NTNU
Norwegian University of
Science and Technology

Master's thesis

2022

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M.Sc. in Biology	
Submission date:	May 2022
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Acknowledgement

First, I want to thank my fantastic team of supervisors. Thanks to Henrik, my main supervisor, for letting me work on this fantastic dataset, for introducing me to the CBD-network and facilitate further collaborations. I thank Bernt-Erik for all the outstanding theoretical impot, and my super-duper-visor Thor Harald for introducing me to the house sparrow project and being my only supervisor with a (good) sense of humour. A special thanks to Jon for always being brutally honest, challenging me, supporting all my work, and taking interest in what is best for me. A special thanks also to Yimen, for being the genius that you are, for making me enjoy statistics and generally being an awesome guy. And last, but definitely not least, thanks to Peter, for not only being my supervisor, but also my mentor, colleague, and dear friend.

I would like to thank to Niels Dingemanse for helpful comments and discussions on the study.

I would also like to thank Bernt Rønning, for teaching me more about science and the scientific process during two weeks of fieldwork in October 2017, than any course ever thought me. Also, you thought me that in order to survive in science, I would need to be patient, consistent and work my ass of. For making me realize this at such an early stage, I am forever grateful.

Also, I would like to thank Eivin, Bård (and the rest of the Femund-team) and Anders for giving me opportunities to develop scientific qualities and get hand-on experience from studies outside my M.Sc.

I am also grateful to the SQuID-team, for letting me attend the course, introducing me to a larger aspect of mixed models and letting me get to know outstanding people. You rock!

Thanks to Henriette Vaagland and Anita Kaltenborn for all the laughs and collaborations. The daily encounters in the hallway and the nice chats in the office made my day for almost five years. Thank you!

In addition, I would like to thank Jon, Michael and Fredrik (and Curtis Jackson) for effective scientific collaboration, for listening to my ideas and doing so simply for the joy of doing research.

At last, I will thank Simen and Per for reading all my silly first drafts and Fredrik for (not) holding the ladder. Cheers!

This study was supported by the Norwegian Research Council Grant 302619 and Grant 223257 to the Centre for Biodiversity Dynamics (CBD) at NTNU.

Sammendrag

De drivende mekanismene bak fordelingen av polygami hos sosialt monogame fuglearter har fått mye oppmerksomhet de siste tiårene. Alder og morfologi, og særlig sekundære seksuelle trekk, har ofte blitt pekt på som viktige faktorer bak hvilke hanner som får avkom med flere hunner innen samme kullperiode (multippelt farskap). Likevel har få studier separert underliggende prosesser bak alder, som selektivt frafall og en plastisk effekt av alder, mens man kontrollerer for morfologi. Samtidig er det viktig å kontrollere for alder når man skal forstå rollen til morfologi, siden morfologi kan være aldersavhengig. Ved å bruke stianalyse på et langtids-datasett fra en metapopulasjon med gråspurv *Passer domesticus*, separerte jeg effektene bak alder på multippelt farskap for hanner, både uavhengig av morfologi, og gjennom alderens effekt på morfologiske trekk. En innen-individs plastisk effekt av alder, men ikke mellom-individs selektivt frafall, var en viktig faktor for multippelt farskap når morfologi ble kontrollert for. Størrelsen på brystflekken påvirket ikke multippelt farskap etter effekten av alder var kontrollert for, selv om den økte med alder både gjennom en plastisk komponent og selektivt frafall. Disse resultatene utfordrer hypotesen om «gode gener» for variasjon i utroskap basert på hannens kvalitet eller attraktivitet. Effekten av alder, morfologi og hekkesynkronitet ble også studert fra hunnene sin side, uten å finne noen effekt for om de påvirker om hun tillater utroskap innen kullet sitt eller ikke. Dette studiet illustrerer viktigheten av å benytte korrekt statistisk dekomponering av ulike mekanismer av alder og andre økologiske aldersavhengige prosesser bak multippelt farskap, som min bruk av stianalyse innen et Bayesiansk rammeverk med miksa-modeller. Jeg viser at, når man benytter denne metoden, kan den gi innsikt i økologiske prosesser, og hvordan de samtidig påvirker rollen av morfologi og alder på multippelt farskap og fremhever at denne variasjonen i reproduktiv atferd kan ha ringvirkninger for populasjonsdynamikk gjennom å påvirke demografisk varians.

Abstract

The driving forces behind multiple paternity variation in socially monogamous birds have been widely investigated over the last few decades. Age and morphology, and secondary sexually selected traits in particular, have often been demonstrated to be important factors determining a male's ability to sire offspring with multiple females. However, most studies fail to separate the different processes underlying age effects, such as selective disappearance and an individual plastic effect of age, while controlling for the effects of morphology. Furthermore, it is also important to control for age when understanding the role of morphology, since morphology can also be age-dependent. Using path analysis on a long-term dataset from a house sparrow *Passer domesticus* metapopulation, I disentangle the effects of age on male multiple paternity, both independent from morphology, and through its effect on morphological traits. Within-individual plastic effects of age, but not among-individual selective disappearance by age (i.e., age of last reproduction), was a key determinant of multiple paternity for male sparrows once the effects of morphological traits were accounted for. Badge size did not affect multiple paternity after controlling for the effect of age, although it increased on average with age through both individual plasticity and selective disappearance. These results challenge 'good genes' explanations for variation in extra-pair paternity based upon male quality or attractiveness. The effect of age, morphology and breeding synchrony were also studied from a female's perspective, but little support was found for any effects on multiple paternity within broods. This study illustrates the importance of applying the correct statistical decomposition of the different aspects of age and other ecological age-dependent processes on multiple paternity, such as my use here of path analyses within a Bayesian mixed-effect modelling framework. Doing so provides insight to different ecological processes, and how they simultaneously affect the role of morphology and age on multiple paternity, and such variance in reproductive contributions and any resulting demographic variance that will be important for the species' population dynamics.

Keywords: extra-pair paternity, house sparrow, badge size, age, secondary sexual trait, selective disappearance, plasticity

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Introduction

The study of extra-pair paternity (EPP) in birds has received increased attention over the last few decades, with this behaviour being commonly observed in many avian systems otherwise regarded as socially monogamous, due to the possibility of genetic determination of parents (Griffith et al. 2002; Westneat & Stewart 2003; Brouwer & Griffith 2019). As noted by Trivers (1972), males should seek extra-pair copulations in order to maximize the number of offspring, while females should seek extra-pair copulation to increase offspring genetic quality. The most studied hypotheses describing the potential benefits of this behaviour for females therefore include: (a) “good genes” where offspring fitness benefits from better quality male genes that the female identifies from male phenotypic cues (Westneat 1990; Birkhead & Møller 1992; Hasselquist et al. 1996); (b) “compatible genes” where the offspring fitness increases due to a better genetic match between their mother and the extra-pair male (Kempenaers et al. 1999; Tregenza & Wedell 2000; Griffith & Immler 2009); and (c) fertility assurance by increasing the chance of females producing viable offspring with at least one fertile male partner (Sheldon 1994; Schmoll & Kleven 2016). The costs related to EPP for females may include: (i) reduced investment of paternal care to the brood if partners are instead investing time and energy pursuing extra-pair matings (e.g., Magrath & Elgar 1997); (ii) the costs to females of searching for and pursuing potential extra-pair partners (Dunn & Whittingham 2007); and (iii) parasite and pathogen transmission via microorganisms (Hillgarth 1996; Westneat & Birch Rambo 2000) and sexually transmitted diseases (Sheldon 1993).

The individuals involved in EPP are therefore not expected to be a random subset of the adult population (Sheldon & Ellegren 1999), and the traits of both males and females linked to EPP rates have attracted considerable research attention (Møller 1990; Sheldon & Ellegren 1999; Roeder et al. 2019). In particular, secondary sexual traits have been widely investigated in terms of how they affect male extra-pair mating success (Andersson & Iwasa 1996; Sheldon & Ellegren 1999; Whittingham & Dunn 2016; Roeder et al. 2019). However, although secondary sexual traits have been shown to be important in particular studies, meta-analyses have revealed that publication-bias is a main contributor to any general effect in the literature, bringing into question their relative importance (Akçay & Roughgarden 2007; Sanchez-Tojar et al. 2018). Moreover, few studies have been able to control for age-dependent confounds in these secondary sexual traits, which could also result in misleading conclusions.

The size of the black breast plumage 'bib', or badge, in male house sparrows (*Passer domesticus*) has been used as an example of a signal of status after studies revealed larger badges were associated with higher levels of EPP, suggesting that it is an important secondary sexual trait (Møller 1990). However, other studies failed to support such a relationship (e.g., Whitekiller et al. 2000). A thorough meta-analysis has since demonstrated publication bias towards positive results regarding the effects of male house sparrow badge size as a status signal, with a low overall mean effect size and statistical uncertainties overlapping zero (Sanchez-Tojar et al. 2018). Moreover, badge size in male house sparrows is an age-dependent trait, with older age classes generally displaying larger badge sizes (Jensen et al. 2004, 2006, 2008; Nakagawa et al. 2007). Given that age has been found to be an important factor in determining extra-pair mating success, many studies may report misleading results if the effects of badge size and age are not properly decomposed, and age is not corrected for when testing for an age-independent effect of badge size.

