 doi:10.20944/preprints202203.0388.v1

Review

Evolutionary Ecology of Fixed Alternative Male Mating Strategies in the Ruff (*Calidris pugnax*)

Michel Baguette[1,3], Baptiste Bataille [2] and Virginie M. Stevens [3,*]

[1] Institut Systématique, Evolution, Biodiversité (ISYEB), UMR 7205 Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France; michel.baguette@mnhn.fr
[2] Jozef De Windestraat 17, B-1700 Dilbeek, Belgique; baptiste@baptiste-bataille.com
[3] Centre National de la Recherche Scientifique, SETE Station d'Ecologie Théorique et Expérimentale, CNRS UAR 2029, Moulis, France
* Correspondence: virginie.stevens@sete.cnrs.fr

Abstract: A few empirical examples document fixed alternative male mating strategies in animals. Here we focus on the polymorphism of male mating strategies in the ruff (*Calidris pugnax*, Aves Charadriiformes). In ruffs, three fixed alternative male mating strategies coexist and are signaled by extreme plumage polymorphism. We first present relevant data on the biology of the species. Then we review the available knowledge of the behavioral ecology of ruffs during the breeding season and we detail the characteristics of each of the three known fixed male mating strategies. We next turn to the exceptional quality results accumulated on both the structural and functional genomics of the ruff over the past few years. We show how much these genomic data can shed a new, mechanistic light on the evolution and maintenance of the three fixed alternative male mating strategies. We then look if there are sufficient indication to support frequency-dependent selection as key mechanism in maintaining these three strategies. Specifically, we search for evidences of equal fitness among individuals using each of the three strategies. Finally, we propose three lines of research avenues that will help to understand the eco-evolutionary dynamics of phenotypic differences within natural populations of this iconic model species.

Keywords: phenotypic polymorphism; structural genomics; chromosomal inversion; supergene; functional genomics; hormonal plasticity; frequency-dependent selection; cryptic female choice of sperm; sexual selection; eco-evolutionary dynamics

1. Introduction

The most common occurrence of alternative male mating strategies (AMMS) in animals involves the coexistence of two extreme behaviors: (1) males that defend either a territory or a group of females and gain access to mates through their aggressive behavior, and (2) males that perform various forms of sneaking behavior by parasitizing the attractiveness of others [1]. In many animal species, AMMS are signaled by phenotypic polymorphism [2-5]. Earlier works focused on the mechanisms and evolutionary stability of alternative phenotypes coexistence in populations, while speculating on the environmental *vs.* genetic origin of this polymorphism. Most reported variation in reproductive behavior within populations is nongenetic, and even when genetically determined most strategies turn out to be conditional and reversible [1,6,7]. In conditional strategies the behavior an individual adopts is determined by some aspect of their state (*e.g.*, age, size, conditional). However, in a few cases genetically determined strategies are fixed all over individual's lifetime [8]. Conditional strategies correspond thus to behavioral plasticity: individuals adopt well-defined behavioral tactics but can change them according to their state (*e.g.*, age, size, condition). In genetically determined fixed strategies, behavioral tactics that the individual can adopt remain constant throughout its life, regardless of its condition. Theory suggests that conditional male mating strategies are most frequent at intermediate levels of variance in male mating success, whereas fixed strategies evolve

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 March 2022 doi:10.20944/preprints202203.0388.v1

mainly when male mating success is highly skewed, but also when the costs and limits of being conditional are very high, or the benefits of being conditional are very low [8]. Importantly, if there is either fitness costs or limits to behavioral plasticity, conditional strategies are never able to entirely replace fixed strategies, and equilibrium populations may frequently consist of a mixture of conditional and fixed strategies [8].

According to the evolutionary game theory, the maintenance of fixed AMMS based on genetic polymorphism should depend on negative frequency-dependent selection that provides each strategy with equal fitness [2]. However, only but a few empirical examples document genetic-based, fixed alternative strategies, and the evidence of frequency-dependent selection and/or of equal fitness between strategies remains elusive [2]. A noticeable exception is the side-blotched lizard *Uta stansburiana*, in which three differently colored male phenotypes each have a distinct mating strategy. In this species, color phenotypes, and thus mating strategies are genetically determined and have high heritability [2]. Each of them has a fitness advantage over one of the other two, and is inferior to the last. There is frequency-dependent selection on these phenotypes, which translates into cycles of morph frequency in populations over time [2]. Sinervo & Lively [2] suggest that "frequency-dependent selection maintains substantial genetic variation in alternative male strategies, while at the same time prohibiting a stable equilibrium in morph frequency".

This well-documented study remains dramatically isolated, however. In particular, the scarcity of empirical demonstration that fixed AMMS are maintained by frequency-dependent selection raises the questions of their origin, maintain and evolution. Austad in 1984 [6] mentions that in the ruff (*Calidris pugnax* [LINNÉ, 1758]), a Palearctic breeding shorebird that is a classic case of male mating strategy polymorphism, a genetic mechanism could interfere with frequency-dependent selection to maintain the different strategies documented in this species [6]. In the light of current knowledge, the simple genetic scenario of two alleles at a single locus proposed by Austad [6] does not correspond to the autosomal inversion and recombination that are at work to generate and retain the ruff male polymorphism [9,10]. However, it is a premonitory vision of the importance of the joint role of ecological (frequency-dependent selection) and evolutionary (complex genomic mechanisms) as drivers of fixed AMMS that deserves to be highlighted.

