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„Sexual conflict in European House Martins (*Delichon urbicum urbicum*)“

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Contents

I General Introduction

With the advent of molecular parental analyses about three decades ago, increasing numbers of studies revealed that more than 80% of all known bird species exhibit extra-pair paternity (EPP), i.e. part or all offspring not sired by the social father (for review see: Griffith et al. 2002). This contrasts with the fact that 93% of avian species are socially monogamous (Lack 1968) and about 81% show biparental care. This contrast is even more remarkable when one considers the potential costs of EPP for both sexes (reviewed in: Westneat and Stewart 2003). Beside negative direct (physiological) impacts like injuries, predation, transmitted diseases and energetic costs of mating itself, it might impose indirect (fitness) costs. Cuckolded males lose genetic offspring and misdirected paternal care incurs further fitness relevant costs. Females may also suffer when their cuckolded partner reduces its contribution in parental care due to perceived uncertainty of paternity. In short, males of social monogamous species with paternal care are vulnerable to cuckoldry and females are vulnerable to abandonment by their partner (Trivers 1972).

This complex situation inspired copious research to explain the evolution of EPP behaviour. To understand the evolution of behavioural and other traits, research has to consider intraspecific variation, without which neither natural nor sexual selection would work. Another approach, the comparative one, takes advantage of interspecific variation. In this context it was shown that about 50% of the variance could be explained by phylogenetic dependencies and reflect adaptations and fundamental life-history styles that were acquired in a distant evolutionary past (Arnold and Owens 2002). Ecological correlates like breeding density and breeding synchrony contribute to contemporary variation in EPP rates, but numerous studies have found many more additional influences. An important aspect turned out to be male parental care which is negatively correlated with EPP rates (Møller 2000; Arnold and Owens 2002). Causalities, however, are far from being resolved because in practice the need for paternal care and male reduction of investment due to doubtful paternity are difficult to discern. Hence, it is quite obvious that a single factor alone appears to be insufficient to explain variance in EPP rates (Griffith et al. 2002). Bennett and Owens (2002) therefore proposed a hierarchical concept of life history predisposition and ecological facilitation when looking at variation of EPP in monogamous birds. As indicated above, trying to explain interspecific variation of EPP rates is only one approach to investigate the discrepancy between social and genetic monogamy in birds, and is maybe misleading as it puts the cart before the horse.

Darwin's sexual selection theory (Darwin 1871) forms the theoretical framework for understanding how extra-pair strategies evolved and are maintained in socially monogamous species. In a nutshell, this theory views sexual selection, as an adaptive outcome (Bock 2003), has its source in the interplay between male-male competition for access to mates and female choice. Since all extant animal species exhibit anisogamy, with females producing few large, costly gametes, and males very small, and thus presumably cheap gametes even when both attributes are combined in one organism like in hermaphrodites. This leads to different kinds of reproductive strategies. In a famous, but recently contested (Tang-Martinez 2012) experiment Bateman (1948) showed that in fruit flies (*Drosophila sp*.) males can improve their reproductive success with increasing number of mates. In males of social monogamous species this means that additional matings outside the pair bond increase reproductive success without providing paternal care (Trivers 1972). With focus on the females' perspective, several hypotheses derived from sexual selection theory have been proposed to explain why probable benefits of extra-pair mating can outweigh potential costs. Females may obtain direct and indirect benefits. First, females can accrue direct benefits when engaging in extra-pair copulations (EPC) by securing fertilization of their eggs to avoid infertility of their partner (e.g.: Wetton and Parkin 1991; Sheldon 1994), or gain resource benefits by extra-pair males (e.g. Gray 1997). Second, more evidence could be found for indirect (fitness) benefits of females that engage in EPCs which are related to genotype variation among males. The assumption is that females can increase their fitness through extra-pair mating by optimizing genetic compatibility with their mates (Griffith and Immler 2009) -including inbreeding avoidance (Foerster et al. 2003), higher genetic diversity (Petrie et al. 1998), or genetic quality (Kempenaers et al. 1992). Because of these findings, female choice was widely accepted as main driving force of sexual selection (Parker and Birkhead 2013). However, these genetic benefits have been recently called into question, and in the course of corresponding research it turned out that extra-pair behaviour cannot solely be explained by those indirect benefits for females (e.g.: Arnqvist and Kirkpatrick 2005; Akçay and Roughgarden 2007). Mathematic models too could not clarify whether extra-pair behaviour is female or male driven and the issue remains largely unresolved (Eliassen and Kokko 2008).

Despite substantial research effort to explain extra-pair frequencies and their variation, mainly mixed results have been attained and their evolutionary consequences still remain unclear (Westneat and Stewart 2003). Westneat and Stewart (2003) therefore suggested that pursuing extra-pair behaviour should be considered as a three player game (i.e. female,

social mate and extra pair mate), in which the fitness of each player is influenced by behavioural actions of the others, at the prevailing ecological and social circumstances.

Because reproductive interests of participants in that game are most likely different, an intersexual conflict is generated. More generally, this conflict of interest can prime an evolutionary arms race between the sexes and is intimately interwoven with sexual selection (Trivers 1972; Parker 1979; Arnqvist and Rowe 2005). "Sexual conflict theory" therefore can be seen as enhancement of the original "Sexual Selection theory" and has a stronger focus on the involved divergent reproductive strategies of the sexes. Primarily, hypotheses derived from conflict theory were corroborated in taxa that exhibit an overt sexual conflict with non-consensus mating behaviour like male coerciveness (reviewed in: Arnqvist and Rowe 2005; Kokko 2005). In birds e.g., aggressive male mating strategies like coercion of females through forced copulations (FEPC) of males were particularly investigated in waterfowl (e.g.: Adler 2010). This is not surprising as males of this bird order feature an intromittent organ which enhances fertilization success. In comparison to that, males of most extant bird orders are lacking such a penis which fostered the assumption that fertilization success through FEPCs is negligible (McKinney and Evarts 1997; Gowaty and Buschhaus 1998). Nevertheless, there is increasing evidence showing that forced copulations can indeed lead to successful fertilizations, even in species without such organs, like passerines (Low 2005; Townsend 2009; Greenlaw and Post 2012) and seabirds (Jones et al. 2012). In waterfowls, Brennan and Prum (2012) developed a "concept of sexual conflict in a broader sense" to describe male reproductive traits that are harmful to females. They suggested that these traits cannot be adequately explained by considering female choice and resistance as identical phenomena, and by neglecting intra-sexual competition (like male-male competition) as well as indirect effects. However, waterfowl mating systems are characterised by overt sexual conflict, and it remains unknown how this broader concept of sexual conflict applies to systems with less apparent sexual conflict and a more balanced parental investment.

The aim of my study therefore was to test if hypotheses derived from sexual conflict in a wider sense are feasible to explain variation and adaptive functions of extra-pair behaviour in a species with an apparent consensus between socially monogamous partners. I chose to study the European House Martin (*Delichon urbicum urbicum*) a migratory, colonially breeding and sexually monomorphic passerine species. In my opinion the lack of dimorphism is important to emphasize as it indicates low levels of overall sexual selection. House Martin colonies allow easy access to a sufficient number of birds, with little

predator pressure on nests, and presumably high levels of male-male competition and extra pair mating opportunities. House Martins form seasonal pair bonds, display mate guarding (Lifjeld and Marstein 1994; Riley et al. 1995) as well as male parental investment like engagement in nest building, incubation of eggs as well as brooding and feeding of the altricial offspring (Bryant 1988; Whittingham and Lifjeld 1995b). Additionally, Whittingham and Lifjeld (1995a) reported that 19% of chicks (35% of broods) were of extra-pair origin. It was still not known to which extent female or male House Martins have control over extra-pair fertilization (McKinney and Evarts 1997). This indicates a potential sexual conflict in this species.

To investigate the extant of this conflict and how mating strategies of the sexes are shaped by it, I carried out a three-year field study on two House Martin colonies. I collected reproductive as well as individual phenotypic parameters, behavioural data and blood samples for molecular paternity analyses. Furthermore I conducted an experiment to increase uncertainty of paternity by retaining males during the fertile phase of their female partner.

In Chapter 1, we first investigated whether a fundamental sexual conflict over reproductive success exists in the two House Martin colonies, which would be characterized by a distinct reproductive skew. In general, the outcome of diverging reproductive interests between the sexes can be seen in sex-specific variances in mating and reproductive success and resulting thus in a reproductive skew (Bateman 1948). The observations of Bateman (1948) had a big impact on the sexual selection theory (Jones 2009) and were summarized as “Bateman’s principles” (e.g.: Arnold 1994). They indicate that the sex under higher sexual selection has a higher variance in mating as well as reproductive success and a steeper regression slope of fitness. Therefore this slope is the key parameter which is also called “Bateman’s gradient” (Andersson and Iwasa 1996). Although recent work has highlighted problems with testing and interpreting Bateman’s principles (Parker and Tang-Martinez 2005; Gerlach et al. 2012; Gowaty et al. 2012) there is evidence for their robustness (e.g.: Collet et al. 2014). However, the principles are generally used as a measurement for the opportunity for sexual selection. Furthermore, even when polyandry (reproducing with more than one male) reduces the sex difference in Bateman´s gradients, sexual conflict over mating and parental investment can still be prevalent (Parker and Birkhead 2013). In this context we think that the use of the gradients was appropriate to estimate the basic situation of a prevailing inter-sexual conflict, although they cannot fully describe its magnitude.

Secondly, we tested theoretical predictions by relating individual reproductive success (RS) derived from breeding data, combining them with parental analyses and individual phenotypic traits assuming that these traits represent individual quality. According to conflict theory, we expect that the sex with higher parental investment (females) exerts a choice on traits reflecting the perceived quality of potential partners. Additionally, we expect that female House Martins should defend against EPCs, and should engage in extra-pair copulations (EPC) only to compensate for potential (genetic) shortcomings of their social partners. They also should be presumably capable to evaluate the phenotypic traits of extra-pair partners. Therefore females which are able to exert choice should have higher RS. If sexual conflict is strong, we expect that male House Martins are persistent if they get a possibility for an EPC. Therefore males which are able to subdue females, e.g. due to superior morphology, should have a higher RS.

In Chapter 2 we aimed to test if sexual conflict might explain variation in genetic heterozygosity and viability of offspring in House Martins. There is evidence for increased reproductive success in female birds due to higher offspring heterozygosity through extra-pair mating (Hansson et al. 2001; Foerster et al. 2003; Suter et al. 2007). Despite this, results so far were mixed, and researchers still debate whether indirect (genetic) benefits can outweigh potential costs of extra-pair mating (Arnqvist and Kirkpatrick 2005). Nevertheless it is possible that females may gain indirect benefits when mating with extra-pair mates with higher heterozygosity even when it imposes potential costs. In the context of sexual conflict this means that if females are free to choose potential extra-pair mates they should prefer genetically more dissimilar males. Thus, extra-pair offspring should have higher heterozygosity which may affect viability of nestlings. If the system is more male driven such an effect would not be pronounced as male individual quality and their ability to outcompete potential rivals is unlikely directly linked to genetic dissimilarity with potential extra-pair females. Therefore in Chapter 2 we test if individual heterozygosity in House Martins is related to fitness benefits due to extra-pair mating by analysing genetic variation of microsatellites in comparison with nestling survival.

In Chapter 3 we wanted to manipulate the propensity of females to engage in extra-pair copulations by experimentally detaining males during their fertile phase. We expected that females actively pursue EPCs when they are “released” from guarding constraints imposed by their male partners. As a consequence, this would increase variation in extra-pair paternity. This could be found in other studies that carried out male detainment experiments (e.g.: MacDougall-Shackleton et al. 1996; Lifjeld et al. 1997; Chuang-Dobbs

et al. 2001). Moreover, if extra-pair mating is mainly female driven, we expected that males with individually preferred phenotypes should sire more extra-pair offspring than others. However, this could be also the case when males increase persistence in EPC attempts and successful males can outcompete potential rivals. This would refer to male-male competition as main driving force. In Chapter 3 we therefore aimed to test experimentally if females are more likely to engage in EPCs when they are unguarded by their social mates or if males increase their persistence on unguarded fertile females. We did this by relating observational data derived from the experiment with phenotypic and genetic data.

REFERENCES

Adler, M. (2010) Sexual conflict in waterfowl: why do females resist extrapair copulations? *Behavioral Ecology* **21**: 182-192. doi: 10.1093/beheco/arp160

Akçay, E., Roughgarden, J. (2007) Extra-pair paternity in birds: review of the genetic benefits. *Evolutionary Ecology Research* **9**: 855–868.

Andersson, M., Iwasa, Y. (1996) Sexual selection. *Trends in Ecology & Evolution* **11**: 53-58. doi: 10.1016/j.tree.2006.03.015

Arnold, K.E., Owens, I.P.F. (2002) Extra-pair paternity and egg dumping in birds: life history, parental care and the risk of retaliation. *Proceedings of the Royal Society B* **269**: 1263-1269. doi: 10.1098/rspb.2002.2013

Arnold, S.J. (1994) Bateman's principles and the measurement of sexual selection in plants and animals. *American Naturalist* **144**: S126-S149. doi: 10.1086/285656

Arnqvist, G., Kirkpatrick, M. (2005) The Evolution of Infidelity in Socially Monogamous Passerines: The Strength of Direct and Indirect Selection on Extrapair Copulation Behavior in Females. *The American Naturalist* **165**: S26-S37. doi: 10.1086/429350

Arnqvist, G., Rowe, L. (2005) *Sexual conflict*. Princeton University Press, Princeton

Bateman, A.J. (1948) Intra-sexual selection in *Drosophila*. *Heredity* **2**: 349-368. doi: 10.1038/hdy.1948.21

Bennett, A.F., Owens, I.P.F. (2002) *Evolutionary Ecology of Birds: Life Histories, Mating Systems and Extinctition*. Oxford University Press, Oxford

Bock, W.J. (2003) Ecological Aspects of the Evolutionary Processes. *Zoological Science* **20**: 279-289. doi: 10.2108/zsj.20.279

Brennan, P.L.R., Prum, R.O. (2012) The limits of sexual conflict in the narrow sense: new insights from waterfowl biology. *Philosophical Transactions of the Royal Society B* **367**: 2324-2338. doi: 10.1098/rstb.2011.0284

Bryant, D.M. (1988) *Lifetime Reproductive Success of House Martins*. In: Clutton-Brock, T.H. (ed) Reproductive Success: Studies of Individual Variation in Contrasting Breeding Systems. University of Chicago Press, Chicago, pp 173-188

Chuang-Dobbs, H.C., Webster, M.S., Holmes, R.T. (2001) The effectiveness of mate guarding by male black-throated blue warblers. *Behavioral Ecology* **12**: 541-546. doi: 10.1093/beheco/12.5.541

Collet, J.M., Dean, R.F., Worley, K., Richardson, D.S., Pizzari, T. (2014) The measure and significance of Bateman's principles. *Proceedings of the Royal Society B* **281**: 20132973. doi: 10.1098/rspb.2013.2973

Darwin, C.R. (1871) *The Descent of Man, and Selection in Relation to Sex*. John Murray, London

Eliassen, S., Kokko, H. (2008) Current analyses do not resolve whether extra-pair paternity is male or female driven. *Behavioral Ecology and Sociobiology* **62**: 1795-1804. doi: 10.1007/s00265-008-0608-2

Foerster, K., Delhey, K., Johnsen, A., Lifjeld, J.T., Kempenaers, B. (2003) Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature* **425**: 714-717. doi: 10.1038/nature01969

Gerlach, N.M., McGlothlin, J.W., Parker, P.G., Ketterson, E.D. (2012) Reinterpreting Bateman gradients: multiple mating and selection in both sexes of a songbird species. *Behavioral Ecology* **23**: 1078-1088. doi: 10.1093/beheco/ars077

Gowaty, P.A., Buschhaus, N. (1998) Ultimate Causation of Aggressive and Forced Copulation in Birds: Female Resistance, the CODE Hypothesis, and Social Monogamy. *American Zoologist* **38**: 207-225. doi: 10.1093/icb/38.1.207

Gowaty, P.A., Kim, Y.K., Anderson, W.W. (2012) No evidence of sexual selection in a repetition of Bateman's classic study of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America* **109**: 11740-11745. doi: 10.1073/pnas.1207851109

Gray, E.M. (1997) Female red-winged blackbirds accrue material benefits from copulating with extra-pair males. *Animal Behaviour* **53**: 625-639. doi: 10.1006/anbe.1996.0336

Greenlaw, J.S., Post, W. (2012) Apparent forced mating and female control in Saltmarsh Sparrows. *Wilson Journal of Ornithology* **124**: 253-264. doi: 10.1676/11-072.1

Griffith, S.C., Immler, S. (2009) Female infidelity and genetic compatibility in birds: the role of the genetically loaded raffle in understanding the function of extrapair paternity. *Journal of Avian Biology* **40**: 97-101. doi: 10.1111/j.1600-048X.2009.04562.x

Griffith, S.C., Owens, I.P.F., Thuman, K.A. (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology* **11**: 2195-2212. doi: 10.1046/j.1365-294X.2002.01613.x

Hansson, B., Bensch, S., Hasselquist, D., Åkesson, M. (2001) Microsatellite diversity predicts recruitment of sibling great reed warblers. *Proceedings of the Royal Society B* **268**: 1287-1291. doi: 10.1098/rspb.2001.1640

Jones, A.G. (2009) On the opportunity for sexual selection, the Bateman gradient and the maximum intensity of sexual selection. *Evolution* **63**: 1673-1684. doi: 10.1111/j.1558-5646.2009.00664.x

Jones, M.G.W., Techow, N.M.S.M., Ryan, P.G. (2012) Dalliances and doubtful dads: what determines extra-pair paternity in socially monogamous wandering albatrosses? *Behavioral Ecology and Sociobiology* **66**: 1213-1224. doi: 10.1007/s00265-012-1374-8

Kempenaers, B., Verheyen, G.R., Van den Broeck, M., Burke, T., Van Broeckhoven, C., Dhondt, A.A. (1992) Extra-pair paternity results from female preference for high-quality males in the blue tit. *Nature* **357**: 494-496. doi: 10.1038/357494a0

Kokko, H. (2005) Treat 'em mean, keep 'em (sometimes) keen: evolution of female preferences for dominant and coercive males. *Evolutionary Ecology* **19**: 123-135. doi: 10.1007/s10682-004-7919-1

Lack, D. (1968) *Ecological Adaptions for Breeding in Birds*. Methuen, London

Lifjeld, J.T., Marstein, B. (1994) Paternity assurance behavior in the House Martin *Delichon urbica*. *Journal of Avian Biology* **25**: 231-238. doi: 10.2307/3677080

Lifjeld, J.T., Slagsvold, T., Ellegren, H. (1997) Experimental mate switching in pied flycatchers: male copulatory access and fertilization success. *Animal Behaviour* **53**: 1225-1232. doi: 10.1006/anbe.1996.0430

Low, M. (2005) Female resistance and male force: context and patterns of copulation in the New Zealand stitchbird *Notiomystis cincta*. *Journal of Avian Biology* **36**: 436-448. doi: 10.1111/j.0908-8857.2005.03460.x

MacDougall-Shackleton, E.A., Robertson, R.J., Boag, P.T. (1996) Temporary male removal increases extra-pair paternity in eastern bluebirds. *Animal Behaviour* **52**: 1177-1183. doi: 10.1006/anbe.1996.0265

McKinney, F., Evarts, S. (1997) Sexual coercion in waterfowl and other birds. *Ornithological Monographs*: 163-195. doi: 10.2307/40166723

Møller, A.P. (2000) Male parental care, female reproductive success, and extra-pair paternity. *Behavioral Ecology* **11**: 161-168. doi: 10.1093/beheco/11.2.161

Parker, G.A. (1979) *Sexual selection and sexual conflict*. In: Blum, M.S., Blum, N.A. (eds) Sexual selection and reproductive competition in insects. Academic Press, New York, pp 123-166

Parker, G.A., Birkhead, T.R. (2013) Polyandry: the history of a revolution. *Philosophical Transactions of the Royal Society B* **368**: 20120335. doi: 10.1098/rstb.2012.0335

Parker, P.G., Tang-Martinez, Z. (2005) Bateman Gradients in Field and Laboratory Studies: A Cautionary Tale. *Integrative and Comparative Biology* **45**: 895-902. doi: 10.1093/icb/45.5.895

Petrie, M., Doums, C., Møller, A.P. (1998) The degree of extra-pair paternity increases with genetic variability. *Proceedings of the National Academy of Sciences of the United States of America* **95**: 9390-9395. http://www.pnas.org/content/95/16/9390.full

Riley, H.T., Bryant, D.M., Carter, R.E., Parkin, D.T. (1995) Extra-pair fertilizations and paternity defence in house martins, *Delichon urbica*. *Animal Behaviour* **49**: 495-509. doi: 10.1006/anbe.1995.0065

Sheldon, B.C. (1994) Male phenotype, fertility, and the pursuit of extra-pair copulations by female birds. *Proceedings of the Royal Society B* **257**: 25-30. doi: 10.1098/rspb.1994.0089

Suter, S.M., Keiser, M., Feignoux, R., Meyer, D.R. (2007) Reed bunting females increase fitness through extra-pair mating with genetically dissimilar males. *Proceedings of the Royal Society B* **274**: 2865-2871. doi: 10.1098/rspb.2007.0799

Tang-Martinez, Z. (2012) Repetition of Bateman challenges the paradigm. *Proceedings of the National Academy of Sciences of the United States of America* **109**: 11476-11477. doi: 10.1073/pnas.1209394109

Townsend, A.K. (2009) Extrapair copulations predict extrapair fertilizations in the American Crow. *The Condor* **111**: 387-392. doi: 10.1525/cond.2009.090010

Trivers, R.L. (1972) *Parental Investment and Sexual Selection*. In: Campbell, B. (ed) Sexual selection and the descent of man 1871-1971. Aldine, Chicago, pp 136-179

Westneat, D.F., Stewart, I.R.K. (2003) Extra-Pair Paternity In Birds: Causes, Correlates, and Conflict. *Annual Review of Ecology Evolution and Systematics* **34**: 365-396. doi: 10.1146/annurev.ecolsys.34.011802.132439

Wetton, J.H., Parkin, D.T. (1991) An association between fertility and cuckoldry in the house sparrow, *Passer domesticus*. *Proceedings of the Royal Society of London B* **245**: 227-233. doi: 10.1098/rspb.1991.0114

Whittingham, L.A., Lifjeld, J.T. (1995a) Extra-pair fertilizations increase the opportunity for sexual selection in the monogamous House Martin *Delichon urbica*. *Journal of Avian Biology* **26**: 283-288. doi: 10.2307/3677042

Whittingham, L.A., Lifjeld, J.T. (1995b) High paternal investment in unrelated young: extra-pair paternity and male parental care in house martins. *Behavioral Ecology and Sociobiology* **37**: 103-108. doi: 10.1007/bf00164155

Chapter 1

The dark side of sex: sexual conflict over reproductive success

ABSTRACT

Although extra-pair mating is a common reproductive strategy in socially monogamous birds a comprehensive explanation for this phenomenon is still missing. In our study we applied a broader concept of sexual conflict as basic mechanism to explain individual variation of reproductive success in relation to paternity in European House Martins (*Delichon urbicum urbicum*). We used measurements of phenotypical traits and related these to individual reproductive success (RS). Indeed from our analyses we could verify on one hand a fundamental sexual conflict in RS between the sexes and an even more pronounced conflict in extra-pair mating strategies. In our study colonies of House Martins we found that males increase their RS with their number of (sexual) mating partners. This is only rudimentary true for females as they cannot produce additional offspring by having more than three mating partners. Offspring numbers of broods were increasing with fathers' age and males were increasing their total RS with age. Proportion of extra-pair paternity was mainly influenced by male age but also by female age and male body condition. More generally, extra-pair mating was related to male body condition and female wing length, indicating that extra-pair fertilizations were more likely to happen when the social father is in weak body condition and the female partner has short wings. As male House Martins are known to persistently pursue extra-pair copulations our results therefore provide evidence that extra-pair mating in House Martins is mainly male induced.

