Female Reproductive Strategies in the Ruff (*Philomachus pugnax*)

BY

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Abstract

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Traditionally, females have been considered to be strictly monogamous. Today, we know that females in the majority of species actively seek out and mate with several males. Trying to understand female preferences, including what benefits mate choice entails, has been the focus of intense research during the last decades. Females can gain both direct (e.g. access to better territories or paternal care) and indirect (i.e. genetic) benefits. The aim of this thesis is to further our understanding of the female reproductive strategies in the ruff, *Philomachus pugnax* (Aves, Scolopacidae). The ruff is a lekking wader, where males gather on leks to display to females that come there to mate. Males do not provide any paternal care to the offspring. Lekking systems are ideal for studying indirect benefits of female choice, as females do not gain any direct benefits from males.

Females mated with several males and 50% of the broods were fathered by at least two males. The level of genetic similarity between two parents has previously been shown to be an important source of variation in offspring fitness. Males that were less closely related to the female fathered more offspring in broods with multiple paternity, such that females that mated multiply gained in terms of receiving more outbred offspring. There did not, however, appear to be an overall female preference for less closely related males. There are two genetically determined male reproductive strategies in the ruff, that differ in behaviour and morphology. There was no evidence for females taking male strategy into account when choosing a partner.

Female post-fertilisation strategies may also influence fitness, e.g. through differential investment in eggs, gender of the offspring and choice of breeding habitat. Females allocated sex in a non-random manner dependent upon body condition, such that females in good condition had more daughters.

Females were found to nest in higher than average vegetation and in areas with higher than average abundance of insects, factors likely to influence predation rates on both eggs and young, as well as foraging opportunities for the precocial young. Further, females were faithful to their previous breeding site and usually nested within meters of their previous nest. Hatching success did not, however, affect a female's decision to return or not.

Keywords: sexual selection, female reproductive strategies, indirect benefits

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In loving memory
of Trudi

PAPERS INCLUDED IN THE THESIS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I Griffith Simon C, Owens Ian PF, Thuman Katherine A. (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology* 11: 2195-2212.

II Thuman Katherine A, Widemo Fredrik, Piertney Stuart B. Ruff reproductive strategies revisited: Paternity data supports mixed ESS. (Manuscript)

III Thuman Katherine A, Widemo Fredrik, Borg Lotta. Nest site selection and breeding area fidelity in a lek- breeding wader, the ruff (*Philomachus pugnax*). (Manuscript)

IV Thuman Katherine A, Widemo Fredrik, Griffith Simon C. (2003) Condition-dependent sex allocation in a lek-breeding wader, the ruff (*Philomachus pugnax*). *Molecular Ecology 12*: 213-218.

V Thuman Katherine A. Cryptic female choice in a lekking shorebird. (Submitted)

VI Thuman Katherine A, Widemo Fredrik, Piertney Stuart B. (2002) Characterisation of polymorphic microsatellite DNA markers in the ruff (*Philomachus pugnax*). *Molecular Ecology Notes* 2: 276-277.

Introduction

"Sexual selection is incompletely understood, especially in species where females choose mates and males contribute only genes" (Andersson 1994)

It is not that long ago that birds in general (Lack 1968) and females in particular, were thought to be strictly monogamous, and dating back to Darwin, females were almost considered to be passive bystanders (Birkhead & Møller 1992). Observations of extra-pair copulations were mostly ignored until an evolutionary reason for the behaviour came when Trivers (1972) first formalised the idea that due to the different levels of investment by males and females in offspring, alternative-mating strategies would be adaptive. The concept that females are merely passive bystanders has been replaced by the knowledge that females of many species actively seek out and mate with several males (Birkhead & Møller 1992). For instance, a substantial proportion of socially monogamous species have turned out to be sexually promiscuous, and true monogamy has actually been found in less than 25% of the socially monogamous bird species studied to date (Paper **I**). Although most of the work to date on multiple mating and paternity has been performed on monogamous species, most of the theory and conclusions apply to non-monogamous species as well.