Age has been found to be a key factor predicting both the loss of within-pair paternity by young males and the acquisition of extra-pair paternity by older males (Richardson & Burke 1999; Gil et al. 2007; Hsu et al. 2015). Although, the expected individual correlation across males between the loss of within-pair paternity and gaining of extra-pair paternity turns out to be non-significant across species in general (Cleasby & Nakagawa 2012). Meanwhile, female age does not seem to affect levels of extra-pair paternity (Dunn et al. 1994; Barber et al. 1996). In the house sparrow, older males have previously not been reported to gain more extra-pair matings, but to have more extra-pair offspring, indicating an important role of (possibly female mediated) post-copulatory advantage for older males (Girndt et al. 2018, 2019). Older males may also be better at obtaining copulations because of experience, or they might be better at convincing or forcing copulations with females, identified as "the manipulation hypothesis", which thus predicts that older and larger males will obtain more EPP (Westneat & Stewart 2003; Hsu et al. 2015).

Although studies have statistically investigated the role of age in itself, there are different underlying processes by which age could influence EPP rate. Age can be important through increased within-individual reproductive investment with age, or through among-individual selective appearance and disappearance, where individuals that survive to reach higher ages are simply higher quality individuals (van de Pol et al. 2006). Whilst age has been identified as a key driver of multiple paternity, most studies do not disentangle such within- versus among-individual effects (Rebke et al. 2010), despite both of these being shown to be important drivers of life-history trait variation (Bouwhuis et al. 2009). Unfortunately, few long-term studies have quantified the relative importance of the within-individual plastic age component and selective disappearance in wild populations (Hayward et al. 2013), and only a few have investigated how these mechanisms affect the distribution of extra-pair paternity. A recent study of the cooperative breeding Seychelles warbler (*Acrocephalus sechellensis*) indicates that within-individual change was the main driver behind the effect of age on EPP (Raj Pant et al. 2020). Conversely, Segami et al. (2021) found that the among-individual effect of age due to selective disappearance was most important for EPP in collared flycatchers (*Ficedula albicollis)*. However, neither of these studies focused upon the combined role of secondary sexual traits and these different age effects, or relationships between them.

In addition to morphological traits, other effects such as breeding synchrony of a female (when a female breeds relative to the other females in the population) have also been shown to be important drivers of rates of extra-pair paternity (Stutchbury & Morton 1995). Breeding synchrony can affect levels of multiple paternity by regulating the availability of potential extra-pair mates that might themselves be performing parental duties during different stages of the breeding period. The synchrony hypothesis states that levels of multiple paternity will increase with breeding synchrony, because it facilitates female comparisons of different males (Stutchbury & Morton 1995), although breeding synchrony may possibly inhibit EPP by increasing the intensity of mate guarding behaviour in pair males (Møller 1991). Either way, these population-level effects of breeding synchrony have been difficult to demonstrate (Dunn et al. 1994; Stewart et al. 2006).

The objectives of the current study are to quantify the potential relationships between age and age-dependent morphological traits such as badge size, and their relative contributions in explaining individual variance in multiple paternity using long-term data from a house sparrow metapopulation in northern Norway collected between 1993-2014. This study therefore aims to investigate the underlying within- versus among-individual effects of age by explicitly modelling selective disappearance versus age-related plasticity both with respect to morphology and obtaining multiple paternity. I am able to address these questions due to access to a detailed long-term genetic pedigree in these house sparrows, which enabled me to disentangle the effects of age and morphology on multiple paternity. I quantify the independent and total effects of these variables on multiple paternity using a path analysis (Wright 1934). This makes it possible to study the causal pathways affecting multiple paternity and to obtain precise estimates and associated uncertainty for the total effects of the paths of age and morphology on multiple paternity.

Methods

Data collection

I used data from a long-term study of house sparrows in an insular metapopulation on the Helgeland archipelago (66.30°–66.80°N, 12.00°–13.10°E), including 15 different islands (Figure 1) in northern Norway between 1993–2014. The population on the island of Ytre Kvarøy went extinct in 2000 after occupying the islands for several decades (Ringsby et al. 2006), and the population on Aldra was established by four individuals (one female and three males) in 1998 (Jensen et al. 2007). In this study system, the house sparrow breeds mostly in farms on the islands closer to the coast, and mainly in artificial nest boxes on the islands farther from the coast (Pärn et al. 2012). The breeding season in this area lasts from early May to mid-August. Throughout the breeding season, new nests were thoroughly searched for, and previously used nest sites were visited regularly to check if they were reused. During incubation, the nests were visited two or three times in order to estimate first egg-laying date, assuming one egg was laid each day, and the maximum number of eggs recorded was used as the clutch size. After hatching, the age of nestlings was evaluated based upon developmental stage, and at age 7-12 days individual measurements were taken and nestlings ringed with a unique combination of one metal and three colour rings. Based on aged nestlings, hatch-date was back-calculated. Additionally, 25 µL blood was drawn from the brachial vein for use in genetic analysis.

Fledged juveniles and adults were caught using mist nets, and a blood sample was collected ensuring blood sample and morphological measurements for previously non-marked individuals. Body mass was measured using a Pesola spring balance to nearest 0.1 g. When adult males were captured, both the potential and realized height and width of the badge was measured to nearest mm (only potential height and width was used in the analysis). Potential height is measured in the same way as described by Møller (1987) but using the potential area of the badge rather than the realized area. These two ways of measuring the badge show similar tendencies with age in this metapopulation, although the realized badge size is more sensitive to environmental conditions (Jensen et al. 2006). As a result of the intensive search for nests and mist nets capture, the percentage of marked adults on the islands often exceeded 80% (see Ringsby et al. 1999).

Pedigree

I used the number of identified genetic fathers within a clutch to confirm the presence-absence of multiple paternity. Hence, I did not distinguish between multiple paternity for clutches with two or more genetically assigned fathers. In this population, the sparrows can have up to three clutches within a season. Here, only the first clutch is included in the analysis to avoid the problem of assigning multiple genetic partners for the males in case of replacement of partner between the clutches. In order to assign genetic parentage of the sparrows, I used polymerase chain reaction (PCR) amplification of up to 14 polymorphic microsatellites and the parentage assignment software CERVUS, following the procedure described by Holand et al. (2015). There are several reasons why one or both parents were not assigned for a minority of offspring (772 of 2607 clutches). For example, genetic data on one or both parents can be missing, or it can be issues assigning these also when the genetic data is available. Therefore, I only used clutches with genetically known mother and where at least two different genetically known fathers were assigned to denote multiple paternity (MP) within the clutch. When additional clutches with assumed MP due to uncertainty about the assignment of fathers were also included in the analyses, the results generally provided similar effect sizes and conclusions, although perhaps with slightly different interpretations based upon modified p-values (Table S1). I therefore rely for the main results here on the data where I am confident regarding the assignment of fathers to offspring. Females were assigned MP if their clutch had multiple fathers (yearly overview given in Table S2), but not if their males had MP. Males, on the other hand, were assigned MP if they were assigned offspring in at least two different first clutches where they could not potentially be the social father in both nests, because of strong overlap of all the first clutches (yearly overview given in Table S3). In most cases, MP directly reflects EPP, unless the male has no within-pair offspring and/or sired only extra-pair offspring.

Treatment of variables

In order to investigate how nest hatch date relative to other nests in the population that year influenced the occurrence of MP for females, each clutch's hatch date was mean standardized within each year and island. I only included the first clutch in my analyses in order to also avoid any confusion with measures of breeding synchrony across clutches, since population-level synchrony is expected to decrease throughout the season with multiple clutches.

Morphology

The body mass of the individuals was used as a measure of body size, as this has previously been shown to be a reliable proxy for body size in this metapopulation (Araya-Ajoy et al. 2019).

For males, the total badge area was calculated based on the measurements of height and width of the potential area of black badge plumage for each adult male. The calculation was preformed according to the formula by Møller (1987):

$$\text{Badge size} = 166.67 + (0.45 \times X \times Y), \tag{1}$$

Where X is the potential badge height and Y is the potential badge width.

All morphological traits were log-transformed to facilitate allometric comparisons.

Statistical analysis

<u>*Pathways to multiple paternity*</u>

I used path analyses to study a set of *a priori* hypothesized relationships between aspects of age, morphological traits, and MP. To facilitate comparisons and to provide an overview of all of the hypotheses, they are summarized in a path diagram (Figure 2). These relationships were then parametrized as a joint likelihood path model in a Bayesian mixed-model framework. The total effect of the predictors was calculated based upon the path rules established by Wright (1934) and extensions to nonlinear relationships (Morrissey 2015; Henshaw et al. 2020), adding the direct effects with the indirect effects (Table 1 & 2). Indirect effects were calculated as the product of the effects of the indirect paths. In this way, I aimed to describe the causal relationship between morphological traits, age, and the occurrence of MP.