INTRODUCTION

The vast majority of bird species is socially monogamous (Lack 1968). Increasing research has, however, revealed that the pursuit of extra-pair fertilizations (EPF), resulting in extra-pair paternity (EPP), is a common reproductive strategy in birds and that it occurs in approximately 70% of species (for review see: Griffith et al. 2002). Yet no consensus exists on why the pursuit of EPF is such a widespread strategy amongst birds, and why high interspecies variation in EPP rates exists. Bennett and Owens (2002) proposed a hierarchical framework of phylogenetic and life history predisposition to EPP in birds, in addition to ecological facilitation (e.g. food availability and nesting density). As considerable variation in life histories and ecological facilitations exists even in closely-

related species, it is additionally necessary to account for different behavioural strategies in reproduction when exploring variation in the prevalence of EPP.

The pursuit of EPF by males was traditionally thought of as overtly beneficial (Trivers 1972). However, several costs have now been identified including sperm depletion (Edler and Friedl 2008), sexually transmitted infections (Sheldon 1993), reduced time for mate guarding (Hasselquist and Bensch 1991) or reduced paternal investment (Westneat et al. 1990; Magrath and Elgar 1997). Since around 1980 the major part of research on EPP in birds has instead focused on female perspective of pursuing EPCs (Parker and Birkhead 2013), revealing that females may gain both direct (Wetton and Parkin 1991; Sheldon 1994; Gray 1997) and indirect benefits (Møller 1988; Kempenaers et al. 1992; Petrie 1994; Veen et al. 2001; Foerster et al. 2003; Rubenstein 2007; Pryke et al. 2010). In any case, the current prevalent view that females have freedom of choice among displaying males, has recently been called into question as an increasing number of studies shows that extra-pair behaviour cannot solely be explained by these benefits for females (e.g.: Arnqvist and Kirkpatrick 2005; Hadfield et al. 2006; Qvarnström et al. 2006; Akçay and Roughgarden 2007; Kotiaho and Puurtinen 2007; Sardell et al. 2012). That's why, despite many studies supporting the role of female choice in determining EPP levels, the question whether extra-pair behaviour is female or male driven remains largely unresolved (Eliassen and Kokko 2008).

No matter which sex is the main driving force in this system, to elucidate causes and consequences of EPP it may be helpful to primarily focus on potential divergence of reproductive interests between the sexes. These contrasting reproductive concerns are ultimately predetermined by the fact that mating partners are genetically different (Lessells 1999) and anisogamy influences the evolution of sex roles (Schärer et al. 2012). If this imbalance increases, females and males show differential mating strategies as well as different parental investment which may lead to an intersexual conflict (Trivers 1972; Parker 1979; Arnqvist and Rowe 2005). Moreover, the pursuit of EPCs should be considered a three player game (i.e. female, social mate and extra-pair mate), where the fitness of each player is influenced by the behavioural actions of the others (Westneat and Stewart 2003). Therefore, intraspecific conflicts of interest, (e.g. male-male competition) also play an important role in EPF rates. Mating strategies adopted by the sex with the higher parental investment (i.e. females) should involve choice and denial (or resistance), while sexual display and persistence (coercion) should be adopted by the sex with the lower parental investment (i.e. males). Thus, extra-pair behaviour adopted by males can

range from persistence to coercion, depending on the level of intraspecific competition. Although fertilization success through forced extra-pair copulations (FEPC) is highly doubted in most bird species, mainly due to the lack of an intromittent organ in most orders of extant birds (McKinney and Evarts 1997; Gowaty and Buschhaus 1998), recent work shows that forced copulations can indeed lead to successful fertilizations, even in species without such organs, like passerines (Low 2005; Townsend 2009; Greenlaw and Post 2012) and seabirds (Jones et al. 2012). In case of species with intromittent organs, like waterfowl, Brennan and Prum (2012) referred to a "concept of sexual conflict in a broader sense" to describe harmful male reproductive traits. They suggested that these traits cannot be adequately explained by considering choice and resistance as identical phenomena, and neglecting intra-sexual competition as well as indirect effects. However, waterfowl mating systems are often characterised by overt sexual conflict (e.g. coercive behaviour of males), and it remains unknown how this broader concept of sexual conflict applies in systems with less apparent sexual conflict and a more balanced parental investment.

In comparison to waterfowl, in which males often hardly invest in their offspring, the majority of passerines exhibits biparental care of offspring (Clutton-Brock 1991). In these systems, the main intersexual conflict for females is vulnerability to male desertion, while males are vulnerable to cuckoldry (Trivers 1972). Females should avoid fitness costs due to male desertion, by assuring paternal investment. If the reduction in male care due to doubtful paternity is effectively costly, females should be reluctant to pursue EPCs or at least not obtrusive. Actually there is evidence that high paternal investment is correlated with low or zero EPP rates (e.g.: Morton et al. 1998; Matysiokova and Remes 2013; Tarwater et al. 2013). Females therefore face a trade-off between pursuing or accepting EPCs and ensuring investment from their social partners (Møller 2000; Arnold and Owens 2002; Seki et al. 2007). From the males' perspective, mate guarding is a common strategy for paternity assurance (Birkhead and Møller 1992). In addition to other costs of mate guarding (e.g. energetic – Komdeur 2001), it decreases the opportunities for males to pursue EPCs. Investigations on the effectiveness of mate guarding have provided mixed results (Chuang-Dobbs et al. 2001; Komdeur et al. 2007), and males that actively seek EPCs also suffer a higher risk of cuckoldry (Marthinsen et al. 2005; Woolfenden et al. 2005). Consequentially, they have to balance their within-pair (e.g.: mate guarding, paternal investment) and their extra-pair behaviour (Westneat et al. 1990; Wright and Cotton 1994).

However, if a fundamental conflict due to diverging reproductive interests between the sexes of a species exists, sex specific variances in mating and reproductive success should be found. This variances are characterized by differences between the regression slopes of each sex (Andersson and Iwasa 1996) and results in a reproductive skew (Bateman 1948). The usefulness of these regression slopes as gradients for sexual selection has been debated (e.g.: Gowaty et al. 2012), but nevertheless proofed to be robust (Collet et al. 2014), at least to describe a basal sexual conflict and the opportunity for sexual selection (Parker and Birkhead 2013).

To get a more detailed picture of how sexual conflict shapes extra-pair strategies and its sex-specific variances we tested this issue in European House Martins (*Delichon urbicum urbicum*), a socially monogamous, sexually monomorphic passerine species with biparental care. This long distance migrant and colonially breeding species forms seasonal pair bonds, displays mate guarding (Lifjeld and Marstein 1994; Riley et al. 1995) as well as paternal investment where males engage in nest building, incubation of eggs as well as brooding and feeding of the altricial offspring (Bryant 1988; Whittingham and Lifjeld 1995b). Additionally, Whittingham and Lifjeld (1995a) reported that 19% of chicks (35% of broods) were of extra-pair origin, but it is still not known to which extent female or male House Martins have control over extra-pair fertilization (McKinney and Evarts 1997). This refers to a potential sexual conflict in this species.

Our study therefore was aimed to test if adopting a wider range of sexual conflict can provide insights into the role of EPP and how mating strategies of sexes are shaped by this conflict. For this reason we made the following predictions:

(1) We expect fundamental differences in reproductive success (RS) between females and males due to their different levels of parental investment. The sex with generally lower parental investment (males) should increase its RS with quantity of mating partners.

(2) We assume that certain phenotypic traits indicate quality in both sexes alike. Furthermore, the sex with higher parental investment (females) should exert a choice on such traits reflecting the quality of potential partners. Therefore individuals showing characteristics of traits assigning high quality should have high RS and in males with these traits additionally increased RS can be found.

(3) We expect that female House Martins are reluctant to EPCs and should accept "EPC-offers" to e.g. compensate potential disadvantages of social partners. They

do this by evaluating phenotypic traits of extra-pair partners. Therefore females which are able to exert choice should have higher RS.

(4) We expect that male House Martins are persistent if they get a possibility for an EPC. Therefore males which are able to subdue females, e.g. due to predominant morphology, should have a higher RS.

METHODS

Fieldwork

We studied two colonies of House Martins in Illmitz, Burgenland, Austria (WGS 84: 47.76887N, 16.76619E) from 2002 until 2004. Both colonies are located at buildings surrounded by reed beds, small channels and pasture landscape. Colony 1 was attached to the roof of the boat house of the biological station Illmitz (henceforth BSI) and comprised between 42 and 78 mud-built nests per year. Due to a harsh winter in 2002/2003, remains of natural nests were replaced by 63 artificial nest cups. Natural nests at colony 2 were attached to roof awnings of buildings at the Seebad Illmitz (henceforth Seebad) and comprised up to 30 nests. The colonies were located 2.5km away from each other. The numbers of successful nests (i.e. with at least one chick surviving until ringing) were: 2002: 39 first and six second broods at colony 1, 19 first and one second brood at colony 2; 2003: 25 first and two second broods at colony 1, eleven first and one second brood at colony 2; 2004: 42 first and 15 second broods at colony 1 (no data for colony 2).

All nests were monitored weekly from end of April until mid of September.

We mainly trapped adult birds when emerging from their nests by holding small basket-nets in front (majority of birds were caught when finishing the clutch) and occasionally with mist nets blocking the approach path to the colony for a maximum of one hour. We individually ringed the birds, marked the foreheads and tail feather tips of adult females with white fluid (Tipp-Ex®) and the white rump of adult males with different colours of Edding® permanent markers. Adult birds were sexed based on absence (male) or presence (female) of a brood patch following the method of Bryant (1975). As we caught the majority of adults after the clutch was finished, the differences in brood patch development between sexes of each pair was easy to discern. Late- and non-breeding individuals with unclear brood patch development could be sexed at later recaptures or with molecular methods following the protocol of Griffiths et al. (1998). The social parents of each nest were confirmed by checking individual markings on multiple observations during incubation and nestling phase.

Eight to 19 days post hatching nestlings were ringed, weighed and the wing length was measured. If adults were not ringed as nestlings (and hence having known age), we estimated their minimum age (i.e. newly ringed adult birds are at least one year old, birds recaptured in two subsequent years are at least two years old, etc.). In adults we measured wing length (maximum chord of folded wing) with a stopped ruler. The longest wing feather (eighth primary - P8) and the tail length to nearest 0.5mm were measured with a pinned ruler. Including wing pointedness which can affect flight performance we measured Kipp's Distance (length from the tip of the longest secondary cover to the tip of the longest primary) at the folded wing with a calliper to 0.1mm nearest (see: Kipp 1959). As predictor for body size we took keel length (breast bone extension, see: Bryant and Westerterp 1983) with a calliper to 0.1mm nearest. As predictors for body condition we measured body mass with an electronic pocket balance (Model 1479 Tanita, max. 100g, accuracy level 0.1g with an accuracy of 0.1g). Further we estimated body fat extent by following the classifications after Kaiser (1993). As additional condition index we calculated body mass/keel length residuals of males, because our study focused on extra-pair paternity. For validity of morphometric indices as indicator for body condition see Labocha and Hayes (2011). Related to body condition it could be shown earlier that ectoparasites like louse flies can transmit blood parasites in birds (Baker 1967). These endoparasites like avian malaria can negatively affect survival in House Martins (Marzal et al. 2008). Therefore we also noted the number of louse flies (*Stenepteryx hirundinis*) on each individual. Blood samples (approximately 25µl) were obtained from the brachial vein and stored in Queen's buffer (for basic protocol see: Seutin et al. 1991).

Microsatellite Genotyping

We extracted DNA from 735 blood samples (215 adults and 520 nestlings) and 17 tissue samples (unhatched eggs and dead nestlings) using DNeasy® Blood and Tissue kit (Qiagen) per manufacturer's recommendation. DNA purity and concentration were checked on a NanoDrop 2000 (Thermo Scientific). The parental status of each nestling was determined using six independent microsatellite markers: Aar4 (Hansson et al. 2000), Ase18 (Richardson et al. 2000), Escµ6 (Hanotte et al. 1994), HrU6 and HrU7 (both: Primmer et al. 1995), Ltr6 (McDonald and Potts 1994).

Ase18, HrU6 and HrU7 were amplified in a 11µl multiplex reaction containing 1.1µl 10x PCR Buffer II (Applied Biosystems), dNTPs 200µM each (Fermentas), 2.5mM $MgCl_2$ (Fermentas), Ase18 forward and reverse primer 2pmol each, HrU6 forward and reverse

primer 1pmol each, HrU7 forward and reverse primer 3pmol each, 0.4U AmpliTaq DNA Polymerase (Applied Biosystems), approximately 25ng template DNA and 5.42µl ddH_2O. Aar4, Escµ6 and Ltr6 were amplified in 11µl singleplex reactions containing 1.1µl 10x PCR Buffer II (Applied Biosystems), dNTPs 200µM each (Fermentas), 2.5mM $MgCl_2$ (Fermentas), forward and reverse primer 2pmol each, 0.4U AmpliTaq DNA Polymerase (Applied Biosystems), approximately 25ng template DNA and 6.22µl ddH_2O. PCR reactions were performed using a T-Gradient thermocycler (Biometra) with the following amplification conditions: 2min initial denaturation followed by either 35 cycles for the singleplex reactions or 30 cycles for the multiplex reaction of 94°C for 30sec, corresponding annealing temperature for 90sec, 72°C for 90sec and a final extension step at 72°C for 10min. For primer sequences, fragment sizes and annealing temperatures see Table 1. Amplified products were prepared for fragment analyses by diluting with ddH_2O (Multiplex HrU7, Hru6 & Ase 18: 1/65, singleplexes Aar4 & Escµ6: 1/20, Ltr6: 1/10). 1µl of diluted product was added to 20µl sample loading solution/+400 DNA size standard mixture (Beckman Coulter). Samples were run on a CEQ™ 8000 Beckman Coulter automated sequencer and analysed using Genetic analyses software Version 9.0.25, Beckman Coulter Copyright® 2004. The output was independently scored by two persons who had no knowledge of age (adults and nestlings) or nest ID of each individual.

Parental analyses

For 154 broods (518 offspring) we obtained genetic data where we sampled both social parents. For each year, parentage analysis was done using CERVUS 3.0 (Marshall et al. 1998; Kalinowski et al. 2007). We first calculated the maternity status of all possible mothers in the population within a given year to check for any possible brood parasitism and to allow mothers to be assigned as known parent during the paternity analyses. For both maternity and paternity analyses we used the following parameter settings: a 1% error rate due to mutation or low rates of null alleles at various loci, 100% of loci typed, and an estimated 90% of all candidate parents were sampled. In 2002 we analysed data for 209 offspring along with 69 candidate mothers and 68 candidate fathers, in 2003: 132 offspring, 41 candidate mothers, and 53 candidate fathers and in 2004: 195 offspring, 41 candidate mothers and 44 candidate fathers. Microsatellite loci showed a mean of 20.5 alleles (SD: ±9.9, range: 13-40 alleles/locus) and a combined non-exclusion probability of $4.1x10^{-4}$ for a putative father if the mother is known. Allele frequencies deviated from Hardy-Weinberg equilibrium at two loci, Hru6 and Escµ6, with an estimated frequency of null alleles of 0.067 and 0.046 respectively. A nestling was considered as

sired by another parent part than the social ones according to the results given by CERVUS. This was the case if the LOD (maximum likelihood ratio) score was higher or positive for the alternative parent. The egg dumping mother or the cuckolder could be identified when this female or male showed none or one mismatch (i.e. due to null alleles), a higher LOD score and a significant Pair or Trio confidence.

Statistical Analyses

If sexual conflict between sexes in House Martins is fundamental, a pronounced reproductive skew should be detected. Therefore we first compared distribution and skew of offspring numbers of females and males in each breeding season. Then we looked for possible correlations between offspring numbers of individuals with the number of genetic mates in females and males for each breeding season. As the regression slope of fitness against mating (Bateman's gradient, see: Andersson and Iwasa 1996) is the key measurement for sexual conflict we applied a bootstrap calculation to look for differences in reproductive success of individual females and males related to the number of genetic partners.

To investigate which differential characteristics of phenotypic traits in adults are correlated with reproductive success and how this shapes extra-pair strategies we used generalised linear mixed models (GLMM). Individual ID was incorporated as a random effect in all models to reduce the effects of pseudoreplication when an individual had multiple broods within or between years. We used a number of different estimates of reproductive success for females and males respectively: 1) individual numbers of offspring 2) individual numbers of within-pair offspring (WPO) 3) the percentage of extra-pair offspring (EPO) within the attended nest 4) if the individual cuckolds (female) or was cuckolded (male), 5) individual numbers of offspring fledged from the nest. For males we additional included: individual numbers of offspring sired (WPO+EPO), individual numbers of extra-pair offspring sired and individual numbers of sired offspring fledged. The probability of being cuckolded followed a binomial distribution, with a logit link function, whereas all other variables followed a Poisson distribution, with a logarithmic link function.

We tested the effect of the following phenotypic parameters of each adult on its reproductive success: minimum age, length of eighth primary feather (P8), wing length, tail length, Kipp's distance and keel length. Parameters related to condition were body mass, fat class, residuals of body mass and keel length (Mass-Res) as body condition index and number of ectoparasites (louse flies) of each individual. Further we tested for effects of the following parameters: year, colony site (BSI or Seebad), trapping date of adults and

hatching date of chicks as estimator for seasonality in timing of breeding. We first created a range of candidate models including these independent variables and then selected the most parsimonious based on the Akaike information criterion (AIC). AIC was calculated as AIC = Deviance (measure of fit) + 2*K, where Deviance = -2 * log Likelihood (*L*), for each model, and K is the number of parameters in the model. Due to low samples sizes in number of parasites from AIC values we calculated a second order bias correction called AIC_c (see: Burnham et al. 2011) with the following formula AIC+ value+(2* Deviance (Deviance+1))/number of values-number parameters-1). The model with the lowest AIC_c was selected as the most parsimonious. The model resulting in the lowest AIC_c was considered the most parsimonious model, and competing models with differences in AIC_c values of more than two were considered significantly different. When the model with the lowest AIC_c differed by less than two from the model with the second lowest AIC_c, we selected the model with the fewest number of parameters (Quinn and Keough 2002). GLMMs were performed using GenStat software package 14.0 (for review see: Bolker et al. 2009).

Additionally we looked separately on phenotypical traits predicting variation in individual RS revealed from model selection. As age seems to be a key factor in variation of individual numbers of pair offspring we correlated individual numbers of pair offspring with age of mothers and fathers of social pairs.

Further we wanted to know if age plays an equal role in mating patterns like it does in RS. We first looked for a correlation of social partners' age. To proof if social pairing in relation to age was not biased by the majority of yearling pairs (social mother and father were both one year of minimum age), we developed a computer programme called "Lolita" (© Hans Winkler, 2015). First it calculates a contingency index with the observed pairings applying a Chi^2 Test. Then it computes randomized sampling (10.000 iterations) and compares contingencies of new configurations with the observed pairings.

We then investigated extra-pair mating and tested for correlations of age of mothers and their extra-pair partners and additionally tested if there is a correlation of minimum age of fathers. Single correlations apart from GLMM testing were calculated applying IBM SPSS software package 20.0.

RESULTS

Microsatellite genotyping

We identified three cases of intraspecific brood parasitism in 2002 and one in 2003, where the genetic profile of nestlings neither matched the social mother nor the social father. One of these three chicks in 2002 could not be assigned to any of the sampled adults. These four "egg dumping chicks" aside, 96% of nestlings could be assigned at 95% confidence level to one of the sampled males. On average 20.5% (107/521) of offspring did not match the genetic profile of the social father and 42.9% of broods (67/156) contained 1.7 offspring sired by an extra-pair male. For detailed distribution of the results per year see Table 2.

Reproductive skew

When looking at individual genetic offspring produced per breeding season for all years (Figure 1 a + b), males showed almost double the skewness (0.638, Kurtosis: -0.235) than females (0.346, kurtosis: -0.414). Nevertheless, comparison of means of chicks produced per season (males: N = 170, mean ± SD = 3.0 ± 2.53, females: N = 168, 3.14 ± 2.39) showed no significant difference (Mann-Whitney *U*-test $z = -0.656$, $p = 0.512$) between the sexes. We then calculated the Bateman's gradient by comparing the number of (genetic) mates and number of offspring per individual for females and males. Applying a bootstrap analysis we found a significant correlation between number of mates and offspring for males (N = 170, $R^2 = 0.68$, b-value: 2.23, 10000 iterations, $p < 0.00001$) and females (N = 113, $R^2 = 0.53$, b-value: 1.58, 10000 iterations, $p < 0.00001$) respectively. The optimum number of genetic partners to increase individual offspring for males is 6.43 whereas for females it is 3.21 (see Figure 2).

Reproductive success and mating patterns

From AICc model selection (see Tables 3-6) we found six out of 14 different factors which predict reproductive success in House Martins. The most parsimonious model explaining variation in individual numbers of offspring in females included hatching date of offspring and year, indicating that earlier breeders have higher RS, but depending on the year. In males minimum age is the dominant factor explaining variation in RS into the direction that older males produce more offspring in total as well as with their social partners than younger males. The same is true for females, as number of offspring produced with their social partners increases with their partner's age. As the factor male minimum age best explains variation in numbers of pair offspring both in females as in males, we separately

tested for correlations of female and male minimum age with number of within pair offspring (see Figure 3). We found that the number of pair offspring was strongly correlated with minimum age in males (Two-tailed Spearman correlation, N = 133, r = 0.302, $p \leq 0.0001$) but not in females (Two-tailed Spearman correlation, N = 133, r = 0.166, $p \leq 0.057$).