Multiple mating patterns
It has become evident that extra-pair paternity, EPP, is important because, not only does it influence the strength of sexual selection (Møller & Ninni 1998;

Sheldon & Ellegren 1999), but it plays a fundamental role in the evolution of many other aspects of life-history strategies (Gowaty 1996; Mauck *et al.* 1999; Møller 2000; Møller & Cuervo 2000; Slagsvold & Lifjeld 1997). Many hypotheses have been put forward to explain interspecific variation in the level of EPP, but until recently there has been relatively little success in identifying robust, biologically sensible correlates of this interspecific variation (Birkhead & Møller 1996, Ligon 1999, Bennett & Owens 2002). One explanation for this paradox is that most attempts to identify biological correlates of interspecific variation in the rate of EPP have been based on the assumption that the level of EPP shown by a species is determined by contemporary ecological factors, such as breeding density and/or breeding synchrony. We, however, found little evidence that interspecific variation in the rate of EPP is due to variation in breeding density or breeding synchrony (Paper **I**). However, phylogenetic analysis of the EPP data showed that estimates of extra-pair paternity are not distributed randomly with respect to phylogeny. In fact, over 50% of the interspecific variation in the level of EPP occurs between families or between orders, rather than among closely-related species. This suggests that many differences between species in terms of EPP rate are likely to have been determined in the ancient evolutionary history of avian lineages, and that explanations based on contemporary ecology alone will prove insufficient.

The limited explanatory success of the two most popular ecological explanations for interspecific variation in EPP have led to a new generation of

hypotheses that high rates of EPP could be associated with little need for paternal care (Mulder *et al.* 1994, Birkhead & Møller 1996, Gowaty 1996), as females should be more likely to seek extra-pair copulations when they can rear offspring with little help from their male partner. Birkhead and Møller (1996), show that, as predicted, EPP rates tend to be comparatively low in species where male care was 'essential' (Birkhead and Møller 1996, Møller 2000). Further, interspecific variation in the extent of female constraint appears to vary across the same phylogenetic levels as does interspecific variation in the level of EPP (see Owens & Bennett 1997). Another variable that has recently been suggested to explain interspecific variation in the rate of EPP, and is known to vary considerably among ancient avian lineages (Owens & Bennett 1996; Bennett & Owens 2002), is the rate of adult mortality (Mauck *et al.* 1999, Wink & Dyrcz 1999). Both Mauck *et al.* (1999) and Wink & Dyrcz (1999) found that species with high annual mortality rates have high levels of EPP.

There appears to be a hierarchical explanation for EPP, with variation at different phylogenetic levels being associated with different ecological, genetic and social correlates (Paper **I**). Variation in the rate of EPP among major avian lineages is due to variation in the likely costs of extra-pair behaviour as determined by gross differences in the form of parental care (see Mulder *et al.* 1994; Gowaty 1996; Birkhead & Møller 1996) and reproductive lifespan (see Mauck *et al.* 1999; Wink & Dyrcz 1999). It now seems likely that differences between species in the rate of EPP are due to a combination of differences in life history, i.e. patterns of parental care,

and the local opportunities for promiscuity. However, revealing the function of EPP remains the most obvious ongoing challenge.

The question of why females should engage in extra-pair copulations, or seek to mate with more than one male, has received much theoretical treatment and been thoroughly reviewed several times (e.g. Westneat *et al.*1990; Birkhead & Møller 1992; Birkhead 1998; Møller 1998; Petrie & Kempenaers 1998; Ligon 1999, Paper **I**). The main types of explanation for why females may mate with several males are summarised in Table 1 (from Birkhead & Møller 1992; Møller 1998) (Paper **I**).

Considering that an individuals genetic makeup somehow determines the future ability of that individual to reproduce and compete for mates, there will be individuals that are better than others, i.e. have so called good genes (Birkhead 1998). Some of the most convincing evidence for good genes comes from monogamous species where females choose to perform extra-pair copulations with males of better quality, and these benefits are passed on to offspring (Paper **I**). If females were only mating for obtaining good genes there should be a strong directional selection that decreases genetic variation, "the lek paradox" (Taylor & Williams 1982). However, instead of choosing males with inherently "superior" genes, females may instead mate with males that complement their genotype the best, i.e. genetic compatibility (Tregenza & Wedell 2000, Johnsen *et al.* 2000), i.e. what is the best match for one individual is not necessarily the best match for another.

Table 1. Hypotheses on the function of EPP in birds (adapted from Birkhead & Møller 1992, Møller 1998).