Here we focus on the evolutionary ecology of the polymorphism of mating strategies in the ruff. We first present relevant data on the biology of the species. Then we review the available knowledge of the behavioral ecology of ruffs during the breeding season, and we detail the characteristics of each of the three known fixed male mating strategies. We next turn to the exceptional quality results accumulated on structural and functional genomics of the ruff over the past few years. We show how much these genomic data can shed a new, mechanistic light on the origin and evolution of phenotypic polymorphism associated to the three alternative male mating strategies. We then examine whether currently available data do support frequency-dependent selection as key mechanism in maintaining alternative male mating strategies. Specifically, we search for evidences of equal fitness among individuals using each of the three strategies. Finally, we propose three lines of research avenues that will help to understand the eco-evolutionary dynamics of phenotypic differences within natural populations in this iconic model species.

2. Biology of ruffs

2.1. Systematics and taxonomy

The ruff *Calidris pugnax* (Aves, Charadriiformes) is a shorebird belonging to the Scolopaci clade (suborder), the Scolopacini tribe, the Scolopacidae family and the Calidridinae subfamily (*e.g.*, 11-14). Ruff was initially described as belonging to the polytypic genus *Tringa* LINNÉ 1758. The species was then placed into different monotypic genera (*Philomachus* MERREM 1804, *Pavoncella* LEACH 1816, *Machetes* CUVIER 1817). This mon-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 March 2022 doi:10.20944/preprints202203.0388.v1

otypic treatment is probably due to the many particularities of this species, which distinguish ruffs from other Charadriiformes. However, recent phylogenetic studies based on nuclear and mitochondrial genes [15,16] revealed that the species was closely related to birds belonging to the genus *Calidris* MERREM 1804. Accordingly, *Calidris pugnax* is now acknowledged as the valid scientific name of the species [17-19].

2.2. Adult description

The most conspicuous particularity of ruffs is the polymorphism of male nuptial plumages. These plumages are progressively acquired by a nuptial molt during the spring migration, and lost during a post-breeding molt until mid-autumn [20]. Individual males can be assigned unambiguously to three different categories of nuptial plumages (Figure 1). The "darkish" males may wear a blackish-reddish-bluish ruff of elongated neck-feathers, two tufts on top of their head and a collection of small facial wattles between bill and eyes [21]. The "whitish" males may wear the same ornamentation but their color is here predominantly white [21]. The coloration patterns of individuals are highly variable, making reliable identification possible with human eyes [20-22]. A third category of males does not develop male nuptial plumage. Those males that are similar to females in nuptial plumages were first called "naked-nape males" by Hogan-Warburg [21,23] and then "faeder" by Jukema & Piersma [24]. Males maintain a single nuptial plumage phenotype throughout their adult lifetime [20,21,25]. Female nuptial plumage is only slightly different from their winter plumage, which is also similar to the prenuptial plumage of males [21]. Variation among female nuptial plumage exists in females but is usually too vague to allow individual identification of females [21].

Figure 1. Illustration of ruff plumages: variable darkish males, a whitish male and a female. The dark male in the background adopts the squat posture (see text). Painting by Johann Friedrich Naumann (1780–1857), public domain.

Another particularity of ruffs is a sexual dimorphism in size and weight: males are larger and heavier (10% to 70% [14]) than females, which is unusual in birds [27]. As shown by the extensive analysis of such sexual size dimorphism in shorebirds, this reversal from the general pattern is related to polyandrous mating system [27], which is indeed observed in ruffs, (*e.g.*, [21,28-30]). There are also size and weight differences among the three male plumage phenotypes: darkish males are heavier with longer wings, tarsus and overall body size than whitish males [31], whereas wing length and weight of naked-nape males males fall in-between females and darkish and whitish males [24].

2.3. Distribution and habitats

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 March 2022 doi:10.20944/preprints202203.0388.v1

The ruff breeds in lowlands of high and low Arctic and subarctic, in boreal and temperate zones in Europe and Siberia almost to Pacific, towards oceanic fringe of west Palearctic, and overlaps steppe zone in continental interior (Figure 2) [14,26,32-34]. The main wintering area is Africa, where most Fennoscandian breeders overwinter in Sahel zone, and most Siberian breeders overwinter in east and southern Africa [32]. Some western breeders spend the winter in western, south-western and central Europa, whereas some Siberian breeders overwinter in the Middle East, India and easternmost Asia (Figure 2) [14,26,32-34]. Sexes are not distributed evenly on the wintering grounds. Males tend to overwinter northerly, whereas females go further south (26,32,36]; the proportion of females wintering in Africa exceeds the female proportion in breeding populations, whereas almost all birds overwintering in western Europe are males [32]. This sex bias is explained by sexual selection for earlier return of males on breeding grounds [35], which is a general pattern in birds in which male fitness depend on the number of mating (*e.g.* [36]). Migration departure of both sexes also differs in time, males leaving their wintering areas sooner than females in March, and their breeding areas in July several weeks before females. Juveniles fly northwards simultaneously or even later than females [35]. The ruff total world population is estimated at over 2 million individuals, with 1 million overwintering in western Africa and 1 million in east and south Africa, and in Asia [14].

