Infidelity in Birds – Causes and Consequences of Extra-pair Paternity

Jakob Augustin, 2012

ABSTRACT

Forty years ago, more than 90% of bird species were classified as monogamous and not very exciting systems for studies of e.g. sexual selection. Since then, the discovery of extra-pair paternity (EPP) in more than 75% of surveyed monogamous bird species has made avian monogamy, and the interaction between social and genetic mating systems in general, a challenging and attractive area of research. Despite three decades of research on EPP in birds, however, many questions and controversies remain unresolved. This thesis contributes to the understanding of mechanisms and adaptive reasons, primarily from the female's perspective, for the highly diverse frequencies of EPP in birds.

First, in a population of the common redshank (*Tringa totanus*), a wader for which the genetic mating system has not been described previously, a surprising absence of EPP is demonstrated (**I**). Presumably, some female pre- or postcopulatory resistance to extra-pair fertilisations is present. The potential mechanisms and adaptive significance of this is discussed in relation to redshank ecology and behaviour.

In the three following papers (**II-IV**), assumptions and predictions of hypothesised female benefits from EPP are addressed. In sand martins (*Riparia riparia*), there were no indications that extra-pair fertilisations resulted in genetic benefits (e.g. heterozygosity or 'good genes') (**II**). Paper **III** tests an assumption related to the genetic compatibility hypothesis, i.e. that overall heterozygosity leads to increased chick survival; this did not seem to be the case in Kentish plovers (*Charadrius alexandrinus*). In northern lapwings (*Vanellus vanellus*), the indirect benefits hypothesis is partly supported by a positive association between EPP and brood sex ratio (**IV**). As predicted by the differential sex allocation hypothesis, broods with extra-pair offspring contained a higher proportion of sons than broods without extra-pair offspring. As for the yet unknown mechanism of sex determination in birds, an unusual case of a fertile, triploid Kentish plover female is presented and discussed with regard to the two present major hypotheses for sex-determination (**VI**). Finally, as an alternative or additional interpretation of what appears to be brood sex ratio adjustment by the female, the often neglected effect of differential mortality is discussed (**V**).

Keywords: Extra-pair paternity, genetic benefits, heterozygosity, sex ratio, *Riparia riparia, Tringa totanus, Vanellus vanellus, Charadrius alexandrinus*

LIST OF ARTICLES & MANUSCRIPS

This thesis is based on the following articles and manuscripts:

I   **Augustin J**, Isaksson D, Pauliny A, Wallander J & Blomqvist D. No evidence of extra-pair paternity in the common redshank (*Tringa totanus*). *Manuscript*

II **Augustin J**, Blomqvist D, Szép T, Szabó ZD & Wagner RH (2007). No evidence of genetic benefits from extra-pair fertilizations in female sand martins (*Riparia riparia*). *Journal of Ornithology* 148: 189-198

III Küpper C, Kosztolányi A, **Augustin J**, Dawson DA, Burke T & Székely T (2010). Heterozygosity-fitness correlations of conserved microsatellite markers in Kentish plovers *Charadrius alexandrinus. Molecular ecology* 19: 5172-5185

IV **Augustin J**, Grønstøl GB, Pauliny A, Wagner RH & Blomqvist D. Variation in brood sex ratio associated with extra-pair paternity in a wader, the northern lapwing (*Vanellus vanellus*). *Manuscript*

V   **Augustin J** & *Bartoszek K.* Are you sure you have shown primary sex ratio adjustment? The problem of differential mortality revisited. *Manuscript*

VI Küpper C, **Augustin J**, Edwards S, Székely T, Kosztolányi A & Janes AE. Triploid plover female provides support for a role of the W chromosome in avian sex determination. *Manuscript*

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INTRODUCTION

Once two birds fall in love, they stick together for the rest of their lives. Every year they work hard to build a cosy nest, in which they raise a bunch of babies with care and discretion. Something along these lines may be the way a lot of people think about birds. Ornithological research never had such a romantic view on birds, but not so long ago, 90% of bird species were classified as being monogamous (Lack 1968). The lack of enthusiasm felt by some researchers for this mating system was (jokingly) summarized by Mock (1985) as follows, '*Monogamous birds do not establish spectacular leks and only occasionally are highly ornamented. On the surface, monogamy has seemed relatively tame and uniform, with a single male mating routinely with a single female. Not only has sexual selection appeared feeble, but the whole package seems bland.*'

From what we know today, things became exciting rather than bland for avian monogamy. As molecular genetic tools to determine parentage became available and applied to wild bird populations, it turned out that the social parents are not necessarily the genetic parents of all their offspring. In fact, true genetic monogamy has been found in less than 25% of surveyed potentially monogamous bird species (reviewed by Griffith et al. 2002). Therefore, when considering mating system in birds today, researchers distinguish between a social and a genetic mating system. The two systems rarely match, predominately because of male and/or female 'sexual infidelity', which often results in what behavioural ecologists usually refer to as extra-pair paternity.

Extra-pair paternity

Extra-pair paternity means that an offspring is fathered by a male (the so called extra-pair male) other than the female's social mate. The finding of significant levels of extra-pair paternity has been suggested as one of the most important empirical discoveries in avian mating systems over the last

30 years (e.g. Bennett & Owens 2002). However, despite three decades of research on extra-pair paternity, we know surprisingly little about its causes and consequences (e.g. Westneat & Stewart 2003). One thing we know for certain, however, is that there is striking variation among species and even among populations in frequencies of extra-pair paternity as well as much diversity in behaviours associated with extra-pair paternity.

Extra-pair paternity ranges from zero percent of broods in e.g. the northern fulmar (for scientific names see: List of scientific and common names, pages 52-53) (Hunter et al. 1992) to 95% in the superb fairy-wren (Mulder et al. 1994). Variation in extra-pair paternity also exists among populations of the same species such as in the willow warbler, where one study reported no extra-pair offspring (Gyllensten et al. 1990) while another study found extra-pair offspring in 50% of the broods (Bjørnstad & Lifjeld 1997). Also within populations, the rate of extra-pair paternity may vary widely between years (Johnsen & Lifjeld 2003). Finally, even within single females the proportion of extra-pair offspring within successive broods may range from zero to 100% (e.g. Charmantier et al. 2004; Dietrich et al. 2004; Bouwman et al. 2006).

Variation in behaviours associated with extra-pair paternity is also remarkable. In bearded tits, for example, females appear to solicit extra-pair males to be chased by them before gaining copulations, maybe to incite male-male competition (Hoi 1997). In contrast, in some waterfowl, copulations by extra-pair males (extra-pair copulations) are often forced and females try to resist them (McKinney et al. 1983). Sometimes such forced copulations lead to the female being injured or even drowned (Adler 2010). In the Seychelles warbler, extensive mate guarding (the male following the female closely during her fertile period) decreases the loss of paternity for the social mate (Komdeur et al. 1999). In bluethroats, however, males that guarded more nevertheless lost more paternity (Johnsen et al. 1998b), which probably was related to a guarding strategy of less attractive or less competitive males (Johnsen et al. 1998a).

To explain this diversity of extra-pair paternity frequencies and associated behaviours has been challenging and difficult. Numerous studies on various aspects of extra-pair paternity have been conducted but many

basic questions remain, such as: Is extra-pair mating behaviour typically male- or female-driven? How do females benefit from soliciting, or at least not resisting, extra-pair copulations (or, subsequently, extra-pair fertilisation)? In other words, what is the adaptive function of extra-pair paternity from a female perspective?