Hypothesis	Description	Refs.
Fertility A	Females seek EPP in order to guard against infertility in their own social mate, but females have no way of assessing the fertility of males.	Wetton & Parkin (1991)
Fertility B	Females seek EPP in order to guard against infertility in their own social mate, and females are able to assess male fertility through phenotypic cues.	Sheldon (1994)
Genetic diversity	Females seek EPP to maximise genetic diversity among their offspring, but females cannot assess the extent of genetic similarity between themselves and males.	Williams (1975); Westneat *et al.* (1990)
Genetic compatibility	Females seek EPP to maximise genetic compatibility between themselves and the father of the offspring, and females can assess the extent of genetic similarity between themselves and males through phenotypic cues.	Kempenaers *et al.* (1999); Tregenza & Wedell (2000)
Good genes	Females seek EPP to obtain 'good genes' for their offspring, and females can assess the genetic quality of males through phenotypic cues.	Møller (1988); Hamilton (1990); Westneat *et al.* (1990); Birkhead & Møller (1992)
Direct benefit	Females seek EPP to obtain (non-genetic) resources for their offspring, and females can assess the resources held by males.	Wolf (1975); Burke *et al.* (1989); Colwell & Oring (1989);

Matings between genetically similar individuals can have fitness costs, leading to reduced reproductive success and survival (Bensch *et al.* 1994, Kempenaers *et al.* 1996). Mechanisms by which individuals avoid mating with close relatives should therefore have evolved through natural selection, by either pre-mating mechanisms, i.e. mate choice and kin recognition (Bateson 1982) and/or post-mating mechanisms, i.e. cryptic female choice or sperm selection (Birkhead 1998). However, mating with a too distantly related individual can result in outbreeding depression (Lynch 1991, LeBas 2002), so the optimum may lie somewhere in the middle (Bateson 1982, Peacock & Smith 1997, Höglund *et al.* 2002).

has been found both in insects (Stuart & Herbers 2000, Edmands 2002) and in birds

(Bensch *et al.* 1994). Recently, Blomquist *et al.* (2002) showed that in several socially monogamous waders, females paired with genetically similar males had a higher frequency of extra-pair offspring. The authors implied that birds are able to recognize distant relatives. However, in birds, there is no conclusive evidence of mate choice due to genetic similarity to date, for example MHC (Tregenza & Wedell 2000), or choice of extra-pair partner and relatedness (Bench *et al.* 1994, Kempenars *et al.* 1996). Several studies have suggested that birds have the

potential to recognize kin, but this has not been shown in relation to mate choice (Höglund *et al.* 1999, Petrie *et al.* 1999, Shorey *et al.* 2000).

Lekking species and indirect benefits
The lek mating system is one of the most extravagant and extreme where males gather in tight aggregations to attract females. There are four criteria defining a lekking system; males aggregate on arenas, or leks, to display; there are no resources on the lek; there is no paternal care; females choose their mate on the basis of male attributes (Höglund & Alatalo 1995). As females do not gain any direct benefits by visiting a lek and mating with a certain male, lekking species are excellent for studying indirect benefits, i.e. genetic benefits ("good genes", genetic compatibility and genetic diversity (Westneat *et al.* 1990, Jennions & Petrie 2000) of female choice.

What one would expect in terms of multiple paternity rates in lekking species is not straightforward. As females are free to choose to mate with whom they prefer, one might expect very low levels of multiple paternity (Birkhead & Møller 1992) as found in black grouse (*Tetrao tetrix*) (Alatalo et al 1996). However, if females want to maximise genetic benefits or increase genetic diversity among offspring, lekking females would suffer no cost of for example reduced paternal care, by mating multiply. If there are no costs to the female of mating with several males, one might expect high levels of multiple paternity as found in the buff-breasted sandpiper (*Tryngites subrufcollis)* (Lanctot *et al.* 1997) and the ruff (*Philomachus pugnax*) (Lank *et al.* 2002). As there is a highly skewed

variation of copulation rates on the lek, where few males gain a majority of the matings (Höglund & Alatalo 1995) and copulation rates can be high (Widemo 1998), sperm depletion could potentially be a problem. Females could therefore mate with several males to ensure that she has enough sperm to fertilise her eggs (Sheldon 1994, Sax *et al.* 1998).

Most of the work done on sexual selection in lekking species has focused on male characteristics (Höglund & Alatalo 1995). This is not surprising as lekking males are the more extravagant and noticeable of the sexes. However, without understanding why females choose, and what affects her choice of certain males, it is impossible to fully understand lekking behaviour.

The ruff
I have studied female reproductive strategies in the ruff (*Philomachus pugnax*), a lekking wader (van Rhijn 1991). The fieldwork was carried out in the breeding seasons of 1999 – 2002 on southern Gotland (57°10′ N, 18° 20′ E), an island off the coast of Sweden in the Baltic Sea. The study site is a flat open peninsula, approximately 410 hectares of open shore meadows that have been used for grazing by cows and sheep for decades. The vegetation is low, and dominated by different species of grasses (Graminae spp.) and sedges (*Carex* spp.), with very few trees and bushes.