Statistical implementation

I used the RSTAN package (Stan Development Team 2016) in the R environment (R Core Team 2015) to estimate the joint likelihood of the different models that were part of each path analysis. This approach allowed us to obtain appropriate measures of the uncertainty of compound pathways and efficiently using partially missing data., to model missing data. When morphological measurements were missing, the mean of the metapopulation was used when they were fitted as a response variable (Nakagawa & Freckleton 2008). I were then able to correct for this missing information when the morphological measures were used as a predictor, because I used the model predictions of one sub-model as the predictor for the subsequent sub-model. I also corroborated my results using a stepwise frequentist approach (“lmer” package, Bates et al. 2007) for the different hypothesized direct relationships, and I fitted models including the number of first clutches at the given island-year to control for potential effects of clutch-availability (see Supplementary Materials for details).

Modelling age effects

Age can affect MP directly or indirectly through different paths of cause and effect. First, older individuals may be of higher quality, purely as a result of the selective disappearance of 'lower quality' males, which will cause an among-individual effect of age on MP. Second, individuals may improve their abilities, levels of investment and/or state with age, reflected as a within-individual plastic effect age on MP. In order to decompose the effect of age into either within- or among-individual effects, I used age of last reproduction (ALR) per individual to estimate selective disappearance (van de Pol & Verhulst 2006). I also fitted age itself to allow for the effect of a plastic effect with age, and quadratic age to test for any non-linear effects (Bouwhuis et al. 2009). Age was measured in years, with intercept (age-category 0) representing 1-year old individuals. Since basically all individuals start breeding after one year, I did not include age of first reproduction to model selective appearance. Since age and ALR were on the same unit scale (age in years), the direct effects of these variables can be compared. The compound paths (direct and indirect effect combined) can also be compared as their effects are still biologically relevant units. Hence, testing both for among-individual (ALR) and within-individual (age and quadratic age) changes allowed me to disentangle within-individual plasticity versus selective disappearance (van de Pol & Verhulst 2006) when both were included in the different models (described in detail below). Only individuals with known age were used in the analysis.

Model for males

I used mixed-effect models in the path analysis framework for males based upon three different sub-models (with numbers in parenthesis here referring to path links given in Figure 2). In the first sub-model, the direct effects on body mass from ALR (1), age (2) and quadratic age (3) were estimated by fitting them as fixed effects, with body mass as the response variable with a Gaussian error distribution. Individual and island-year (combination of island and year) were fitted as random effects. In the second sub-model, badge size was treated as response variable with a Gaussian error distribution, and ALR (4), age (5), quadratic age (6) and body mass (7) were fitted as fixed effects with individual and island-year as random effects. In the last sub-model, male MP was fitted as a response variable with a binomial error distribution, and ALR (8), age (9), quadratic age (10), body mass (11) and badge size (12) were fitted as fixed effects, with individual and island-year as random effects. I included a quadratic age effect to allow for a non-linear effect of age on MP, a relationship that has been demonstrated with EPP previously in other species (Raj Pant et al. 2020). The compound paths were then calculated following the path rules by Wright (1934) and presented in Table 2.

Whenever both the linear and quadratic effects of age were supported by the model, the peak trait age was calculated as

$$\text{Peak age} = \frac{-\beta(linear)}{2\,\beta(quadratic)} \qquad (2)$$

Model for females

The mixed-effect model path analysis for females was based upon two sub-models (Figure 3). First, I modelled body mass as response variable with ALR (1), age (2) and quadratic age (3) as fixed effects, with Gaussian error distribution. Second, female MP as response variable with binomial error distribution was tested with ALR (4), age (5), quadratic age (6), body mass (7) and breeding synchrony (8) as fixed effects. Both models included individual and island-year as random effects. The compound paths were then calculated as given in Table 2, again following the path rules by Wright (1934).

Results

Multiple paternity in the population

In this population, 30.77% (*n*=877) of the males obtained multiple paternity (MP) in the first clutch at some point during their lifetime. For females, 129 of the 614 (21.01%) that had at least one first clutch did so with multiple fathers. The proportion of clutches with more than one genetic father varied among years (Tables S2 and S3), from 0% in a few island-years to 100% on a few island-year occasions (Figure S1).

Age-dependent body mass for males

There was a positive relationship between age-at-last-reproduction (ALR) and body mass, indicating that larger individuals were more likely to reach the older age-classes than smaller individuals (Table 3 and Figure 2). However, I found no support for linear or non-linear effects of age on body mass, implying that there were no systematic increases or decreases in adult body mass with age.

Age and body mass dependent badge size

The model supported both the effect of age and quadratic age (with 95% credible interval (CrI) just overlapping zero, and frequentist models supported the effect). Hence, badge size plastically increased non-linearly with age, and there was also a positive effect of ALR on badge size (Table 3, Figure 4). Therefore, a within-individual plastic effect of age increased the badge size, until a peak of badge size at age of 5.54 years, although few individuals reached this age. At the same time, high-quality males, with larger badge sizes, had a higher probability to reach older age classes. This was captured by the among-individual effect of ALR, also contributing to larger badge sizes in older age classes. Although the point estimate for body mass affecting badge size was positive, the 95% CrI overlapped zero, I thus find weak support for the expected allometric relationship between body size and badge size, after correcting for the effects of age. The model also revealed that the compound path of age and quadratic age affected badge size, again with badge size increasing most during younger ages as a within-individual plastic effect of age. In addition, the compound path of ALR positively affecting badge size was supported by the model (Table 3). Therefore, both among- and within-individual effects of age were found to directly affect badge size when controlling for body mass, and when I estimated the total effect of those age parameters through their effects of body size. Running the same models using a stepwise approach in a frequentist framework showed similar results (Table S4), as did models controlling for the number of available first clutches in the population at the given year (Table S5).

Age and morphological traits affecting MP for males

ALR had no direct effect on MP for males (Table 3 and Figure 5). However, the model supported age positively affecting MP directly, with a negative effect of quadratic age. This non-linear relationship was also supported by the total path calculations. Hence, the chance of obtaining MP for males increased non-linearly as a result of within-individual plastic effect of age, until it reached the highest probability at age of 3.40 (3-4) years old (Figure 6). In addition, body mass did not have any direct or indirect effects (via badge size) on MP. Badge size showed no effect on MP (Table 3, Figure 5) once any direct effects of age were controlled for. The compound path of ALR and body mass was also not supported by the model. The frequentist-based model for MP, and models including the number of first clutches in the population in a given year, showed similar results (Table S4 and Table S5).

Age-dependent morphological traits for females

ALR positively affected body size for females (Table 4 and Figure 7), but a non-linear plastic effect of age on body mass was not supported by the model. Hence, the increase in female body mass with age was related to an among-individual effect of larger individuals reaching those older age classes. Models with a frequentist approach and controlling for number of first clutches showed similar relationships (Table S6 and Table S7).

Age and morphological traits affecting multiple paternity for females

There was no evidence for body mass affecting female MP directly (Table 4, Figure 7). In addition, no effects of age, quadratic age or ALR were supported by the model in affecting female MP directly (Table 4, Figure 7). Moreover, the compound path including the direct effects of both age components and their indirect links via body mass was not supported. Therefore, there was no sign of either an among-individual quality-dependent effect of age, or a within-individual plastic effect of age on the probability of MP for females. When controlling for the effect of body mass and all age components, the model also revealed little evidence for an effect of breeding synchrony on female MP (Table 4), thereby providing no support for the synchrony hypothesis. Frequentist models again showed similar results (Table S6 and Table S7).

Discussion

The present study shows that age was an important factor in determining multiple paternity (MP) for males, in line with findings across numerous bird species (Cleasby & Nakagawa 2012). However, most studies do not disentangle the two age-related processes of within-individual age-based plasticity versus among-individual selective disappearance with age. In this study system, males obtained more MP with increasing age, and this was through a plastic within-individual effect, whilst controlling for any effects of among-individual age-at-last-reproduction (ALR) and morphological traits. Interestingly, badge size as a secondary sexual trait did not appear to influence MP, as predicted by hypotheses where badge size is a trait reflecting male quality and/or dominance (Møller 1990). However, there was no sign of ALR, through selective disappearance, affecting MP (van de Pol & Verhulst 2006). Hence, I show that disentangling the effects of age into known age-related processes can provide valuable information regarding these types of functioning ecological mechanisms in the wild.

The finding that age as an important driver for males obtaining MP, both directly and through the total compound path, is informative as to why females might seek MP and EPP. If females seek EPP for the genetic quality via EPP, through either "good genes" (Hamilton & Zuk 1982; Westneat et al. 1990), or "compatible genes" (Kempenaers et al. 1999; Tregenza & Wedell 2000; Griffith & Immler 2009), one might expect high-quality males to consistently obtain more EPP. However, I found no support for ALR, an among-individual effect of age on MP, showing no pattern of the same males constantly obtaining MP throughout their life. Contrary to the expectation from these hypotheses, I found the within-individual plastic effect of age to be an important factor, illustrating a positive, but non-linear plastic effect of age. This in itself is not enough to rule out the compatibility hypothesis, but if this hypothesis is the key driver behind MP and EPP, one might also expect certain males to obtain more MP/EPP throughout their lifetime, irrespective of age. However, the compatibility hypothesis can still be important but hidden under the finding of an effect of a plastic component of age. In accordance with Raj Pant et al (2020) and another study of house sparrows by Hsu et al (2017), the results of this study do not support the effect of male quality, through selective disappearance, on MP. A within-individual plastic effect of age, rather than selective disappearance, may reflect different underlying processes, for example increased male investment or a trade-off between within-pair paternity and extra-pair paternity. However, male house sparrows have also been found to increase within-pair paternity with age (Hsu et al. 2015), indicating that increased male investment in both types of paternity may be the most feasible explanation.