Adaptive explanations for extra-pair paternity

Trivers (1972) pointed out that monogamous males should retain promiscuous tendencies to try fertilise other females that they do not raise young with, thus increasing reproductive success without the costs of parental investment. The first studies on extra-pair paternity focused mainly on the male perspective on extra-pair mating behaviour, e.g. male responses to being cuckolded, both towards his female and towards potential extra-pair males (e.g. Barash 1976; Barash 1977; Beecher & Beecher 1979).

Only about 3% of bird species possess a so called intromittent organ (an external organ, analogous to the mammalian penis, to deliver sperm during copulation) (Briskie & Montgomerie 1997) while most birds copulate by pressing their cloacae together. Therefore, it has been argued that sperm transfer depends so much on female cooperation that forced copulations should be unlikely (Fitch & Shugart 1984) and exceptional (reviewed by Thornhill & Palmer 2000). However, a few exceptions do exist; for example in waterfowl (McKinney & Evarts 1998), and in the stitch bird where males force copulations by a unique face to face copulation technique (Low 2005). Nevertheless, it seems increasingly clear that females are usually not passively subjected to extra-pair copulations, but rather play an active part in soliciting and participating in extra-pair copulations, as shown in a range of species, e.g. northern fulmar (Hatch 1987), zebra finches (Birkhead et al. 1988), black-capped chickadees (Smith 1988), house sparrows (Møller 1990), blue tits (Kempenaers et al. 1992) and tree swallows (Lifjeld & Robertson 1992). This raised the question of how and if females benefit from extra-pair copulations (Birkhead & Møller 1992). Several types of female benefits have been proposed (reviewed by e.g. Westneat et al. 1990; Kempenaers & Dhondt

1993; e.g. Zeh & Zeh 1996; Zeh & Zeh 1997; Petrie & Kempenaers 1998; Griffith et al. 2002; Westneat & Stewart 2003; Akçay & Roughgarden 2007; Slatyer et al. 2012) which can be assigned to either direct or indirect fitness benefits.

Female benefits from extra-pair fertilizations

Direct (non-genetic) benefits refer to benefits that directly enhance female survival or fecundity (Kirkpatrick & Ryan 1991). Birds are breeding on all continents, under very diverse environmental and ecological circumstances. Hence, there is a large number of ecological and behavioural factors that influence a female's survival or fecundity. Direct benefits include e.g. assuring fertilization of the eggs in case the within pair male is infertile, access to resources provided by males, defence against predation (e.g. Reynolds 1996). Insurance against mate infertility (Wetton & Parkin 1991) might be the most universal type of direct benefits from extra-pair copulations. A recent study in Norway reported frequencies of azoospermia (a lack of sperm) of 4% in willow warblers and 2% in bluethroats (Lifjeld et al. 2007). Azoospermia, however, is only one cause of male infertility. Other causes of male infertility include dysfunctional copulation behaviour (i.e. unsuccessful sperm transfer), low sperm counts, and low sperm quality (e.g. Johnson 1986; Birkhead & Møller 1992).

As a safeguard against all the above, frequent copulations with the same or more mates can therefore increase fertilisation success (Walker 1980; Gibson & Jewell 1982). Another type of direct benefits is some form of resource acquisition: In common terns, for example, females beg for food from any male that approach them and both observed extra-pair copulation attempts were preceded by courtship feeding (Gonzalez-Solis et al. 2001). In a study on great grey shrikes, males offered more valuable nuptial gifts before extra-pair copulations than before within-pair copulations (Tryjanowski & Hromada 2005). Direct benefits from extra-pair copulations that are not resource based are scarce. However, one example is the red-winged blackbird. In this species extra-pair males assist in mobbing nest

predators (Gray 1997b), which may explain the higher fledging success of females engaging in extra-pair copulations (Gray 1997a).

Indirect (or genetic) benefits, which are the focus of this thesis, arise because certain genes or gene combinations, gained from extra-pair matings, raise the mean offspring fitness (Jennions & Petrie 2000). Indirect fitness benefits include 'viability genes', 'attractiveness genes', and 'compatible genes' (e.g. Jennions & Petrie 2000). They enhance the viability and/or mating success of the offspring (e.g Westneat et al. 1990; Kirkpatrick & Ryan 1991; Birkhead & Møller 1992; Andersson 1994; Reynolds 1996; Jennions & Petrie 2000; Tregenza & Wedell 2000; Griffith et al. 2002; Akçay & Roughgarden 2007).

'Viability genes' (or 'good genes') refer to heritable traits that improve offspring survival. 'Attractiveness genes' increase the future mating success of the offspring. To gain such genes for her offspring, a female has to find a social mate or an extra-pair mate that possesses these genes, i.e. males of superior genetic quality. Male traits that might express genetic quality, and that have been correlated with paternity, including e.g. body size and song strophe length in blue tits (Kempenaers et al. 1997), large song repertoires in great reed warblers (Hasselquist et al. 1996) and body condition in barn swallows (Møller et al. 2003). Another trait that might reflect genetic quality is age, because age demonstrates directly an individual's viability (e.g. Trivers 1972; Manning 1985; Kokko & Lindström 1996). A positive relationship between within-pair paternity and male age has been found in several species, such as purple martins (Morton et al. 1990; Wagner et al. 1996), Bullock's orioles (Richardson & Burke 1999), lazuli buntings (Greene et al. 2000) and reed buntings (Bouwman et al. 2007), although these correlations may also reflect that old males are better at mate guarding (e.g. Johnsen et al. 2003).

Females might also engage in extra-pair copulations to gain 'compatible genes', which refer to how well the male and female genome match each other (Zeh & Zeh 1996; Tregenza & Wedell 2000), but often it refers to avoiding homozygote disadvantages in the offspring (Pusey & Wolf 1996). Thus, by mating with extra-pair males that are genetically more dissimilar to the female than her social mate, the female can reduce the risk

of producing offspring of low genetic compatibility (e.g. Tregenza & Wedell 2000).

In birds, individual heterozygosity has been found to be positively associated with offspring survival (Hansson et al. 2001; Foerster et al. 2003), reproductive success (Foerster et al. 2003; Seddon et al. 2004), and sexually selected traits such as plumage ornamentation (Foerster et al. 2003), song repertoire size (Marshall et al. 2003) and song structure (Seddon et al. 2004). Support that females might seek extra-pair fertilizations in order to have their offspring fathered by genetically dissimilar males comes from recent studies showing a positive relationship between extra-pair fertilizations and genetic similarity between social mates in three shorebird species (Blomqvist et al. 2002), Mexican jays (Eimes et al. 2005) and splendid fairy wrens (Tarvin et al. 2005). A study of blue tits also found that extra-pair offspring were more heterozygous than their maternal half-siblings (Foerster et al. 2003).

However, genetically disassortative mating might sometimes lead to outbreeding depression in the offspring by breaking up coadapted gene complexes, or locally adapted gene combinations (Bateson 1983; Pusey & Wolf 1996; Kokko & Ots 2006). Thus, females might seek extra-pair copulations in order to have their offspring sired by genetically more similar males to decrease outbreeding costs. Such a relationship was found in pied flycatchers in which breeding pairs with low genetic similarity had more extra-pair young in their broods and produced fewer fledglings (Rätti et al. 1995).

In general, relationships between heterozygosity and fitness related traits are referred to as heterozygosity fitness correlations. Heterozygosity itself can refer to a single locus or an overall measurement of the genome and deserves closer examination.