Male ruffs arrive in late April and begin displaying on traditional leks, soon thereafter the first females arrive on the leks (Widemo 1997). Active leks were observed from permanent or portable hides to determine which males were territory holders. Males were caught

using cannon nets (see Widemo 1995 for details). Ruffs are size dimorphic, males being considerably larger than females, called reeves. While the male plumage is exaggerated with the large ruff that varies in colour, reeves are dull and inconspicuous. Cryptic colouration of females is essential as they are ground nesting birds and rely on camouflage to avoid predation.

Reeves mate repeatedly with one or several males within a short time span (van Rhijn 1991, Widemo 1998) and levels of multiple paternity are high (Lank *et al.* 2002, Paper **III**) giving potential for both sperm competition and sperm selection. Reeves make their nest in tussocks were they, like most waders, lay four eggs (occasionally three, 12%), in a well concealed nest on the ground. After 21-22 days of incubation the young hatch and are precocial, i.e. leave the nest shortly after hatching and receive no care from the male and very little from the female (van Rhijn 1991). Nests were located by observing females from portable or permanent hides. Females were caught on the nest using a walk in cage. Both males and females were colour-ringed with an unique combination, measured (tarsus, wing and bill length and weight)

and a small blood sample was taken and stored in ethanol. After hatching and before leaving the nest, all chicks were ringed and bled as for adults. Unhatched eggs were collected to obtain DNA for molecular work. The exact location of nests and leks was determined using GPS. In total 209 nests were found (Table 2).

Multiple paternity
All individuals were genotyped using seven microsatellite primers (Paper **VI**). Potential fathers were found for each chick manually by matching paternal alleles in chicks to males. Potential fathers were then tested in Cervus 2.0 (Marshall *et al.* 1998) for level of significance.

In paper **III**, paternity was assigned to 221 out of 333 chicks (67%) in 66 out of 97 nests (Table 2). In 66 broods where at least partial paternity was assigned, 32 nests had single paternity, and there was multiple paternity in 34 broods (51.5%). The high levels of multiple paternity in ruff broods appears to be consistent in Europe, as practically the same result was previously found in a Finnish ruff population (Lank *et al.* 2002).

Table 2. Number of nests found and hatched each year and in total. Numbers of nests where the female was caught and chicks were ringed. Numbers of nests and chicks where paternity was assigned.

Year	Nests found	Females caught	Nests hatched	No. of chicks	Hatched nests with mother caught	No. of chicks with mother caught	Paternity assigned in nests	Chicks with assigned fathers
1999	33	28	18	62	18	63	13 (72%)	41 (65%)
2000	76	59	36	130	33	117	29 (88%)	102 (87%)
2001	61	43	39	113	29	100	18 (62%)	59 (59%)
2002	39	29	18	55	17	53	6 (35%)	19 (36%)
Total	209	159	121	360	98	340	66 (68%)	221 (67%)

In lekking species, due to the potential low costs to females of mating multiply, genetic benefits may drive the evolution of polyandry.

Cryptic female choice
Recently, it has been suggested that females can assess the level of genetic similarity (even low levels of similarity) of their mate, and adapt their reproductive strategy accordingly (Blomquist et al. 2002). Three previous studies have revealed genetic structuring across or within leks, and implied that males can assess genetic similarity of other males (Höglund *et al.* 1999 Petrie *et al.* 1999, Shorey *et al.* 2000).

In paper **IV**, I show that reeves possibly mate randomly with respect to genetic similarity as males that fertilised eggs were no more or less genetically similar to the female than the other males in the population (randomisation test, p = 0.955) (randomisation tests were performed by drawing expected relatedness values, 10000 times, from a pool of all potential male/female values and comparing the observed value to the expected distribution). Further, males gaining paternity were no more nor less genetically similar to the female than the other males on the same lek, in 11 out of 22 cases the successful male was less related to the female than was the average male on the lek.

The level of genetic similarity between two parents may be an important source of variation in offspring fitness. Selection should favour the evolution of mechanisms for avoiding inbreeding and optimising outbreeding by either pre-mating mechanisms, i.e. mate choice through kin recognition (Petrie *et al.* 1999) and/or post-mating mechanisms

for sperm selection. The incidence of sperm competition is high (Lank *et al.* 2002, Paper **III**) thereby providing an opportunity to test the hypothesis that cryptic female choice through sperm selectiveness may operate as a mechanism to select for genetically dissimilar fathers.