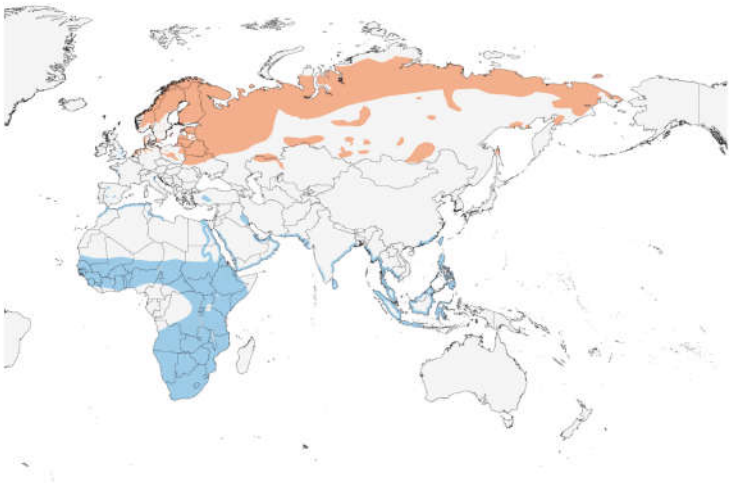

Figure 2. Distribution map of ruff (*Calidris pugnax*) breeding (orange) and wintering (blue) areas [14], © BirdLife International.

The breeding habitats of the ruff are coastal or inland wetlands with adjacent feeding, courtship and nesting areas; usually in coastal tundra to forest tundra near small lakes, in marshes and deltas with shallow-water margins in boreal zones; in damp to swampy meadows in Western and Central Europa (*e.g.* [14,26,34]). Outside breeding season, the proximity between feeding, resting and roosting areas is relaxed. The ruff prefers wetlands, but feeds on grass, wheat or rice fields, not always close to water. Birds use nighttime roosts on shallow water along lake edges (*e.g.* [14,26,34]).

2.4. Food and foraging

The diet consists chiefly of invertebrates during the breeding season, mainly larval and adult insects (aquatic and terrestrial), but the ruff feeds also on molluscs and earthworms. Outside the breeding season birds feed on a wider range of animals, including amphipod Crustaceans, spiders, frogs and small fish, and on vegetal material, mainly seeds but also vegetative parts of plants (*e.g.* [14,26,34]). Individuals forage for food by

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 March 2022 doi:10.20944/preprints202203.0388.v1

walking on the ground or by wading in shallow water; their bill probes mud or soft soil, or picks up material from soil or water surface and from vegetation. When foraging in water, birds sometimes immerse their heads [14,26].

2.5. Demography

The yearly survival is 0.52 ± 0.04 with a type II survivorship (constant over time) and no sex difference reported. The oldest ringed bird is 10 year 11 months [26]. The age at first reproduction is 2 years, but probably older for males [14]. There is one brood per year. The mean clutch size is 3.72 eggs ± 0.31 (n=18) [37]. The hatching success is 0.92 (n=62) [37]. The fledging success is not yet documented. The sex ratio in juvenile and adult populations is ♂:♀ 0.34 [38].

3. Behavioral ecology of breeding ruffs

3.1. Male strategies on leks

Lekking behavior is the most striking particularity of the ruff. Males aggregate on mating arenas ("leks") and display close together [39]. Females visit the lek and are free to mate with any male on the lek. After mating, the female leaves the lek whereas the male stays and continues to display towards other females. The female incubates the eggs and attend the young all by herself. Males provide no resource, except the sperm necessary to fertilize the egg, and no parental care [39]. Lekking is most prevalent in avian across the Animal kingdom, even if there is a strong taxonomic bias towards a few bird families [39]. Apart the ruff only a handful of Scolopacidae species that use this mating system (the great snipe [*Gallinago media*] [(40], the buff-breasted sandpiper [*Calidris subruficollis*] [41], and the pectoral sandpiper [*Calidris melanotos*] [42]). However, ruff's lekking behavior is even more particular because the three plumage phenotypes of males described above (darkish, withish and naked-nape males) correspond each to different courtship behaviors on the lek [20,21,29,30]. We will therefore use from now on the term "phenotype" to designate both the plumage and the courtship behaviors subsuming the three fixed AMS used by males.