The most parsimonious models explaining variation in the proportion of extra-pair offspring in females' nests are minimum age of mother, their partner's minimum age and their partners' mass-residuals. The effects of each factor show that proportion of extra-pair offspring increases when older females are paired with younger males in weak condition. In males the factor mass-residuals is correlated with the proportion of extra-pair offspring in the direction that males with weak condition have more extra-pair chicks in their nests. Similar, mass residuals explain variation in the amount of extra-pair offspring produced by males. The effect of this factor indicates that males in good condition are more likely to sire extra-pair offspring. To complete the picture in male extra-pair strategies we looked if there is a trade-off between producing within- or extra-pair offspring in males. In that case a negative correlation of individual numbers of pair offspring with extra-pair offspring should be found, which we could not (Two-tailed Spearman correlation, N = 135 males, r = 0.147, $p \leq 0.089$).

When looking at extra-pair behaviour more generally by simply checking whether extra-pair offspring is present or not, in females the factors mass residual of their social partners and length of the eighth wing feather (P8) were included in the most parsimonious model. The effects of the factors indicate that females with shorter wing feathers paired to males in weak condition are more likely to cuckold. For males the factor influencing the probability to get cuckolded is mass-residuals indicating that males with weak condition are more likely to be cuckolded. When looking at individual numbers of fledged offspring, in females the factors hatching date and minimum age of partner are the best determinants to explain variation. Here earlier breeders with older males increase the number of fledged offspring. The same is true when looking at males, where more pair offspring is fledging when they hatch early in season and more offspring from older males (within-pair offspring and extra-pair offspring) is fledging.

As AICc model selection shows that the factor minimum age was included in seven out of 12 measures of RS we separately checked if age predicts mating patterns. We could find a strong correlation between female and male age of social pairs in our study population (N = 133 pairs, r = 0.510, $p \leq 0.001$, see Figure 3). The "Lolita" test showed independency

from the majority of "yearling pairs" which proofs that there is age assortative social mating (see Figure 4, Chi^2 = 64.09912 [24 d.f.], Contingency coefficient = 0.570274, 10 configurations showed close or equal contingency, corresponds to a P value of 0.001100).
We then also looked at extra-pair mating and selected females which had at least one extra-pair chick in their nest. We found that young females (minimum age: one) had significantly more often older extra-pair mates (Paired sample Test, t = -2.179, d.f. = 19, $p \leq 0.042$) than older females (Paired sample Test, t = 1.624, d.f. = 23, $p \leq 0.118$, see Figure 5).

DISCUSSION

In our study population of House Martins males show a higher reproductive skew than females. In detail this means that there are more males than females siring only one or two chicks, although both sexes on average produce about three chicks per breeding season. On the other hand males can approximately double their reproductive success compared to females by mating with additional partners. Nevertheless females increase their RS when they mate with more than one male, although there seems to be an optimal number of three partners.
The factors which explain variation of RS in female House Martins best are hatching date of offspring, minimum age of their social partners, the mass-residuals of their social partners, their own minimum age and length of P8. In the total amount of produced offspring we additionally found an effect of the study year. However, minimum age of their social partners affects females' RS in three from five different measures of RS (numbers of within-pair offspring, proportion of extra-pair offspring, and numbers of fledged offspring). Hatching date of offspring in females affects individual number of offspring and fledged offspring. Variation in RS of females in relation to extra-pair strategies are best explained by their social partners´ mass residuals (proportion of extra-pair offspring) and is connected with length of the longest wing feather when cuckolding.
In RS of males our results show a similar picture. Male minimum age affects RS in case of numbers of sired offspring, numbers of sired within-pair offspring and number of sired offspring fledged. Males' mass-residuals best explain numbers of sired extra-pair offspring, proportion of extra-pair offspring in nest and if they get cuckolded by their females. Only the number of social offspring is related to a non-phenotypic factor namely hatching date of offspring.
Therefore, male age and body condition are the predominant factors to explain variation of RS in House Martins and age predicts mating strategies as we could find age assortative

social mating. In case of extra-pair mating we found that extra-pair partners from young females (minimum age: one year) were significantly older than themselves. For older females (two years and more) we could not find this correlation.

Our first hypothesis predicted a fundamental sexual conflict which can be shown by the distinct reproductive skew we found. Furthermore, males, the sex with in principle lower parental investment, can definitely increase their RS with increasing number of sexual partners. These results confirm early theories about sexual conflict over RS due to sex-dependant parental investment (e.g.: Trivers 1972). Moreover also females, the sex with generally higher parental investment, can increase their RS with more partners than one but seem to be bound to maximally three different mates. From this we infer that for females it is important with whom they mate in social and extra pairings and therefore should exert a choice if possible. In our second hypothesis we expected that the decision process of females follows the evaluation of values of specific phenotypical traits representing male quality. Males carrying these traits should therefore have higher RS. In general, we could show that males' age and body condition (represented by mass-residuals) are the main phenotypical traits affecting RS in House Martins.

When looking at mating patterns we found age assortative social mating. This finding is not new, as already earlier capture-recapture studies on House Martins could show this effect (Rheinwald et al. 1976; Hund and Prinzinger 1985). Additionally we found that the number of pair offspring produced and fledged increases with age of social fathers but focused on females it is linked to hatching date of offspring. Nevertheless, as breeding start is negatively correlated with age both in male and female House Martins (Bryant 1979; Hund and Prinzinger 1985), we could show the importance of age in case of social mating and RS in our study population.

Having a closer look on RS from extra-pair mating, according to our third hypothesis we found that young females seem to compensate their social partners' youth by choosing older partners as extra-pair mates. Thus, if age assigns male quality this pattern should be also found in extra-pair partners of older females, which we did not. However, AICc model selection showed that the proportion of extra-pair offspring in a female´s nest increases when older females are paired with younger males in weak body condition. These findings on the first glance could be interpreted as proof for female choice as main reason of variation in EPP in House Martins. But if we look at factors influencing whether females cuckold their partners at all, we found that beside mass residuals of their social partners, the length of their longest wing feather predicts extra-pair behaviour best. This result needs

more attendance as its causal dependency is not trivial. In that context we want to stress the issue that from other species forced extra-pair copulations (FEPCs) are known as alternative male mating tactic. Beside waterfowl (McKinney et al. 1983) or seabirds (Jouventin et al. 2007) it was also described in other non-passerines (Emlen and Wrege 1986) and passerines (Castro et al. 1996). Furthermore, in Cliff Swallows (*Petrochelidon pyrrhonota*), which are closely related to House Martins, Brown and Bomberger Brown (1996) described that at places where they gather nest material (mud-holes) more than half of EPCs were forced. From our own observations we can affirm that also male House Martins regularly show this assaults on females when collecting nest material and seem to employ FEPCs as additional strategy to increase mating success. When observing these FEPCs, we can confirm that females mounted by males always tried to get rid of the mounting males by wriggling out and a quick start to fly off. In this context a longer P8 could be beneficial for a faster take off. According to our fourth hypothesis in which we predicted that males which can subdue females due to superior morphological traits would have higher RS this result makes sense into two directions. First, males with weaker body condition are not able to restrain females on the ground and second, females with longer wings can fly off more easily.

Our results meet the four predictions we deployed and we can summarize that sexual conflict is shaping variation of RS in House Martins. As age is a main factor influencing variation in mating and RS we want to incur at this point that we only could tell the exact age in adults which were banded as nestlings in our colonies. We assigned age of new recruits in the colony to a minimum age of one year. However, this is in accordance with an earlier field study on House Martins where it could be shown that new colony recruits were most likely in their first summer (Bryant 1979). Further we banded over 90% of nestlings as well as adults in our colonies the two previous years, additionally decreasing the amount of unmarked individuals. For this reason we think that our age determination is valid, even in cases when we only knew the minimum age.

An increasing number of studies points to age related improvement in reproductive success (Reid et al. 2003). Especially for male age there are examples from birds (e.g.: Bouwman et al. 2007; Schmoll et al. 2007; Mitrus et al. 2014) as well as from mammals (Rasmussen et al. 2008; Dugdale et al. 2011). Though explanations why RS increases with age are hard to state because of confounding proximate factors. A plausible reason is that it is often not possible to discern variation in male strategies (e.g.: congregation of experience or differential allocation) from different levels of selection (e.g.: disappearance of inferior

phenotypes or active female choice). Also on the basis of our results we cannot estimate to which extent males improve their mating and reproductive skills with increasing age. Additionally, to our knowledge there exist only few studies that focussed on male experience in reproductive performance and found appropriate evidence (Horie and Takagi 2012; Yasukawa 2013). Nevertheless as we could show that male age is mainly affecting RS in social pairs we act on the assumption that accruing experience with age is also influencing extra-pair success in our study population.

Further, when we look at selection processes we are not able to tell from our results to which extant yearly increasing absence of individuals with lower quality or other selection processes like active female choice contribute to the detected age affect. Actually there is evidence from a study on a species closely related to House Martins, the Barn Swallow, where disappearance of poor quality males could be inferred (Balbontín et al. 2012). Also because of the similar ecology and phylogenetic relatedness of the two swallow species we assume that this is also true for our colonies.

Proposing that males increase their experience in mating and reproductive skills over years and there is (natural) selection on disadvantageous phenotypes or individuals in bad physiological condition there is not much room left for other selective mechanisms like active female choice. In that context we refer to a study on the American subspecies of Barn Swallows in which age-related variation of trait expression impede interpretation into the direction of female choice (Lifjeld et al. 2011). In detail the authors could show that male tail length was a significant predictor of male fertilization success in older but not in younger males. Older males in our study population had higher overall RS but extra-pair performance was best explained by male body condition. However, despite this apparent discrepancy we cannot disentangle the two phenotypically traits and therefore the role of active female choice in this game remains indefinable. Furthermore, the fact that the probability of getting cuckolded - beside weak male condition - is negatively correlated with females' primary length is not suitable to interpret it as the consequence of female choice, not even when both factors are directly linked. In contrast we suppose that it is a consequence of male persistent extra-pair behaviour. Accordingly only males in good condition can overpower females in EPC attempts when females are resistant and additionally females with shorter wings cannot fly off so easily when mating outside the nest. Actually there is evidence from inter- and intra-individual comparisons of wing morphology on different bird species which indicate that more rounded wings are better for a steep angle take off whereas longer and therefore more pointed wings perform better in

increasing speed from a flat angle (Swaddle and Lockwood 2003; Fernández-Juricic et al. 2006). Thus the correlation between poor males' condition and females with shorter primaries triggering cuckoldry leads more into the direction of male than female induced extra-pair behaviour. This is in accordance with early observational studies on mating behaviour on House Martins showing that males invoke copulations outside the nest (Lind 1960). However, as Lifjeld and Marstein (1994) could show in House Martins it is more likely that the majority of copulations occur in the nest. Additionally, they found that nest intrusions of foreign males are most intense during the fertile period of the female and males at this phase spent more time in the nest with their partner in comparison to other breeding stages. Although they video recorded (within-pair) copulations in one nest induced by the female, we would not deduce that females have full control over all copulations they get offered. We agree that inside the nest it might not be easy for the male to obtain cloacal contact without the female being cooperative. Nevertheless the intensive mate guarding of males in the nest shown by Lifjeld and Marstein (1994) and our finding that cuckoldry is more likely when females are paired to males in weaker body condition highlights male-male competition rather than active female choice as weaker males are not able to continuously repel foreign males. For an ultimate support of this conclusion an elaborate data set of behavioural observation in combination with DNA- fingerprinting would be needed which we cannot provide due to tremendous increase of effort in field work.

In our study we could show that mating and reproductive success in House Martins are related to (male) age and body condition. Furthermore we could find that variation in extra-pair fertilizations is predicted by male body condition. As age implies different factors like gathering experience or disappearance of inferior phenotypes selective mechanisms on other phenotypical traits like e.g. female choice for extra-pair mates are confounded. Therefore, we infer from our results that extra-pair behaviour in our population is male induced and variation of this behaviour is shaped by male-male competition. We cannot tell to which extant females have control over extra-pair copulations although we found a correlation that EPP increases when older females are mated with younger males in weaker body condition. Neither can't we proof that females are not actively searching for EPCs although from our data and earlier investigations in this species it seems unlikely. Furthermore, from our results we cannot infer how females could evaluate age and condition of potential extra-pair partners. However, we suppose that resistance of females to EPCs could be nevertheless beneficial for them as it provokes that

e.g. mainly males in good body condition can overcome their defence. Given that better body condition is related to better individual quality of males an indirect benefit of intrinsic factors like genetic quality should be found in the subsequent generation. Accordingly related variance in offspring survival or other nestling performance should be found between within-pair and extra-pair offspring. A test in this direction could be the comparison of nestling survival with genetic data like e.g. heterozygosity values which are known to effect offspring performance.

Table 1: Primer sequences and PCR conditions used for DNA fingerprinting

Locus	Primer sequence	Annealing temp. [°C]	Fragment length [bp]	Reference
Aar4	Fw: 5'-GATGACTAAGGTCTCTGGTGTG Rev: 5'-GTTTGTGCATCAATTAGTCATG	56	150 – 157	Hansson et al. 2000
Ase18	Fw: 5'-CTACAGTCTTCGCAAAAGCC Rev: 5'-TGCCCCAGAGGGAAGAAG	52	184 – 202	Richardson et al. 2000
Escμ6	Fw: 5'-CATAGTGATGCCCTGCTAGG Rev: 5'-GCAAGTGCTCCTTAATATTTGG	50	109 – 141	Hanotte et al. 1994
HrU6	Fw: 5'-GCTGTGTCATTTCTACATGAG Rev: 5'-ACAGGGCAGTGTTACTCTGC	52	185 – 237	Primmer et al. 1995
HrU7	Fw: 5'-GCATTCACAGTGTAGACAATG Rev: 5'-GATCACTATGAGTCCCTGGAA	52	128 – 167	Primmer et al. 1995
Ltr6	Fw: 5'-GCCATGCCACAGGAGTGAGTC Rev: 5'-AGTCATCTCCATCAAGGGCAT	55	184 – 200	McDonald & Potts 1994

Table 2: Number of sampled broods and individuals used for and results from DNA fingerprinting

Parental analyses	**2002**	**2003**	**2004**	**Total**
Broods_sampled	65	39	57	161
Broods_social_mother_sampled	62	36	57	155
Broods_social_father_sampled	62	37	57	156
Broods_parasitized	3	1	0	4
Broods_cuckolded	25	14	28	67
Offspring (OS)_sampled	209	131	196	536
OS_social_mother_sampled	204	121	196	521
OS_social_father_sampled	204	125	196	525
OS_genetic_mother_assigned	203	121	196	520
OS_genetic_father_assigned	198	121	191	510
OS_from_parasitizing	3	1	0	4
OS_from_cuckolding	35	26	46	107
Average_number_of cuckoldings / brood	1.4	1.9	1.6	1.7

Table 3: Sources of inter-individual variation of five different measures of reproductive success (RS) in female House Martins.

Factor	**Effect**	**Wald**	***P***
Numbers of offspring			
Constant	1.317 ± 0.0494		
Hatch_Date	-0.01356 ± 0.002073	42.61	<0.001
Year (2002, 2003, 2004)	0.0000/-0.0280/0.3393	30.52	<0.001
Numbers of within-pair offspring			
Constant	1.146 ± 0.0464		
Social_Father_Minimum_Age	0.1689 ± 0.04225	15.98	<0.001
Proportion of extra-pair offspring in nest			
Constant	3.197 ± 0.1664		
Mother_Minimum_Age	0.2465 ± 0.17736	0.14	0.708
Social_Father_Minimum_Age	-0.4563 ± 0.20412	5.78	<0.020
Social_Father_Mass-Residuals	-0.01072± 0.169069	0.00	0.90
Cuckolding			
Constant	0.03514 ± 0.267642		
Social_Father_Mass-Residuals	-0.2332 ± 0.27157	0.61	0.437
Mother_P8	-0.08951 ± 0.125822	0.51	0.48
Number of fledged offspring			
Constant	1.380 ± 0.0314		
Hatch_Date	-0.01178 ± 0.002132	48.29	<0.001
Social_Father_Minimum_Age	0.09844 ± 0.031158	9.98	0.002

Results are from GLMMs including individual identity of females as a random factor and hatch date, year, minimum age of mothers and social fathers, mass-residuals of fathers and length of P8 of mothers as fixed factors. The most parsimonious model explaining variation in each measure was selected via the Akaike's information criterion (see Table 4 for list of most parsimonious models for each test).

Table 4: The top three parsimonious candidate models explaining variation in five measures of RS of female House Martins, as determined via the corrected Akaike's information criterion. Results are from GLMMs including individual identity of females as a random factor and Hatch_Date, Year, Colony, SF_Min_Age (Minimum age of social father), M_LF (Louse flies-load of mother), M_Min_Age (Minimum age of mother), SF_Mass-Res (Mass-residuals of social father) and M_P8 (Length of primary 8 of mother) as fixed factors.

Reproductive Parameter	**Candidate Model**	**AIC_c**
Total number of offspring	Hatch_Date + Year	-104.69
	Hatch_Date + Colony + Year	-97.86
	M_LF + Hatch_Date + Colony * Year	-91.30
Number of within–pair offspring	SF_Min_Age	-36,67
	Hatch_Date	-34,53
	Hatch_Date + SF_Min_Age	-32,59
Proportion of extra-pair offspring	M_Min_Age + SF_Min_Age + SF_ResMass	101.07
	SF_ Mass-Res + M_P8 + Hatch_Date	109.81
	M_Min_Age + SF_Min_Age + Hatch_Date + SF_Mass-Res	114.68
Cuckolding	SF_ Mass-Res + M_rP8	153.06
	M_Min_Age + SF_Min_Age + SF_ Mass-Res	156.56
	SF_Min_Age + SF_ Mass-Res + Hatch_Date	167.02
Number of fledged offspring	Hatch_Date + SF_Min_Age	-105.72
	Hatch_Date	-104.12
	Hatch_Date + M_Min_Age	-97.23

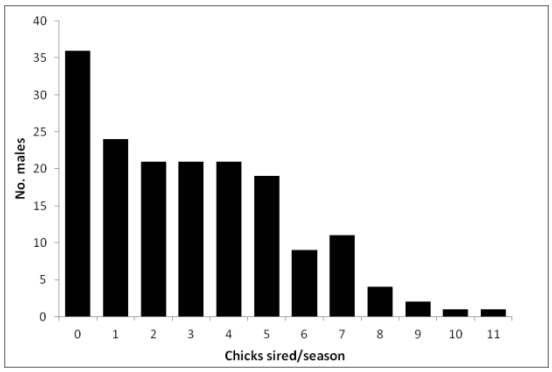
Table 5: Sources of inter-individual variation of seven different measures of reproductive success (RS) in male House Martins.

Factor	**Effect**	**Wald**	***P***
Numbers of sired offspring			
Constant	1.326 ± 0.0502		
Father_Minimum_Age	0.1874 ± 0.04766	15.46	<0.001
Numbers of sired within-pair offspring			
Constant	1.153 ± 0.0456		
Father_Minimum_Age	0.1717 ± 0.04272	16.16	<0.001
Numbers of sired extra-pair offspring			
Constant	-0.2229 ± 0.22745		
Father_Mass-Residuals	0.1640 ± 0.22752	0.52	0.474
Proportion of extra-pair offspring in nest			
Constant	3.099 ± 0.1577		
Social_Father_Mass-Residuals	-0.1199 ± 0.15555	0.59	0.444
Cuckolded by social partner			
Constant	-0.1501± 0.22938		
Social_Father_Mass-Residuals	-0.1876± 0.23100	0.66	0.420
Number of fledged offspring from own nest			
Constant	1.357 ± 0.0343		
Hatch_Date	-0.01345 ± 0.002080	41.80	<0.001
Number of sired offspring fledged			
Constant	1.283 ± 0.0515		
Father_Minimum_Age	0.1999 ± 0.04829	17.13	<0.001

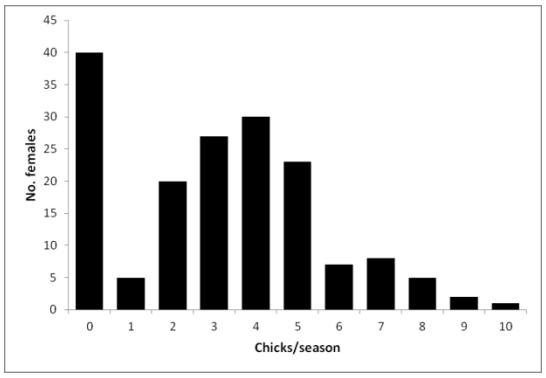
Results are from GLMMs including individual identity of males as a random factor. Minimum age and mass-residuals of genetic (Father) and social fathers (Social Father) as well as hatch date were set as fixed factors. The most parsimonious model explaining variation in each measure was selected via the Akaike's information criterion (see table 6 for list of most parsimonious models for each test).

Table 6: The top three parsimonious candidate models explaining variation in seven measures of reproductive success of male House Martins, as determined via the corrected Akaike's information criterion. Results are from GLMMs including individual identity of males as a random factor and F_Min_Age (Minimum age of genetic father), F_LF (Louse flies-load of genetic father), Year, Hatch_Date, F_Mass-Res (Mass-residuals of genetic father), F_Fat (Body fat of genetic father), F_Mass (Body mass of genetic father), SF_Mass-Res (Mass-residuals of social father) and SF_Min_Age (Minimum age of social father) as fixed factors.

Reproductive Parameter	**Candidate Model**	**AIC_c**
Numbers of sired offspring	F_Min_Age	-10.30
	F_LF	-9.34
	F_LF * Year	-8.15
Numbers of sired within-pair offspring	F_Min_Age	-36.49
	Hatch_Date	-34.99
	Hatch_Date + F_Min_Age	-32.76
Numbers of sired extra-pair offspring	F_Mass-Res	127,55
	F_Mass-Res * Year	129,97
	F_Fat + F_Mass + F_Mass-Res	130,06
Proportion of extra-pair offspring in nest	SF_Mass-Res	127.47
	SF_Mass-Res * Year	131.27
	SF_Mass-Res + SF_Min_Age	132.05
Cuckolded	SF_Mass-Res	197.32
	SF_Mass-Res * Year	201.63
	SF_Mass-Res + SF_Min_Age	203.50
Number of fledged offspring from own nest	Hatch_Date	-106.75
	Hatch_Date + SF_Min_Age	-105.97
	Hatch_Date + SM_Min_Age	-99.18
Number of sired offspring fledged	F_Min_Age	-9.39
	F_LF	-6.56
	F_LF * Year	-0.20



(a)



(b)

Figure 1: Number of males (a) and females (b) producing 0- n offspring per season.