As body mass was not related to MP for males, this partly contradicts the manipulation hypothesis, where larger males may force copulations with females (Westneat & Stewart 2003; Hsu et al. 2015). However, older males may still have had improved time-management during breeding periods, for example through experience or increased frequency of copulations, regardless of the effect of body mass, which might be the underlying mechanism when plasticity is involved in the house sparrow (Westneat & Stewart 2003; Hsu et al. 2015). Note, however, that is remains unknown if the plastic component of age reflects increased male investment, reproductive trade-offs or simply the process of improved optimal time allocation. A meta-analysis by Akçay & Roughgarden (2007) indicated that larger males often obtained more EPP than smaller males, but they identified a lack of empirical support for whether this effect appeared via an influence of secondary sexual traits. However, both body size and badge size has previously been demonstrated to affect mating success in this metapopulation, although these studies did not correct for the effect of age. (Jensen et al. 2008). Akçay & Roughgarden (2007) also concluded that the genetic benefits alone fail to explain the distribution of EPP, due to inconsistencies with the hypothesis and expected relationships. This is in line with the present findings, particularly as the effects of both ALR and badge size were not supported by the model explaining MP, which would have been expected if there were good genes benefits of MP. However, this does not completely rule out genetic benefits of MP, as cues regarding male quality could work through other (unmeasured) traits as well. Hence, the results of this study are overall more in line with the manipulation hypothesis as the causal driver EPP in this study system.

When controlling for the effect of among- and within-individual effects age, badge size did not appear to increase the chances of a male of obtaining MP (Table 1). This is surprising, given that badge size has been hypothesised to affect EPP in house sparrows (Møller 1990; Sanchez-Tojar et al. 2018). Furthermore, badge size has been shown to be important for lifetime reproductive success (Jensen et al. 2004), as well as mating success and recruit production (Jensen et al. 2008) in this population (although age was included these analyses, and the authors did not disentangle the underlying processes of any age effects). Hence, the effect of badge size on lifetime reproductive success demonstrated in this population may not be due to more frequent MP in the first clutches, but it could still work through greater within-pair paternity and/or higher survival of males with lager badges. Although the badge size in house sparrows has been used as example of an important secondary-sexual trait (Møller 1990), a recent meta-study showed no effect of badge size on male EPP (Sanchez-Tojar et al. 2018). This study therefore supports the findings of Sanchez-Tojar et al. (2018), as the effect of ALR which was used as a possible measure of male quality was not supported, further questioning the role of badge size as an important secondary sexual trait in house sparrow mating systems. This also demonstrates the importance of disentangling age-dependent effect and badge size on MP, because age was positively related to badge size and to MP itself. However, in this dataset, not including any age component in the analysis did not change the interpretation of the lack of an effect of badge size on MP (Table S8). Nevertheless, failing to correct for age-dependency can potentially lead to misleading conclusions in other datasets (van de Pol & Verhulst 2006).

The results of this study show a positive relationship between ALR and body mass. This indicates that the selective disappearance of smaller males is the reason why there is a larger body mass in individuals that reach the older age-classes, which is in line with the previous demonstration of positive survival-selection on body mass in this population (Jensen et al. 2008). Neither age nor quadratic age was found to affect body mass. Furthermore, ALR was positively associated with badge size. Simultaneously, badge size increased non-linearly with age, with decreasingly positive effects at older ages. Therefore, both selective disappearance and within-individual plasticity led to larger badge sizes at older age classes. This clearly demonstrates that these two among- and within-individual effects of age can act simultaneously. Moreover, it opens the possibility that females may, in some cases, use badge size as a cue for male age. Furthermore, I found no relationship between body mass and badge size when controlling for the effects of age. This is interesting, given that body mass is found to be a reliable proxy for body size in this population (Araya-Ajoy et al. 2019). Hence, in this study, there seems to be no direct links between body size and badge size, two traits that has previously been shown to be genetically correlated in this population (Jensen et al. 2008), and which are both expected to affect the chance of MP through the manipulation hypothesis (Hsu et al. 2015). The absence of body mass as an important factor for obtaining MP for males is particularly interesting. The manipulation hypothesis predicts that larger males may be better at forcing copulations or convincing females to copulate. However, I find no such support of an effect of body mass, which can indicate that the other part of this hypothesis regarding the increase in experience of the individuals with age is the most probable explanation for why males gain more MP with age.

Despite demonstration here of a within-individual plastic effect of age on male MP, it is important to note that within- and among-individual effects of age on MP are not mutually exclusive, and these two different processes may vary in strength under different conditions in different times and locations. Few studies have disentangled the effect of among-individual quality-based and within-individual plastic effect of age on EPP, both in socially monogamous species (Segami et al. 2021) and cooperative breeders (Raj Pant et al. 2020). These found different results, indicating selective disappearance and a plastic component of age to be important for obtaining EPP, respectively. Therefore, more studies on this are needed in order to understand under which conditions these different patterns apply.

The variation shown here in MP, particularly for males, is also interesting given that demographic variance strongly affects effective population size in this metapopulation (Stubberud et al. 2017). MP may therefore be one of the drivers behind this demographic variance. Furthermore, the support for a quadratic effect of age also indicates reproductive senescence (Bouwhuis et al. 2009). However, due to few datapoints after the calculated peak, a post-peak decline could not be tested effectively, although a quadratic effect of age on reproduction (measured as recruits produced pr. year) has previously been demonstrated in this metapopulation (Araya-Ajoy et al. 2021).

For females, ALR affected body size, demonstrating that older females had larger body sizes because larger females were more likely to reach these older age classes via the process of the selective disappearance of smaller females. Neither age nor quadratic age affected body size, suggesting no systematic increase in female mass with age. Furthermore, none of the parameters, including all the age-components, body mass and breeding synchrony, directly affected female MP. The absence of any effect of breeding synchrony on female MP in the studied house sparrow metapopulation is interesting given that it has been found to be an important in other species via the availability of extra-pair mating opportunities (Stutchbury & Morton 1995), although other studies have failed to find support for the synchrony hypothesis (von Schantz et al. 2004). As the synchrony hypothesis has divergent predictions regarding the effect of breeding synchrony, the two directions of effects could possibly contradict each other.

If the female breeds close to the breeding onset of other females, this could make their breeding male easier to compare against other extra-pair males, and therefore increase the level of EPP (Stutchbury & Morton 1995). At the same time, it might enhance mate guarding from pair males and lower the level of EPP (Møller 1991), particularly in the time of season before both parents are provisioning offspring. If these two processes act simultaneously, they may cancel each other out. In addition, although different underlying processes related to the synchrony hypothesis could potentially cancel each other out, the intensity of these mechanisms may also vary temporally and spatially. Therefore, although there was no support here for the predicted relationships reflecting strong influence from breeding synchrony on MP, this hypothesis needs to be tested further in study systems where sufficient additional information on these underlying processes is available.

Conclusions

This study show that older males obtained more MP as a result of a within-individual increase in MP with age. No evidence was found for selective disappearance as the driver behind this relationship between age and MP. There was therefore little support for hypotheses explaining EPP in this system in terms of benefits to offspring of good genes or parental genetic compatibility, although the latter could be hidden under the finding of within-individual increase in MP with age. Instead, the current findings support alternative hypotheses, such as the manipulation hypothesis via increased effort and/or experience in males in obtaining successful copulations both within- and extra-pair. In addition, age, but not body size was positively related to male badge size, through both within-individual age-based plasticity and among-individual selective disappearance. However, badge size was not associated with MP, whether or not the models controlled for the effects of age, yet again bringing into questioning the signal of status hypothesis regarding badge size in MP house sparrows. This study emphasises the utility of statistically disentangling the different biological effects of age and morphology through path analysis in order to properly investigate the causal pathways of female choice and male-male competition within extra-pair mating systems. Moreover, I emphasise that MP and EPP may be important drivers regarding the population dynamics of this house sparrow system.