Heterozygosity fitness correlations

Many studies have reported positive associations between genetic diversity and fitness parameters such as parasite resistance, reproductive success or

survival in wild populations (David 1998; Hansson & Westerberg 2002; Piertney & Oliver 2006; Kempenaers 2007; Chapman et al. 2009). Microsatellite markers have been the most popular markers to estimate genetic diversity in non-model organisms (Hansson & Westerberg 2002; Coltman & Slate 2003; Chapman et al. 2009). It is not yet clear how such positive associations between microsatellite heterozygosity and fitness come about, but three mechanisms have been suggested (Hansson & Westerberg 2002): First, microsatellite markers may directly affect fitness when located in a coding region e.g. by causing a deleterious shift of the reading frame (Metzgar et al. 2000; Tóth et al. 2000). Second, the markers might be linked to functional loci and different microsatellite alleles associated with certain alleles of the functional locus ('local effect', Hansson & Westerberg 2002). Third, multiple genetic markers scattered across the whole genome may provide an estimate of overall (genome-wide) heterozygosity, which may serve as a proxy for the inbreeding coefficient (Hansson & Westerberg 2002). A positive heterozygosity fitness correlation would then be directly related to inbreeding depression ('general effect', Hansson & Westerberg 2002). Recently a debate whether heterozygosity fitness correlations describe the impact of inbreeding through general or local effects was reignited by suggestions that the significance of local effects is overestimated by inappropriate statistical testing and multilocus heterozygosity estimators provide a better proxy to investigate inbreeding (Szulkin et al. 2010).

Sex ratio and extra-pair paternity

It may take some time until we know if, and to what extent, females gain genetic benefits from extra-pair copulation. In the meantime, we might assume that they do, and test follow up hypotheses e.g. by combining sex allocation theory with paternity data.

In natural populations, a primary sex ratio close to 1:1 is predicted if the costs to produce male and female offspring are equal (Darwin 1871; Düsing 1884, cited by Edwards 1998; Fisher 1930). However, under some circumstances, the expected fitness returns from sons and daughters might

differ so that parents would benefit from skewing the offspring sex ratio towards the more favourable sex (e.g. Trivers & Willard 1973; Charnov 1982). For example, given that a male's ability to compete for mates is partly inherited by sons, then females that are paired to males with high mating success might benefit from producing more sons (e.g. Burley 1981). Similarly, if sons of attractive and/or high quality extra-pair males have a higher fitness potential than their sisters, then extra-pair offspring should be male biased. Indeed, due to molecular sex determination techniques, evidence of skewed brood sex ratio in birds is rapidly accumulating (reviewed by Alonso-Alvarez 2006). To date, there is mixed evidence of an association between extra-pair paternity and brood sex ratio bias in birds. In blue tits (Kempenaers et al. 1997) and house wrens (Johnson et al. 2009) for example, extra-pair young were more likely to be sons. A number of other studies, however, could not find such a sex bias in extra-pair young (e.g. Magrath et al. 2002; Abroe et al. 2007; Delmore et al. 2008). Birds are ideal to investigate predictions from differential sex allocation theory because in birds, the female is the heterogametic sex and therefore (in theory) able to skew the sex ratio of the offspring (Charnov 1982).

Primary sex ratio?

One key variable in sex allocation theory is the primary sex ratio (the sex ratio of offspring at fertilization) and should be estimated accurately. However, since sex ratios are usually measured long after the ('primary') zygote stage, such measurements have to be interpreted with great caution. One reason for that is that an observed skewed brood sex ratio might as well be the result of differential mortality during the preceding stages (Fiala 1980). Differential mortality means that the rate of mortality differs between the sexes. In great tits, blue tits and collared flycatchers it has been shown that unhatched eggs were male biased (Cichon et al. 2005). Unhatched eggs or dead nestlings prior to sampling are common in field studies and so primary sex ratio adjustment is not an exclusive explanation for an observed skewed sex ratio. Some studies have tried to circumvent this problem by

sampling only complete broods (i.e. broods in which no offspring died or got lost prior to sampling) but as e.g. Fiala (1980) pointed out '...*if differential mortality exists then the sample of broods which escape mortality will be biased in favour of the sex with greater survivorship.*' Yet, the misconception that a sample of complete broods represent the primary sex ratio has persisted in the literature (Krackow & Neuhäuser 2008).

Sex determination in birds

With females being the heterogametic sex in birds, the sex of the offspring is determined by the female gamete. In contrast, in most mammals the male's gamete determines whether an offspring is male or female. In humans, for example, the presence of the gene *SRY*, located on the Y chromosome, triggers male development (Koopman et al. 1991). Whether the development of sex in birds is triggered in a similar way is still debated (Teranishi et al. 2001; Smith et al. 2009; Ellegren 2011).

In birds, males possess two Z sex chromosomes whereas females have one Z and one W sex chromosome. It is however debated, how the phenotypic sexual dimorphism is initiated (Teranishi et al. 2001; Smith et al. 2009; Ellegren 2011). Two models have been proposed to explain sex determination in birds (Clinton 1998). The 'Z dosage' model postulates that the main determinant for sex is located on the Z chromosome. This sex determinant interacts with an autosomal gene and, depending on the ratio between copies of Z chromosomes and autosomes (Z:A ratio), the embryo will develop as male or female. If the Z:A ratio equals 1, the embryo will develop into a male and, if the Z:A ratio equals 0.5, the embryo will develop into a female. The other model, the so called 'dominant W' model postulates that female development is triggered by a still unknown W-linked 'female-gene', similar to the *SRY* mentioned above. Chromosomal aberrations, such as aneuploidy with sex chromosomes being present in extra or fewer copies than normally, result in autosomes ratios that might help to clarify the sex determination mechanism. Unfortunately, aneuploidy is often lethal at the embryonic stage in birds (Forstmeier & Ellegren 2010).

FOCUS OF THIS THESIS

Paternity studies over the last 30 years have raised many questions and new ideas about extra-pair paternity. The most interesting unresolved question is probably if, and how, females benefit from extra-pair fertilizations. In this thesis I contribute to the field in two ways. First, I investigate paternity in one monogamous species with so far unknown genetic mating system. Second, I test hypotheses and assumptions related to possible female benefits.

More specifically, together with my co-authors of each particular paper, I:

- reveal the genetic mating system of a semi colonial wader, the common redshank (*Tringa totanus*; **Manuscript I**).

- investigate if females gain genetic benefits from extra-pair copulations in a colonial, short lived passerine, the sand martin (*Riparia riparia;* **Article II**)

- test whether increased heterozygosity increases survival in the offspring as predicted by one 'compatible genes' model (**Article III**).

- investigate a predicted association between brood sex ratio and extra-pair paternity (**Manuscript IV**).

- remind the reader to be careful when analyzing primary sex ratios (**Manuscript V**), report an interesting case of a triploid Kentish plover female and discuss this observation in relation to sex determination in birds (**Manuscript VI**).