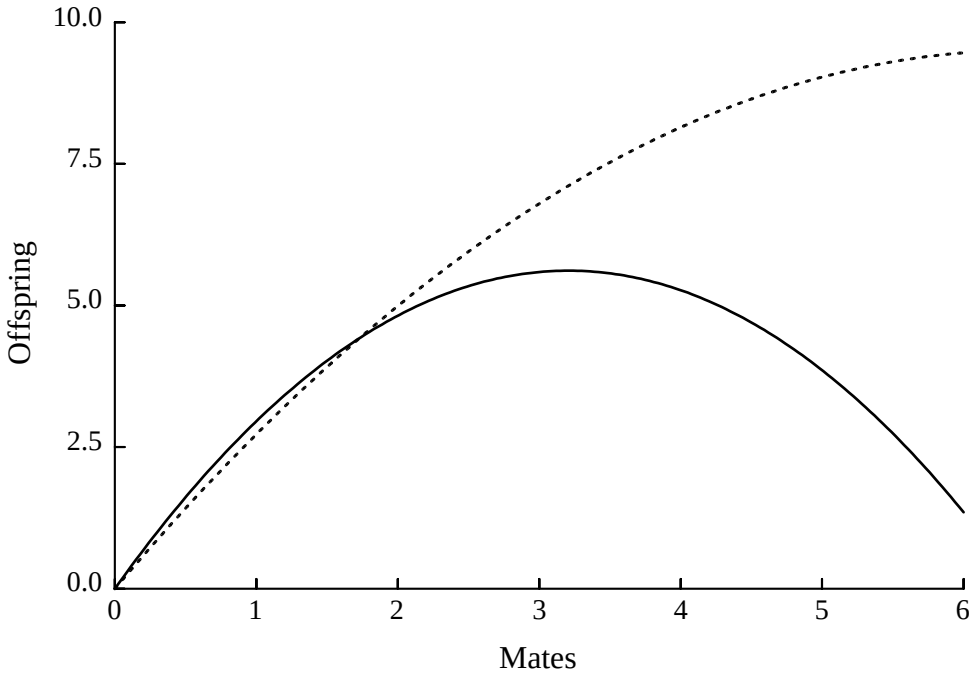


Figure 2: Number of sexual partners involved to produce maximum number of offspring expressed in Bateman's gradients resulting from a bootstrap analysis. Gradients of males (dashed line) and females (solid line) show different optima of partners and produced offspring.

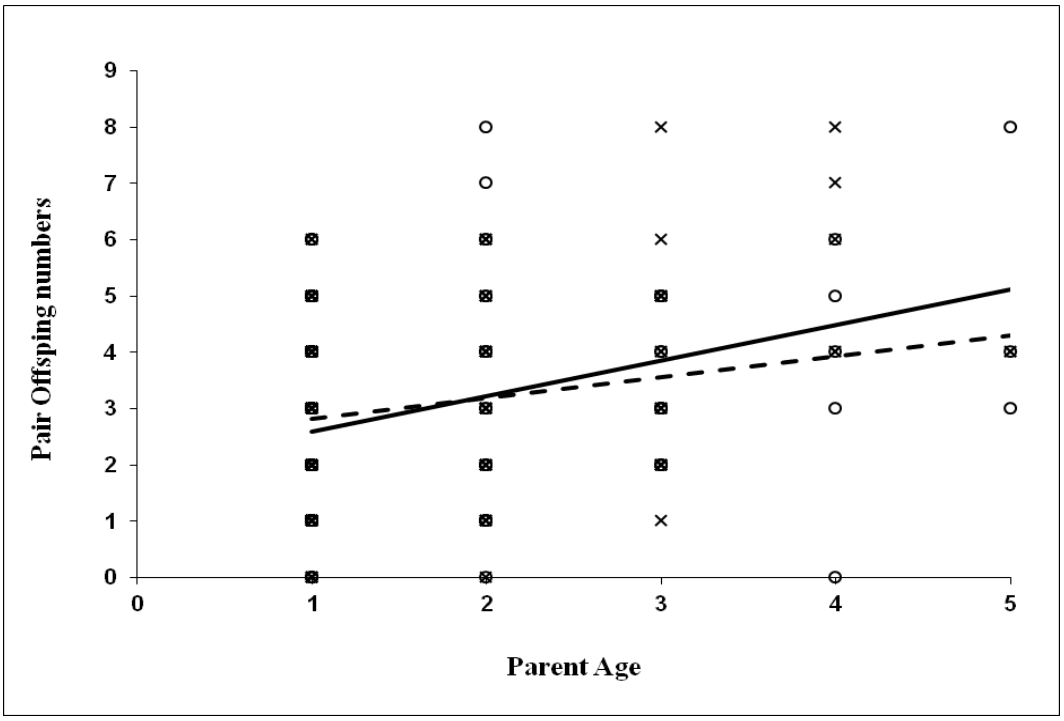


Figure 3: Relationship of male and female age and the number of produced within-pair (social) offspring. Circles and dashed line represent female and crosses and solid line male data.

Figure 4: Relationship of female and male age of social pairs implying age assortative mating (N = 133 pairs).

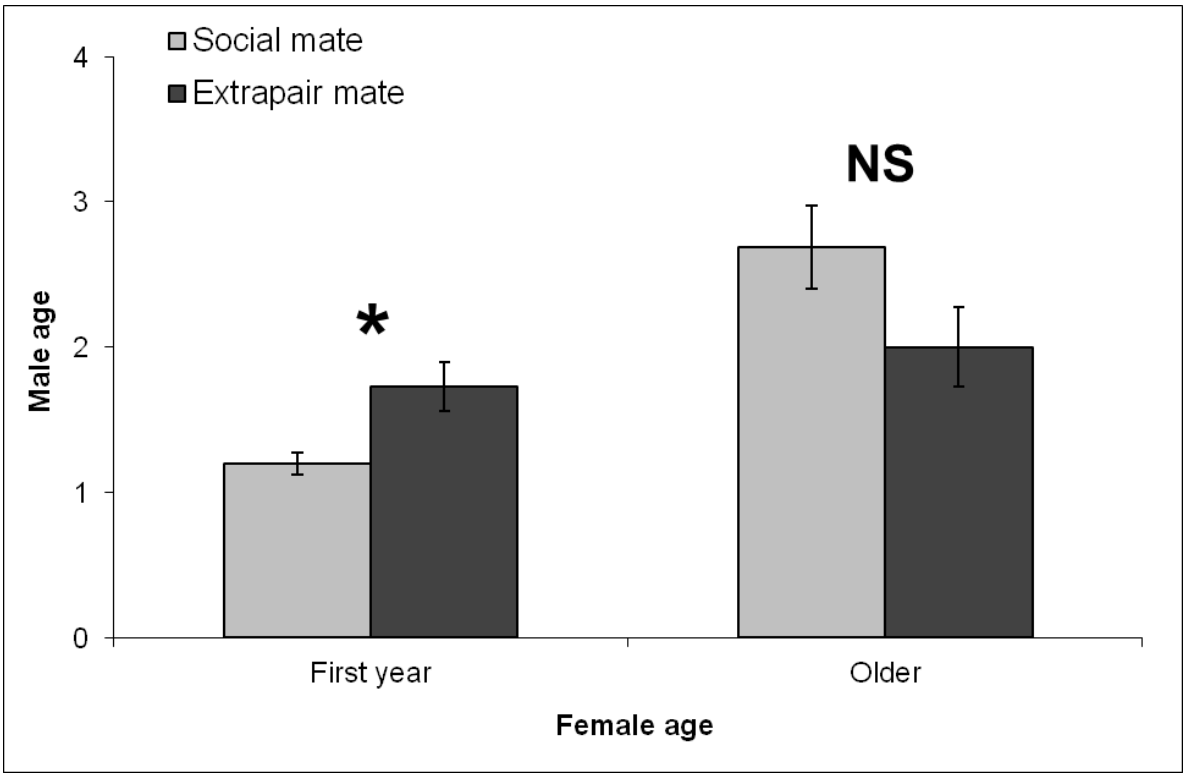


Figure 5: Relationship between young (minimum age 1) and older (minimum age 2+) females and age of their respective social and extra-pair mates.

REFERENCES

Akçay, E., Roughgarden, J. (2007) Extra-pair paternity in birds: review of the genetic benefits. *Evolutionary Ecology Research* **9**: 855–868.

Andersson, M., Iwasa, Y. (1996) Sexual selection. *Trends in Ecology & Evolution* **11**: 53-58. doi: 10.1016/j.tree.2006.03.015

Arnold, K.E., Owens, I.P.F. (2002) Extra-pair paternity and egg dumping in birds: life history, parental care and the risk of retaliation. *Proceedings of the Royal Society B* **269**: 1263-1269. doi: 10.1098/rspb.2002.2013

Arnqvist, G., Kirkpatrick, M. (2005) The Evolution of Infidelity in Socially Monogamous Passerines: The Strength of Direct and Indirect Selection on Extrapair Copulation Behavior in Females. *The American Naturalist* **165**: S26-S37. doi: 10.1086/429350

Arnqvist, G., Rowe, L. (2005) *Sexual conflict*. Princeton University Press, Princeton

Baker, J.R. (1967) A review of the role played by the Hippoboscidae (Diptera) as vectors of endoparasites. *The Journal of Parasitology* **53**: 412-418. doi: 10.2307/3276603

Balbontín, J., Møller, A.P., Hermosell, I.G., Marzal, A., Reviriego, M., de Lope, F. (2012) Geographical variation in reproductive ageing patterns and life-history

strategy of a short-lived passerine bird. *Journal of Evolutionary Biology* **25**: 2298-2309. doi: 10.1111/j.1420-9101.2012.02606.x

Bateman, A.J. (1948) Intra-sexual selection in *Drosophila*. *Heredity* **2**: 349-368. doi: 10.1038/hdy.1948.21

Bennett, A.F., Owens, I.P.F. (2002) *Evolutionary Ecology of Birds: Life Histories, Mating Systems and Extinctition*. Oxford University Press, Oxford

Birkhead, T.R., Møller, A.P. (1992) *Sperm competition in birds. Evolutionary causes and consequences*. Acad. Press, Harcourt Brace Jovanovich Publishers, London, San Diego, New York, Boston, Sydney, Tokyo, Toronto

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.-S.S. (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution* **24**: 127-135. doi: 10.1016/j.tree.2008.10.008

Bouwman, K.M., Van Dijk, R.E., Wijmenga, J.J., Komdeur, J. (2007) Older male reed buntings are more successful at gaining extrapair fertilizations. *Animal Behaviour* **73**: 15-27. doi: 10.1016/j.anbehav.2006.01.031

Brennan, P.L.R., Prum, R.O. (2012) The limits of sexual conflict in the narrow sense: new insights from waterfowl biology. *Philosophical Transactions of the Royal Society B* **367**: 2324-2338. doi: 10.1098/rstb.2011.0284

Brown, C.R., Bomberger Brown, M. (1996) *Coloniality of the Cliff Swallow: the effect of group size on social behavior*. University of Chicago Press, Chicago, London

Bryant, D.M. (1975) Changes in Incubation Patch and Weight in the Nesting House Martin. *Ringing & Migration* **1**: 33-36. doi: 10.1080/03078698.1975.9673696

Bryant, D.M. (1979) Reproductive costs in the House Martin (*Delichon urbica*). *Journal of Animal Ecology* **48**: 655-675. doi: 10.2307/4185

Bryant, D.M. (1988) *Lifetime Reproductive Success of House Martins*. In: Clutton-Brock, T.H. (ed) Reproductive Success: Studies of Individual Variation in Contrasting Breeding Systems. University of Chicago Press, Chicago, pp 173-188

Bryant, D.M., Westerterp, K.R. (1983) Short-Term Variability in Energy Turnover by Breeding House Martins *Delichon urbica*: A Study Using Doubly-Labelled Water (D218O). *Journal of Animal Ecology* **52**: 525-543. doi: 10.2307/4570

Burnham, K.P., Anderson, D.R., Huyvaert, K.P. (2011) AIC model selection and multimodel inference in behavioral ecology: some background, observations, and

comparisons. *Behavioral Ecology and Sociobiology* **65**: 23-35. doi: 10.1007/s00265-010-1029-6

Castro, I., Minot, E.O., Fordham, R.A., Birkhead, T.R. (1996) Polygynandry, face-to-face copulation and sperm competition in the Hihi *Notiomystis cincta* (Aves: Meliphagidae). *Ibis* **138**: 765-771. doi: 10.1111/j.1474-919X.1996.tb08834.x

Chuang-Dobbs, H.C., Webster, M.S., Holmes, R.T. (2001) The effectiveness of mate guarding by male black-throated blue warblers. *Behavioral Ecology* **12**: 541-546. doi: 10.1093/beheco/12.5.541

Clutton-Brock, T.H. (1991) *The evolution of parental care*. Princeton University Press, Princeton, N.J.

Collet, J.M., Dean, R.F., Worley, K., Richardson, D.S., Pizzari, T. (2014) The measure and significance of Bateman's principles. *Proceedings of the Royal Society B* **281**: 20132973. doi: 10.1098/rspb.2013.2973

Dugdale, H.L., Pope, L.C., Newman, C., Macdonald, D.W., Burke, T. (2011) Age-specific breeding success in a wild mammalian population: selection, constraint, restraint and senescence. *Molecular Ecology* **20**: 3261-3274. doi: 10.1111/j.1365-294X.2011.05167.x

Edler, R., Friedl, T.W.P. (2008) Within-pair young are more immunocompetent than extrapair young in mixed-paternity broods of the red bishop. *Animal Behaviour* **75**: 391-401. doi: 10.1016/j.anbehav.2007.05.004

Eliassen, S., Kokko, H. (2008) Current analyses do not resolve whether extra-pair paternity is male or female driven. *Behavioral Ecology and Sociobiology* **62**: 1795-1804. doi: 10.1007/s00265-008-0608-2

Emlen, S.T., Wrege, P.H. (1986) Forced Copulations and Intra-specific Parasitism: Two Costs of Social Living in the White-fronted Bee-eater. *Ethology* **71**: 2-29. doi: 10.1111/j.1439-0310.1986.tb00566.x

Fernández-Juricic, E., Blumstein, D.T., Abrica, G., Manriquez, L., Adams, L.B., Adams, R., Daneshrad, M., Rodriguez-Prieto, I. (2006) Relationships of anti-predator escape and post-escape responses with body mass and morphology: a comparative avian study. *Evolutionary Ecology Research* **8**: 731-752.

Foerster, K., Delhey, K., Johnsen, A., Lifjeld, J.T., Kempenaers, B. (2003) Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature* **425**: 714-717. doi: 10.1038/nature01969

Gowaty, P.A., Buschhaus, N. (1998) Ultimate Causation of Aggressive and Forced Copulation in Birds: Female Resistance, the CODE Hypothesis, and Social Monogamy. *American Zoologist* **38**: 207-225. doi: 10.1093/icb/38.1.207

Gowaty, P.A., Kim, Y.K., Anderson, W.W. (2012) No evidence of sexual selection in a repetition of Bateman's classic study of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America* **109**: 11740-11745. doi: 10.1073/pnas.1207851109

Gray, E.M. (1997) Female red-winged blackbirds accrue material benefits from copulating with extra-pair males. *Animal Behaviour* **53**: 625-639. doi: 10.1006/anbe.1996.0336

Greenlaw, J.S., Post, W. (2012) Apparent forced mating and female control in Saltmarsh Sparrows. *Wilson Journal of Ornithology* **124**: 253-264. doi: 10.1676/11-072.1

Griffith, S.C., Owens, I.P.F., Thuman, K.A. (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology* **11**: 2195-2212. doi: 10.1046/j.1365-294X.2002.01613.x

Griffiths, R., Double, M.C., Orr, K., Dawson, R.J. (1998) A DNA test to sex most birds. *Molecular Ecology Notes* **7**: 1071-1075. doi: 10.1046/j.1365-294x.1998.00389.x

Hadfield, J.D., Burgess, M.D., Lord, A., Phillimore, A.B., Clegg, S.M., Owens, I.P.F. (2006) Direct versus indirect sexual selection: genetic basis of colour, size and recruitment in a wild bird. *Proceedings of the Royal Society B* **273**: 1347-1353. doi: 10.1098/rspb.2005.3459.

Hanotte, O., Zanon, C., Pugh, A., Greig, C., Dixon, A., Burke, T. (1994) Isolation and characterization of microsatellite loci in a passerine bird: the reed bunting *Emberiza schoeniclus*. *Molecular Ecology* **3**: 529-530. doi: 10.1111/j.1365-294X.1994.tb00133.x

Hansson, B., Bensch, S., Hasselquist, D., Lillandt, B.G., Wennerberg, L., Von Schantz, T. (2000) Increase of genetic variation over time in a recently founded population of great reed warblers (*Acrocephalus arundinaceus*) revealed by microsatellites and DNA fingerprinting. *Molecular Ecology* **9**: 1529-1538. doi: 10.1046/j.1365-294x.2000.01028.x

Hasselquist, D., Bensch, S. (1991) Trade-off between mate guarding and mate attraction in the polygynous great reed warbler. *Behavioral Ecology and Sociobiology* **28**: 187-193. http://www.jstor.org/stable/4600534

Horie, S., Takagi, M. (2012) Nest positioning by male Daito White-eyes *Zosterops japonicus daitoensis* improves with age to reduce nest predation risk. *Ibis* **154**: 285-295. doi: 10.1111/j.1474-919X.2011.01204.x

Hund, K., Prinzinger, R. (1985) Die Bedeutung des Lebensalters für brutbiologische Parameter der Mehlschwalbe (*Delichon urbica*). *Journal für Ornithologie* **126**: 15-28. https://link.springer.com/article/10.1007/BF01640440

Jones, M.G.W., Techow, N.M.S.M., Ryan, P.G. (2012) Dalliances and doubtful dads: what determines extra-pair paternity in socially monogamous wandering albatrosses? *Behavioral Ecology and Sociobiology* **66**: 1213-1224. doi: 10.1007/s00265-012-1374-8

Jouventin, P., Charmantier, A., Dubois, M.P., Jarne, P., Bried, J. (2007) Extra-pair paternity in the strongly monogamous Wandering Albatross *Diomedea exulans* has no apparent benefits for females. *Ibis* **149**: 67-78. doi: 10.1111/j.1474-919X.2006.00597.x

Kaiser, A. (1993) A new multi-category classification of subcutaneous fat deposits of songbirds. *Journal of Field Ornithology* **64**: 246-255. http://hdl.handle.net/11858/00-001M-0000-0028-0B84-8

Kalinowski, S.T., Taper, M.L., Marschall, T.C. (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* **16**: 1099-1106. doi: 10.1111/j.1365-294X.2007.03089.x

Kempenaers, B., Verheyen, G.R., Van den Broeck, M., Burke, T., Van Broeckhoven, C., Dhondt, A.A. (1992) Extra-pair paternity results from female preference for high-quality males in the blue tit. *Nature* **357**: 494-496. doi: 10.1038/357494a0

Kipp, F.A. (1959) Der Handflügel-Index als flugbiologisches Maß. *Die Vogelwarte* **20**: 77-86. http://www.zobodat.at/publikation_articles.php?id=259573

Komdeur, J. (2001) Mate guarding in the Seychelles warbler is energetically costly and adjusted to paternity risk. *Proceedings of the Royal Society B* **268**: 2103-2111. doi: 10.1098/rspb.2001.1750

Komdeur, J., Burke, T., Richardson, D.S. (2007) Explicit experimental evidence for the effectiveness of proximity as mate-guarding behaviour in reducing extra-pair fertilization in the Seychelles warbler. *Molecular Ecology* **16**: 3679-3688. doi: 10.1111/j.1365-294X.2007.03420.x

Kotiaho, J.S., Puurtinen, M. (2007) Mate choice for indirect genetic benefits: scrutiny of the current paradigm. *Functional Ecology* **21**: 638-644. doi: 10.1111/j.1365-2435.2007.01286.x

Labocha, M.K., Hayes, J.P. (2011) Morphometric indices of body condition in birds: a review. *Journal of Ornithology* **153**: 1-22. doi: 10.1007/s10336-011-0706-1

Lack, D. (1968) *Ecological Adaptions for Breeding in Birds*. Methuen, London

Lessells, C.M. (1999) *Sexual Conflict in Animals*. In: Keller, L. (ed) Levels of Selection. Princeton University Press, Princeton, pp 75-99

Lifjeld, J.T., Kleven, O., Jacobsen, F., McGraw, K.J., Safran, R.J., Robertson, R.J. (2011) Age before beauty? Relationships between fertilization success and age-dependent ornaments in barn swallows. *Behavioral Ecology and Sociobiology* **65**: 1687-1697. doi: 10.1007/s00265-011-1176-4

Lifjeld, J.T., Marstein, B. (1994) Paternity assurance behavior in the House Martin *Delichon urbica*. *Journal of Avian Biology* **25**: 231-238. doi: 10.2307/3677080

Lind, E.A. (1960) *Zur Ethologie und Ökologie der Mehlschwalbe, Delichon U. Urbica (L.)*, vol 21. Societas Zoologica Botanica Fennica "Vanamo", Helsinki

Low, M. (2005) Female resistance and male force: context and patterns of copulation in the New Zealand stitchbird *Notiomystis cincta*. *Journal of Avian Biology* **36**: 436-448. doi: 10.1111/j.0908-8857.2005.03460.x

Magrath, M.J.L., Elgar, M.A. (1997) Paternal care declines with increased opportunity for extra-pair matings in fairy martins. *Proceeding of the Royal Society of London B* **264**: 1731-1736. doi: 10.1098/rspb.1997.0240

Marshall, T.C., Slate, J., Kruuk, L.E., Pemberton, J.M. (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**: 639-655. doi: 10.1046/j.1365-294x.1998.00374.x

Marthinsen, G., Kleven, O., Brenna, E., Lifjeld, J.T. (2005) Part-Time Mate Guarding Affects Paternity in Male Reed Buntings (*Emberiza schoeniclus*). *Ethology* **111**: 397-409. doi: 10.1111/j.1439-0310.2005.01079.x

Marzal, A., Bensch, S., Reviriego, M., Balbontin, J., de Lope, F. (2008) Effects of malaria double infection in birds: one plus one is not two. *Journal of Evolutionary Biology* **21**: 979-987. doi: 10.1111/j.1420-9101.2008.01545.x

Matysiokova, B., Remes, V. (2013) Faithful females receive more help: the extent of male parental care during incubation in relation to extra-pair paternity in songbirds. *Journal of Evolutionary Biology* **26**: 155-162. doi: 10.1111/jeb.12039

McDonald, D.B., Potts, W.K. (1994) Cooperative display and relatedness among males in a lek-mating bird. *Science* **266**: 1030-1032. doi: 10.1126/science.7973654

McKinney, F., Derrickson, S.R., Mineau, P. (1983) Forced copulation in waterfowl. *Behaviour* **86**: 250-294. doi: 10.1163/156853983x00390

McKinney, F., Evarts, S. (1997) Sexual coercion in waterfowl and other birds. *Ornithological Monographs*: 163-195. doi: 10.2307/40166723

Mitrus, J., Mitrus, C., Rutkowski, R., Sikora, M. (2014) Extra-pair paternity in relation to age of the Red-breasted Flycatcher *Ficedula parva* males. *Avian Biology Research* **7**: 111-116. doi: 10.3184/175815514X13948188185179

Møller, A.P. (1988) Female choice selects for male sexual ornaments in the monogamous swallow. *Nature* **332**: 640-642. doi: 10.1038/332640a0