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Figures and Tables

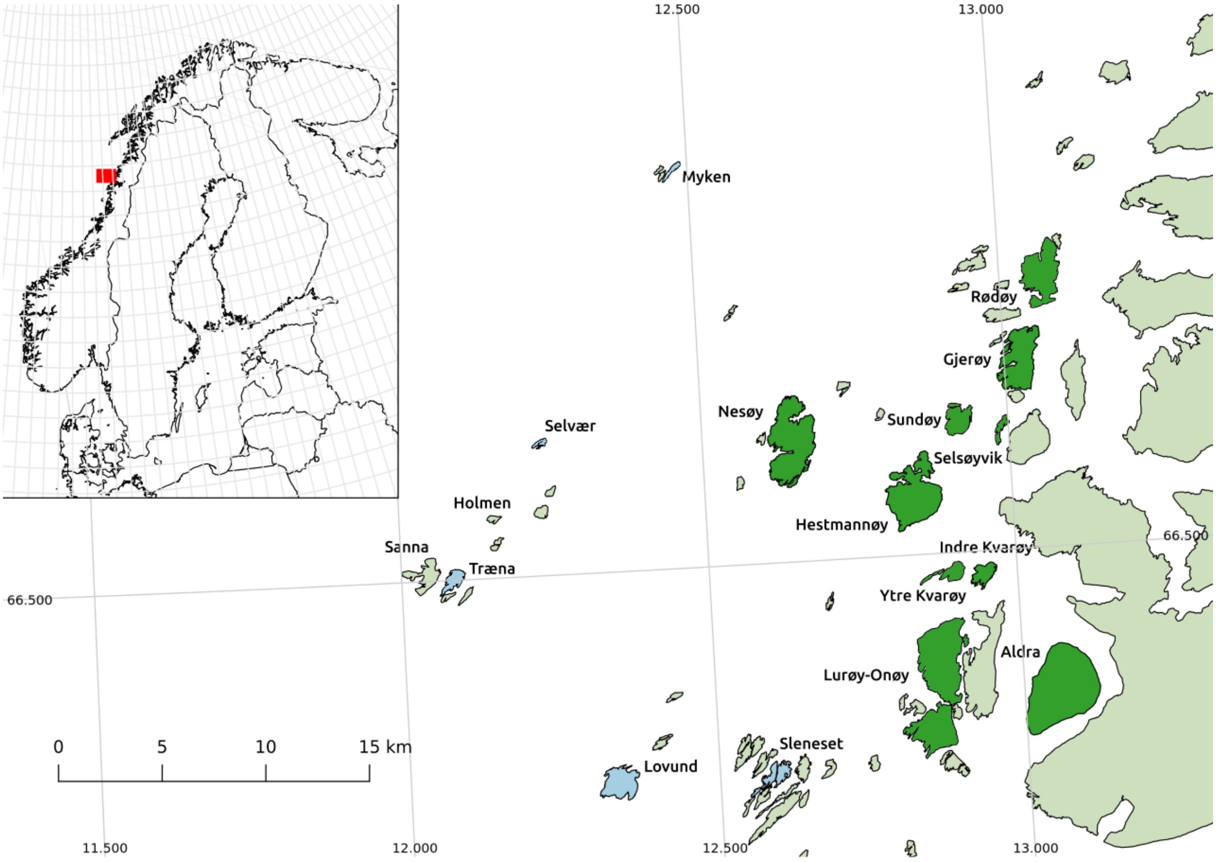

Figure 1: The study system in the Helgeland archipelago showing the islands included in this study. Inner islands where house sparrows reside close to farms are marked in green, while outer islands without farms are marked in blue.

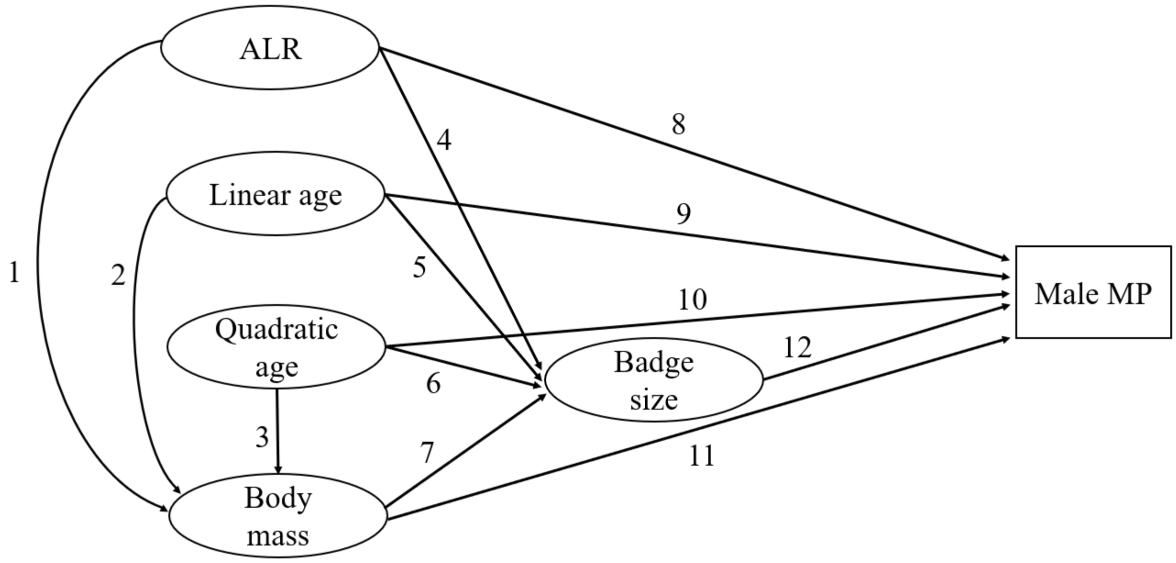

Figure 2: Path analysis of the effects of ALR (age of last reproduction) age, quadratic age and morphological traits, with numbers representing the different predictors in the different sub-models, with body mass, badge size and male MP as response variables, respectively (see Table 1, number 1-12).

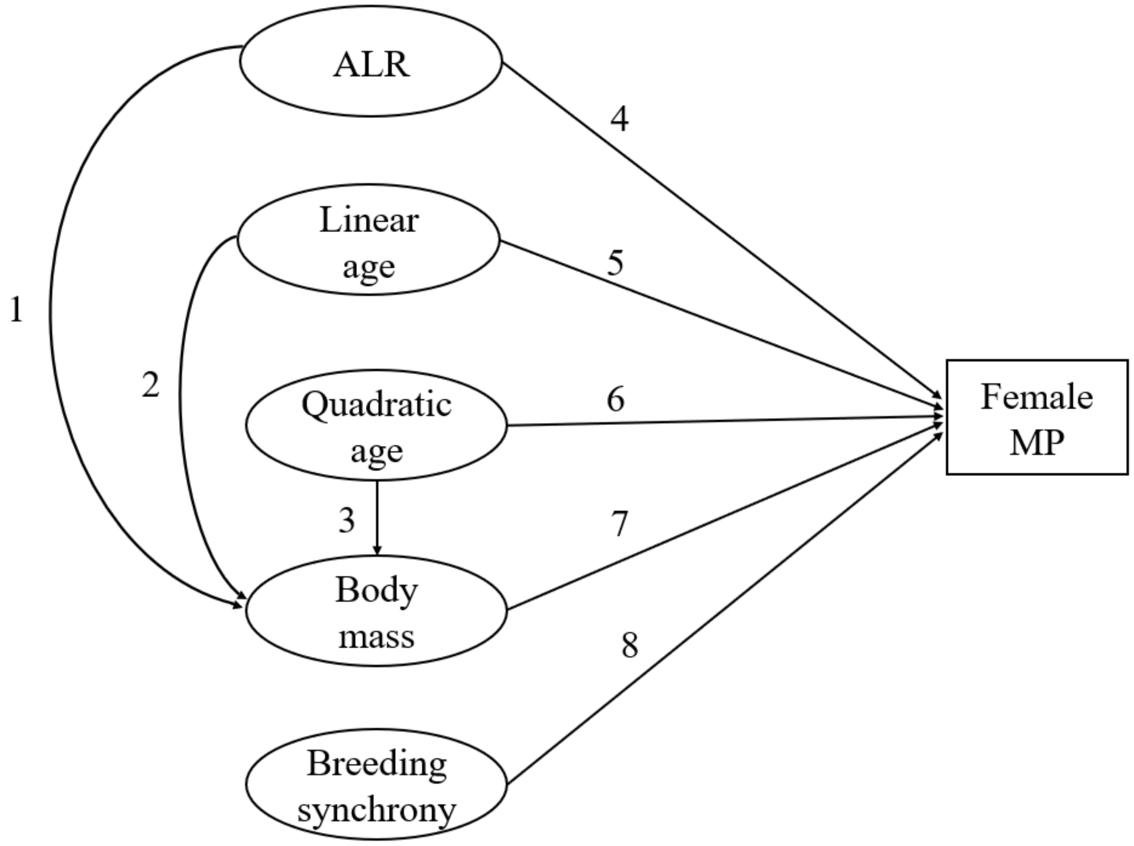

Figure 3: Path diagram showing the hypothesised links between age components, morphology and breeding synchrony on female MP. Numbers correspond to the predictors in the models. The total model includes two sub-models with body mass and female MP as response variables, respectively.