STUDY ANIMALS

The sand martin (*Riparia riparia*, Linnaeus, 1758, Sweden)

Sand martins are socially monogamous swallows, coloured brown on the upper side and white on the underside with a brown breast band. The sexes are monomorphic. Their lifespan is approximately 1.7 years (T. Szép, unpublished data). They dig nest holes in sand walls (usually river banks) and breed in small to large colonies. Both parents incubate the 4-5 eggs and provide for the nestlings. Fieldwork was carried out in eastern Hungary at the Tisza River (48.18°N, 21.05°E) near the village of Szabolcs. Our study colony contained about 4900 nest borrows along a riverbank of approximately 300 meters in length.
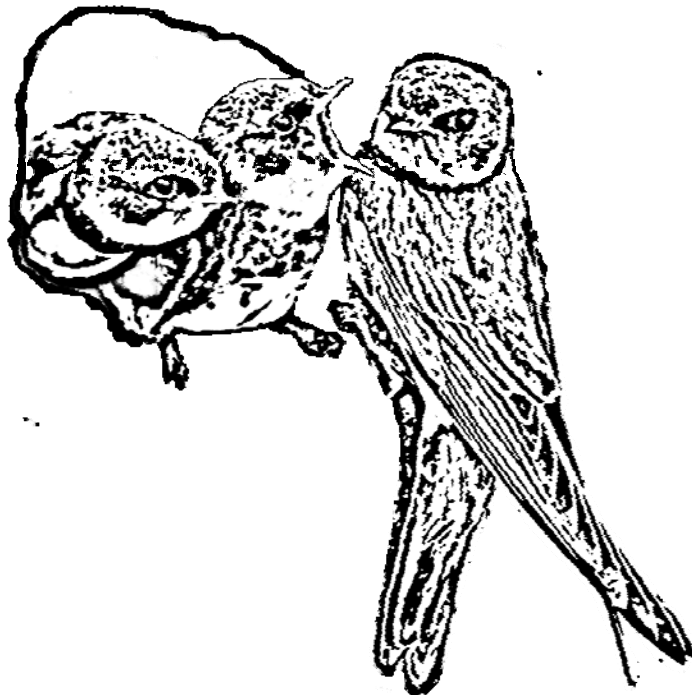
Sand martin, by Jakob Augustin

The common redshank (*Tringa totanus*, Linnaeus, 1758, Sweden)

The common redshank is a medium sized migratory wader, which in breeding plumage is coloured ashy-brown with dark spots all over the body and super-bright red-orange legs and bill. The sexes are monomorphic. The birds breed in open, moist or wet grassland throughout the Palaearctic (Cramp & Simmons 1983). Like in most waders the modal clutch size is four eggs. Redshanks are socially monogamous with bi-parental care and often nest semi-colonially with densities up to 10 nesting pair/ha (Cramp & Simmons 1983; own study: up to 8 nest/ha). The study was carried out at the south-western coast of Sweden (main location Båtafjorden: 57°14´N 12°08´E).
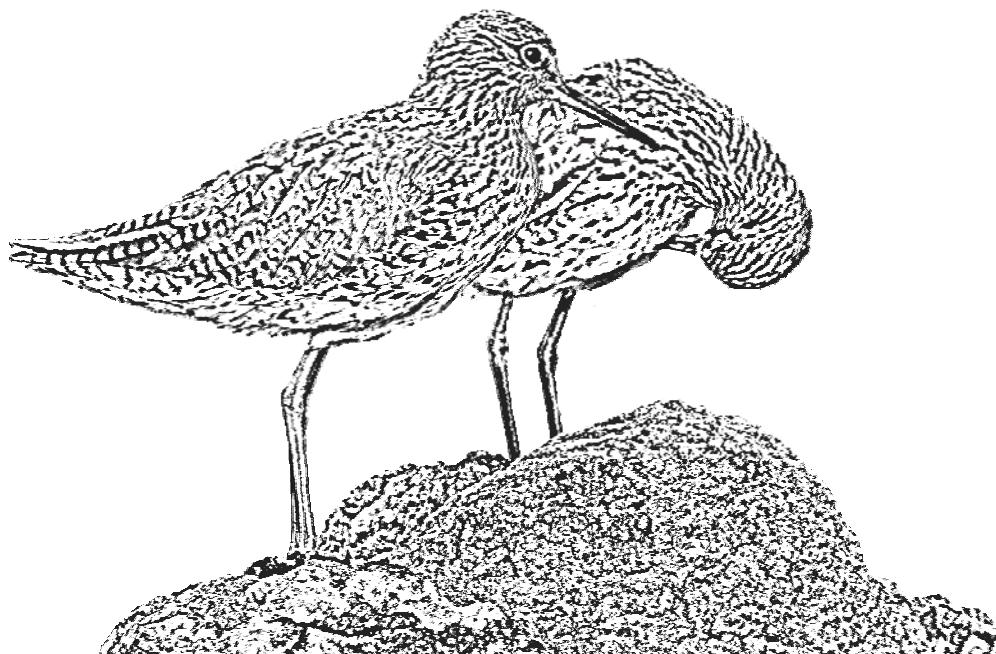
Common redshank, by Jakob Augustin

The Kentish plover (*Charadrius a. alexandrinus,* Linnaeus 1758, Egypt)

The Kentish plover is a small and inconspicuous, precocial, cosmopolitan, migratory wader, coloured greyish brown on upperparts and white on underparts. It breeds on sandy (mostly saltwater) beaches. In breeding plumage, the male is slightly more colourful then the female. The modal clutch size is three eggs. The social mating system includes monogamy, polygyny and polyandry with bi- or uni-parental care (mostly the male). A genetic parentage study revealed a low rate of extra-pair paternity (3.4% of broods; in Küpper et al. 2004). In this thesis, a breeding population of Kentish plovers was studied in the salt marsh of Tuzla, Turkey (36°42 N, 35°03 E).

Kentish plover, by Jakob Augustin

The northern lapwing (*Vanellus vanellus*, Linnaeus 1758, Sweden)

Lapwings are intermediate-sized migratory waders, coloured black on upperparts, with green and purple iridescence, a white belly and red-pink legs. They often breed on cultivated grassland or fields with short vegetation and wet areas close by. Their social mating system includes monogamy and polygyny. Most of females lay four eggs that hatch into precocial chicks. Northern lapwings show a moderate rate of extra-pair paternity (approx. 20% of broods in our study population, own unpublished data). Fieldwork was carried out at several locations on the island of Öland, in south-eastern Sweden (56°31'N, 16°36'E).

Northern lapwing, by Jakob Augustin

METHODS

This methodology section serves primarily to give a general idea about the methods used in this thesis and to provide information that is otherwise not given in the articles or manuscripts.

Investigating the genetic mating system

To investigate genetic paternity (**I, II**), one needs a DNA sample from the father, his offspring and preferentially also from the mother. In birds, red blood cells contain a nucleus and so DNA can be easily gained by taking a small blood sample from the individual. From all adult birds and sand martin nestlings we took a small blood sample by puncturing the brachial vein (located on the wing's underside). From wader chicks, we collected a blood sample by puncturing the metatarsal vein (located on the leg). In some cases, embryonic tissue was recovered, for example, if the nest was abandoned or destroyed.

The next step is to extract DNA from the blood or tissue sample using one of various available extraction methods (e.g. ammonium acetate (Nicholls et al. 2000), salt acetate (Bruford et al. 1998), or an adapted phenol–chloroform method (Krokene et al. 1996). The DNA is then used to generate a genetic profile of the individual by employing genetic markers. To analyse paternity in sand martins and lapwings, minisatellites were used as genetic markers and in the redshank microsatellites. Both markers are also referred to as VNTRs (variable number tandem repeats). They refer to locations in the genome consisting of repeat units. The number of repeats of these units is often highly variable among individuals resulting in alleles that are distinguishable in length (Avise 1994). If there is a sufficient number of markers available for different locations in the genome and all markers have a number of alleles, they will yield a 'unique' combination (genetic profile) of

alleles at different locations for each individual. Since the offspring is the genetic product of its parents, the genetic profile of the offspring is a combination of its parents' genetic profiles. One part of the offspring's profile should be identical with the mother's genetic profile and the other part with the father's profile. If the offspring shows mismatches with the social father's profile then one can exclude the offspring from being a true (within-pair) offspring of the social father. The offspring in this case would be an extra-pair young.