Multiple paternity was resolved in 34 broods, although all fathers were not assigned. For 15 broods at least two fathers were assigned, and in all but two cases the paternity was unevenly divided with one male fathering a majority of the offspring. In these broods, where sperm competition inevitably must have occurred, genetic similarity between potential parents affected the outcome of competition, as in 11 of 13 broods the male most genetically dissimilar to the female (based on relatedness values (Wang 2002)) sired more offspring (paired t-test: t = 3.28, df = 12, p = 0.007). Given that the outcome of sperm competition is related to the genetic similarity between a male and female, a variable extrinsic to both individuals, these findings will not be caused by any intrinsic male characteristic such as testes mass or ejaculate size.

These results indicate that genetic similarity between male and female may affect cryptic female choice of sperm. Such cryptic female choice has previously been demonstrated in a sand lizard (*Lacerta agilis*) (Olsson *et al.* 1996), fruit fly (*Drosophila melanogaster*) (Mack *et al.* 2002), and field cricket (*Gryllodes supplicans*) (Stockely 1999). These results show that cryptic female choice and genetic similarity can play an important role in higher taxa as well, and given the high prevalence of polyandrous mating in

birds (Paper **I**) have important implications for sexual selection in this group, where post-mating female choice may be just as important to determining male reproductive success as pre-mating choice.

Mating success of male strategies

There are two genetically determined male mating strategies in the ruff (Hogan-Warburg 1966, van Rhijn 1991, Lank *et al.* 1995). *Independents*, are males that fight to attain a territory. The other strategy, S*atellites,* are males that never attempt to establish a territory, but instead act submissively towards territory holding males on the lek and can thereby attain access to a lek. The two strategies are thought to constitute a mixed evolutionarily stable strategy (ESS) and previous studies have shown that both strategies benefit from each other (Widemo 1998). There are approximately 16-19% satellites in ruff populations (van Rhijn 1991, Widemo 1998).

Recently, Lank *et al.* (2002) proposed that females may attempt to diversify offspring with respect to morph, as females were more likely to mate multiply with males of both morphs than by chance. Satellite-Independent cooperation on leks increases copulation rates for both morphs (Widemo 1998). Thus, females may also benefit by having offspring of both morphs through half-sib cooperation, as co-display by half-sibs would decrease the fitness costs of sharing matings with another male (Lank *et al.* 2002). In paper **III** we show that females are not genetically diversifying broods with respect to morph. In multiple fathered broods, two independents fathered chicks in 12 out of 34 (58.8%) of the broods. Both an

independent and a satellite fathered in four broods (11.8%), and two satellites fathered in 3 broods. None of the observed mating combinations differ from what was expected calculated from the proportion of males assigned as fathers to the different strategies (single father broods Fisher's exact: p = 0.42) (Table 3a) and multiple paternity broods (single father broods Fisher's exact: p = 0.32) (Table 3b). Thus, females did not appear to discriminate against males belonging to either strategy.

Table 3a. Numbers of offspring assigned to the two morphs in single paternity broods and the expected number of offspring calculated from the proportion of males assigned to the different strategies.(Fishers exact).

	Ind	Sat	p
Obs	30	2	
Exp prop	0.846	0.154	
Exp	27	5	0.42

Table 3b. Contingency table of morph combinations in multiple paternity broods with proportion of males assigned to the different strategies used to calculate expected values (Fishers exact).

	Ind-Ind	Ind-Sat	Sat-Sat	p
Obs	12	4	3	
Exp prop	0.72	0.26	0.02	
Exp	13.6	5.0	0.4	0.32

Sex allocation

Effects on female fitness do not, however, end with mate choice. Trivers & Willard (1973) suggested that females in good condition should invest more in the sex with the highest variance in reproductive success, given that the fitness of this sex is likely to be most

positively affected by good maternal condition.

In paper **V** we investigated if reeves allocate sex in a non-random way in relation to body condition (residual of weight on tarsi) (Bachman & Widemo 1999) (Trivers & Willard 1973). In lekking species a female's choice of mate (or mates) is relatively unconstrained where each female copulates with the male(s) they perceive to be most attractive. Therefore we would not actually expect females to allocate sex in relation to any male characteristic, as shown in monogamous species (Sheldon *et al.* 1999). Due to the early maternal care, and invariable clutch size a female's largest investment comes with laying and incubating the eggs. Therefore, the principal way in which a female can influence the fitness returns of her reproductive effort is likely to be through the allocation of resources (e.g. Cordero *et al.* 2000; Petrie *et al.* 2001) and, potentially gender (Nager *et al.* 1999) to her eggs.