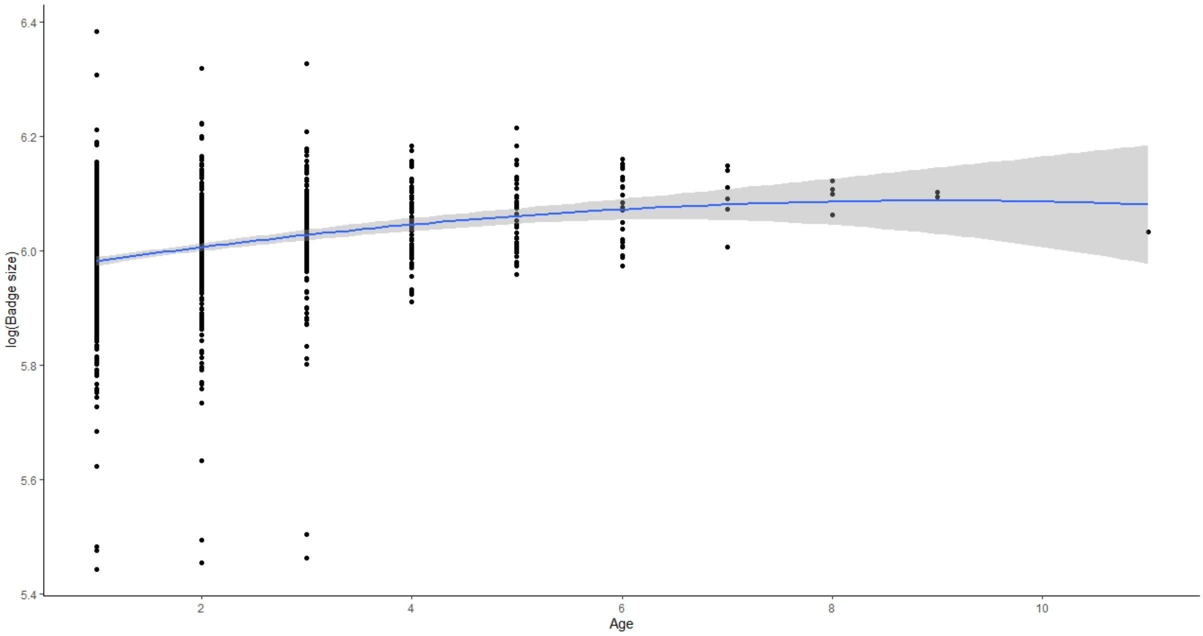

Figure 4: Effect of age on log-transformed badge size for males based upon the frequentist model. Here, each individual has a marginalized predicted badge size for a given age, and does not take random effects of individual and island-year into account, causing the deviation from the calculated peak based on the Bayesian approach. Shaded area represents 95 % CI.

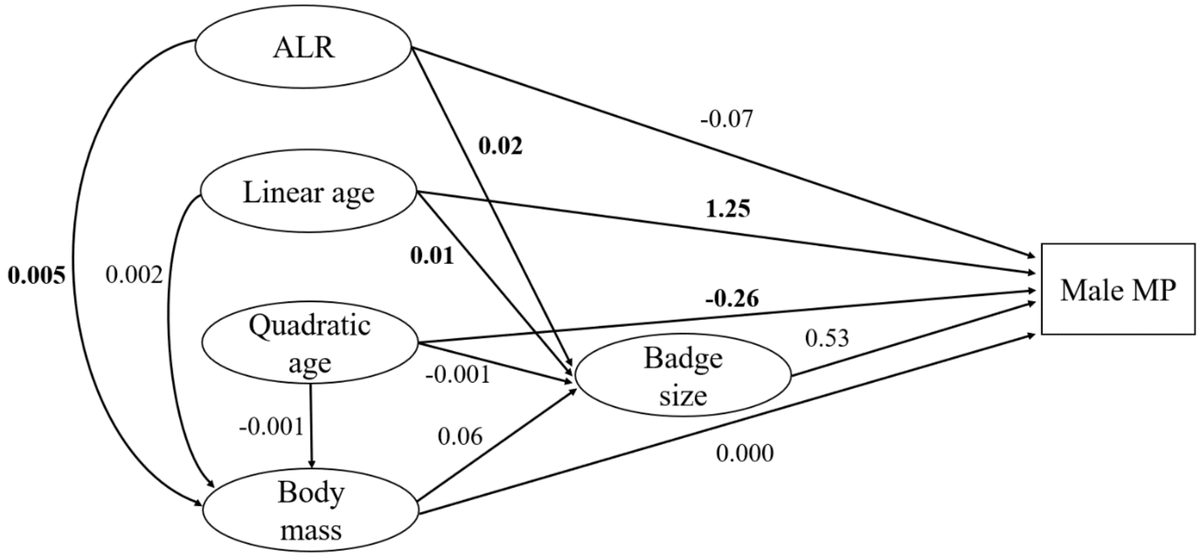

Figure 5: Path diagram illustrating the direct and indirect pathways of age-at-last-reproduction (ALR), age, quadratic age and age-dependent traits affecting multiple paternity for males (Male MP). Point estimates in bold had strong support by the model (95% CrIs non-overlapping zero).

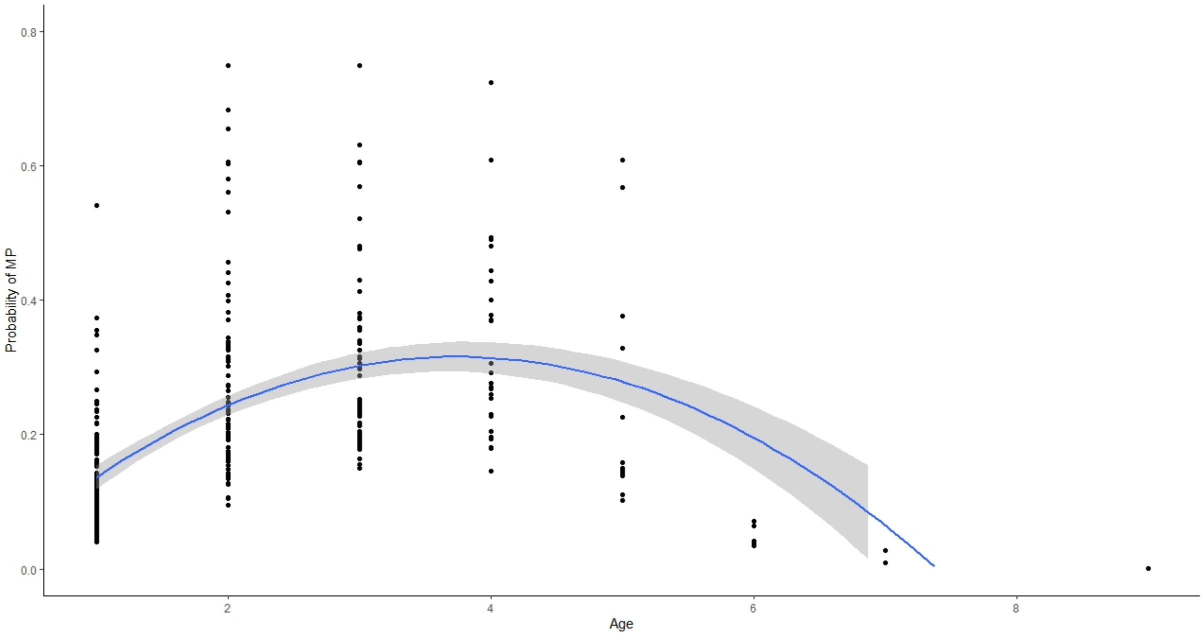

Figure 6: Effect of age on the probability of obtaining multiple paternity (MP) for males, predicted from sub-model 3 with a frequentist approach. Each male here has a marginalized predicted value of probability of MP, which does not take the random effects of individual and island-year into account. Shaded area represents 95% CI.