The *independent strategy* is used by darkish males. Some independent males set up small display territories (approximatively 30-60 cm in diameter) within a main arena. Those males are called *resident independents* [20,21]. The main arena (about 10 m in diameter) is located close (300-400 m) to suitable breeding sites, and may be used for several dozens of years [20,22]. Display territories are grassy areas that are trampled up to becoming bare ground at the end of the breeding season [20]. Each display territory has its own independent resident [21]. A peculiarity of display territories of residents is that they are not contiguous: there is a space in between, which is not specifically claimed by any of the resident males [21]. The size of this space is variable (100-150 cm) and seems directly dependent on the size of the lek[21]. Resident males defend vigorously their display territories against territorial independent neighbors or any independent males moving in between territories. Residents returns to their display territory after absence from the lek; they arrive before dawn, even when completely dark and stay until dusk, or even spend night there ([21], overall spending most of their time (*ca.* 90% of daylight) during the 6-8 weeks of the lekking season in their display territories [20,26]. Residents may leave the lek to forage nearby around midday, though any conspecific interaction on the lek entails immediate back flight [21]. The number of attending residents can vary greatly in space (across arenas) and time (over the season), but average between 3 and 8 [21]. Many residents males remain faithful to the same lek for up to five breeding seasons [20].

All independent males do not succeed in establishing a display territory on the arena. Those males that fail to own a display territory are called *marginal independents* because they are located at the margin of the arena, out of the area occupied by the display territories [21]. They spend less time on the lek, and are less regular there than their resident counterparts. Marginals are frequently attacked by residents during their visit on

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 March 2022 doi:10.20944/preprints202203.0388.v1

the lek, but usually lose their fights with territory owners [43]. These floater individuals are ususally young, unexperienced low ranking males that may become territorial in subsequent years [20,21,43]. Others are territory prospectors that may establish new territories usually at the border of the lek. These new residents try to move towards the center of the arena if vacancies occur, which gives rise to fights with prospective neighbors [20]. More rarely, territory prospectors may oust resident males during the course of the breeding season [20,21]. Ousted resident males behave then as marginals, and visit the lek even more rarely than other marginal males [21]. Whereas resident males rarely if ever visit another arena, they behave there as marginal males [21]. Marginal males are less faithful to the lek from one year to the next [44].

The *satellite strategy* is used by withish males. Satellites are smaller than independents, and usually do not engage in aggressive behaviors or set up display territories. Rather, a satellite male will tend to form a coalition with a resident to perform a joint, ritualized display when females visit the lek (see below). Satellite males usually prefer high ranking residents, and high ranking residents can be visited by up to four satellites [20]. However, satellites behave opportunistically: they follow females visiting the arena and doing so, they can move among territories [21]. Satellites spend less time on the lek than residents [20, 21]. Resident males have variable reactions towards satellites: they can attack and try to expel them, or on the contrary they may allow the resident coming into their display territories and to stay there in their company [21]. The adoption of a given satellite by a resident male seems a progressive process: if the resident male is not tolerant, the satellite will "freeze" in a distinct posture (the "squat" posture) and accept pecks by the resident to possibly acquire a position on the display territory. If the resident male is tolerant, the satellite will adopt distinct postures ("oblique" and "upright" postures) [20]. Different satellites may have different preferences for display territories on the lek [20] and for resident males (one or two by satellite individual [21]), which suggests the establishment of a privileged relationship with the resident owner of the display territory, and therefore reciprocal individual recognition.

Similar to what happens with independents, there are *central* and *peripheral satellite* males. Central satellites visit the display territories and the area in between them more often and for longer period than marginal satellites. Central satellites are also more easily accepted by residents than peripheral ones. Peripheral satellites spend less time on the lek than central ones [21,44], and seem less faithful to the lek from one year to the next [44].

The *sneaker strategy*, i.e. the parasitism by some males of the attractiveness of others is used by naked-nape males. Sneaker males have no ornementation and do not perform display behavior [9,24,34]. They wander through the lek where their female-like phenotype inhibits aggressive interactions with other males. Sneaker males rapidly sneak copulations when females solicit matings from ornemanted displaying males [9,34]. However, precise and detailed quantitative data on the behaviors of sneaker males are still missing (*e.g.* [44]).

The frequency of these three male phenotypes is rather constant over time and across space [7,20,21,39], even if there may be some discrepancies between frequency estimates on leks and within populations due to differences in patterns of lek attendance between resident independents, marginals independents, and satellites [45]. The respective proportions of the three phenotypes within populations are *ca.* 83-85% of independents, 14-16% of satellites and 1% of sneaker males (*e.g.* [23,25,34]). Importantly, the relative constancy of these phenotypes do not support the dramatic cyclic changes in phenotype proportions within populations as predicted by frequency-dependent selection of alternative male-mating strategies (*e.g.* [3,46].

3.2. Male-female interactions on leks

Females return from migration and arrive later than males on breeding grounds [21]. They form mixed flocks with males that forage on meadows and visit lekking areas. While males progressively install on leks, females began their solitary life in the surroundings

 doi:10.20944/preprints202203.0388.v1

[21]. Males on leks have to incit flying flocks or single females to land. Usually most ornamented males on leks whatever their phenotype perform a *reception ceremony* [20, 21], in which they flap both wings and display their white underside,which results in flash-like signals showing unambiguously the location of the lek. Females visiting leks land in between territories. Resident males on their territory display up-down movements oriented towards females, and then "freeze" in a "*squat*" posture, Figure 1). A chain reaction in the lek may occur from the movements of a resident male that drive all other males to resume their displays, including postures or attacks or residents that are directed towards their neighbors or towards satellites (21, 20). Females observe male displays, walking on the leks and either fly away or sollicit copulation by crouching while installed on a display territory [21]. The installation of a female on a display territory is favored when the resident interrupts his squat posture. Van Rhijn [20] showed that this interruption, and the subsequent series of short movements is higher when the resident male is accompanied by one or more satellites.