Further, in species with little post-hatching care, for example those with precocial young, a female's largest investment comes with laying and incubating her eggs. There seems to be three ways a female could differentially invest in her eggs, one adjusting clutch size depending on condition and mate, as found in some bird studies (Bolton *et al.* 1993, Pietiäinen & Kolunen 1993, Sasvari & Hegyi 2001), the second being that females could differentially invest in provisioning male and female embryos through differential egg size (Cunningham & Russell 2000) or composition (Petrie *et al.* 2001), or females might be able to adjust the number of sons and daughters they have

dependent upon their condition (Wiebe & Bortolotti 1992, Nager *et al.* 1999, Sheldon *et al.* 1999).

Egg width and length was measured, and eggs were numbered using a permanent marker pen. Egg volume was calculated using Hoyts formula, (volume = 0.51x length x width) (Hoyt 1979). The sex of the chicks was determined molecularly (Griffiths *et al.* 1998).To determine which chick came from which egg, when hatching water-soluble food dye was carefully squirted through the hole that appeared in the egg. Three different colours of dye therefore allowed the subsequent identification of which chick came from which egg.

We did not find that females invested differentially in sons and daughters through egg size (paired t-test, $t = 0.60$, $n = 30$, $p = 0.55$), or weight (paired t-test, $t = 0.23$, $n = 29$, $p = 0.82$). Egg size was independent of female condition estimate ($r^2 = 0.01$, $n = 68$, $p > 0.41$). These results indicate that females are not allocating resources differently between the sexes.

We did find evidence of non-random sex allocation by reeves. At the population level, females adjusted sex ratios between years as overall female condition varied ($\chi^2 = 12.65$, $df = 6$, $p < 0.05$) (Fig. 1a). In the year when females were in overall poor condition and nested earlier, the population as a whole produced more daughters (2000: G test, $G = 4.83$, $n = 120$, $p < 0.05$) (Fig. 1b).
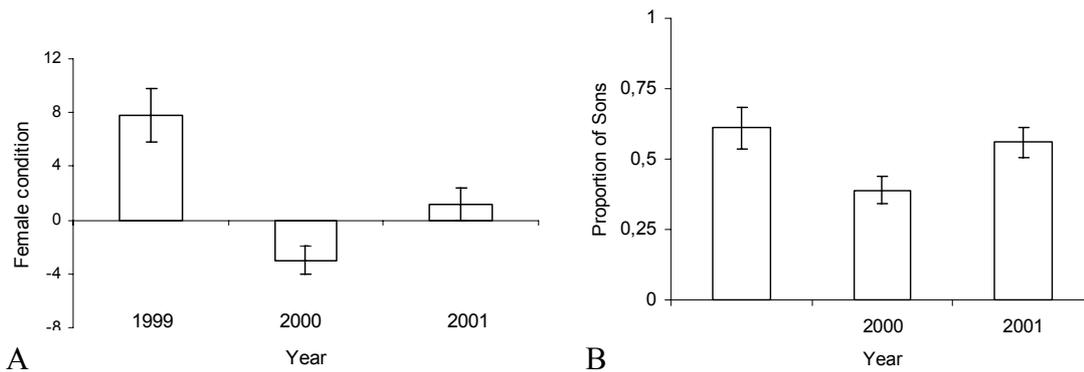
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Figure 1. A)Average female condition (± s e) in three study years, calculated as weight residuals, corrected for time of day and season, on tarsus length. B) Median proportion of sons per clutch (± s e) in three study years.

Conversely, in years when females were in better condition we could not find a deviation from an equal sex ratio (1999: G test, G = 2.26, n = 64, p > 0.05 resp. 2001: G = 0, n = 102, p > 0.05). These results highlight the shortcomings of short term studies, as theoretically non-random allocation is only likely to occur when the difference in fitness returns between the sexes is sufficiently great to outweigh the costs of allocation, for example in extreme years, and therefore one would not necessarily expect to see effects every year.

Looking within years, a relationship between individual female condition and sex ratio was only found in one of three years. In the year when the female population was in overall poor condition, individual females in better than average condition still produced more daughters (2000 r_s = -0.43, n = 32, p = 0.015), which is the opposite of what might initially be expected from ideas presented by Trivers & Willard (1973).