Møller, A.P. (2000) Male parental care, female reproductive success, and extra-pair paternity. *Behavioral Ecology* **11**: 161-168. doi: 10.1093/beheco/11.2.161

Morton, E.S., Stutchbury, B.J.M., Howlett, J.S., Piper, W.H. (1998) Genetic monogamy in blue-headed vireos and a comparison with a sympatric vireo with extrapair paternity. *Behavioral Ecology* **9**: 515-524. doi: 10.1093/beheco/9.5.515

Parker, G.A. (1979) *Sexual selection and sexual conflict*. In: Blum, M.S., Blum, N.A. (eds) Sexual selection and reproductive competition in insects. Academic Press, New York, pp 123-166

Parker, G.A., Birkhead, T.R. (2013) Polyandry: the history of a revolution. *Philosophical Transactions of the Royal Society B* **368**: 20120335. doi: 10.1098/rstb.2012.0335

Petrie, M. (1994) Improved growth and survival of offspring of peacocks with more elaborate trains. *Nature* **371**: 598-599. doi: 10.1038/371598a0

Primmer, C.R., Møller, A.P., Ellegren, H. (1995) Resolving genetic relationships with microsatellite markers: A parentage testing system for the swallow *Hirundo rustica*. *Molecular Ecology* **4**: 493-498. doi: 10.1111/j.1365-294X.1995.tb00243.x

Pryke, S.R., Rollins, L.A., Griffith, S.C. (2010) Females Use Multiple Mating and Genetically Loaded Sperm Competition to Target Compatible Genes. *Science* **329**: 964-967. doi: 10.1126/science.1192407

Quinn, G.P., Keough, M.J. (2002) *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge

Qvarnström, A., Brommer, J.E., Gustafsson, L. (2006) Testing the genetics underlying the co-evolution of mate choice and ornament in the wild *Nature* **441**: 84-86. doi: 10.1038/nature04564

Rasmussen, H.B., Okello, J.B.A., Wittemyer, G., Siegismund, H.R., Arctander, P., Vollrath, F., Douglas-Hamilton, I. (2008) Age- and tactic-related paternity success in male African elephants. *Behavioral Ecology* **19**: 9-15. doi: 10.1093/beheco/arm093

Reid, J.M., Bignal, E.M., Bignal, S., McCracken, D.I., Monaghan, P. (2003) Age-specific reproductive performance in red-billed choughs *Pyrrhocorax pyrrhocorax:* Patterns and processes in a natural population. *Journal of Animal Ecology* **72**: 765-776. http://www.jstor.org/stable/3505358

Rheinwald, G., Gutscher, H., Hormeyer, K. (1976) Einfluss des Alters der Mehlschwalbe (*Delichon urbica*) auf ihre Brut. *Die Vogelwarte* **28**: 190-206. http://www.zobodat.at/pdf/Vogelwarte_28_1976_0190-0206.pdf

Richardson, D.S., Jury, F.L., Dawson, D.A., Salgueiro, P., Komdeur, J., Burke, T. (2000) Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. *Molecular Ecology* **9**: 2226-2231. doi: 10.1046/j.1365-294X.2000.105338.x

Riley, H.T., Bryant, D.M., Carter, R.E., Parkin, D.T. (1995) Extra-pair fertilizations and paternity defence in house martins, *Delichon urbica*. *Animal Behaviour* **49**: 495-509. doi: 10.1006/anbe.1995.0065

Rubenstein, D.R. (2007) Territory quality drives intraspecific patterns of extrapair paternity. *Behavioral Ecology* **18**: 1058–1064. doi: 10.1093/beheco/arm077

Sardell, R.J., Arcese, P., Keller, L.F., Reid, J.M. (2012) Are There Indirect Fitness Benefits of Female Extra-Pair Reproduction? Lifetime Reproductive Success of Within-Pair and Extra-Pair Offspring. *American Naturalist* **179**: 779-793. http://www.jstor.org/stable/10.1086/665665 .

Schärer, L., Rowe, L., Arnqvist, G. (2012) Anisogamy, chance and the evolution of sex roles. *Trends in Ecology & Evolution* **27**: 260-264. doi: 10.1016/j.tree.2011.12.006

Schmoll, T., Mund, V., Dietrich-Bischoff, V., Winkel, W., Lubjuhn, T. (2007) Male age predicts extrapair and total fertilization success in the socially monogamous coal tit. *Behavioral Ecology* **18**: 1073-1081. doi: 10.1093/beheco/arm082

Seki, M., Wakano, J.Y., Ihara, Y. (2007) A theoretical study on the evolution of male parental care and female multiple mating: Effects of female mate choice and male care bias. *Journal of Theoretical Biology* **247**: 281-296. doi: 10.1016/j.jtbi.2007.03.010

Seutin, G., White, B.N., Boag, P.T. (1991) Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology* **69**: 82-90. doi: 10.1139/z91-013

Sheldon, B.C. (1993) Sexually transmitted disease in birds: occurence and evolutionary significance. *Philosophical Transactions of the Royal Society of London B* **339**: 491-497. doi: 10.1098/rstb.1993.0044

Sheldon, B.C. (1994) Male phenotype, fertility, and the pursuit of extra-pair copulations by female birds. *Proceedings of the Royal Society B* **257**: 25-30. doi: 10.1098/rspb.1994.0089

Swaddle, J.P., Lockwood, R. (2003) Wingtip shape and flight performance in the European Starling *Sturnus vulgaris*. *Ibis* **145**: 457-464. doi: 10.1046/j.1474-919X.2003.00189.x

Tarwater, C.E., Brawn, J.D., Maddox, J.D. (2013) Low Extrapair Paternity Observed in a Tropical Bird Despite Ample Opportunities for Extrapair Mating. *The Auk* **130**: 733-741. doi: 10.1525/auk.2013.13117

Townsend, A.K. (2009) Extrapair copulations predict extrapair fertilizations in the American Crow. *The Condor* **111**: 387-392. doi: 10.1525/cond.2009.090010

Trivers, R.L. (1972) *Parental Investment and Sexual Selection*. In: Campbell, B. (ed) Sexual selection and the descent of man 1871-1971. Aldine, Chicago, pp 136-179

Veen, T., Borge, T., Griffith, S.C., Saetre, G.P., Bures, S., Gustafsson, L., Sheldon, B.C. (2001) Hybridization and adaptive mate choice in flycatchers. *Nature* **411**: 45-50. doi: 10.1038/35075000

Westneat, D.F., Sherman, P.W., Morton, M.L. (1990) The ecology and evolution of extra-pair copulations in birds. *Current Ornithology* **7**: 331-369.

Westneat, D.F., Stewart, I.R.K. (2003) Extra-Pair Paternity In Birds: Causes, Correlates, and Conflict. *Annual Review of Ecology Evolution and Systematics* **34**: 365-396. doi: 10.1146/annurev.ecolsys.34.011802.132439

Wetton, J.H., Parkin, D.T. (1991) An association between fertility and cuckoldry in the house sparrow, *Passer domesticus*. *Proceedings of the Royal Society of London B* **245**: 227-233. doi: 10.1098/rspb.1991.0114

Whittingham, L.A., Lifjeld, J.T. (1995a) Extra-pair fertilizations increase the opportunity for sexual selection in the monogamous House Martin *Delichon urbica*. *Journal of Avian Biology* **26**: 283-288. doi: 10.2307/3677042

Whittingham, L.A., Lifjeld, J.T. (1995b) High paternal investment in unrelated young: extra-pair paternity and male parental care in house martins. *Behavioral Ecology and Sociobiology* **37**: 103-108. doi: 10.1007/bf00164155

Woolfenden, B.E., Stutchbury, B.J.M., Morton, E.S. (2005) Male Acadian flycatchers, *Empidonax virescens*, obtain extrapair fertilizations with distant females *Animal Behaviour* **69**: 921-929 doi: 10.1016/j.anbehav.2004.06.030

Wright, J., Cotton, P.A. (1994) Experimentally Induced Sex Differences in Parental Care: an Effect of Certainty of Paternity. *Animal Behaviour* **47**: 1311-1322. doi: 10.1006/anbe.1994.1179

Yasukawa, K. (2013) Effects of age and experience on the responses of territorial and floater male Red-winged Blackbirds to models of receptive females. *Journal of Field Ornithology* **84**: 377-388. doi: 10.1111/jofo.12037

Chapter 2

Extra-pair paternity does not increase fitness benefits due to offspring heterozygosity in European House Martins (*Delichon urbicum urbicum*)

ABSTRACT

One major explanation to account for variances of extra-pair paternity in social monogamous birds is that females benefit through indirect (genetic) fitness effects when engaging in copulations beyond the social mating. The most prominent hypothesis is the genetic compatibility (GC) hypothesis which states that females increase their fitness through extra-pair mating with mates that show more compatible genes than their social partners. Compatibility expresses how well the genes of the biological parents function together in their offspring. One aspect of this compatibility is the heterozygosity of the offspring which should thus be higher in extra-pair offspring. We employed microsatellite markers to determine paternity and genetic relatedness in European House Martins (*Delichon u. urbicum*) and related them to phenotypical parameters of the putative parents, and offspring performance. For this we calculated two indices to assess if genetic similarity of parents affects homozygosity of offspring. Firstly, we tested whether the probability that a social pair produces homozygous offspring (*Phm* index) predicts occurrence of extra-pair offspring, and secondly whether within-pair offspring is more likely to be homozygous than extra-pair offspring (*HL* index). Our analyses show that genetic relatedness of social parents is negatively affecting within-pair offspring, but does not influence proportion or total number of extra-pair offspring. We could not show that extra-pair offspring is more likely to be heterozygous than within-pair offspring. Offspring performance was not affected by genetic relatedness of parents, but it was linked to the age of the social father. We therefore suggest that the hypothesis females' pursue extra-pair mating to receive genetic benefits contributes little to explain variation in extra-pair paternity in House Martins.

INTRODUCTION

Although the majority of birds show socially monogamous mating, in more than 70% of 150 analysed species extra-pair behaviour could be detected (Westneat and Stewart 2003). More than that, since the advent of DNA-fingerprinting and the improvement of methods using molecular markers, parental analyses revealed extra-pair paternity in more than 80% of tested social monogamous species (Griffith et al. 2002).

Obviously, the benefit of extra-pair behaviour for males lies in fitness enhancement at low paternal costs. Benefits for females engaging in extra-pair mating are not that apparent (Arnqvist and Kirkpatrick 2005). Direct benefits for females could be coverage in case of infertility of the social partner, supplement with resources (e.g.: food) and assistance in parental effort. However evidence for these direct benefits is more than limited. Additionally only a small number of species has been studied by now (Westneat and Stewart 2003).

The most frequent explanation proposed is that females enhance their fitness by gaining indirect, namely genetic, benefits with producing extra-pair matings. Females paired with genetically suboptimal partners try to "correct for" their initial mate choice with this strategy (Møller 1992). Two hypotheses have been postulated how females might enhance genetic quality of their offspring fitness, the "good genes hypothesis" and the "genetic compatibility hypothesis" (reviewed in: Neff and Pitcher 2005). According to the first hypothesis females accrue additive genetic benefits if extra-pair mates have "higher" genetic quality than their pair males. The second hypothesis is related to non-additive genetic effects where females gain fitness benefits if extra-pair mates have a more compatible genomic contribution to their own than their social partner (reviewed in: Tregenza and Wedell 2000; but see also: Puurtinen et al. 2005). This comprises loci-specific genetic complementarity as well as genome-wide heterozygosity. Actually, most studies addressing the genetic compatibility hypothesis apply genetic similarity or relatedness as test variable and there is some evidence for fitness benefits in producing more heterozygous offspring through extra-pair mating (e.g.: Hansson et al. 2001; Foerster et al. 2003; Suter et al. 2007).

However, as extra-pair mating also imposes (indirect) costs on females, selection favouring extra-pair behaviour in females seems to be weaker than the directional selection against it (Arnqvist and Kirkpatrick 2005). Convincing evidence that accumulation of genetic benefits of females is the main driving force in extra-pair behaviour is still lacking (Akçay and Roughgarden 2007).

Nevertheless, several studies showed that extra-pair mates are more heterozygous or genetically less related to the females than their social partner (Richardson et al. 2005; Oh and Badyaev 2006; Lindstedt et al. 2007), and that extra-pair offspring (EPO) shows higher heterozygosity than within-pair offspring (WPO) (e.g.: Stapleton et al. 2007; Lampila et al. 2011). However, only few studies could answer the question how female birds can assess genetic dissimilarity of potential extra-pair partners (e.g.: Ryder et al.

2010). Though, we think that if females actively engage in extra-pair behaviour with preferred types of males it should be reflected in improved attributes of EPO versus WPO.

We investigated this hypothesis by studying extra-pair paternity in European House Martins (*Delichon u. urbicum*). Using microsatellite markers we determined paternal status and genetic diversity. As a measure of fitness we documented survival of nestlings.
House Martins are socially monogamous, sexually monomorphic passerines with altricial offspring and biparental care. They are long distance migrants, breed colonially in self-built mud nests and maintain seasonal pair ponds. House Martins are classified as species with high paternal investment (Bryant 1988). Both sexes invest equally in parental duties like nest construction and feeding of the offspring (Whittingham and Lifjeld 1995b). Earlier studies revealed extra-pair rates of 19%, apparently without negative consequences on paternal investment suggesting that females have control over extra-pair mating (Whittingham and Lifjeld 1995a). This fact makes them an ideal study system to test for possible benefits that females gain by engaging in extra-pair mating.
We hypothesized that in nests of genetically similar or less heterozygous partners extra-pair offspring is more likely to occur. Additionally we expect that extra-pair offspring is more heterozygous than within-pair offspring. Beyond that we presume that fitness advantages of heterozygous offspring will be reflected in a better nestling survival.

METHODS

Study site

The study was conducted from 2002 until 2004 at two House Martin colonies. One was located under the roof of the boat house at the biological station Illmitz (BSI)/Lake Neusiedl in Austria (47°46’07.8’’N, 16°45’59.0’’E) and from 2002 until 2003 there was another one located in 2.5km flight distance at the Seebad Illmitz (47 45’14.8’’N, 16 44’30.2’’E) from April until September each year. At BSI the colony had 42-78 mud nests/year and at Seebad up to 30 natural nests. Additionally, in 2003 we provided 63 artificial nest cups because during winter 2002/2003 most of the natural nests were destroyed. A more detailed description of breeding success of the colonies is given in Chapter 1.

Field methods

Adult birds were mainly trapped when emerging from their nests or with mist nets installed in front of the colony. We ringed them individually with uniquely numbered aluminium

rings provided by the "Vogelwarte Radolfzell", Germany. Further we marked females with a tip of white correction fluid (Tipp-Ex®) on their foreheads and males with different colours of Edding® permanent markers on the white rump. Birds were sexed by brood patch development (Bryant 1975). Nestlings were ringed between eight to 19 days after hatching. In 2004 we additionally collect unhatched eggs and or dead nestlings. Blood samples for microsatellite genotyping were taken (up to 25µl) from the brachial vein and stored in Queen's lysis buffer (Seutin et al. 1991). For a more detailed description of morphological and fitness related measurements see Chapter 1.

Genetic analyses

DNA was extracted from 735 blood and 17 tissue samples using DNeasy® Blood and Tissue kit (Qiagen). We amplified DNA from individual samples at six microsatellite loci, Aar4 (Hansson et al. 2000), Ase18 (Richardson et al. 2000), Escµ6 (Hanotte et al. 1994), HrU6 and HrU7 (both: Primmer et al. 1995), Ltr6 (McDonald and Potts 1994), where we could type 99.6% of individuals at all loci. After controlling DNA purity and concentration on Nano Drop 2000 (Thermo Scientific) we performed polymerase chain reactions (PCR) in a multiplex-reaction and three singleplex reactions with 11µl volume. For all reactions we used 25ng genomic DNA. The multiplex setup contained 1.1µl 10x PCR buffer, dNTPs 200µM each, 2.5mM $MgCl_2$, 0.4U polymerase, 1, 2 and 3pmol of the three different forward and reverse primers and 5.42µl H_2O. The singleplex set up contained 1.1µl 10x PCR buffer, dNTPs 200µM each, 2.5mM $MgCl_2$, 0.4U polymerase, forward and reverse primer 2pmol each and 6.22µl H_2O. For primer specifications, manufacturers of used chemicals as well as details of PCR cycling conditions see Chapter 2. PCR products were run on a CEQ™ 8000 Beckman Coulter automated sequencer and analysed using genetic analyses software provided by the manufacturer.

Parentage analysis

From 752 sampled individuals for 518 offspring we could genotype both respective social parents for robust parental analyses. Using CERVUS 3.0 software package (Kalinowski et al. 2007), for each year we first calculated maternity probability to look for occurring intraspecific brood parasitism and allow assignment of known mothers for subsequent paternity calculations. The parameter settings we used for parental analyses were: a 1% error rate due to mutation or low rates of null alleles at various loci, 100% of loci typed, and an estimated 90% of all candidate parents were sampled. At two loci, allele frequencies deviated from Hardy-Weinberg equilibrium with an estimated null allele

frequency of 0.067 and 0.046 each. Primer loci showed a mean of 20.5 alleles (SD: ±9.9, range: 13-40 alleles/locus). Offspring was assigned to another parent than the social one if the alternative candidate shows none or one allelic mismatch (i.e. due to null alleles), a higher or positive LOD (maximum likelihood ratio) score at strict 95% confidence level and a significant Pair or Trio confidence calculated by CERVUS 3.0. The combined non-exclusion probability was 4.1×10^{-4} for a putative father if the mother was known.

Genetic similarity and homozygosity

To test if homozygosity of parents predicts frequency of extra-pair offspring we compared genetic similarity between social partners. Genetic similarity was estimated as the probability that a given social pair (*x*,*y*) produces homozygous offspring (Phm). The Phm index first proposed by Mathieu et al. (1990) for each locus (*l*) is equal to

$$\mathrm{Phm}_{xy}(l) = \frac{s_{ac}+s_{ad}+s_{bc}+s_{bd}}{4}.$$

At locus *l* individual *x* shows the alleles *a* and *b* and individual *y* shows the alleles *c* and *d*. If both alleles are the same *S* equals 1, if not 0.For a value across all loci we calculated the weighted average of Phm_{xy} (*l*) using the formula:

$$\mathrm{Phm}_{xy} = \frac{\sum_l \frac{1}{p_l} Phm_{xy}(l)}{\sum_l \frac{1}{p_l}},$$

where p_l is the probability of an individual being homozygous by chance at locus *l*. Phm_{xy} converges 1 when the pair members are genetically similar and therefore more likely to produce homozygous offspring. We calculated Phm_{xy} using the IDENTIX software (Belkhir et al. 2002).

Genetic similarity between social pairs was analysed via generalised linear mixed models (GLMM) using IBM SPSS software package 20.0. We set individual ID of parents as random factor with mother*father as subject combination, unstructured covariance type and included a constant term. Our fixed effects were year, minimum age of mother and father and Phm_{xy} index. We tested these for effects of genetic similarity on reproductive parameters like proportion of EPO in the brood, total number of EPO, total number of offspring and total number of WPO. For males we included number of extra-pair offspring sired, total number of offspring sired and number of extra-pair offspring in the brood not sired by the social father. Further we looked on number of sired offspring fledged and number of social offspring fledged. Additionally we included hatching date of offspring and trapping date of adults to control for possible seasonal effects in our analyses. As phenotypical parameters of males and females wing length, length of eighth primary

feather (P8), keel length, and weight were included. According to the distribution of parameters a logit link function was set for gamma distributed and an identity link function for normally distributed parameters.

To test if within-pair offspring is more likely to be homozygous we decided for *HL* (homozygosity by loci) as homozygosity index proposed by Aparicio et al. (2006). In comparison to other relatedness indices, like e.g. Internal relatedness (IR) (see Amos et al. 2001), it weights contribution of each locus to overall homozygosity depending on the allelic variability. The weight to the given loci is proportional to their expected heterozygosity (*E*) as suggested by Queller and Goodnight (1989) to estimate relatedness among groups or individuals. From this follows that $E = 1 - \sum f_i^2$, where f_i is the frequency of the i^{th} allele in the population. Taking this term into account, *HL* would be:

$$HL = \frac{\sum E_h}{\sum E_h + \sum E_j}$$

Eh and *Ej* are the expected heterozygosities at the loci that an individual has in homozygosis (*h*) and in heterozygosis (*j*), respectively. It approaches 1 when all loci are homozygous and 0 if they are heterozygous. Intermediate values depend on the expected heterozygosity of the loci involved in homozygosis or heterozygosis. Expected heterozygosity values for each locus in our population were calculated with CERVUS. Therefore we could simplify the formula to calculate individual *HL* index to

$$HL = \frac{\sum Lo_{ho}}{\sum E_j},$$

where Lo_{ho} is the amount of homozygote loci and E_j the sum of expected heterozygosity values.

To see if there is a correlation between within-pair offspring and homozygosity, we tested the calculated *HL* indexes for individuals applying generalised linear mixed models (GLMM) using IBM SPSS software package 20.0. We set brood as random factor, unstructured covariance type and included a constant term. Our fixed effects were year and *HL* index. Our target data were whether offspring was within-pair or extra-pair. As data were not normally distributed we set a logit link function.

RESULTS

Microsatellite genotyping

We found our microsatellites to be highly polymorphic, with a mean of 20.5 alleles per locus and a mean of 83.2% of heterozygotes. Allele frequencies revealed that two loci might contain null-alleles, detected as general excess of homozygotes. Detailed description

of used loci, heterozygosis calculations and non-exclusion probabilities are given in Table 1.

Parental analyses

Despite the possible existence of null-alleles, offspring with the respective social parent equally genotyped, could be assigned to 99.8% (521/522) of females and 97.1% (510/525) of males at 95% confidence level. Our analyses revealed four cases of intraspecific brood parasitism. Beside these four nestlings, on average 20.5% (107/521) of offspring across 42.9% of broods (67/156) could not be assigned to their social father. Nests where offspring's genotype did not match the genotype of the social father contained on average 1.7 nestlings sired by an extra-pair male. For a detailed summary of the genotyping results of each year see Table 2.

Genetic similarity and heterozygosis

We found a negative correlation of genetic similarity between social pair mates, represented as the probability of producing homozygous offspring (Phm_{xy}), with the number of (social) offspring in the brood (GLMM; k = -1.011, p = 0.001 n =133) and WPO (GLMM; k = -0.753, p = 0.019, n =133). In both cases offspring numbers were affected by the social fathers minimum age (GLMM; social offspring: k = 0.136, p = 0.001, and WPO: k = 0.135, p = 0.002, n =133). However, presence of extra-pair offspring (total number and proportion) could not be predicted by the Phm_{xy} index of social pair mates (GLMM; k = -0.168, p = 0.383, and k = -0.766, p = 0.448, n =133) or minimum age of social parents. Additionally we could not find an effect of the Phm_{xy} index of males siring extra-pair offspring (GLMM; k = 0.066, p = 0.898, n =133) but it was negatively correlated with the offspring number (WPO + EPO) a male sired (GLMM; k = -0.799, p = 0.024, n =133). Further survival of social offspring (fledging success of nestlings in the brood) was negatively affected by the Phm_{xy} index (GLMM; k = -2.857, p = 0.011, n =133) which is also true for survival of genetic offspring of males although the effect is not as strong (GLMM; k = -0.712, p = 0.049, n =133). Nevertheless, also here we have to remark that both measurements of offspring survival are not only affected by Phm_{xy} but also positively correlated with minimum age of the social father (GLMM; social offspring: k = 0.498, p = 0.001 and genetic offspring: k = 0.156, p = 0.002, n =133).

