de los individuos pueden medirse a través de las variables de medida de red social, basadas en el nodo (individuo). La consistencia interindividual en tales variables sociales persiste a través del tiempo y en distintos contextos, sugiriendo que pueden ser consideradas rasgos de personalidad en las aves paseriformes.
- Las hembras con altos niveles de sociabilidad medidos a través de las variables individuales de red social, muestran mayor proporción de cópulas extra-pareja durante la fase fértil. Ello sugiere que la sociabilidad es la estrategia a través de la cual muchas hembras de aves gregarias se enfrentan a sus entornos durante la reproducción, permitiendo así incrementar su éxito reproductor, aumentando la proporción de cópulas extra-pareja con machos preferidos. Sin embargo, las hembras más sociales también podrían estar utilizando las cópulas extra-pareja como una estrategia para evitar la agresión por parte de otros machos.
- Las distintas variables de red social utilizadas como medidas de centralidad individual, proporcionan diferente información sobre los individuos y deben tener distinto efecto en su comportamiento reproductor. Mientras la variable “fuerza” muestra una relación positiva con la proporción de cópulas extra-pareja, y representa el sumatorio de las interacciones directas de un individuo focal con los otros en la población, la “cercanía” no muestra ninguna relación y

la “intermediación” muestra una relación negativa, siendo ambas variables indicadoras de la posición de un individuo focal en la red social.

- Es necesario incluir el contexto social, entendido como las relaciones entre los individuos de una población, en los estudios de comportamiento reproductor y especialmente al investigar las razones del comportamiento extra-pareja.

GENERAL CONCLUSIONS

- Female white wing patches act functionally as badges of social dominance mediating female-female social interactions in competition for nest cavities in pied flycatchers *Ficedula hypoleuca*. This entails that female plumage signals are the product of social selection acting on them during the breeding season. As their size shows a positive association with incubation attentiveness, they may be selected as traits that signal female competitive ability through enhancing their reproductive success.
- Extra-pair paternity (EPP) in territorial species may be driven by a sexual conflict, in which males have a stronger drive to copulate with extra-pair females than females have to avoid these matings, or by female choice of preferred mates outside the social pair bond (female choice). Experimentally flight-handicapped pied flycatcher females during the fertile phase present increased levels of EPP compared with un-handicapped females. This supports the sexual conflict scenario rather than the female choice hypothesis.
- More body reserves at laying means a higher potential fecundity but also higher levels of EPP if body mass affects female extra-pair activity through either improved condition for active search of extra-pair mates, or through reduced flight capacity for avoiding harassment by extra-pair males. A reduced nest construction effort leading to higher mass during the fertile phase in female pied flycatchers is associated with higher EPP compared with control females, which can be attributed to either adaptive scenario. The evolution of mass change strategies in breeding altricial birds has implications for EPP patterns which have not been stressed before.

- Dependence of EPP on the social context may make it an individually non-repeatable trait. In two populations of pied flycatchers, EPP was not individually repeatable between years in either sex. The social context where extra-pair matings occurred was not identified and its variation for different individuals within and across years can potentially explain the absence of individual repeatability. This has strong implications for future research as fluctuations of the social context may make results from different studies of the same population or of different populations non-repeatable.
- Individual social phenotypes can be measured through their node-based metrics in the social population network. Individual consistency in these social metrics persists through time and context in wild and captive populations of the House sparrow. This suggests that social metrics can be considered animal personality traits in socially gregarious passerines.
- Females with higher levels of sociality measured through node-based metrics, show higher EPC during the breeding phase. This suggests that sociality is the strategy most females in gregarious species use to cope with their environments during breeding, allowing them to increase their fitness by copulating with preferred extra-pair males. However an alternative explanation would be that more sociable females can use EPCs as a strategy to avoid aggression by extra-pair males.
- Social metrics used as measures of individual centrality provide different information from individuals and may enforce different effects on their reproductive behaviour. While strength shows a positive relationship with EPC, and represents the summary of direct interactions of a focal individual with others in the population, closeness shows no relationship and betweenness shows a negative relation with EPC. Both later social metrics are indicators of the specific position of an individual in the social network.
- Including the social context (namely social relations among individuals in a population) when studying mating behaviour (and especially extra-pair behaviour) is needed.

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