The advantage for a resident of having satellite is thus the raise of his activity level that incits a female visit on his territory. In that sense, satellite males can be considered as "kingmakers" [34]. However, Van Rhjn [20] suggests that the presence of satellites is no longer advantageous to the resident male after the arrival of a female on his territory. Satellites perturb the stimulation of females to crouch, and even when the female crouches resident males are almost unable to copulate when a satellite is present [20]. Residents use two tactics to avoid having both satellites and females on their territory. Firstly, resident males who frequently copulate are strongly intolerant to the presence of satellites and prevent their presence on their display territory. Secondly, resident males become intolerant to the presence of satellites when a female visit their territory. During the interruption of the squat posture, these males harrass their satellite by turning around them, pecking at their wattles and even actually attacking them. Some satellites manage to resist such harrassment and hinder the resident copulation, even to the extent to mount themselves the crouching female [20,21]. The behavioral interactions between residents and satellites on leks include thus cooperative and competitive elements [45]. Hugie and Lank [45] emphasize that residents do not merely tolerate satellites, but appear sometimes to actively recruit them onto their courts. Furthermore, most intolerant behavior appears to be an attempt to control rather than to evict satellites. Such behavior suggests that resident males benefit from having a satellite on their court, even though they are reproductive competitors that must be controlled [45]. Another tactic used by satellites is to follow females walking on the lek. The visit of a female to a display territory often results in the resident male owner being attacked by a neighbor. On this occasion, a central satellite present in the immediate vicinity may take advantage of the presence of a receptive female attracted to the resident and mate with her, taking advantage of the resident owner settling accounts with his neighbor [43]. The sneaker mating tactic seems to follow the same scenario [9,20, 21,24,34].

The comparison of independent-satellite copulation success on leks shows that males copulating with female are mostly resident independents (61-80% in [21], 82%-95% in the extensive study of Vervoort & Kempenaars [44]. The latter study reveals that among satellites, only central males manage to copulate on leks. The rarity of sneakers has so far precluded a quantitative assessment of the mating success of this phenotype [44]. Another limitation of the comparison of phenotype mating success is the lack of data on possible copulation out of the leks. Satellite and marginal independent males frequently move with females between leks and foraging sites, and often display to foraging females. Such highly mixed groups of birds are hard to follow, but observations at suitable foraging sites show that copulations do occur outside leks [23].

Widemo [23] mentions that satellites are remarkably quick to mount a female soliciting an independent male. Satellites complete a copulation in less than a second, which means that in many cases females are probably not able to terminate the copulation before its completion. Van Rhijne [20] reports that the mating duration of satellite males is shorter than that of residents, and Küpper et al. [9] makes the same observation for

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 March 2022 doi:10.20944/preprints202203.0388.v1

sneakers. But at this stage, there does not seem to be a formal comparative study of the copulation duration between the three male phenotypes. Nonetheless, the faster copulation of both satellite and sneakers can be related to the larger testes of males belonging to these two categories. Jukema & Piersma [24] mention that sneakers had testes 2,5 times the size of "normal" males in April. Küpper *et al.* [9] showed that testes of satellites can be even larger than those of sneakers. They suggest that these differences in testes size might explain the persistence of the satellite and the sneaker phenotypes via successful sperm competition despite their lower mating success as measured on leks [23,45]. Ruffs have the longest sperm of any shorebird yet measured, which supports an evolutionary history of sperm competition [47,48]. Accordingly, it should be noticed that more than 50% of female ruffs are polyandrous, which is the highest rate of polyandry known in a lekking bird [48]. This observation leads Lank *et al.* to suggest that female ruffs actively genetically diversify their offspring [48]. A pedigree study actually demonstrates that in direct sperm competition, the male siring the majority of offspring is most often the least genetically similar to the female [49]. Thuman & Griffith [49] speculate that their results provide support for preferential female cryptic choice of sperm from the least genetically similar male. As we will see, recent genetic and genomic investigations shed new light on what can be the proximate mechanism of this offspring genetic diversification, and the ultimate mechanism of the maintenance of stable genetic polymorphism for alternative reproductive strategies [9,10].