Genetic profiles can also be used to gain an estimate of genetic similarity between two individuals, e.g. social mates, in that genetic similarity increases with similarity between the genetic profiles.

Heterozygosity

Heterozygosity was investigated in paper **III**. At a specific location in the genome, one or more forms of a gene, so called alleles, are present. If an individual shows two allelic variants (coming from a set of homologous chromosomes in a diploid organism) at a single locus, it is heterozygous at that specific locus. If it shows only one variant (in which case both variants of the homologous chromosomes are the same) it is termed homozygous.

Different alleles ('gene variants') at a locus are originally created by mutations, i.e. changes in the nucleotide sequence, which in most cases reduce or destroy the gene function, resulting in so called deleterious alleles. Over time, and as long as the other allele (in diploid organisms) is functional, such deleterious alleles can accumulate in heterozygotes, whereas homozygotes (with both alleles deleterious) carry the costs. One might therefore expect that individuals preferentially mate with genetically dissimilar mating partners to increase heterozygosity in their offspring, which in turn lowers the risk of two deleterious alleles coming together in an individual. A genetically too dissimilar mating partner may, however, also be disadvantageous. For example, if selection favours small individuals in one and large individuals in another population, then matings between members

of these two populations will result in intermediate-sized offspring that are not favoured by selection in either population.

With co-dominant markers (markers that allow analysing the profile of one single marker) it is possible to determine if an individual is homozygous or heterozygous at the marker locus. Microsatellites are for example co-dominant markers and usually assumed to be neutral (not functional). However, recently a significant proportion of microsatellites have been found to be located in functional genomic regions (Li et al. 2002; Li et al. 2004) which raised the question if estimates of microsatellite heterozygosity reflect an overall heterozygosity effect on fitness or rather a single locus effect.

Primary sex ratio

In birds, especially in monogamous species, it is often not possible to discriminate between the sexes based on adult plumage characteristics because male and female are monomorphic (i.e. they look alike). In the offspring, plumage or other sex-specific characteristics are very rare. Molecular genetic tools made it possible to determine the sex of an individual based on a small DNA sample. Most studies nowadays employ the method described by Fridolfsson and Ellegren (1999) or Griffiths et al. (1998). In both methods, homologues sections of the CHD-Z gene (located on the W chromosome) and the CHD-W gene (located on the Z chromosome) are amplified via PCR. The amplified fragments of the CHD-Z and CHD-W gene section can be distinguished by length. Females have both sex chromosomes (Z and W) and show therefore both fragment lengths in the analysis. Males have two Z sex chromosomes and show only one fragment length in the analysis.

The potential ability of a female bird to manipulate the sex ratio of her offspring, by ovulating the desired gamete is very intriguing (**IV**). Recent experimental studies suggest that primary sex-ratio adjustment is mediated via hormones (Pike & Petrie 2006; Bonier et al. 2007; Gam et al. 2011; Pinson et al. 2011; e.g. Pryke et al. 2011). However, it is important to account for differential mortality, to make sure primary sex ratio is indeed recorded (**V**)

Sex determination

In paper **VI** sex determination was investigated. The exact mechanism that triggers male and female development in birds is debated (Teranishi et al. 2001; Smith et al. 2009; Ellegren 2011). Chromosomal aberrations can help to clarify the sex determination mechanism in birds, although they are often lethal already at the embryonic stage (Forstmeier & Ellegren 2010). Two models, the 'Z dosage' and the 'dominant W' have been proposed to explain avian sex determination.

Only two chromosomal aberrations would unambiguously discriminate between the two models, ZZW:2A and Z0:2A. The 'Z dosage' model predicts a male phenotype based on a ZZW:2A genotype and a female phenotype based on Z0:2A genotype, whereas in the 'dominant W' model the predictions would be reversed (Ellegren 2000). Unfortunately, The genotype of the Kentish plover female in paper **VI** showed a ZZW:3A chromosomal aberrations, which does not unambiguously discriminate between the two models. Nevertheless, the case might be instructive.

RESULT SUMMARIES OF ARTICLES AND MANUSCRIPTS WITH DISCUSSION

I **Augustin J**, Isaksson D, Pauliny A, Wallander J & Blomqvist D. No evidence of extra-pair paternity in the common redshank (*Tringa totanus*)

This is the first study investigating the genetic mating system of the socially monogamous common redshank. The rate of extra-pair paternity in monogamous waders is thought to be low (ca. 5% of broods) compared to other birds (ca. 20% of broods; Thomas et al. 2007). However, only a few socially monogamous species (8 out of 102 species; see Thomas & Szekely 2005; Casey et al. 2011) have been examined so far and two recent findings of moderate (18.5% and 30.4% of broods, respectively) extra-pair paternity rates in the monogamous common sandpiper (Mee et al. 2004) and the upland sandpiper (Casey et al. 2011) suggest that not all species or populations of socially monogamous waders fit the description of low extra-pair paternity. Thus, clearly, more studies are needed before a complete picture can be painted, which is where this study fits in.

Based on three polymorphic microsatellite loci and 22 broods, this study did not detect any cases of extra-pair parentage. Although sample size was limited, the indication of low levels of extra-pair paternity is consistent with most of the other studies of monogamous waders.

The result is also somewhat surprising considering some social behaviours in redshanks. In this study population, pairs were breeding at high densities (up to 8 pairs/ha), presumably with frequent extra-pair mating opportunities. Indeed, based on casual observations, I did observe several extra-pair copulation attempts and also one successful extra-pair copulation. In addition, there were no indications of mate-guarding by the social mate.

Why there is no or little extra-pair paternity in redshanks, remains unresolved. A low rate of extra-pair paternity in spite of ample opportunities for extra-pair matings may suggest a lack of genetic benefits from extra-pair

fertilizations. Extra-pair copulations in redshank might be rare but they do exist, which might indicate that females receive at least some benefits from extra-pair copulations. Occasional extra-pair copulations, for example, might serve as an insurance against infertility of the within-pair mate (Sheldon 1994). If so, extra-pair paternity should be detected given a larger sample size.

II **Augustin J**, Blomqvist D, Szép T, Szabó ZD & Wagner RH. No evidence of genetic benefits from extra-pair fertilizations in female sand martins (*Riparia riparia*)

In this study I investigated possible indirect female benefits from extra-pair fertilizations in the sand martin (*Riparia riparia*), a socially monogamous and colonially breeding swallow.

I assessed whether male traits, which might reflect genetic quality (i.e. viability or 'good' genes) such as male body condition and male age, were associated with paternity losses. Further, I investigated genetic similarity (or 'compatible genes') between mates of broods with and without paternity losses.