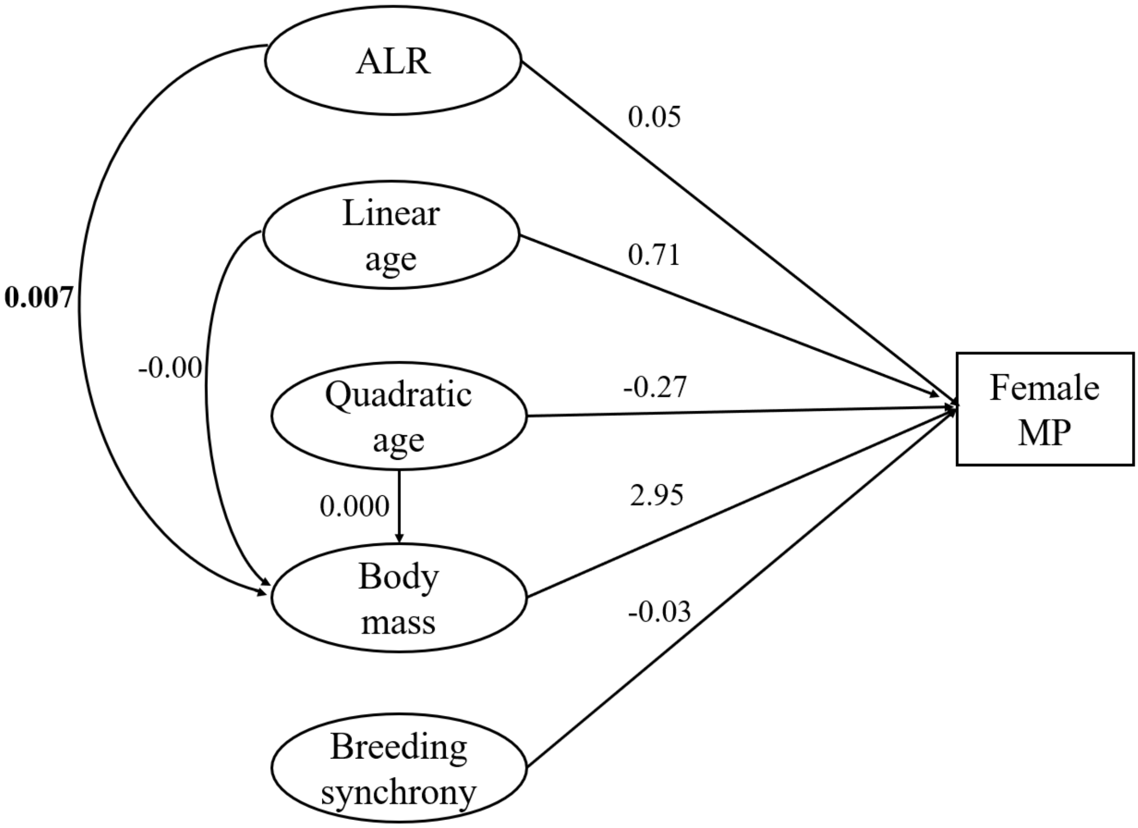

Figure 7: Path diagram showing the direct paths of different components of age (including age-at-last-reproduction ALR), age-dependent morphological trait and breeding synchrony and their effect on multiple paternity for females (Female MP). Values marked in bold received strong support by the model.

Table 1: Direct and compound paths calculation based upon the different sub-models (on body mass, badge size and multiple paternity for Male multiple paternity, MP). The values correspond to the parameter estimates described in the text and further to the links numbered in Figure 2. The compound paths are calculated as the direct effect plus the product of the parameter estimates in the other paths.

Direct effects	***Body mass***	***Badge size***	***Male MP***
ALR	1	4	8
Age	2	5	9
Quadratic age	3	6	10
Body mass	-	7	11
Badge size	-	-	12
Compound path			
ALR	-	4 + 1*7	8 + 1*11 + 4*12 + 1*7*12
Age	-	5 + 2*7	9 + 2*11 + 5*12 + 2*7*12
Quadratic age	-	6 + 3*7	10 +3*11 + 6*12 + 3*7*12
Body mass	-	-	11 + 7*12

Table 2: Direct and compound path calculation for models of body mass and multiple paternity for females (Female multiple paternity, MP). The values correspond to the parameter estimates described in the text and further to the links numbered in Figure 3. The compound paths are calculated as the direct effect plus the product of the parameter estimates in the other paths.

Direct effects	**Body mass**	**MP**
ALR	1	4
Age	2	5
Quadratic age	3	6
Body mass	-	7
Breeding synchrony	-	8
Compound path		
ALR	-	4 + 1*7
Age	-	5 + 2*7
Quadratic age	-	6 + 3*7

Table 3: Effects of morphological traits and two age-components (including age-at-last-reproduction ALR) on body mass, badge size and multiple paternity for males (Male MP). Values in bold are supported by the model (95% CrIs not overlapping zero). The parameter estimates of the direct effects represent the estimates given in Figure 5. The results are based on the Bayesian model with sub-models described in the text.

	Body mass	**Badge size**	**MP**
Direct effects [a]	***β*** **[95% CrI]**	***β*** **[95% CrI]**	***β*** **[95% CrI]**
Intercept [b]	**3.44 [3.43, 3.46]**	**5.73 [5.05, 6.37]**	**-7.80 [-35.70, 28.30]**
ALR	**0.005 [0.002,0.008]**	**0.02 [0.01, 0.02]**	-0.07 [-0.35, 0.18]
Age	0.002 [-0.002, 0.006]	**0.01 [0.01, 0.02]**	**1.25 [0.50, 2.02]**
Quadratic age	-0.001 [-0.001, 0.000]	-0.001 [-0.003, 0.000]	**-0.26 [-0.43, -0.09]**
Body mass	-	0.06 [-0.13, 0.26]	0.000 [-6.80, 6.34]
Badge size	-	-	0.53 [-3.90, 4.07]
Compound path			
ALR	-	**0.02 [0.01, 0.02]**	-0.06 [-0.32, 0.19]
Age	-	**0.01 [0.01, 0.02]**	**1.26 [0.52, 1.94]**
Quadratic age	-	-0.001 [-0.003, 0.000]	**-0.26 [-0.42, -0.09]**
Body mass	-	-	0.03 [-6.67, 6.27]
Random effects	**σ [95% CrI]**	**σ [95% CrI]**	**σ [95% CrI]**
Plot Year	0.008 [0.002, 0.012]	0.006 [0.000, 0.015]	0.83 [0.19, 1.46]
Individual	0.056 [0.053, 0.061]	0.108 [0.101, 0.117]	0.62 [0.04, 1.36]
Sample sizes	**n**	**n**	**n**
Plot Year	171	171	106
Individuals	484	484	235
Observations	1025	1025	345

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[a] Estimates rounded to two decimals, unless the third decimal is needed to identify positive or negative values, in which three decimals are given

[b] Estimates with age 1 as reference (1-year olds set to 0) for all traits

Table 4: Direct path coefficients of age (including age-at-last-reproduction ALR) and morphology on multiple paternity for females (Female MP) using a frequentist approach, using a dataset where I am not certain of the assignment of fathers. Values in bold are supported by the model (95% CI not overlapping zero). The parameter estimates of the direct effects represent the estimates given in Figure 6. The results are based on the Bayesian model with sub-models described in the text.

	Body mass	**MP**
Direct effect [a]	***β* [95% CI]**	***β* [95% CI]**
Intercept [b]	**3.46 [3.45, 3.47]**	-12.31 [-61.77, 33.67]
ALR	**0.007 [0.002, 0.012]**	0.05 [-0.25, 0.35]
Age	-0.00 [-0.01, 0.01]	0.71 [-0.41, 1.88]
Quadratic age	0.000 [-0.002, 0.003]	-0.27 [-0.63, 0.02]
Body mass	-	2.95 [-10.40, 17.18]
Breeding synchrony	-	-0.03 [-0.57, 0.49]
Compound path		
ALR	-	0.07 [-0.24, 0.37]
Age	-	0.71 [-0.41, 1.87]
Quadratic age	-	-0.27 [-0.62, 0.01]
Random effects	**σ [95% CrI]**	**σ [95% CrI]**
Plot Year	0.02 [0.01, 0.03]	0.67 [0.04, 1.47]
Individual	0.05 [0.04, 0.05]	0.38 [0.01, 1.12]
Sample sizes	**n**	**n**
Plot Year	161	87
Individual	466	164
Observations	728	200

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[a] Estimates rounded to two decimals, unless the third decimal is needed to identify positive or negative values, in which three decimals are given

[b] Estimates with age 1 as reference (1-year olds set to 0) for all traits

Supplementary materials

In addition to a Bayesian framework, I also used a frequentist approach for the direct path coefficient based on the same models as for the Bayesian framework, using the “lme4” package (Bates et al. 2007). For models with mass and badge size as response-variables, I used the “lmer”-function with Gaussian error distribution, as they are both continuous variables with approximately normally distributed residuals. For the models with multiple paternity as response-variables I used the “glmer” function with binomial error distribution.

Table S1: Direct path coefficients of age (including age-at-last-reproduction ALR) and morphology on multiple paternity (MP) for females using a frequentist approach with the lme4-package, using a dataset where I am not certain of the assignment of fathers. Values in bold are supported by the model (95% CrI not overlapping zero).

	MP
Direct effect	***β* [95% CI]**
Intercept	6.96 [-7.46, 21.17]
ALR	**0.27 [0.03, 0.51]**
Age	0.65 [0.19, 1.51]
Quadratic age	**-0.26 [-0.49, -0.04]**
Body mass	-2.42 [-6.50, 1.65]
Breeding synchrony	0.04 [-0.38, 0.43]
Random effects	**σ**
Plot Year	0.69
Individual	0.49
Sample sizes	**n**
Plot Year	87
Individual	164
Observations	200

Table S2: Yearly count of number of individually identified reproducing females, and the number of females with multiple paternity (MP) in their nest, irrespective of whether morphological data were available or not.

Year	Number of reproducing females	Number of females with MP
1993	28	5
1994	33	3
1995	20	2
1996	22	7
1997	23	5
1998	29	5
1999	31	8
2000	22	4
2001	26	7
2002	33	11
2003	35	6
2004	43	4
2005	61	5
2006	69	12
2007	72	16
2008	67	15
2009	73	17
2010	99	17
2011	106	24
2012	71	8
2013	52	11
2014	30	7

Table S3: Number of males with genetically sired offspring and number of males with multiple paternity (MP) over the study period, irrespective of whether morphological data were available or not.