4. Genetics and genomics of male alternative strategies in ruffs

Male plumages are thus reliable signals of the fixed behavioral strategies they will use on leks to gain successful copulations, whereas there is no obvious parallel expression in females [50]. To investigate if there is a genetic determinism in the transmission of these morphological and behavioral phenotypes would help to gain insights on the evolution and maintenance of the alternative mating strategies. Suprisingly, pedigree data of male phenotypes support an autosomal model of genetic inheritance, which contrasts with the sex-linked inheritance reported in other taxa using sex-limited alternative mating strategies [25,46,50]. As predicted by this autosomal model of genetic inheritance, testosterone-implanted females of known lineages show subsequent male mating behaviors that parallel those of their brothers and half-brothers [50]. Pedigree analyses reveal that the three male phenotypes are controlled by a single Menedelian locus with three alleles. Two alleles, *Faeder* (coding for the sneaker strategy of naked-nape males) and *Satellite* (coding for the independent strategy of whitish males), are dominant to *Independent* (coding for the independent strategy of blackish males) but the precise genetic architecture of phenotype determination remained unclear until the use of genomic methods [9,10,25,46,50].

4.1. Structural genomics

Single loci controlling the polymorphism of complex phenotypes (known as supergenes) are indeed a target of of choice for genomic studies [51]. Two independent studies published in 2016 in *Nature Genetics* manage to unravel the location and the structure of the ruff supergene that directs the three male phenotypes [9,10]. It consists in a block of ca. 90-125 genes located on a single chromosome (*ca.* 20% of chromosme 11) inherited together, mostly without recombination [9,10,34,51]. The structure of the block differs between the three alleles. The *Faeder* allele derives from the *Independent* allele by an inversion of the supergene [9,10] that probably happened *ca.* 3.8 million years ago [10] (Figure 3). The *Satellite* allele results from an exceptional recombination event between the *Independent* and the *Faeder* alleles that occurred later, *ca.* 500,000 years ago [10] (Figure 3). Satellite males have indeed intermediate phenotypic characters between the independent males such as a ruff, and the sneaker males such as a small size and a nonagonistic behavior. Satellite males have also their own characteristic like the whithish color of the ruff [34,51]. Importantly, these two studies report that the inversion disrupts the gene

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 March 2022 doi:10.20944/preprints202203.0388.v1

coding for a protein playing an essential role in the formation of centromeres (the *CENPN* gene encoding centromere protein N) [9,10]. Homozygosity for the inversion is lethal ([9]), which means that there is no carrier of two *Satellite*/*Satellite* alleles, of two *Faeder*/*Faeder* alleles, but also of the *Faeder*/*Satellite* alleles. Loveland et al. [52] suggest that the inversion is homozygous lethal because reduced expression from two inversion alleles would be insufficient to sustain cell division.

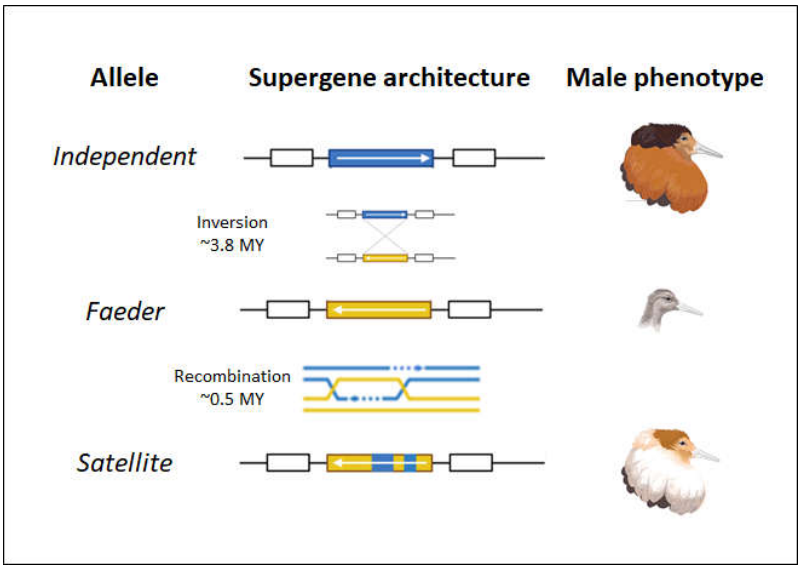

Figure 3. Schematic overview of the evolution of the ruff supergene that regulates the male phenotypes. The inversion of the ruff supergene that occurs *ca*. 3.8 million years ago on the chromosome 11 gives rise to the sneaker phenotype. An exceptional viable recombination event between the *Independent* and *Faeder* allele (*ca.* 500.000 years ago) produces the *Satellite* allele that gives rise to the satellite phenotype. Modified from [52].

4.2. Functional genomics

The two studies that document the inversion and the recombination of the ruff supergene provides a list of candidate genes located in this genomic region that could explain differences in male phenotypes [9,10]. These candidate genes can be assigned to three broad functional categories: (1) genes with roles in the metabolism of sex hormones, (2) genes controlling for either color polymorphism or feather morphogenesis in birds, and (3) genes associated with sperm motility and gonadal expression [9,10]. Altogether, the functions allocated to the genes linked on the supergene and their molecular variation resulting from the inversion and recombination events are coherent with the variation of the ruff male phenotypes [9,10]. The analyses of nucleotide sequence divergence of candidate genes provided new insights on the evolution of the three phenotypes. Gene sequences involved in steroid metabolism are more divergent between sneaker and the two other phenotypes than between independent and satellite males. A gene involved in sperm motility was more divergent between independent and the two other phenotypes than between satellite and sneaker males [9]. A candidate genes for variation in pigmentation, *MC1R* (encoding melanocortin 1 receptor), is located within the inverted region. The three male phenotypes have distinct alleles of the single *MC1R* exon [10]. Theses authors suggest that the *MC1R* allele on *Satellite* supergenes, possibly together with altered metabolism of sex hormones, underlies the white color of ornamental feathers in satellite males [10].