With regard to seasonality we found no effect of Phm_{xy} on hatching date of nestlings but again a (negative) effect of minimum age of social parents (GLMM; mother: k = -0.047, p = 0.019 and genetic offspring: k = -0.060, p = 0.004, n =133).

All other phenotypical variables, like P8, wing length, Kipp's distance, keel length, weight and fat class seem to be not affected by Phm_{xy}.

Analogue to the results of genetic relatedness of social parents we did not find an effect of homozygosity probability *HL* if offspring was sired by a genotyped extra-pair male or the social father (GLMM; k = 0.017, p = 0.199, n = 521), the same was true for comparison of offspring sired by an unknown extra-pair male or the social father (GLMM; k = -0.012, p = 0.703, n = 521).

Nestling survival

A possible fitness consequence of increased homozygosis in WPO would be higher mortality in comparison to EPO. In terms of offspring which did not hatch or died as nestling before fledging we could not find any significant correlation between WPO and mortality (x^2 = 1.449, p = 0.229, n =196).

DISCUSSION

In our study population of House Martins (*Delichon urbicum*) genetic similarity of social partners had a negative effect on the number of offspring produced and the number of WPO. Further, fledging success of social offspring is lower when a pair has higher probability to produce homozygous offspring. Additionally males paired to genetically more similar females produce fewer offspring (WPO + EPO) overall. These results fit to the genetic compatibility (GC) hypothesis (see: Tregenza and Wedell 2000) because according to the hypothesis, mating with genetically more similar partners leads to reduced heterozygosis and therefore reduced offspring fitness (e.g.: Spottiswoode and Møller 2004). Hence, extra-pair mating should be an appropriate strategy to increase offspring fitness if the social partner is genetically (too) similar, which is especially essential for the sex with higher parental investment, namely females (for review see: Jennions and Petrie 2000; Kempenaers 2007). Therefore, the underlying assumption is that polyandry based on genetic complementary enhances offspring fitness due to higher heterozygosity. Indeed there is growing evidence for genetic compatibility driving mate choice in other taxa (reviewed in: Tregenza and Wedell 2000). This seems also true for birds (see: Griffith and Immler 2009), although results are conflicting (e.g.: Freeman-Gallant et al. 2006; reviewed in: Mays et al. 2008) and still remain controversial (Akçay and Roughgarden 2007). Likewise in our study, where on one hand we found reduced reproductive success being related to homozygosity, but on the other hand genetic relatedness between social mother and father did not predict the occurrence of EPO. Additionally we could not find evidence

that probability of homozygosity in offspring is higher in WPO than EPO regardless of whether the extra-pair father was a genotyped colony member or an unknown transient. The latter results are not concordant with the GC hypothesis.

Wetzel and Westneat (2009) argued that the use of the same genetic markers for parental and heterozygosity analyses leads to biased results supporting (majority of derived predictions) or opposing the GC hypothesis without actual effect of heterozygosity. According to the results of GLMMs and given that the mean expected allelic frequency in our population was 83.2% (>0.75, Figure 3b in Wetzel and Westneat 2009) we conclude that our results are unlikely to be affected by this bias. Nevertheless, it was criticised that molecular markers are insufficient to estimate genome-wide heterozygosity (DeWoody and DeWoody 2005). Yet, heterozygosity at microsatellite loci is proposed as surrogate for overall genetic quality (e.g.: Mulard et al. 2009; but see: Boerner et al. 2013) inferring that if certain loci are selected this enhances fitness and increases overall heterozygosity. Although we did not find an effect of heterozygosity on offspring we are aware that we could test this only for the set of markers we used and therefore cannot explicitly exclude certain heterozygosity effects.

However, there is growing evidence showing that fitness enhancement is increased through polyandry not only from birds (e.g.: Johnsen et al. 2000; Foerster et al. 2003; Garvin et al. 2006; Suter et al. 2007; Lampila et al. 2011), but also from mammals where social monogamy is rare (Cohas et al. 2007; Schwensow et al. 2008; but see: Leclaire et al. 2013). Nevertheless, only a few studies could simultaneously affirm several predictions derived from the GC hypothesis, like e.g. that extra-pair mates are more heterozygous than social mates and EPO is more heterozygous than WPO (e.g.: Lindstedt et al. 2007; Fossøy et al. 2008). Even within the Hirundininae subfamily, to which House Martins belong, results from two phylogenetically closely related species like Tree Swallows (*Tachycineta bicolor, T. b.*) and White-rumped Swallows (*Tachycineta leucorrhoa, T. l.*) are different if not oppositional. Both species show unusual high frequencies of extra-pair paternity, i.e.: 47% of offspring resulted from extra-pair fertilizations in *T. bicolor*. (Stapleton et al. 2007) and 56% in *T. leucorrhoa.* (Ferretti et al. 2011). For instance, Whittingham and Dunn (2010) found that in Tree Swallows experienced females (breeding in two consecutive years) produced broods with more sires when they were genetically more similar to their social mates. In White-rumped Swallows on the other hand Ferretti et al. (2011) found that females were less genetically related to their social than to their extra-pair partners. This raises the question why females of two highly related species, both with high rates of

EPOs, should differ in mate choice when heterozygosis is generally thought to be beneficial? Should EPP rates in *T. leucorrhoa* not be lower than in *T. bicolor* due to higher genetic dissimilarity of social mates? One explanation might be post-copulatory mechanisms like sperm competition and cryptic female choice (for review see: Birkhead and Pizzari 2002). There is compelling evidence from other taxa, e.g.: insects (see: Birkhead 2010) but in case of birds mainly studies on domestic fowl (e.g.: Pizzari et al. 2004) and water fowl (e.g.: Brennan et al. 2007) are convincing. But we admit that only because there is little known about this topic in passerines we do not want to exclude post-copulatory mechanisms also in our species.

However we could find that age is a dominant factor related to reproductive success in males, meaning older males produce more social and within-pair offspring. Additionally also fledging success of offspring (social and genetic) is positively correlated with age of the social father. On the other hand we could not find an effect of age on the occurrence of extra-pair offspring in relation to homozygosity. Taking all results together it seems that male age is a good predictor for male parental quality in our study population and extra-pair mating in both sexes occurs not due to the attempt to increase heterozygosity of offspring. There are two explanations for this.

One is that males are more strongly selected on heterozygosity and therefore older males are of higher overall genetic quality. Despite an increasing number of papers, evidence for heterozygosity on adult survival is scarce. In Pied Flycatchers (*Ficedula hypoleuca*) researchers (Canal et al. 2014) could not find any effect of heterozygosity on juvenile or adult survival directly, but relatedness of parents seemed to have influenced survival of their offspring during adult life. In contrast, Olano-Marin et al. (2011) found a positive effect of standardized heterozygosity measures of neutral markers on adult survival of both sexes in Blue Tits (*Cyanistes caeruleus*). Although we could not test for genetic effects on adult survival with our data set we cannot preclude selection of heterozygotes in our population.

However a second explanation is that males increase their paternal qualities with age due to experience. To show this, a long-term study would be required which we could not conduct with our population. But, as such investigations in other species have accumulated large data sets in the last decades, a link between male age and improvement of parental experience enhancing reproductive success has been established. Beside long-living raptors and sea-birds (Espie et al. 2000; Pyle et al. 2001; Riechert et al. 2012) it was also shown for socially monogamous passerines like Brown Thornbills (Green 2001), Barn Swallows

(Balbontín et al. 2007) and Wood Thrushes (Brown and Roth 2009). Comparing these findings with ours it seems very likely that also male House Martins improve their reproduction with experience.

Therefore we conclude that in our study population of House Martins negative effects of homozygosis are not balanced by extra-pair behaviour. It is more likely that over time unfavourable male genotypes are sorted out and males improve their overall reproductive skills and may thus compensate for possible negative effects of homozygous social pairing.

Table 1: Allele frequencies of six polymorphic microsatellite loci for parental analyses in House Martins (*Delichon urbicum*).

Locus	N_A	H_E	N_E	H-W	F_{N-A}
Aar4	20	0.892	0.218	NS	-0.0035
Ase18	13	0.790	0.393	NS	+0.0038
Escμ6	17	0.888	0.227	***	+0.0465
HrU6	40	0.927	0.147	***	+0.0676
HrU7	18	0.780	0.393	NS	+0.0227
Ltr6	15	0.807	0.360	NS	-0.0212

N_A: number of observed alleles; H_E: expected heterozygosity; N_E: Non exclusion probability of second parent; H-W: deviations from Hardy-Weinberg equilibrium (NS denotes non-significant, * denotes a significant deviation); F_{N-A}: estimated frequencies of null-alleles.

Table 2: Numbers of broods and offspring resulting from parental analyses from microsatellite genotyping.

	2002	**2003**	**2004**	**Total**
Broods sampled	65	39	57	161
Broods parasitized	3	1	0	4
Broods cuckolded	25	14	28	67
Broods cuckolded %	40.3	37.8	49.1	42.9
Offspring (OS) sampled	209	132	196	537
OS genetic mother assigned	203	121	196	520
OS genetic father assigned	198	121	191	510
OS from parasitizing	3	1	0	4
OS from cuckolding	35	26	46	107
OS from cuckolding %	17.4	21.0	23.5	20.5
Mean extra-pair OS/brood	1.4	1.9	1.6	1.7

REFERENCES

Akçay, E., Roughgarden, J. (2007) Extra-pair paternity in birds: review of the genetic benefits. *Evolutionary Ecology Research* **9**: 855–868.

Amos, W., Worthington Wilmer, J., Fullard, K., Burg, T.M., Croxall, J.P., Bloch, D., Coulson, T. (2001) The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London B* **268**: 2021-2027. doi: 10.1098/rspb.2001.1751

Aparicio, J.M., Ortego, J., Cordero, P.J. (2006) What should we weigh to estimate heterozygosity, alleles or loci? *Molecular Ecology* **15**: 4659-4665. doi: 10.1111/j.1365-294X.2006.03111.x

Arnqvist, G., Kirkpatrick, M. (2005) The Evolution of Infidelity in Socially Monogamous Passerines: The Strength of Direct and Indirect Selection on Extrapair Copulation Behavior in Females. *The American Naturalist* **165**: S26-S37. doi: 10.1086/429350

Balbontín, J., Hermosell, I.G., Marzal, A., Reviriego, M., De Lope, F., Møller, A.P. (2007) Age-related change in breeding performance in early life is associated with an increase in competence in the migratory barn swallow *Hirundo rustica*. *Journal of Animal Ecology* **76**: 915-925. doi: 10.1111/j.1365-2656.2007.01269.x

Belkhir, K., Castric, V., Bonhomme, F. (2002) IDENTIX, a software to test for relatedness in a population using permutation methods. *Molecular Ecology Notes* **2**: 611-614. doi: 10.1046/j.1471-8286.2002.00273.x

Birkhead, T.R. (2010) How stupid not to have thought of that: post-copulatory sexual selection. *Journal of Zoology* **281**: 78-93. doi: 10.1111/j.1469-7998.2010.00701.x

Birkhead, T.R., Pizzari, T. (2002) Postcopulatory sexual selection. *Nature Reviews Genetics* **3**: 262-273. doi: 10.1038/nrg774

Boerner, M., Hoffman, J.I., Amos, W., Chakarov, N., Kruger, O. (2013) No correlation between multi-locus heterozygosity and fitness in the common buzzard despite heterozygote advantage for plumage colour. *Journal of Evolutionary Biology* **26**: 2233-2243. doi: 10.1111/jeb.12221

Brennan, P.L.R., Prum, R.O., McCracken, K.G., Sorenson, M.D., Wilson, R.E., Birkhead, T.R. (2007) Coevolution of Male and Female Genital Morphology in Waterfowl. *PLoS ONE* **2**: e418. doi: 10.1371/journal.pone.0000418

Brown, W.P., Roth, R.R. (2009) Age-specific reproduction and survival of individually marked Wood Thrushes, *Hylocichla mustelina*. *Ecology* **90**: 218-229. doi: 10.1890/07-2061.1

Bryant, D.M. (1975) Changes in Incubation Patch and Weight in the Nesting House Martin. *Ringing & Migration* **1**: 33-36. doi: 10.1080/03078698.1975.9673696

Bryant, D.M. (1988) *Lifetime Reproductive Success of House Martins*. In: Clutton-Brock, T.H. (ed) Reproductive Success: Studies of Individual Variation in Contrasting Breeding Systems. University of Chicago Press, Chicago, pp 173-188

Canal, D., Serrano, D., Potti, J. (2014) Exploring Heterozygosity-Survival Correlations in a Wild Songbird Population: Contrasting Effects between Juvenile and Adult Stages. *PLoS ONE* **9**: e105020. doi: 10.1371/journal.pone.0105020

Cohas, A., Bonenfant, C., Gaillard, J.M., Allainé, D. (2007) Are extra-pair young better than within-pair young? A comparison of survival and dominance in alpine marmot. *Journal of Animal Ecology* **76**: 771-781. doi: 10.1111/j.1365-2656.2007.01253.x

DeWoody, Y.D., DeWoody, J.A. (2005) On the Estimation of Genome-wide Heterozygosity Using Molecular Markers. *Journal of Heredity* **96**: 85-88. doi: 10.1093/jhered/esi017

Espie, R.H.M., Oliphant, L.W., James, P.C., Warkentin, I.G., Lieske, D.J. (2000) Age-dependent breeding performance in Merlins (*Falco columbarius*). *Ecology* **81**: 3404-3415. doi: 10.2307/177503

Ferretti, V., Massoni, V., Bulit, F., Winkler, D.W., Lovette, I.J. (2011) Heterozygosity and fitness benefits of extrapair mate choice in White-rumped Swallows (*Tachycineta leucorrhoa*). *Behavioral Ecology* **22**: 1178-1186. doi: 10.1093/beheco/arr103

Foerster, K., Delhey, K., Johnsen, A., Lifjeld, J.T., Kempenaers, B. (2003) Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature* **425**: 714-717. doi: 10.1038/nature01969

Fossøy, F., Johnsen, A., Lifjeld, J.T. (2008) Multiple genetic benefits of female promiscuity in a socially monogamous passerine. *Evolution* **62**: 145-156. doi: 10.1111/j.1558-5646.2007.00284.x

Freeman-Gallant, C.R., Wheelwright, N.T., Meiklejohn, K.E., Sollecito, S. (2006) Genetic similarity, extrapair paternity, and offspring quality in *Savannah sparrows* (*Passerculus sandwichensis*). *Behavioral Ecology* **17**: 952–958. doi: 10.1093/beheco/arl031

Garvin, J.C., Abroe, B., Pedersen, M.C., Dunn, P.O., Whittingham, L.A. (2006) Immune response of nestling warblers varies with extra-pair paternity and temperature. *Molecular Ecology* **15**: 3833-3840. doi: 10.1111/j.1365-294X.2006.03042.x

Green, D.J. (2001) The influence of age on reproductive performance in the Brown Thornbill. *Journal of Avian Biology* **32**: 6-14. doi: 10.2307/25161504

Griffith, S.C., Immler, S. (2009) Female infidelity and genetic compatibility in birds: the role of the genetically loaded raffle in understanding the function of extrapair paternity. *Journal of Avian Biology* **40**: 97-101. doi: 10.1111/j.1600-048X.2009.04562.x

Griffith, S.C., Owens, I.P.F., Thuman, K.A. (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology* **11**: 2195-2212. doi: 10.1046/j.1365-294X.2002.01613.x

Hanotte, O., Zanon, C., Pugh, A., Greig, C., Dixon, A., Burke, T. (1994) Isolation and characterization of microsatellite loci in a passerine bird: the reed bunting *Emberiza schoeniclus*. *Molecular Ecology* **3**: 529-530. doi: 10.1111/j.1365-294X.1994.tb00133.x

Hansson, B., Bensch, S., Hasselquist, D., Åkesson, M. (2001) Microsatellite diversity predicts recruitment of sibling great reed warblers. *Proceedings of the Royal Society B* **268**: 1287-1291. doi: 10.1098/rspb.2001.1640

Hansson, B., Bensch, S., Hasselquist, D., Lillandt, B.G., Wennerberg, L., Von Schantz, T. (2000) Increase of genetic variation over time in a recently founded population of great reed warblers (*Acrocephalus arundinaceus*) revealed by microsatellites and DNA fingerprinting. *Molecular Ecology* **9**: 1529-1538. doi: 10.1046/j.1365-294x.2000.01028.x

Jennions, M.D., Petrie, M. (2000) Why do females mate multiply? A review of the genetic benefits. *Biological Reviews* **75**: 21-64. doi: 10.1111/j.1469-185X.1999.tb00040.x

Johnsen, A., Andersen, V., Sunding, C., Lifjeld, J.T. (2000) Female bluethroats enhance offspring immunocompetence through extra-pair copulations. *Nature* **406**: 296-299. doi: 10.1038/35018556

Kalinowski, S.T., Taper, M.L., Marschall, T.C. (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* **16**: 1099-1106. doi: 10.1111/j.1365-294X.2007.03089.x

Kempenaers, B. (2007) Mate Choice and Genetic Quality: A Review of the Heterozygosity Theory. *Advances in the Study of Behavior* **37**: 189-278. doi: 10.1016/S0065-3454(07)37005-8

Lampila, S., Orell, M., Kvist, L. (2011) Willow tit *Parus montanus* extrapair offspring are more heterozygous than their maternal half-siblings. *Journal of Avian Biology* **42**: 355-362. doi: 10.1111/j.1600-048X.2011.05349.x

Leclaire, S., Nielsen, J.F., Sharp, S.P., Clutton-Brock, T.H. (2013) Mating strategies in dominant meerkats: evidence for extra-pair paternity in relation to genetic relatedness between pair mates. *Journal of Evolutionary Biology* **26**: 1499-1507. doi: 10.1111/jeb.12151

Lindstedt, E.R., Oh, K.P., Badyaev, A.V. (2007) Ecological, social, and genetic contingency of extrapair behavior in a socially monogamous bird. *Journal of Avian Biology* **38**: 214-223. doi: 10.1111/j.2007.0908-8857.03889.x

Mathieu, E., Autem, M., Roux, M., Bonhomme, F. (1990) Épreuves de validation dans l'analyse de structures génétiques multivariées: comment tester l'équilibre

panmictique? *Revue de Statistique Appliquée* **38**: 47-66. http://www.numdam.org/item?id=RSA_1990__38_1_47_0

Mays, H.L., Jr., Albrecht, T., Liu, M., Hill, G.E. (2008) Female choice for genetic complementarity in birds: a review. *Genetica* **134**: 147-158. doi: 10.1007/s10709-007-9219-5

McDonald, D.B., Potts, W.K. (1994) Cooperative display and relatedness among males in a lek-mating bird. *Science* **266**: 1030-1032. doi: 10.1126/science.7973654

Møller, A.P. (1992) Frequency of Female Copulations with Multiple Males and Sexual Selection. *The American Naturalist* **139**: 1089-1101. http://www.jstor.org/stable/2462368 .

Mulard, H., Danchin, E., Talbot, S.L., Ramey, A.M., Hatch, S.A., White, J.F., Helfenstein, F., Wagner, R.H. (2009) Evidence that pairing with genetically similar mates is maladaptive in a monogamous bird. *BMC Evolutionary Biology* **9**: 147. doi: 10.1186/1471-2148-9-147

Neff, B.D., Pitcher, T.E. (2005) Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Molecular Ecology* **14**: 19-38. doi: 10.1111/j.1365-294X.2004.02395.x

Oh, K.P., Badyaev, A.V. (2006) Adaptive genetic complementarity in mate choice coexists with selection for elaborate sexual traits. *Proceedings of the Royal Society B* **273**: 1913-1919. doi: 10.1098/rspb.2006.3528

Olano-Marin, J., Mueller, J.C., Kempenaers, B. (2011) Heterozygosity and survival in blue tits (*Cyanistes caeruleus*): contrasting effects of presumably functional and neutral loci. *Molecular Ecology* **20**: 4028-4041. doi: 10.1111/j.1365-294X.2011.05177.x

Pizzari, T., Lovlie, H., Cornwallis, C.K. (2004) Sex-specific, counteracting responses to inbreeding in a bird. *Proceedings of the Royal Society of London B* **271**: 2115-2121. doi: 10.1098/rspb.2004.2843

Primmer, C.R., Møller, A.P., Ellegren, H. (1995) Resolving genetic relationships with microsatellite markers: A parentage testing system for the swallow *Hirundo rustica*. *Molecular Ecology* **4**: 493-498. doi: 10.1111/j.1365-294X.1995.tb00243.x

Puurtinen, M., Ketola, T., Kotiaho, J.S. (2005) Genetic compatibility and sexual selection. *Trends in Ecology & Evolution* **20**: 157-158. doi: 10.1016/j.tree.2005.02.005

Pyle, P., Sydeman, W.J., Hester, M. (2001) Effects of age, breeding experience, mate fidelity and site fidelity on breeding performance in a declining population of Cassin's auklets. *Journal of Animal Ecology* **70**: 1088-1097. doi: 10.1046/j.0021-8790.2001.00567.x

Queller, D.C., Goodnight, K.F. (1989) Estimating relatedness using genetic markers. *Evolution* **43**: 258-275. doi: 10.2307/2409206

Richardson, D.S., Jury, F.L., Dawson, D.A., Salgueiro, P., Komdeur, J., Burke, T. (2000) Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. *Molecular Ecology* **9**: 2226-2231. doi: 10.1046/j.1365-294X.2000.105338.x

Richardson, D.S., Komdeur, J., Burke, T., von Schantz, T. (2005) MHC-based patterns of social and extra-pair mate choice in the Seychelles warbler. *Proceedings of the Royal Society B* **272**: 759-767. doi: 10.1098/rspb.2004.3028

Riechert, J., Chastel, O., Becker, P.H. (2012) Why do experienced birds reproduce better? Possible endocrine mechanisms in a long-lived seabird, the common tern. *General and Comparative Endocrinology* **178**: 391-399. doi: 10.1016/j.ygcen.2012.06.022

Ryder, T.B., Tori, W.P., Blake, J.G., Loiselle, B.A., Parker, P.G. (2010) Mate choice for genetic quality: a test of the heterozygosity and compatibility hypotheses in a lek-breeding bird. *Behavioral Ecology* **21**: 203-210. doi: 10.1093/beheco/arp176

Schwensow, N., Fietz, J., Dausmann, K., Sommer, S. (2008) MHC-associated mating strategies and the importance of overall genetic diversity in an obligate pair-living primate. *Evolutionary Ecology* **22**: 617-636. doi: 10.1007/s10682-007-9186-4