Multi-locus DNA fingerprinting was used to determine parentage. Similar to a previous study (Alves & Bryant 1998), I found a variable mating system, including extra-pair paternity (38% broods) and conspecific brood parasitism (7% broods). To examine paternity and male age I divided males into three age classes: one year old, two years old and three years or older. No differences were found in rates of paternity losses among these age groups. Also, an index of body condition was not significantly different between males that lost paternity and males that obtained complete paternity. There was also no significant difference in mean nestling body condition between extra-pair offspring and their maternal half-siblings.

Genetic similarity of social mates (determined via multi-locus DNA fingerprinting) did not differ between pairs of broods containing extra-pair offspring and pairs of broods without extra-pair offspring.

One possible reason why I found no evidence for genetic benefits of extra-pair fertilizations could be that female sand martins do not pursue

extra-pair copulations. In fact, sexual chases by extra-pair males and apparently forced copulation attempts have been reported in this species (Beecher & Beecher 1979) suggesting that extra-pair copulations might at least sometimes be unwanted. The lack of a relationship between male age or body condition and achieved within-pair paternity is also consistent with a range of alternative explanations, including that male age and body condition do not reflect male quality. Since I did not assign extra-pair offspring to their extra-pair fathers and therefore don't know the identity of extra-pair males, I cannot exclude the possibility that extra-pair males differed from the social males they cuckolded in age, body condition or genetic similarity with the female.

We did, however, find a positive relationship between paternity losses and breeding density, suggesting that high breeding density increase the risks or opportunities for extra-pair copulations.

III Küpper C, Kosztolányi A, **Augustin J**, Dawson DA, Burke T & Székely T. Heterozygosity-fitness correlations of conserved microsatellite markers in Kentish plovers *Charadrius alexandrinus*

Many studies report positive associations between genetic diversity and fitness parameters such as parasite resistance, reproductive success or survival in wild populations (reviewed by Hansson & Westerberg 2002; Chapman *et al.* 2009). One prediction of the 'compatible genes' model is that within populations, females should mate with genetically dissimilar mates to increase overall heterozygosity in the offspring which in turn enhances the offspring's fitness (Tregenza & Wedell 2000). In non-model organisms, microsatellite markers have been the most popular markers to estimate heterozygosity. However, many overall heterozygosity fitness estimates may often be dominated by unproportionally strong effects of single microsatellite loci and not necessarily reflect an overall effect (Hansson et al. 2004; Acevedo-Whitehouse et al. 2006; Lieutenant-Gosselin & Bernatchez 2006; Luikart et al. 2008). Single locus heterozygosity effects may indicate that microsatellite markers directly affect fitness ('direct effect', e.g. David 1998)

or microsatellite markers might be linked to functional loci ('local effect', Hansson & Westerberg 2002).

My co-authors and I examined heterozygosity, offspring development and fitness in a small shorebird, the Kentish plover. The chicks are precocial and exposed to a variable and sometimes hostile environment immediately after hatching: Their thermoregulation is still not fully developed and, although the parents give alarm calls when predators approach, the chicks have to protect themselves from predators. In addition, conspecific adults may injure or kill chicks during territorial conflicts (Székely & Cuthill 1999; Kosztolányi et al. 2006). Heterozygosity was examined in relation to three fitness and development measures: chick survival, and growth in tarsus length and body mass. We employed six 'anonymous' and 11 'conserved' microsatellite markers to estimate multilocus and single locus heterozygosities. The flanking region of 'conserved' markers show sequence homologues in related species for which fully assembled chromosome sequences are available (Küpper et al. 2008). 'Conserved' markers are therefore often embedded in a genomic region with some functionality. The locations of anonymous microsatellite markers in the genome are unknown.

There was no genome-wide effect of heterozygosity on fitness or growth rates. That means multilocus heterozygosity was not related to any of the fitness or development response variables. In contrast, single locus heterozygosity at five conserved markers was associated with chick survival. One conserved marker showed a positive association with chick survival, and another conserved marker was negatively associated with survival. In addition, heterozygosity at three further conserved loci showed significant interaction with non-genetic variables, territory quality, offspring sex and desertion of a parent. The presence of both, negative and positive heterozygosity effects on survival across loci, might explain why multilocus heterozygosity was not suitable to predict chick survival.

Since overall heterozygosity did not correlate with offspring survival, we conclude that heterozygosity fitness correlations in outbred populations seem to be caused by 'direct' or 'local effects' of microsatellite markers.

IV **Augustin J**, Grønstøl GB, Pauliny A, Wagner RH & Blomqvist D. Variation in brood sex ratio associated with extra-pair paternity in a wader, the northern lapwing (*Vanellus vanellus*)

The theory of natural selection predicts a primary sex ratio close to parity in the population when the costs to produce male and female offspring are equal (Fisher 1930). Under some circumstances, however, fitness benefits expected from sons and daughters might differ, which in turn would favour females that are able to skew the sex ratio of their offspring. For example, if sons of attractive and/or high quality extra-pair males have a higher fitness potential than their sisters, then extra-pair offspring should be male biased.

I investigated such a possible relationship between brood sex ratio and paternity in northern lapwings. The northern lapwing shows a moderate rate of extra-pair paternity (approx. 20% of broods in our study population, own unpublished data). Male and female chicks are precocial and do not differ in size or body condition at hatching, suggesting equal costs to produce male and female offspring.

I found a higher proportion of sons in broods containing extra-pair young than in broods with only within-pair young. This difference, however, was caused by a higher proportion of within-pair sons, rather than by extra-pair sons, i.e. contrary to the expectation from differential sex allocation in response to (presumed) higher quality and attractiveness of extra-pair sires. A reason for this could be that females are unable to assign extra-pair sperm precisely to fertilise eggs with a male-determining sex chromosome. That means a mechanism for primary sex ratio adjustment may be present, but not one for differentiating this adjustment between multiple fertilizing males (Sheldon & Ellegren 1996). In this case, within-pair young would be (inadvertently) biased towards sons more often than extra-pair young, simply because within-pair young are more frequent than extra-pair young in most broods with extra-pair young.

The lack of a sex bias in extra-pair young, which is consistent with a body of other studies (e.g. Dietrich-Bischoff et al. 2006; Abroe et al. 2007; Delmore et al. 2008) and supports the idea that female birds are not able to manipulate the sex of an offspring as an immediate response to extra-pair

fertilizations (Sheldon & Ellegren 1996). This is not surprising because extra-pair fertilizations are probably to a large degree unpredictable to females, even when they are wanted.

An alternative explanation for a bias towards sons in extra-pair broods might be female stress. Recent experimental studies suggest that corticosterone levels are linked to primary sex ratio adjustment. For example, an experimental study on Gouldian finches showed that stressed females produced broods with more sons (Pryke et al. 2011). One observed extra-pair copulation and two observed extra-pair copulation attempts in our population seemed to be forced by the male (personal observations) and unwanted by the female. Being coerced to accept extra-pair copulations or being punished by the social male for receiving extra-pair copulation might induce stress and cause lapwing females to produce more sons. Also in this case, within-pair young might be more often the target of stress situations because they are more frequent in mixed broods, resulting in more within-pair sons than extra-pair sons.