This leads us to consider the possible conditions under which daughters would give greater fitness to females in good condition than sons. There may be lots of ways in which daughter fitness could be more affected by female condition than that of sons, affecting variables such as survival to maturity, age at first breeding, longevity and lifetime fecundity. In the ruff very little is known about how these factors affect lifetime reproductive success and indeed the extent to which they might be affected by maternal condition. We cannot rule out that situations can arise where females in good condition should bias sex ratios towards sons. Our results here suggest that understanding the factors affecting lifetime reproductive success is an area worth pursuing.

Nest-site choice and food availability

The choice of nest site and chick rearing area is important for chick survival, and recruitment, and thus may affect female fitness. Predation is typically high for ground nesting birds (Hill 1984) and

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vegetation height and composition are known to affect predation levels (Galbraith 1989). As ruff chicks are precocial and there is little maternal care after hatching, it is important that breeding areas, in addition to suitable nest sites, also include habitats that can provide chicks with food and cover from predators. For most ground-nesting birds, clutch and chick predation is often very high (Hill 1984). Many waders nest in open habitats with little or no vegetation cover and rely on cryptically coloured eggs, chicks and adults for predator protection (Galbraith 1989). Avian predators depend on their vision to localise nests, and vegetation height could therefore be of great importance in choosing nest site and chick rearing areas (Andrén *et al.* 1985, Angelstam 1986).

In 2002, choice of nest site and chick rearing area in relation to food abundance and vegetation height and type was investigated. The vegetation of the open shore meadows on Faludden was classified into four different vegetation types.

I.   **Short grass** - mostly dry ridges with short and herb rich vegetation.
II. **Small wetlands** - patches with short vegetation that during most seasons are more or less filled with water for at least part of the breeding season.
III. **Dry grassland** - drier areas with relatively high and often tufted vegetation.
IV. **Wet tussocks** - wetter areas with a lush tufted vegetation, often around small wetlands with more dense tussocks than dry grassland.

The proportion of each vegetation type were estimated in the study area, using a grid based on markings placed every 50 meters on a map of Faludden (Fig 2). Using coordinates of the grid system, 41 sites were randomly selected. Vegetation type and vegetation height were measured at each site. Vegetation type was recorded and vegetation height was measured at all nests. In order to monitor each brood individually, females were also equipped with radio transmitters (BioTrack, TW-4). Brood movements were monitored daily by radio-tracking the mothers. Food availability was estimated by collecting invertebrates with a sweep net (Ø=25 cm) at eight random sites of each vegetation type (I-IV as mentioned above).
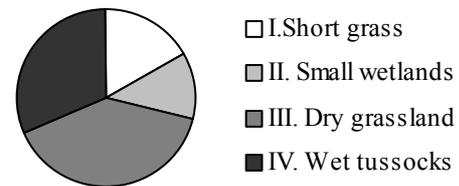

Figure 2. The diagram shows the distribution of the four vegetation types on Faludden. The most common vegetation type was vegetation type III, dry grassland, which covers 39% of the investigated area. Vegetation type I, short grass, covers 17%, vegetation type II, small wetlands, 12% and vegetation type IV, wet tussocks, 32%.

In paper **II**, we found a very clear pattern that reeves choose to nest within areas of suitable habitat. In total 33 nests were found 2002, and all of these were found within the two tufted vegetation types (III and IV). Nest site locations were hence significantly different from the distribution of available vegetation types on the study sites (Fisher's exact

test, p = 0.0022). Between the two tufted vegetation types III-IV there were no differences (G-test, G=0.262, p>0.05). Further, reeves show a strong preference to nest in areas with high vegetation (t-test, all t > 2.48, all p < 0.016) (Fig.3) and a higher than average preference to nest on sites where abundance of insects was high (t-test, t = -2.64, n_1 = 16, n_2 = 16, p= 0.014 Fig. 4), these are areas suitable for offspring to forage in. There was no difference in vegetation height between hatched and predated nests (t = 0.21, =16, p = 0.836).