Seutin, G., White, B.N., Boag, P.T. (1991) Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology* **69**: 82-90. doi: 10.1139/z91-013

Spottiswoode, C., Møller, A.P. (2004) Genetic similarity and hatching success in birds. *Proceedings of the Royal Society of London B* **271**: 267-272. doi: 10.1098/rspb.2003.2605

Stapleton, M.K., Kleven, O., Lifjeld, J.T., Robertson, R.J. (2007) Female tree swallows (*Tachycineta bicolor)* increase offspring heterozygosity through extrapair mating. *Behavioral Ecology and Sociobiology* **61**: 1725-1733. doi: 10.1007/s00265-007-0404-4

Suter, S.M., Keiser, M., Feignoux, R., Meyer, D.R. (2007) Reed bunting females increase fitness through extra-pair mating with genetically dissimilar males. *Proceedings of the Royal Society B* **274**: 2865-2871. doi: 10.1098/rspb.2007.0799

Tregenza, T., Wedell, N. (2000) Genetic compatibility, mate choice and patterns of parentage: Invited Review. *Molecular Ecology* **9**: 1013-1027. doi: 10.1046/j.1365-294x.2000.00964.x

Westneat, D.F., Stewart, I.R.K. (2003) Extra-Pair Paternity In Birds: Causes, Correlates, and Conflict. *Annual Review of Ecology Evolution and Systematics* **34**: 365-396. doi: 10.1146/annurev.ecolsys.34.011802.132439

Wetzel, D.P., Westneat, D.F. (2009) Heterozygosity and extra-pair paternity: biased tests result from the use of shared markers. *Molecular Ecology* **18**: 2010-2021. doi: 10.1111/j.1365-294X.2009.04114.x

Whittingham, L.A., Dunn, P.O. (2010) Fitness benefits of polyandry for experienced females. *Molecular Ecology* **19**: 2328-2335. doi: 10.1111/j.1365-294X.2010.04640.x

Whittingham, L.A., Lifjeld, J.T. (1995a) Extra-pair fertilizations increase the opportunity for sexual selection in the monogamous House Martin *Delichon urbica*. *Journal of Avian Biology* **26**: 283-288. doi: 10.2307/3677042

Whittingham, L.A., Lifjeld, J.T. (1995b) High paternal investment in unrelated young: extra-pair paternity and male parental care in house martins. *Behavioral Ecology and Sociobiology* **37**: 103-108. doi: 10.1007/bf00164155

Chapter 3

Male detainment induces brood abandonment in House Martins (*Delichon urbicum urbicum*)

ABSTRACT

Although extra-pair paternity seems to be prevalent in socially monogamous birds it is still not known which sex represents the main driving force. Male birds in some species intensively guard their female partner during their fertile phase to assure paternity. This is also the case in European House Martins (*Delichon urbicum urbicum*). In our work we aimed to test whether female House Martins are more likely to engage in extra-pair behaviour when temporarily detaining their social partner during their fertile phase. If females were constrained by their guarding mates they should increase extra-pair solicitations or forays when their partner is absent. This should affect variation in extra-pair paternity as consequence. Unfortunately we were not able to obtain paternity data as in 15 experimental pairs egg-laying was stopped. In eleven out of these 15 cases females abandoned their brood and social partner. This implies that females are more vulnerable to disturbances during the start of the breeding period. In comparison, we could not find such a severe effect in control pairs. Although we cannot disentangle which disturbance factor mainly triggers this reaction, we suppose that an increase of nest intrusions and sexual harassment due to temporal male absence lead to this result. Our observations did not indicate that females would pursue extra-pair mating during their social mate's absence. This is in accordance with other studies applying male detainment experiments. We therefore conclude that this methodological approach is only a suboptimal tool to manipulate propensity of females to engage in extra-pair behaviour. It does mainly increase male pressure on females and manipulates male-male competition.

INTRODUCTION

Though the majority of birds are socially monogamous (Lack 1968), genetic monogamy seems to be rather an exception than the norm. Different hypotheses have been proposed why it might be beneficial for both females and males to engage in extra-pair mating beyond their social pair bonding (Griffith et al. 2002). Ever since almost a quarter of century of investigations on paternity using tools of molecular biology revealing different levels of extra-pair paternity, there is still no support for a comprehensive hypothesis explaining variation in extra-pair mating strategies in birds (Akçay and Roughgarden

2007). However, the main driving force behind extra-pair mating is proposed to be an interplay between male-male competition and female choice promoting sexual conflict (Arnqvist and Rowe 2005). Furthermore, the sexual conflict arising as a consequence of extra-pair mating could be better understood if considering a three (mother, social father and extra-pair father) or more players game (Westneat and Stewart 2003; Chaine et al. 2015). Therefore, when investigating causal dependency of variation in extra-pair mating results, different approaches of the involved parties should be defined beforehand.

In this context it is believed that engaging in extra-pair behaviour is costly for females notably in monogamous species as it might entail reduction of paternal investment due to doubtful paternity (Trivers 1972). It was hypothesized that females try to compensate these costs by obtaining indirect (genetic) benefits from extra-pair males with assumed higher (genetic) quality (Westneat et al. 1990) or to spur sperm competition which will affect sperm competitiveness of following male generations (Birkhead and Møller 1992). As a consequence, variances in fitness of within-pair and extra-pair offspring should be found (e.g.: Sheldon et al. 1997). Nevertheless, compelling evidence for this indirect selection is rare. Instead, better arguments could be found for direct processes involving costs to females derived from extra-pair behaviour (Arnqvist and Kirkpatrick 2005; Albrecht et al. 2006).

Therefore it is crucial to have a closer look at the period during which females are fertile and paternity of their social mates is most vulnerable. Due to ensuing fitness costs of their partners´ infidelity males have developed various strategies to ensure paternity. They vary among taxa and mate guarding is common in monogamous birds (reviewed in: Birkhead and Møller 1992). As mate guarding, where individual males follow their mates and increase close proximity during the females' fertile period, is prevalent in birds, it was investigated in various species to determine the efficiency of this paternity ensuring behaviour. Depending on the investigated species, conflicting results could be found and moreover different females' propensity to engage in extra-pair copulations (e.g.: Björklund et al. 1992; Johnsen et al. 1998) precluded any comprehensive conclusions. Hence, to proof effectivity of paternity assurance tactics like mate guarding, experimental manipulation of this strategy in relation to paternity has been proposed (Møller and Ninni 1998). One suitable experimental approach is to prevent males from mate guarding by removing them from their partners during the females' fertile phase. At this stage males' paternity could be threatened by other competitors. A higher extra-pair offspring rate should be found as a consequence. Actually there is evidence that detainment of males in

early breeding stages increases EPP rates in birds (e.g.: MacDougall-Shackleton et al. 1996; Lifjeld et al. 1997; Chuang-Dobbs et al. 2001). As the cited studies were all conducted on sexually dimorphic species with males owing a bright plumage in comparison to the other sex, choice for morphological traits assigning male quality is a probable mechanism for females to evaluate potential extra-pair partners.

Therefore we wanted to apply the detainment experiment in a species that is sexually monomorphic and males don't show ostensible traits which could serve as quality cues. We conducted our experiments in a population of European House Martins (*Delichon urbicum urbicum*), a socially monogamous passerine species for which extra-pair paternity was already demonstrated (e.g.: Whittingham and Lifjeld 1995). House Martin males show intensive defence against nest intrusions by other males during the fertile phase of their mates when accompanying them in the nest. Additionally, males follow their departing females more often at this stage than during other phases of the breeding cycle. This means that mate guarding is a common male strategy for paternity assurance in this species (Lifjeld and Marstein 1994). This is in accordance with another study where Riley et al. (1995) detained male House Martins from the pair female during her fertile phase. However, in this study paternity data could only be obtained for three out of seven experimental pairs revealing conflicting results. Nevertheless, their respectable observational data set showed that males guard their partners in the nest and not the nest itself. Moreover, possible extra-pair copulations most likely occur when extra-pair males enter the nest in the absence of the pair male.

Therefore we judged this manipulation as suitable to shed light on variance in female and male extra-pair behaviour and tried to repeat the experiment in combination with paternity analyses. According to the results of the studies cited above, when keeping away the male thus leaving its pair female unguarded during its fertile period, we predicted following consequences:

(1) Extra-pair males will more frequently enter nests.
(2) Extra-pair males will stay longer inside the nest to obtain successful copulations.
(3) Females will try to advertise that they are alone.
(4) Females will leave the nest to visit other nests to increase the number of potential extra-pair mates.
(5) Returning males will immediately copulate with their partner to ensure paternity.
(6) Extra-pair paternities will increase.

METHODS

Fieldwork

We collected data from two breeding colonies of European House Martins (*Delichon urbicum urbicum*) at Illmitz, Burgenland, Austria (WGS 84: 47,76887 N 16,76619 E) from 2002 until 2004. Both colonies were separated by 2.3km and located at buildings surrounded by reed beds, small channels, vineyards, and pastures. Colony 1 was attached to the roof of the boat house of the biological station Illmitz (henceforth BSI) and comprised between 42 and 78 mud-built nests per year. Due to a harsh winter in 2002/2003, remains of natural nests were replaced by 63 artificial nest cups. Natural nests at colony 2 were attached to roof awnings of buildings at the Seebad Illmitz (henceforth Seebad) and comprised up to 30 nests. The numbers of successful nests (i.e. with at least one chick surviving until ringing) were: 2002: 39 first and six second broods at colony 1, 19 first and one second brood at colony 2; 2003: 25 first and two second broods at colony 1, eleven first and one second brood at colony 2; 2004: 42 first and 15 second broods at colony 1 (no data for colony 2). All nests were monitored weekly from end of April until mid of September.

For the standard trapping as well as measurement and sampling procedures see detailed description in the methods section of Chapter 1.

Male Removal experiment

We randomly selected 30 (15 control and 15 experimental) pairs in colony 1 at the time when the female laid the first egg (day 0 in the breeding cycle). The detainment experiment was carried out on the following morning on one pair, another pair was used as a matched control to see if trapping and handling themselves were affecting the pair bond and therefore confounding the experimental results. It is supposed that the fertile period of female House Martins lasts from day -5 until the day the penultimate egg is laid (Lifjeld and Marstein 1994). Moreover from day 0 on extra-pair fertilizations occur more often as they go hand in hand with a reduction of mate guarding (Riley et al. 1995). In this study each egg was laid on average between 0600 and 0900 hours in the morning and according to Lifjeld and Marstein (1994), intensity of nest intrusion is the highest in the morning hours. Therefore, we plugged nest entrances of an experimental and a control nest with cotton wool one hour before dawn to prevent birds from escaping before the start of the actual experiment. We removed trapped birds from nests at dawn (between 0400 and 0500 hours) and immediately released females of the experimental group, as well as the pairs of

the control group together, upon marking and individually ringing them. We kept males of experimental pairs in cotton bird bags for one hour and released them in front of the colony afterwards. Foreheads and tail feather tips of females were marked with white fluid (Tipp-Ex®) and the white rump of adult males with different colours of Edding® permanent markers. Adult birds were sexed based on absence (male) or presence (female) of a brood patch following the method of Bryant (1975). The differences in brood patch development between sexes of each pair were easy to discern already at this early breeding stage, Additional measurements and sampling of individuals were done when birds were caught again later in the season.

Nests were checked daily and for each experimental nest observational monitoring was done by a person who had not participated in trapping and handling. The monitoring was continued for one hour after males were released, as well as in the evening from 1900 until 2100 hours latest and the day after the experiment. In total we invested approximately 115 hours into the observational monitoring of the nests.

RESULTS

Unfortunately, for none of the 15 experimental pairs we could gain genotyping data because all females stopped egg-laying directly after the experiment. In eight cases we recorded that females abandoned the nest and could no longer be observed in the colony. Their males found a new mate and a new nest within the colony in the following two weeks after the removal experiment. In three cases females deserted the nest and left the colony, their males found a new mate in the same nest between eight and 14 days after the experiment. In two cases the males deserted the nest and the colony and their females found a new mate for the same nest between eight and 14 days after the experiment start. In one case the male and the female left the colony and in one case the couple stayed together in the same nest but we observed the male removing an egg and the female started with a new clutch after eight days.

In comparison to that, egg-laying continued in the normal sequence at control nests and no mate-abandonment or -switching was observed. Additionally, both males and females of control pairs were regularly observed at their nests.

In eleven cases where experimental females abandoned their brood they returned to the nest within six until 35 minutes after release. Females generally entered the nest when returning to the colony and remained there ducked and quiet even when their partner returned. None of them were observed at the nest the day after the experiment. In four

cases where females abandoned the nest we could not observe them at the nest neither in the evening of the experimental day nor the day after.

In the two cases of male nest abandonment, one of the females staid quiet in the nest, while the other experimental female regularly flew out when her partner had left, but always returned within three minutes. She left the nest after her mate returned only for two times followed by him after a few seconds and came back a few seconds later after the male had entered the nest again.

In the case where both male and female of a pair left the colony, the female returned to the nest nine minutes after being released but left the nest again after two minutes. After that we did not observe her in the colony again.

In the case where the couple stayed together, but egg-laying was ceased, the female returned to the nest 18 minutes after her release. In her partners absence she left the nest seven times being outside for no longer than two minutes each. During these outings we could not observe her entering other nests. After her partner had returned, she went out only once followed by him and returned after 13 minutes shortly after he had first returned to the nest. Her partner returned to the nest eight minutes after having been released from detainment. Approaching the nest, he moved in quickly, turned around immediately, and looked out of the nest entrance. We could observe three nest intrusions by neighbouring males which were successfully deterred by the male in the first hour upon his return. Additionally, we recorded a possible (pair-) copulation as we recorded a distinctive "churring" song and grappling movements inside the nest. It was most likely induced by the female as she had been preening his chin for an extended period beforehand. Nevertheless, the next morning we could observe him removing one egg from the nest with his bill.

Detained males returned to their nest between 13 to 48 minutes after release. In all cases present females accepted the males in the nest when they came back, and males immediately turned around and blocked the nest entrance. However, within one hour after returning, five males exhibited regular "out and in flights" from their nest for short periods no longer than 30 seconds. Furthermore two of them (unsuccessfully) tried to enter neighbouring nests at twelve occasions. These were the males who deserted their mate and from the morning after the experiment on we were not able to observe them in the colony again.

Surprisingly, beside the three nest intrusions directed to the pair which remained together, we could observe only seven additional nest intrusions by unmarked individuals. Two

happened when females were alone in the nest. At both events the females left the nest after a short grappling sequence and returned alone after some minutes. The five other intrusions were four times deterred by the male and in one case by male and female together.

DISCUSSION

Because all experimental pairs except one abandoned their nest and partner, we were not able to confirm the predictions we had made. Despite the considerable time we invested in monitoring the behaviour of the martins, we have difficulties in explaining the severe impact our removal experiments clearly had.

Regarding the applied trapping method, we think that it is unlikely that only the fact that we caught the experimental pairs in the nest explains the results because the handling procedure was the same for control pairs which obviously continued to behave normally. There are successful studies in Blue Tits (*Cyanistes caeruleus*) and Black-throated Blue Warblers (*Dendroica caerulescens*) where males were temporally removed during the fertile period of their partner by trapping them in somedistance from the nest using mist nets and male decoy or song (Kempenaers et al. 1995; Chuang-Dobbs et al. 2001). Other studies applying temporal removal of males trapped at the nest in Barn swallows (*Hirundo rustica*) and Great Tits (*Parus major*) did not report ceased egg laying or breaking of pair-ship either (Møller 1987; Björklund et al. 1992). Furthermore, Lifjeld and Robertson (1992) permanently removed ten resident males from the nest box during different stages of female fertility in Tree Swallow pairs (*Tachycineta bicolor*), and reported not a single egg laying suspension. In a comparable study by Riley et al. (1995) House Martins of a population in central Scotland were also caught in the morning hours at the nest. However, these researchers did not plug the nest entrance one hour before the experiment started. It is possible that this additional confinement increased stress and perceived insecurity. On the other hand, Riley et al. (1995) removed males of eleven pairs for five to eight hours and reported laying suspensions only in two nests. In our case, the manipulation from plugging the nest until returning of the retained male lasted for no longer than three hours. Nevertheless, in the cited study they conducted the experiment also on pairs in which the females had already laid their second egg without stating the relative proportion of nests with one or two eggs. This indicates that pair bonding stabilizes with every additional egg.

We conducted our experiments from 5th of May until 15th of May which is right at the beginning of the breeding season in our House Martin population. Breeding season in the study area lasts until beginning of September, and on average 35% of pairs of our

population have at least a second brood. At our study area adverse weather conditions are more frequent in April and May than later in the season. Accordingly, at this time we would expect a higher propensity for nest abandonment or laying suspension as the comparable long breeding season has just started and there is enough time to invest in a second brood. Laying suspensions due to poor weather conditions have been reported from early studies of House Martins (Bryant 1979; Hund and Prinzinger 1979). Therefore, it is possible that external factors would lead more rapidly to breeding interruptions at this particular period of the breeding season.

Females abandoned their nest and partner in 11 out of 15 experimental trials indicating that they are more vulnerable to disturbances at this phase than males. Besides physiological trade-offs related to egg-laying, it is conceivable that the temporal absence of the partner exposes females to a higher affliction through nest intruding extra pair males. However, we recorded only a few nest intrusions directly during the absence of males. Similar to that, Riley et al. (1995) observed no nest intrusions at the three experimental nests for which paternity data could be obtained. They found, however, a higher overall probability of intruders entering the nest when the pair male was absent during the fertile phase of the female. This is in accordance with a study on Violet-Green Swallows (*Tachycineta thalassina*) and Tree Swallows (*Tachycineta bicolor*) in which Beasley (1996) temporally detained males during the assumed fertile period of females. It is very likely that nest intrusions increase also at our colony only later in the morning. As we applied focal observations for a maximum of one hour after the male returned, we therefore would have missed a potential increase of intruders. Because we cannot tell the exact daytime when eggs were laid, it is therefore quite possible that it happened after the temporal absence of the male. When the female lays her egg it is assumed that afterwards the probability increases that the subsequent egg is fertilized. That time, called “insemination window”, is supposed to be relatively short, probably maximally one hour per day (Birkhead and Møller 1993). In that context male absence before egg laying might give the impression to a female that her partner prefers to pursue extra-pair copulations elsewhere and therefore is not likely to protect her when nest intrusions are imminent.

We also assume that nest intrusions increase with colony size and therefore increase pressure on females. Riley et al. (1995), for instance, reported 12 breeding pairs in one colony in one year whereas the minimum number per year in our colony was 27 broods. Accordingly we would expect an increase in nest intrusions by extra-pair males especially at the time after egg-laying, although we cannot support that with our observational data.

Nest approaches by extra-pair females were regularly reported in the House Martin studies cited above and from our own observations, although to a much lesser extent than in males. Also here we would expect an increase with colony size.

Despite the lack of supporting observational data, we suppose that abandonment of nest and partner by females was due to a combination of seasonal timing, trapping disturbance, colony size, and absence of their social partner. Although we cannot disentangle the impact of each factor we assume that temporal removal of pair males was the final straw that led to this result. Beside ethical considerations we would thus not recommend to repeat that type of experiments in House Martins, at least not without methodological adaptations, as it mainly destabilizes pair bonding. Principally, a comparable experiment should not be conducted at the beginning of the breeding season because propensity for brood suspension or abandonment is higher due to more instable weather conditions. Additionally, trapping at the nest should be avoided since it enhances perceived insecurity especially in females. Colony size should also be considered as it increases nest intrusions by those males in the population that pursue extra-pair copulations. Related to that it is arguable that colonial species are principally not suitable for such experiments because male-male competition strategies are influenced by density effects. Anyway, concluding from our experience any replication of the male detainment experiment in House Martins would be only accomplishable in adherence of the following requirements. Generally we highly recommend continuous and not focal observation (on the manipulation and the ensuing day) inside but also outside (to additionally register unsuccessful nest intrusions) the target nest by applying nest cameras. Additional behavioural observations are required before the experiment for at least four hours in the morning and evening. As a second important precondition we suggest that males should be trapped in some distance from their nests e.g. with mist nets in front of the colony or - methodologically more demanding - during their extra-pair forays by presenting an empty nest in combination with a female dummy and/or playback of vocalisations uttered during copulation. Finally, the duration of the detainment should not exceed one hour.

Our results beside, it is questionable more generally whether detainment experiments with males during the fertile phase of their mate can really yield useful data to discern variances of female and male extra pair strategies. There is actually one study on Black-throated Blue Warblers (Chuang-Dobbs et al. 2001), which applied temporal removal of males for one hour at this delicate period. They could show an increase of extra-pair paternity suggesting efficient male mate guarding. Westneat (1994) could find similar results when

retaining male Red Winged Blackbirds (*Agelaius phoeniceus*), although his experiment was combined with food manipulation. Using a slightly altered detainment method (trapped male was presented visibly in a cage centred in his territory) Kempenaers et al. (1995) found that in Blue Tits nest intrusions by extra-pair males increased but this was not related to extra-pair paternity. Dickinson (1997) detained pair males of fertile Western Blue Bird (*Sialia mexicana*) females for one hour and observed an increase of territory intrusions by extra-pair males and extra-pair copulation frequencies, but unfortunately they could not obtain paternity data. The same is true for a somewhat comparable study on Pied Flycatchers (*Ficedula albicollis*) done by Björklund and Westman (1983) and in Great Tits (Björklund et al. 1992). Beasley (1996) temporarily retained males and females in her experiments on Violet Green- and Tree Swallows and could also find an increase of nest intrusions, but in this study paternity data are not available either. Similar to that, Møller (1987) observed an increase of sexual chases and extra-pair copulations after temporal male detainment of 29 Barn Swallow pairs. However, in that study males were removed up to four hours which might induce a "widow effect" in females although there was no mate replacement observed. We would interpret the results of the male detainment experiment conducted on Eastern bluebirds (*Sialia sialis*) by MacDougall-Shackleton et al. (1996) in that direction. They removed males during their females´ egg-laying phase for two days and beside an increase of extra-pair paternity they also reported replacements of the original male in three out of eleven pairs. Therefore we would caution to interpret that the removal of a pair male "releases his female partner from mate guarding constraints". Moreover, it appears to us that if the male is detained for such a long time the "widowed" female accepts extra-pair copulations to secure replacement for later paternal care.

Despite the differences in experimental methods and the expected species-specific variances in mating behaviour, all the above cited studies have an important outcome in common. That is on one hand that if pair males are temporally detained during the fertile phase of their mates territory or nest intrusions by extra-pair males increase significantly and on the other hand that females do not actively foray for extra-pair copulations. The latter would be expected if male mate guarding is definitely a constraint for females to prevent them from cuckoldry. Yet, while two of the studies found few incidences with females accepting extra-pair copulations from intruders (Björklund and Westman 1983; Dickinson 1997), the majority found the opposite. The main part of experimental females of these studies refused to copulate with or even reacted aggressively to intruding extra-pair males. This was actually shown in Blue Tits and Tree Swallows, species for which

female solicitation of extra-pair copulations have been reported (Kempenaers et al. 1992; Venier et al. 1993). The result of our male detainment experiment could most likely be explained in this context. Although we cannot completely discern which disturbance factor lead to abandonment, it is probable that mainly increased sexual harassment by intruding extra-pair males was responsible for our finding.