V **Augustin J** & *Bartoszek K.* Are you sure you have shown primary sex ratio adjustment? The problem of differential mortality revisited

Primary sex ratio is the sex ratio of offspring at fertilization. In theory, female birds (because they are the heterogametic sex) are in the position to directly manipulate the primary sex ratio of their broods. Sex ratios are usually measured long after the ('primary') zygote stage and therefore might not reflect the primary sex ratio. This is because differential mortality (the rate of mortality differs between male and female embryos and/or nestlings) might have occurred prior to sampling. Some studies have tried to circumvent this problem by sampling only complete broods (i.e. broods in which no offspring died or got lost prior to sampling). Unfortunately this doesn't solve the problem, because if one sex suffers higher embryo mortality then broods that contain less of the vulnerable sex will be more likely to stay complete and, hence be sampled, as pointed out by Fiala (1980). Furthermore, if there is missing data that are caused by non-random factors (e.g. offspring were not

sexed due to unhatched eggs or disappeared nestlings), it is not possible to distinguish between differential mortality and primary sex ratio biases (Fiala 1980).

We investigated if fellow researchers are aware that differential mortality rather than primary sex ratio adjustment might be the cause of a biased brood sex ratio, by browsing the literature on primary sex ratio in birds between 2009 and 2012. In total, we investigated 26 studies on primary sex ratio over the last three years. We found more than half (15) of the studies did not seriously consider differential mortality as an alternative explanation. In most of these studies, differential mortality was erroneously excluded as an alternative explanation to primary sex ratio adjustment based on the misconception that differential mortality can be excluded by analysing only complete broods (i.e. broods in which every egg laid was sexed).

To conclude, our results show that one has to be cautious when analysing and interpreting results on primary sex ratio, especially when the proportion of missing data is large and/or effect size is small.

VI Küpper C, **Augustin J**, Edwards S, Székely T, Kosztolányi A & Janes AE. Triploid plover female provides support for a role of the W chromosome in avian sex determination

In this study, we describe an aneuploid female Kentish plover with an abnormal chromosome number of ZZW:3A (two Z sex chromosomes, one W sex chromosome and 3 sets of autosomes), and discuss the insights provided for the debated mechanisms of avian sex determination.

Two models have been proposed to explain sex determination in birds (Clinton 1998). The 'Z dosage' model postulates that the main determinant for sex is located on the Z chromosome. Depending on the ratio of Z chromosomes to autosomes (Z:A ratio), the embryo will develop as a male or a female. If Z:A equals 1 then the embryo will develop into a male, if Z:A equals 0.5 into a female. The second model, the so called 'dominant W' model, postulates that female development is triggered by a still unknown W-linked 'female-gene'. So far there is evidence for both models. Triploid

ZZW chickens, for example, support the 'Z dosage' model; they are sex changers that start as females but assume phenotypic characteristics of males before sexual maturity, and never produce viable gametes (Lin et al. 1995). In contrast, a fertile great reed warbler female with a ZZW:2A genotype clearly supports the 'dominant W' model (Arlt et al. 2004). However, it was suggested that the female's germline was diploid since 12 sons of her inherited the same Z-linked alleles. Therefore, alternative explanations such as a tissue-restricted mosaicism cannot be ruled out (Fechheimer & Jaap 1980).

The aneuploid Kentish plover female in this study showed characteristic 'three allele' genotypes at 14 autosomal markers and all three female alleles were represent in the offspring for six of these 14 markers which rules out any laboratory bias and strongly suggests that aneuploidy was present in the female's germline and tissue. In fact, this is the first reported case of a fertile (most likely) triploid female bird. The female produced normal diploid offspring. Peak height ratio analysis showed that the sex chromosome composition of the female was ZZW. According to the 'Z dosage' model our female showed a ratio of 2:3 (ZZ:3A) which is between 1 (equals male) and 0.5 (equals female). Unfortunately, our result does not unambiguously discriminate between the two sex determination hypotheses. The 'dominant W' model is supported because a change in Z:A ratio did not affect the development of a normal female which, adding the great reed warbler female, suggest that sex determination mechanism might differ between *galliform* and *non-galliform* birds. Alternatively, it could also be that our observed 2Z:3A ratio did not exceed a certain Z:A threshold that is needed in Kentish plovers to trigger male development. In this case, the Z:A threshold to trigger male development would differ between chickens and Kentish plovers, which is an instructive result as well.

Since the 'Z dosage' model cannot be ruled out, it indirectly provides some support for it. In the light of Arlt et al's (2004) result, this indirect support for the 'Z dosage' model is important, as it motivates why it should remain an alternative to the 'dominant W' model also in future studies.

MAIN FINDINGS

In this thesis, I have examined a diverse set of study species and questions regarding sexual infidelity and extra-pair paternity in monogamous birds. My main findings are as follows:

➢ No evidence of female genetic benefits from extra-pair fertilisations in a short-lived passerine, the sand martin.

➢ A surprising absence or extremely low frequency of extra-pair paternity in the socially monogamous common redshank, despite behavioural observations of several attempted and one successful extra-pair copulation.

➢ Contrary to a proposed 'compatible genes' benefit of extra-pair fertilizations, overall heterozygosity in the Kentish plover did not seem to improve offspring survival.

➢ In mixed support of the differential sex allocation hypotheses, female lapwings produced a male-skewed sex ratio in broods with extra-pair paternity. However, since the bias was not among the extra-pair chicks, the mechanism as well as the adaptive significance remains unsolved.

➢ A brief reminder that estimates of primary sex ratio adjustment, especially when slight, may be confounded or even confused with sex-differential offspring mortality.

➢ Based on a chromosomally aberrant (triploid) Kentish plover female, the debated mechanisms of sex determination in birds could be addressed, although only humble support for 'dominant W' model was provided in this case.

GENERAL CONCLUSIONS

Thirty years of research on extra-pair paternity in birds and *'despite the very substantial research effort in the field, there is still no consensus on a number of fundamental questions related to this topic. For example, there is sustained debate about which sex is actually in control of EPC* (extra-pair copulation) *behaviour and, particularly, about the adaptive significance of extra-pair mating behaviour for female birds.'* This is how Schmoll (2011) summarises the views of several reviews on extra-pair paternity (Griffith et al. 2002; Westneat & Stewart 2003; Arnqvist & Kirkpatrick 2005; Akçay & Roughgarden 2007; Griffith 2007; Griffith & Immler 2009) over the last decade.

My thesis aimed to contribute to the understanding of mechanisms and adaptive reasons, primarily from the female's perspective, for the highly diverse frequencies of extra-pair paternity in birds. I started out by exploring the genetic mating system of the common redshank for which the genetic mating system has not been described previously. I could not investigate potential female benefits from extra-pair paternity, because to my surprise I found not a single extra-pair young in my sample. Based on detailed studies on the redshank's sexual behaviour by Großkopf (1959) and by Hale & Ashcroft (1983) and personal observations (unpublished) in the field, I suggest that the lack of extra-pair paternity in this species might be due to a lack of or low indirect benefits, although alternative explanations cannot be ruled out.

In the sand martin, I found extra-pair young in 38% of broods, which allowed me to test predictions in relation to proposed indirect benefits such as 'good genes' and 'compatible genes'. In my sample, there were no indications that female sand martins gain these indirect benefits. In the northern lapwing, on the other hand, a potential for indirect benefits was indicated by a bias towards sons in broods with extra-pair young. However, it turned out to be the within-pair rather than the extra-pair young that were male biased, hence no support for differential sex allocation in response to e.g. genetically superior or more compatible extra-pair sires. The sex ratio adjustment in within-pair young may still have been adaptive (i.e. due to a

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shift in the relative fitness returns from sons vs. daughters), but it does not indicate indirect benefits from extra-pair mating.