fidelity in this population, and how factors such as hatching success affect site fidelity. Reeves are breeding site faithful, we know that from recovery data approximately 30% return several years in a row, and most nest within meters of the previous years nest (χ^2 = 3.92, df = 1, p= 0.047). The probability of a female returning was not dependent on nesting success in previous years (1999: G = 0.24, df = 1, p = 0.62; 2000: G = 0.017, df = 1, p = 0.90; 2001 G = 2.02, df = 1, p = 0.15; all years: G = 1.32, df = 1, p = 0.25).
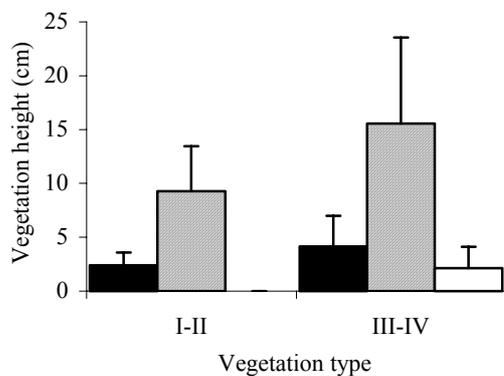

Figure 3. The diagram shows mean values and standard deviations of the two tufted vegetation types where nests and broods were found (III and IV) compared to the other two vegetation types (I and II). Black: mean vegetation height, Pattern: highest vegetation height, White: height of tussocks

Breeding area fidelity is one way that a female can increase nesting success through prior information on depredation levels, food abundance and habitat suitability (Oring & Lank 1984). For birds in general, female hatching success is thought to affect breeding site fidelity, where decreased success would be followed by dispersal to other areas (Oring & Lank 1984). In paper **II** we used recovery data from nesting reeves to estimate levels of breeding area
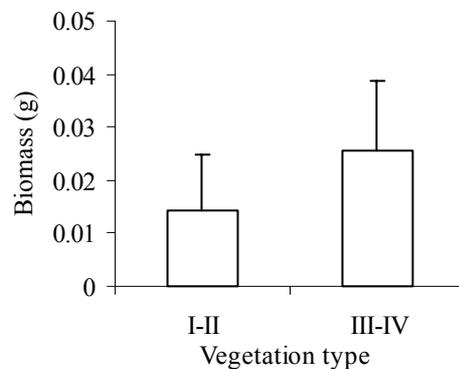

Figure 4. Comparisons between the two vegetation types where nests and broods were found (III and IV) with the other two vegetation types (I-II). The boxes show the mean values of the two sampling times and standard deviations.

Reeves possibly choose nest site location not only with suitable vegetation, but also with high abundances of insects for the precocial offspring. Where they choose to nest is most likely coupled with how the habitat looks the first year, as females nest in the same area even if there are large changes to the habitat, which can decrease hatching success. Our results highlight the risk of habitat degradation, even on a short time scale, affecting populations of breeding ruffs negatively, as nesting females are trapped in sub-optimal environments.

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Conclusions

Females mate with several males resulting in sperm competition and multiple paternity. Although females appear to be mating randomly with respect to genetic similarity, genetic similarity does have an effect on male reproductive success. This can have interesting implications for sexual selection as male reproductive success will vary depending on the female he mated with. A superior male and perhaps the top male on a lek can lose paternity to another less favoured male simply because of his genotype.

Females do not appear to discriminate between males of either strategy, as both strategies father their expected proportion of offspring in the population. Yet another suggestion, that females may induce half-sib cooperation through genetically diversifying broods with respect to morph (Lank *et al.* 2002), was also not supported in this study.

We found condition dependent sex allocation in females, where females that are in relatively better condition had more daughters. However non-random sex allocation may be costly as females that were in relatively worse condition were not deviating from a random sex ratio. However, females in good condition only deviated from random allocation in atypical years, indicating that non-random allocation is only likely to occur when the difference in fitness returns between the sexes is sufficiently great to out-weigh the costs of allocation.

Females prefer to nest in tufted areas with higher than average vegetation and insect abundance. As the offspring are precocial and receive little help from their mother, it is important for the female to nest in areas where the chicks can find food. The probability of a nest being predated was not affected by vegetation height. There could be several reasons for this. Predation could be affected by bird density (Dyrcz *et al.* 1981) and distance to predator perches (Andrén *et al.* 1985, Angelstam 1986) which are both are known to affect predation risk.

A large piece of missing information in this study are the long term effects that female choice and condition have on offspring as I was unable to follow chicks through to maturity. As this was field study with little possibility to manipulate factors affecting female choice, this study has focused on natural variation. To fully understand female choice and its repercussions experiments on a captive population are needed. This study has only scratched the surface in understanding reproductive strategies in ruffs. We do not yet understand why females choose certain males or if and what is the difference between females who only choose one male. Observational data together with genetic data will make the picture clearer but controlled experiments will be needed to fully understand what females gain by mating multiply.

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