Year	Males with sired offspring	Males with MP
1993	41	9
1994	49	13
1995	35	10
1996	39	9
1997	30	7
1998	35	9
1999	42	12
2000	30	5
2001	37	10
2002	51	22
2003	37	11
2004	51	10
2005	55	8
2006	64	15
2007	80	17
2008	73	15
2009	84	24
2010	91	21
2011	121	34
2012	74	20
2013	59	16
2014	34	11

Table S4: Direct path coefficients of age (including age-at-last-reproduction ALR) and morphology on body size, badge size and multiple paternity for males (Male MP) using a frequentist approach with the “lme4” package. Random effects of the binomial male MP model are lacking CIs. Values in bold are supported by the model (95% CI not overlapping zero).

	Body mass	**Badge size**	**MP**
Direct effects	***β* [95% CI]**	***β* [95% CI]**	***β* [95% CI]**
Intercept	**3.45 [3.442, 3.455]**	**5.78 [5.37, 6.18]**	-6.33 [-32.07, 16.85]
ALR	**0.003 [0.0009, 0.008]**	**0.014 [0.007, 0.021]**	-0.08 [-0.34, 0.20]
Age	-0.001 [-0.001, 0.0001]	**0.013 [0.005, 0.019]**	**1.21 [**0.54, 1.91]
Quadratic age	-0.0006 [-0.001, 0.000]	**-0.001 [-0.003, -0.001]**	**-0.25 [**-0.42, -0.07]
Body mass	NA	0.05 [-0.06, 0.17]	0.15 [-5.28, 5.27]
Badge size	NA	NA	0.62 [-2.25, 3.47]
Random effects	**σ [95% CI]**	**σ [95% CI]**	**σ**
Plot Year	0.008 [0.000, 0.012]	0.000 [0.000, 0.016]	0.920
Individual	0.056 [0.052, 0.061]	0.108 [0.099, 0.116]	0.682
Sample sizes	**n**	**n**	**n**
Plot Year	171	171	106
Individuals	484	484	235
Observations	1025	1025	345

Table S5: Effects on multiple paternity in males (Male MP) and morphology by age components (including age-at-last-reproduction ALR), morphology and total number of first clutches on the given island and year. The estimates are based on a frequentist approach, using the “lme4”-package. Values in bold are supported by the model (95% CI not overlapping zero).

	Body mass	**Badge size**	**MP**
Direct effects	***β* [95% CI]**	***β* [95% CI]**	***β* [95% CI]**
Intercept	**3.45 [3.43, 3.46]**	**5.84 [5.43, 6.24]**	-6.81 [-30.49, 17.45]
ALR	**0.005 [0.001, 0.008]**	**0.014 [0.008, 0.021]**	-0.09 [-0.35, 0.18]
Age	0.003 [-0.001, 0.006]	**0.012 [0.005, 0.018]**	**1.20 [0.51, 1.90]**
Quadratic age	-0.001 [-0.001, 0.000]	**-0.001 [-0.003, -0.000]**	**-0.25 [-0.42, -0.08]**
Body mass	NA	0.04 [-0.08, 0.15]	0.17 [-5.09, 4.94]
Badge size	NA	NA	0.75 [-2.21, 3.69]
Number of first clutches	NA	NA	-0.02 [-0.05, 0.02]
Random effects	**σ [95% CI]**	**σ [95% CI]**	**σ**
Plot Year	0.007 [0.000, 0.012]	0.000 [0.000, 0.015]	0.863
Individual	0.056 [0.052, 0.061]	0.108 [0.100, 0.171]	0.636
Sample sizes	**n**	**n**	**n**
Plot Year	171	171	106
Individuals	484	484	235
Observations	1025	1025	345

Table S6: Direct path coefficients of age (including age-at-last-reproduction ALR) and morphology on body size and multiple paternity for females (MP) using a frequentist approach with the "lme4"-package. Values in bold are supported by the model (95% CI not overlapping zero).

	Body mass	**MP**
Direct effect	***β* [95% CI]**	***β* [95% CI]**
Intercept	3.46 [3.45, 3.47]	12.61 [-9.06, 32.72]
ALR	**0.007 [0.002, 0.012]**	0.24 [-0.11, 0.56]
Age	-0.001 [-0.012, 0.010]	0.41 [-0.76, 1.65]
Quadratic age	-0.000[-0.002, 0.003]	-0.19 [-0.51, 0.11]
Body mass	NA	-4.23 [-10.19, 2.23]
Breeding synchrony	NA	-0.17 [-0.81, 0.42]
Random effects	**σ [95% CrI]**	**σ [95% CI]**
Plot Year	0.022 [0.013, 0.030]	1.089
Individual	0.046 [0.035, 0.054]	0.012
Sample sizes	**n**	**n**
Plot Year	161	87
Individual	466	164
Observations	728	200

Table S7: Effects on female multiple paternity MP and morphology by age components (including age-at-last-reproduction ALR), morphology, breeding synchrony and total number of first clutches on the given island and year. The estimates are based on a frequentist approach, using the "lme4"-package. Values in bold are supported by the model (95% CI not overlapping zero).

	Body mass	**MP**
Direct effect	***β* [95% CI]**	***β* [95% CI]**
Intercept	3.46 [3.45, 3.47]	12.57 [-7.42, 35.06]
ALR	**0.007 [0.002, 0.012]**	0.22 [-0.11, 0.56]
Age	-0.001 [-0.012, 0.010]	0.40 [-0.86, 1.55]
Quadratic age	-0.000[-0.002, 0.003]	-0.19 [-0.49, 0.12]
Body mass	NA	-4.10 [-10.72, 1.67]
Breeding synchrony	NA	-0.16 [-0.76, 0.43]
Number of first clutches	NA	-0.02 [-0.07, 0.04]
Random effects	**σ [95% CI]**	**σ [95% CI]**
Plot Year	0.022 [0.013, 0.030]	0.965
Individual	0.046 [0.035, 0.054]	0.003
Sample sizes	**n**	**n**
Plot Year	161	87
Individual	466	164
Observations	728	200

Table S8: The effects of badge size and body mass on multiple paternity (MP) for males without controlling for any age component(s) using a frequentist approach with the "lme4"-package.

	MP
Direct effects	***β* [95% CI]**
Intercept	-10.88 [-32.65, 11.89]
Badge size	1.30 [-1.24, 3.90]
Body mass	0.46 [-4.71, 5.33]
Random effects	**σ**
Plot Year	0.853
Individual	0.575
Sample sizes	**n**
Plot Year	106
Individuals	235
Observations	345

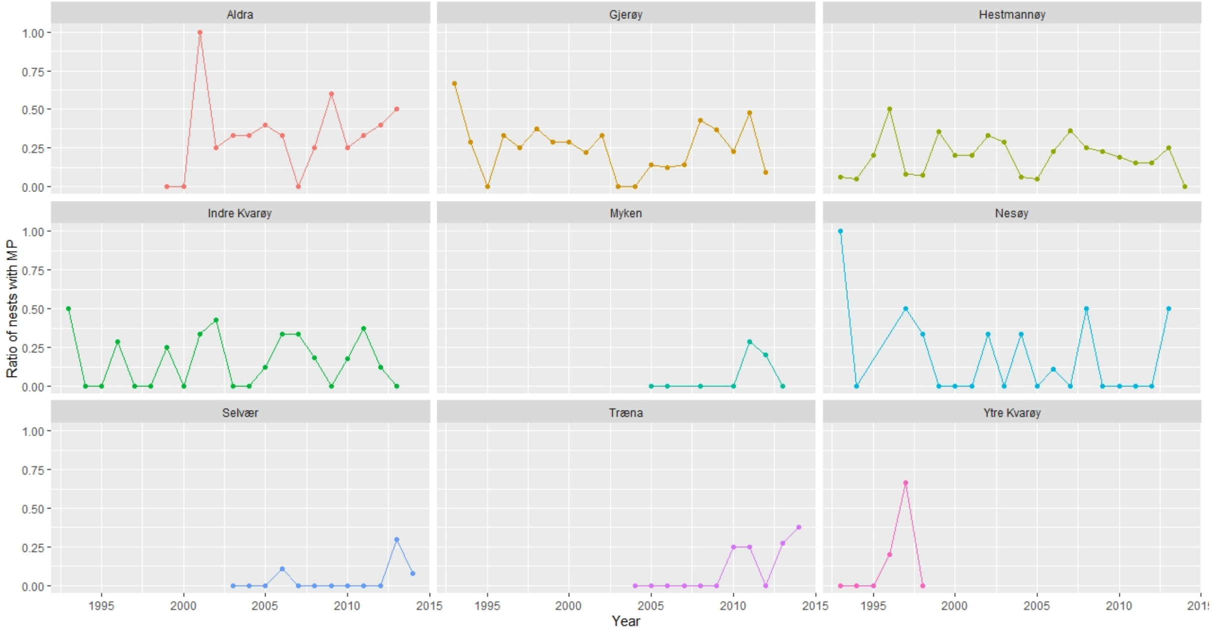

Figure S1: Yearly variation in proportion of nests with multiple paternity (MP) for the different islands over the study period