The above findings are of course no conclusive rejection of indirect benefits from extra-pair fertilizations. They are, however, in line with two recent meta-analyses of extra-pair paternity in passerine birds, which also concluded that there was no strong support for genetic benefits hypotheses (Arnqvist & Kirkpatrick 2005; Akçay & Roughgarden 2007; but see Griffith 2007). On the other hand, Slatyer et al. (2012) found more support in their meta-analysis across a wide range of taxa. In the end, the only general conclusion on adaptive functions of extra-pair paternity maybe the diversity itself, but that is often every bit as interesting.

One cause for the controversies in the field of extra-pair paternity might be methodological challenges (e.g. Schmoll 2011). For example, using the same microsatellite markers to determine paternity and to estimate individual heterozygosity can cause an increase in results supporting the heterozygosity hypothesis also when no effect of heterozygosity actually exists (Wetzel & Westneat 2009). In the Kentish plover, my co-authors and I tested the assumption that overall heterozygosity increases survival in chicks as predicted by one 'compatible genes' model. This did not seem to be the case in Kentish plover chicks; in contrast, a presence of negative and positive effects at different loci suggested that heterozygosity had an antagonistic effect on survival across different loci. Further studies are needed to determine how single microsatellite marker heterozygosities are related to survival; for example, do microsatellite markers directly affect fitness when located in a coding region, or are they associated with certain alleles of the functional locus?

Sometimes, it can be also a persistent misconception that causes trouble. Fiala (1980) and Krakow & Neuhäuser (2008) pointed out that in the case of missing data (caused by non-random factors), a skewed brood sex ratio can be caused by either differential mortality of the sexes or brood sex-ratio adjustment, and that it is not possible to discriminate between the two causes. In a review of the recent literature on primary sex ratio adjustments, I found that the majority of studies neglected this potential source of error. The extent to which this has influenced the conclusions drawn is hard to estimate. However, given that new easy-to-use genetic techniques for sex

determination have resulted in increasing numbers of primary sex ratio studies recently, the most important point of this paper is to increase the awareness for the future.

At least in some systems, especially when females are observed to actively seek extra-pair copulations, it is reasonable to assume that females benefit from extra-pair copulations in one way or another. Maybe future research should focus more on direct benefits, for example assuring fertilization of the eggs in case the within-pair male is infertile (Wetton & Parkin 1991; Sheldon 1994). Also female traits may influence whether she seeks or allows extra-pair copulations or not (e.g. Stutchbury et al. 1997; Bouwman & Komdeur 2005; Whittingham & Dunn 2010).

Finally, even if females in many species benefit to some extent from extra-pair fertilizations, it seems motivated to question whether it is variation in such benefits (and thus selection pressures) that explain the enormous diversity in extra-pair paternity rates? Could it be costs and constraints on female resistance against extra-pair matings, or control of fertilisation, that are the main sources of variation? Or is perhaps the research on extra-pair paternity too focused on female benefits and costs? Thirty years ago, extra-pair paternity was primarily (and maybe because of sexist reason) regarded as a male strategy. For good reasons this has been balanced by the female perspective, but maybe it is time to return to the males to find some of the main behavioural and ecological explanations for the diversity of extra-pair paternity rates.

More research and more detailed studies are needed in this fascinating field of avian reproductive biology, to which I hope to have contributed with some pieces of the puzzle.

ACKNOWLEDGEMENTS

Calum, when I think about it, you are so lucky to know me ;-)!

Judith, danke für die Möglichkeit mal ab und zu steirisch zu quasseln und über die Schweden herzuziehen ;-).

Thank you Krzysztof for the good collaboration.

Mikael, I had a very good time being guest at your house. Thanks for all the help!

Rasmus, Sofia A, Sofia B, Inês, Peter, Daniel, Jörgen, Joacim, Jenny, David and other fellow PhD students, I enjoyed your company, thanks a lot.

Thanks a lot also to all current and former collaborators J.A. Anmarkrud, K.H. Bentz, D. Blomqvist, T. Burke, D.A. Dawson, S. Edwards, K.J. Fernie, J. Figuerola, A.E. Janes, O. Kleven, A. Kosztolányi, J.T. Lifjeld, M.J.L. Magrath, G.K Munimanda, H. Pärn, A Pauliny, R.J. Robertson, S. Quinn, S. Tarof, T. Székely, T. Szép, W.F.D. van Dongen, R.H. Wagner and J. Wallander.

For helping with field work and some good times I also want to thank Zoltan, Jannik, Silke and Johanna.

I am grateful for financial support that was provided by Wilhelm och Martina Lundgrens Vetenskapsfond, Helge Ax:son Johnsons Stiftelse, Rådman och Fru Ernst Collianders Stiftelse and Adlerbert Foreign Student Hospitality Foundation.

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LIST OF COMMON & SYSTEMATIC NAMES

English name	Svensk namn	*Systematic name*
Barn swallow	Ladusvala	*Hirundo rustica*
Black-capped chickadee	Amerikansk talltita	*Poecile atricapillus*
Blue tit	Blåmes	*Cyanistes caeruleus*
Bluethroat	Blåhake	*Luscinia svecica*
Bullock's oriole	Bullocks trupial	*Icterus bullockii*
Collared flycatcher	Halsbandsflugsnappare	*Ficedula albicollis*
Common redshank	Rödbena	*Tringa totanus*
Common sandpiper	Drillsnäppa	*Actitis hypoleucos*
Common tern	Fisktärna	*Sterna hirundo*
Gouldian finche	Gouldsamadin	*Erythrura gouldiae*
Great grey shrike	Varfågel	*Lanius excubitor*
Great reed warbler	Trastsångare	*Acrocephalus arundinaceus*
Great tit	Talgoxe	*Parus major*
House sparrow	Gråsparv	*Passer domesticus*
House wren	Husgärdsmyg	*Troglodytes aedon*
Kentish plover	Svartbent strandpipare	*Charadrius alexandrinus*
Lazuli bunting	Lazulifink	*Passerina amoena*
Mexican jay	Kanjonskrika	*Aphelocoma ultramarina*
New Zealand stitchbird	Skägghonungsätare	*Notiomystis cincta*
Northern fulmar	Stormfågel	*Fulmarus glacialis*
Northern lapwing	Tofsvipa	*Vanellus vanellus*
Pied Flycatcher	Svartvit flugsnappare	*Ficedula hypoleuca*
Purple martin	Blå storsvala	*Progne subis*

English name	Svensk namn	Systematic name
Red-winged blackbird	Rödvingetrupial	*Agelaius phoeniceus*
Reed bunting	Sävsparv	*Emberiza schoeniclus*
Sand martin	Backsvala	*Riparia riparia*
Seychelles warbler	Seychellsångare	*Acrocephalus sechellensis*
Splendid fairy wren	Praktblåsmyg	*Malurus splendens*
Superb fairy-wren	Vitbukad blåsmyg	*Malurus cyaneus*
Tree swallow	Trädsvala	*Tachycineta bicolor*
Upland sandpiper	Piparsnäppa	*Bartramia longicauda*
Willow warbler	Lövsångare	*Phylloscopus trochilus*
Zebra finche	Zebrafink	*Taeniopygia guttata*

CONTRIBUTION REMARKS

I & **II** Jakob Augustin carried out the field work, laboratory work, data analysis and wrote the manuscript.

III & **VI** Jakob Augustin carried out the laboratory work and contributed to the manuscript.

IV Jakob Augustin wrote the manuscript, carried out the data analysis and contributed to field work and laboratory work.

V Jakob Augustin did the data analysis and wrote the manuscript.