Recent studies go further than gene function assignement by showing changes in the expression of genes located on ruff supergene [52,53]. The key point is to investigate if gene expression is altered on alleles of the supergene by comparing the expression of genes located upstream and downstream of the inversion breakpoint [52]. As previoulsy mentioned, the inversion occurs within the *CENPN* gene. In independent males carrying

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 March 2022 doi:10.20944/preprints202203.0388.v1

no inversion, the expression of *CENPN* transcripts is broadly similar upstream and downstream of the inversion breakpoint, whereas in both sneaker and satellite males *CENPN* transcripts from downstream the inversion point have at least twofold lower expression than those from upstream of the inversion breakpoint [52]. The expression of several genes located on the supergene is similarly affected [52].

Changes in both gene sequence and expression were thus observed among the three male phenotypes. However, the identification of the very molecular processes responsible for male morphological and behavioral differences is a complex task, as examplified by a recent experimental study [53]. The role of steroid hormones, especially testosterone, in eliciting morphological and behavioral changes among the three phenotypes has been suggested by three lines of evidences: (1) castration of males prevents the moult of the nuptial plumage in independent and satellite males [54], (2) sub-cutaneous implementation of physiological levels of testosterone to females induces the reversible acquisition of independent- and satellite-like plumage, body mass and behavior [50]; and (3) independent males have higher physiological concentration of testosterone than satellite or sneaker males ([9]). Several genes located on the supergene (namely *HSD17B2*, *SDR42E1*, *CY5B5*) are involved in sex steroid synthesis and metabolism, which suggests a direct effect of supergene alleles on hormone production and regulation [53]. In particular, *HSD17B2* encodes the enzyme that converts testosterone back to its precursor androstenedione. *HSD17B2* is a thus candidate gene to explain the hormonal difference among phenotypes [53]. Sneaker and satellite males have higher concentrations of the circulating precursor androstenedione than independent males [9]. The experimental elevation of circulating androstenedione results in increased aggression in independent males but fails to induce aggressive behavior in satellite males, even if courtship behavior of satellites males raise [53,55]. *HSD17B2* contains several deletions in the *Fader and Satellite* alleles of the supergene and in their immediate surrounding [9,10]. The experimental stimulation of the pituitary gland that should induce sex steroid synthesis in male gonads fails to provide a durable increase in testosterone concentraion in both sneaker and satellite males [53]. A simple explanation could be higher levels of *HSD17B2* enzymes in these males. However, gonadal *HSD17B2* levels are similar across phenotypes [53]. This result suggests thus the existence of an impairment in androstenedione to testosterone conversion in males carrying the *Fader and Satellite* alleles [53]. Based on several similar differences of hormonal production and regulation among phenotypes, these authors and others speculate that genomic rearrangements like inversion generate hormonal plasticity by modifying the expression of genes involved in hormone synthesis and receptivity [53,56,57]. Lovejoy et al. [53] go further by proposing that sequence evolution over time combined with selection on certain supergene haplotypes may further canalize hormonal profiles into a restricted range that becomes associated with specific behaviors and morphological traits [53].

5. Evolution and maintenance of male alternative strategies in ruffs

The evolution of fixed alternative male mating strategiesin ruffs rely thus on genomic rearrangements. that gives rise to the sneaker and the satellite phenotypes. The timing of these rearrangements (3,8 MY for the inversion and 0,5 MY for the recombination, [10]) shows that there is a strong asynchrony in their appearance within ruff populations. There are also strong differences in population frequency of males using each of the strategies, at least on leks (independent: 83-85%, satellite: 14-16% of satellites and sneaker: 1%, *e.g.* [23, 25, 34]) but these differences remain stable across space and over time. Game theory predict that the spread and the long-term maintenance of these strategies from the event of genomic rearrangement is in agreement with the prediction of an equal fitness of the three phenotypes provided by frequency-dependent selection [2]. However, the detailed understanding of how frequency-dependent selection works should be based on a thorough analysis of the fithess costs-benefits associated with each phenotype. We summarize here the available information for such an analysis. We will see to what extent

 doi:10.20944/preprints202203.0388.v1

there is sufficient explanation for the coexistence of the three alternative fixed male strategies. We then propose research avenues that are needed to refine the diagnosis of frequency-dependent selection.