We therefore conclude that experiments involving removal of males during the fertile (egg laying) phase of their female partner in socially monogamous bird species are only a suboptimal tool to disentangle causes and consequences of female and male extra-pair behaviour. The studies cited above have shown that these experiments mainly manipulate male-male competition and create an unsafe environment for females. The goal to facilitate female induced extra-pair behaviour could not be reached. Experiments may nevertheless be helpful in certain cases, but only by carrying out thorough behavioural observations and additional measures to prevent females from being harassed. Such experiments would require a significant increase of study effort which may be the reason why removal experiments are not commonly applied in studies of extra-pair behaviour.

REFERENCES

Akçay, E., Roughgarden, J. (2007) Extra-pair paternity in birds: review of the genetic benefits. *Evolutionary Ecology Research* **9**: 855–868.

Albrecht, T., Kreisinger, J., Pialek, J. (2006) The strength of direct selection against female promiscuity is associated with rates of extrapair fertilizations in socially monogamous songbirds. *American Naturalist* **167**: 739-744. doi: 10.1086/502633

Arnqvist, G., Kirkpatrick, M. (2005) The Evolution of Infidelity in Socially Monogamous Passerines: The Strength of Direct and Indirect Selection on Extrapair Copulation Behavior in Females. *The American Naturalist* **165**: S26-S37. doi: 10.1086/429350

Arnqvist, G., Rowe, L. (2005) *Sexual conflict*. Princeton University Press, Princeton

Beasley, B.A. (1996) Males on guard: paternity defences in violet-green swallows and tree swallows. *Animal Behaviour* **52**: 1211-1224. doi: 10.1006/anbe.1996.0269

Birkhead, T.R., Møller, A.P. (1992) *Sperm competition in birds. Evolutionary causes and consequences*. Acad. Press, Harcourt Brace Jovanovich Publishers, London, San Diego, New York, Boston, Sydney, Tokyo, Toronto

Birkhead, T.R., Møller, A.P. (1993) Why do male birds stop copulating while their mates are still fertile? *Animal Behaviour* **45**: 105-118. doi: 10.1006/anbe.1993.1010

Björklund, M., Møller, A.P., Sundberg, J., Westman, B. (1992) Female great tits, *Parus major*, avoid extra-pair copulation attempts. *Animal Behaviour* **43**: 691-693. doi: 10.1016/s0003-3472(05)81032-2

Björklund, M., Westman, B. (1983) Extra-Pair Copulations in the Pied Flycatcher (*Ficedula hypoleuca*): A Removal Experiment. *Behavioral Ecology and Sociobiology* **13**: 271-275. doi: 10.1007/bf00299674

Bryant, D.M. (1975) Changes in Incubation Patch and Weight in the Nesting House Martin. *Ringing & Migration* **1**: 33-36. doi: 10.1080/03078698.1975.9673696

Bryant, D.M. (1979) Reproductive costs in the House Martin (*Delichon urbica*). *Journal of Animal Ecology* **48**: 655-675. doi: 10.2307/4185

Chaine, A.S., Montgomerie, R., Lyon, B.E. (2015) Sexual conflict arising from extrapair matings in birds. *Cold Spring Harbor Perspectives in Biology* **7**: doi: 10.1101/cshperspect.a017590

Chuang-Dobbs, H.C., Webster, M.S., Holmes, R.T. (2001) The effectiveness of mate guarding by male black-throated blue warblers. *Behavioral Ecology* **12**: 541-546. doi: 10.1093/beheco/12.5.541

Dickinson, J.L. (1997) Male detention affects extra-pair copulation frequency and pair behaviour in western bluebirds. *Animal Behaviour* **53**: 561-571. doi: 10.1006/anbe.1996.0331

Griffith, S.C., Owens, I.P.F., Thuman, K.A. (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology* **11**: 2195-2212. doi: 10.1046/j.1365-294X.2002.01613.x

Hund, K., Prinzinger, R. (1979) Untersuchungen zur Biologie der Mehlschwalbe *Delichon urbica* in Oberschwaben *Ecology of Birds* **1**: 133-158. doi: 10.1007/BF01640440

Johnsen, A., Lifjeld, J.T., Rohde, P.A., Primmer, C.R., Ellegren, H. (1998) Sexual conflict over fertilizations: female bluethroats escape male paternity guards. *Behavioral Ecology and Sociobiology* **43**: 401-408. doi: 10.2307/4601536

Kempenaers, B., Verheyen, G.R., Dhondt, A.A. (1995) Mate guarding and copulation behaviour in monogamous and polygynous blue tits : do males follow a best-of-a-bad-job strategy? *Behavioral Ecology and Sociobiology* **36**: 33-42. doi: 10.1007/BF00175726

Kempenaers, B., Verheyen, G.R., Van den Broeck, M., Burke, T., Van Broeckhoven, C., Dhondt, A.A. (1992) Extra-pair paternity results from female preference for high-quality males in the blue tit. *Nature* **357**: 494-496. doi: 10.1038/357494a0

Lack, D. (1968) *Ecological Adaptions for Breeding in Birds*. Methuen, London

Lifjeld, J.T., Marstein, B. (1994) Paternity assurance behavior in the House Martin *Delichon urbica*. *Journal of Avian Biology* **25**: 231-238. doi: 10.2307/3677080

Lifjeld, J.T., Robertson, R.J. (1992) Female control of extra-pair fertilization in tree swallows. *Behavioral Ecology and Sociobiology* **31**: 89-96. doi: 10.1007/bf00166341

Lifjeld, J.T., Slagsvold, T., Ellegren, H. (1997) Experimental mate switching in pied flycatchers: male copulatory access and fertilization success. *Animal Behaviour* **53**: 1225-1232. doi: 10.1006/anbe.1996.0430

MacDougall-Shackleton, E.A., Robertson, R.J., Boag, P.T. (1996) Temporary male removal increases extra-pair paternity in eastern bluebirds. *Animal Behaviour* **52**: 1177-1183. doi: 10.1006/anbe.1996.0265

Møller, A.P. (1987) Mate Guarding in the Swallow *Hirundo rustica*: An Experimental Study. *Behavioral Ecology and Sociobiology* **21**: 119-123. doi: 10.1007/bf02395439

Møller, A.P., Ninni, P. (1998) Sperm competition and sexual selection: a meta-analysis of paternity studies of birds. *Behavioral Ecology and Sociobiology* **43**: 345-358. https://link.springer.com/article/10.1007/s002650050501

Riley, H.T., Bryant, D.M., Carter, R.E., Parkin, D.T. (1995) Extra-pair fertilizations and paternity defence in house martins, *Delichon urbica*. *Animal Behaviour* **49**: 495-509. doi: 10.1006/anbe.1995.0065

Sheldon, B.C., Merilä, J., Qvarnström, A., Gustaffson, L., Ellegren, H. (1997) Paternal genetic contribution to offspring condition predicted by size of male secondary sexual character. *Proceedings of the Royal Society B* **264**: 297-302. doi: 10.1098/rspb.1997.0042

Trivers, R.L. (1972) *Parental Investment and Sexual Selection*. In: Campbell, B. (ed) Sexual selection and the descent of man 1871-1971. Aldine, Chicago, pp 136-179

Venier, L.A., Dunn, P.O., Lifjeld, J.T., Robertson, R.J. (1993) Behavioural patterns of extra-pair copulation in tree swallows. *Animal Behaviour* **45**: 412-415. doi: 10.1006/anbe.1993.1050

Westneat, D.F. (1994) To guard mates or go forage: conflicting demands affect the paternity of male Red-Winged Blackbirds. *The American Naturalist* **144**: 343-354. http://www.jstor.org/stable/2463165

Westneat, D.F., Sherman, P.W., Morton, M.L. (1990) The ecology and evolution of extra-pair copulations in birds. *Current Ornithology* **7**: 331-369.

Westneat, D.F., Stewart, I.R.K. (2003) Extra-Pair Paternity In Birds: Causes, Correlates, and Conflict. *Annual Review of Ecology Evolution and Systematics* **34**: 365-396. doi: 10.1146/annurev.ecolsys.34.011802.132439

Whittingham, L.A., Lifjeld, J.T. (1995) Extra-pair fertilizations increase the opportunity for sexual selection in the monogamous House Martin *Delichon urbica*. *Journal of Avian Biology* **26**: 283-288. doi: 10.2307/3677042

II Concluding Discussion

The general aim of my study was to test if a broader concept of sexual conflict can explain variation in reproductive success and extra-pair strategies in European House Martins (*Delichon urbicum urbicum*). In Chapter 1, I gave an overview about extra-pair paternity frequencies in our study colonies of House Martins and looked for a basic sexual conflict indicated by different reproductive skews. I provide results from investigations to test adaptive functions of extra-pair paternity in relation to sexual conflict in three chapters as outlined in the general introduction.

In Chapter 1, we report that on average 20.5% (107/521) of offspring did not match the genetic profile of the social father and 42.9% of broods (67/156) contained 1.7 offspring sired by an extra-pair male. These EPP frequencies are comparable to an earlier study on House Martins done by Whittingham and Lifjeld (1995) who reported slightly lower values, with 19% of chicks (35% of broods) of extra-pair origin.

We also found differential reproductive skews and selection gradients in male and female House Martins indicating a fundamental sexual conflict in reproductive interests of the sexes. As males can at least double their reproductive success with increasing the number of matings, females enhance offspring size by mating up to three males, but do not gain fitness by mating with even more partners. These sex-specific differences are in accordance with earlier theories on mating strategies of males and females (Trivers 1972).

Our results suggest that age and body condition of males are the best predictors to explain variance in individual reproductive success. They also corroborate other studies which showed that male age plays a predominant role in reproductive success in various species (e.g.: Bouwman et al. 2007; Schmoll et al. 2007; Mitrus et al. 2014). Yet, because age is confounded with several extrinsic and intrinsic factors (see: Lifjeld et al. 2011) we cannot clearly state which of these factors may have played a role in the age-related variation found. The proportion of extra-pair paternity in our population was related to male age, male body condition, and female age. The number of extra-pair offspring increased when the social father was younger than its mate and in weak body condition. Moreover we found that when looking whether there is extra-pair offspring in a nest or not, male age and length of the longest wing feather of females were the best predictors for the occurrence of extra-pair offspring in a nest. In other words, the likelihood of extra-pair paternity increases with decreasing condition of the social father and decreasing length of wing feathers of the mother. The way we interpret this finding is that males in bad body

condition are not able to properly defend their mates against nest intruding extra-pair males and that females with shorter wings are not able to escape fast enough when getting harassed by extra-pair males outside the nest. This may happen, for instance when they are collecting nest material. We suggest that extra-pair matings in House Martins are mainly male induced. However, females may also gain indirect (genetic) benefit through older males of good heritable body condition siring their extra-pair offspring.

In Chapter 2 we report the results of a study aimed to test whether females might gain indirect (genetic) benefits from mating with extra-pair males. Positive effects may be realized as increased genetic heterozygosity and viability of offspring. We found a negative correlation between the genetic similarity within a pair and the number of their offspring. This effect was not observed for extra-pair offspring. A similar effect was found for nestling survival. Within-pair offspring of genetically similar parents had a lower survival rate, but survival of extra-pair offspring was not affected at all. An important result was that male age related positively to reproductive success (within-pair and extra-pair offspring).
From our results we therefore conclude that although genetic similarity of social parents affects numbers and survival of total amount of offspring but not extra-pair offspring, active female choice for genetic benefits as a possible selective mechanism is unlikely. Moreover we infer from our data that continuous selection on disadvantageous genotypes and the resulting higher average reproductive performance of older males explains most of the reproductive success and extra-pair mating in House Martins. We could not fully reject hidden female choice mechanisms, or genetic benefits accrued by precopulatory female choice as fitness relevant factors. Our methods could detect variation in only a few variables. Because of the fact that extra-pair mating is heavily dependent on the efficacy of male mate guarding we tried to experimentally increase the opportunity for females to actively engage in extra-pair mating. These experiments were addressed in the next chapter.

In Chapter 3 we aimed to test in an experimental approach whether females are more likely to pursue extra-pair copulations when they are free from the constraints exerted by the mate guarding of their social mates. We therefore detained males during the fertile phase of their mates for 60 min during morning hours. Unfortunately all of our 15 experimental pairs stopped egg-laying and in 11 out of 15 cases females left their nest and partner. This is in contrast to other studies which also applied male detainment experiments in House

Martins (Riley et al. 1995) and other passerines (Møller 1987; Björklund et al. 1992; Lifjeld and Robertson 1992). None of these studies reported effects as dramatic as those observed by us. To our knowledge there is only one study that actually demonstrated that extra-pair paternity is increasing when males are not able to guard their pair females during their fertile phase (Chuang-Dobbs et al. 2001). Behavioural observations from our experiment and other studies applying this experimental approach did not show that females are more likely to pursue extra-pair copulations when their social partner is temporarily absent. We therefore conclude that temporal male detainment experiments in general are only a suboptimal tool to test the efficacy of male mate guarding. On the basis of our experiment we rather suggest that it mainly enhanced male-male competition and harassment of females. Our observations provided, however, further evidence that in our House Martin population extra-pair paternity was male driven.

To summarize we can say that this work provided substantial evidence in support of a broader concept of sexual conflict as a framework for investigating sex-specific differences in reproductive success in relation to paternity. Although we could not fully exclude female choice as a possible mechanism for extra-pair paternity, I conclude from our results that sexual conflict over reproductive success in House Martins is more pronounced as it could be shown in previous studies. My work pointed out that extra-pair mating in House Martins is mainly male driven which gives interesting insight into the behavioural ecology of mating strategies in socially monogamous species.

REFERENCES

Björklund, M., Møller, A.P., Sundberg, J., Westman, B. (1992) Female great tits, *Parus major*, avoid extra-pair copulation attempts. *Animal Behaviour* **43**: 691-693. doi: 10.1016/s0003-3472(05)81032-2

Bouwman, K.M., Van Dijk, R.E., Wijmenga, J.J., Komdeur, J. (2007) Older male reed buntings are more successful at gaining extrapair fertilizations. *Animal Behaviour* **73**: 15-27. doi: 10.1016/j.anbehav.2006.01.031

Chuang-Dobbs, H.C., Webster, M.S., Holmes, R.T. (2001) The effectiveness of mate guarding by male black-throated blue warblers. *Behavioral Ecology* **12**: 541-546. doi: 10.1093/beheco/12.5.541

Lifjeld, J.T., Kleven, O., Jacobsen, F., McGraw, K.J., Safran, R.J., Robertson, R.J. (2011) Age before beauty? Relationships between fertilization success and age-

dependent ornaments in barn swallows. *Behavioral Ecology and Sociobiology* **65**: 1687-1697. doi: 10.1007/s00265-011-1176-4

Lifjeld, J.T., Robertson, R.J. (1992) Female control of extra-pair fertilization in tree swallows. *Behavioral Ecology and Sociobiology* **31**: 89-96. doi: 10.1007/bf00166341

Mitrus, J., Mitrus, C., Rutkowski, R., Sikora, M. (2014) Extra-pair paternity in relation to age of the Red-breasted Flycatcher *Ficedula parva* males. *Avian Biology Research* **7**: 111-116. doi: 10.3184/175815514X13948188185179

Møller, A.P. (1987) Mate Guarding in the Swallow *Hirundo rustica*: An Experimental Study. *Behavioral Ecology and Sociobiology* **21**: 119-123. doi: 10.1007/bf02395439

Riley, H.T., Bryant, D.M., Carter, R.E., Parkin, D.T. (1995) Extra-pair fertilizations and paternity defence in house martins, *Delichon urbica*. *Animal Behaviour* **49**: 495-509. doi: 10.1006/anbe.1995.0065

Schmoll, T., Mund, V., Dietrich-Bischoff, V., Winkel, W., Lubjuhn, T. (2007) Male age predicts extrapair and total fertilization success in the socially monogamous coal tit. *Behavioral Ecology* **18**: 1073-1081. doi: 10.1093/beheco/arm082

Trivers, R.L. (1972) *Parental Investment and Sexual Selection*. In: Campbell, B. (ed) Sexual selection and the descent of man 1871-1971. Aldine, Chicago, pp 136-179

Whittingham, L.A., Lifjeld, J.T. (1995) Extra-pair fertilizations increase the opportunity for sexual selection in the monogamous House Martin *Delichon urbica*. *Journal of Avian Biology* **26**: 283-288. doi: 10.2307/3677042

III Summary

Although the majority of contemporary birds mate monogamously, numerous studies using molecular paternity tests showed that the social father frequently is not the genetic father. Such extra-pair paternity was found in about 80% of monogamous bird species studied, with the proportion of extra-pair chicks per brood or offspring numbers showing remarkable interspecific variation. To explain the functional adaptions of this phenomenon several hypotheses have been proposed. Despite extensive descriptive and experimental research conflicting evidence was yielded and no single hypothesis gained sufficient support as a general explanation. Within the framework of my dissertation, I investigated whether hypotheses that propose a fundamental sexual conflict can be applied to a socially monogamous species that mates without overt conflict and can satisfactorily explain extra-pair paternity and its variance.

As study species I chose the European House Martin (*Delichon urbicum urbicum*), a colony breeding passerine species in which earlier studies had already detected extra-pair paternity and biparental care for the altricial offspring. In a study conducted over three years on two House Martin colonies I related reproductive, ecological and individual phenotypic data in a correlational and experimental approach to paternity. Our analyses revealed a different skew of male and female reproductive success (RS) in relation to number of mating partners indicating a fundamental sexual conflict of reproductive interests between the sexes. Furthermore we found that males increase their individual RS with age. In case of extra-pair paternity we could show that the proportion of extra-pair offspring in a nest increases when older females are paired with younger males in poor body condition. More general, the likelihood of extra-pair fertilization increases with disadvantageous male body condition and with shorter wings in females. Taking into account that persistence of extra-pair behaviour in male House Martins is intensive this indicates a strong male effect on variance of reproductive patterns in this species. Nevertheless females might gain indirect (genetic) benefits from such extra-pair encounters which for instance might be expressed in higher genetic diversity of extra-pair offspring. For this hypothesis, however, we could not find clear evidence. We temporarily detained males during the fertile phase of their partner in an experiment. We could not observe that females were more likely to engage in extra-pair matings. In summary, our results indicate that despite the apparently consentient social mating system in this species, sexual conflict is more pronounced as originally expected. Although we cannot completely rule out

cryptic behavioural or post copulatory female choice mechanisms, we found intriguing evidence that extra-pair behaviour in House Martins is primarily male driven.

IV Zusammenfassung

Obwohl sich der überwiegende Teil der rezent vorkommenden Vögel monogam verpaart, konnten zahlreiche Studien mittels molekularer Vaterschaftsanalysen zeigen, dass der soziale Vater nicht immer auch der genetische Vater ist. Diese „Fremdvaterschaften“ wurden bei mehr als 80% der untersuchten monogamen Vogelarten nachgewiesen, wobei der Anteil an „Kuckuckskindern“ pro Brut bzw. Population zwischenartlich deutlich variiert. Um die funktionalen Anpassungen dieses Phänomens zu erklären, wurden verschiedenste Hypothesen vorgeschlagen. Trotz einer Vielzahl an Untersuchungen, die Verhaltensbeobachtungen und Experimente umfassten, waren die Ergebnisse so unterschiedlich, dass keine der Hypothesen allgemeine Gültigkeit erlangte. Im Rahmen meiner Dissertation ging ich der Frage nach, ob Hypothesen, die sich aus einem basalen sexuellen Konflikt ableiten lassen, auch bei sozial monogamen Arten, die sich scheinbar in absoluter Übereinstimmung verpaaren, überprüfbar sind und ob sie Fremdvaterschaften sowie deren Variabilität erklären können.

Meine Studienart war die Europäische Mehlschwalbe (*Delichon urbicum urbicum*), ein in Kolonien brütender Singvogel, bei dem einerseits Fremdvaterschaften, andererseits aber auch hoher elterlicher Aufwand für die nesthockenden Jungen nachgewiesen wurde. In einer dreijährigen Studie zweier Mehlschwalbenkolonien untersuchte ich brutbiologische, ökologische, sowie individuelle phänotypische Parameter und deren Zusammenhang mit Vaterschaften. Erste Analysen unserer Ergebnisse zeigten eine deutliche Schiefe im Fortpflanzungserfolg von Männchen und Weibchen in Verbindung mit der Anzahl der Geschlechtspartner. Dies weist auf einen basalen sexuellen Konflikt zwischen den unterschiedlichen Fortpflanzungsinteressen der beiden Geschlechter hin. Weiters konnten wir bei Männchen eine Zunahme des individuellen Fortpflanzungserfolgs mit fortschreitendem Alter zeigen. In Bezug auf Fremdvaterschaften stellten wir fest, dass das Verhältnis an Jungen, deren genetischer Vater nicht mit dem sozialen übereinstimmt, ansteigt, wenn ältere Weibchen mit jüngeren Männchen in schlechter körperlicher Konstitution verpaart waren. Im Allgemeinen steigt die Wahrscheinlichkeit von Fremdvaterschaften, wenn der soziale Vater in schlechter körperlicher Verfassung ist und die Mutter kurze Flügel aufweist. Wenn man dabei berücksichtigt, dass männliche Mehlschwalben deutliche Hartnäckigkeit in ihrem “Fremdgehverhalten“ zeigen, unterstreichen diese Ergebnisse den Effekt der Männchen auf die Variabilität von Fortpflanzungsmustern bei dieser Art. Nichtsdestotrotz könnten Weibchen indirekte

(genetische) Vorteile durch Kopulationen außerhalb der sozialen Verpaarung gewinnen, zum Beispiel durch Erhöhung der genetischen Diversität der „außerehelichen“ Jungen, was wir jedoch nicht nachweisen konnten. Darüber hinaus konnten wir im Rahmen eines Experiments, bei dem wir Männchen während der fertilen Phase ihrer sozialen Partnerinnen kurzfristig einbehielten, keine Zunahme an außerpartnerschaftlichem Verhalten der Weibchen beobachten. Zusammenfassend zeigen unsere Ergebnisse, dass, trotz offensichtlich übereinstimmender sozialer Verpaarungen, der sexuelle Konflikt bei dieser Art stärker ausgeprägt ist als ursprünglich erwartet. Obwohl wir versteckte Selektionsmechanismen durch prä- und postkopulatorischer Weibchenwahl nicht gänzlich ausschließen können, fanden wir überzeugende Hinweise dafür, dass bei Mehlschwalben Verpaarungen außerhalb der sozialen Bindung in erster Linie durch die Männchen gesteuert werden.