Our starting point is the lethality of homozygous individuals for inversion, *i.e.* the *Satellite*/*Satellite*, *Faeder*/*Faeder* and *Faeder*/*Satellite* genotypes. Such lethality should purge these rare alleles on the long term, a situation that is not observed in the ruff. Some authors therefore conclude that carriers of the inversion in one or both sexes must have a higher fitness than those individuals that are homozygous for the ancestral *Independent* allele [9,10]. For Lamichhaney et al. [10], those individuals that are heterozygous for the *Satellite* allele should have about 5% higher fitness to maintain an allele frequency of about 5% in compensating for the lethality of the homozygote [10]. Küpper et al. [9] propose that the costs related to the lethality of inversion homozygous carriers, and to the alleged low survival of heterogygous individuals at the supergene they observed in experimental crossings might be balanced by a higher reproductive success of sneaker and satellite males [9].

In his review of alternative reproductive strategies, Gross [2] briefly mentions the ruff as a study system for fixed strategies, and indicates that "there are presently no fitness measurements that include both mating success and life history differences, nor are there data to test for frequency-dependent selection" [2]. It must be said that more than 25 years later, such crucial data are still missing. However, some elements might help to understand how this frequency-dependentselection is likely to occur.

Firstly, the copulation success of males on leks is very unevenly distributed among phenotypes, which corresponds to the theoretical prediction of Plainstow et al. [8] that conditional male mating strategies are most frequent at intermediate levels of variance in male mating success, whereas fixed strategies evolve mainly when male mating success is highly skewed. As previoulsy mentioned, resident independent males obtain the vast majority of copulations. Should we conclude that this success is responsible for the greater frequency of the independent phenotype in ruff populations? The answer is no, for several reasons. Copulation success is monitored on leks, for practical facilities but copulations occur outside leks on foraging sites. These observations remain still anecdotal, however. Lank & Smith [58] in a rare study following individual behavior on and off leks mention that over 90% of the social displays they observed occurred aways from leks. So it is critical to compare the frequencies of the copulations by phenotypes outside leks. Besides, even on leks the rarity and the stealth of sneaker males preclude a quantitative analysis of their copulation success. A detailed monitoring of sneaker males reproductive success is also needed.

Secondly and much more important, it remains to be proved that the copulation success of males translates into effective paternity. Indeed, the above mentioned pedigree analysis of Thuman & Griffith [49] provides evidence of dissortative mating in ruffs. The output of direct sperm competition depends mostly on the genetic dissimilarity between the male and the female, and the least similar male usually sires a larger proportion of the offspring [49]. Female cryptic choice by sperm competition therefore appears to be extremely important in maintaining the polymorphism of fixed alternative male mating strategiesin the ruff. This key finding of dissortative mating by post-copulatory sperm competition [49] is supported by indirect evidences. Ruff males have large testes, which is unusual in lekking bird species [59], and both sneaker and satellite males have testes that are even 2,5 larger than those of independents. Intraspecific variation in testis size in birds is positively related to variation in number of sperm per ejaculate [59], and the chances of fertilization for a given male are proportional to the relative number of sperm simultaneously inseminated in a sperm competition situation [59]. Higher number of sperm per ejaculate may thus be viewed here as a considerable advantage for those phenotypes that copulate infrequently and, unlike independent males, cannot keep other males from mating with a female. Satellite and sneaker males are indeed practically certain to face sperm competition [60]. It should be remembered here that among the coding sequences located on the ruff supergene, there is a gene involved in sperm motility

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 March 2022 doi:10.20944/preprints202203.0388.v1

that is more divergent between independent males and the two other phenotypes than between satellite and sneaker males [9], which might result in a difference in sperm motility between phenotypes. As mentioned earlier, female ruffs hold the record of registred polyandry in lekking bird species, with more than 50% of poyandrous females [48]. Females visit leks about one week before laying their first egg and do not visit leks during incubation [48]. So there is ample possibilities and time before egg laying for cryptic mate choice by sperm competition.

In the light of all these elements, we anticipate that everycomponent of the life history of the three ruff phenotypes that can affect dissortative mating by post-copulatory sperm competition is likely to be involved in frequency-dependent selection. Besides, as the supergene is located on an austosomal chromosome, females carry the same alleles than males. As previously mentioned, testosterone implants on females generate the appearance of both independent and satellite male plumages and behaviors [50]. However, little is known about the allelic frequencies of the ruff supergene in females – Mustonen [60] mentions a frequency of 12% of females carrying the inversion in a captive population. How females carrying the inversion from females carrying two ancestral alleles do differ in reproductive success is a key question. Size differences similar to that of males also occur among female genotypes. This difference in size corresponds to a lower reproductive success of the females carrying the *Faeder* allele: in a captive population with known individual pedigrees, females carrying the *Faeder* allele show lower laying rate, smaller egg size and lower offspring survival than females with two ancestral alleles [61]. Females with the *Satellite* allele, which have an intermediate size between the other two phenotypes, show reproductive sucess which is also intermediate [61]. From these data, the lower reproductive success of females carrying the inversion, especially those carrying the *Faeder* allele, questions the maintain of these phenotypee in ruff populations. Confirmation by field studies is essential, however, because captivity is likely to mask compensation mechanisms for these differences in reproductive success, such as, for example, better foraging strategies for female carrying the inversion, or even an earlier return of wintering areas giving them an advantage in the choice of partners and nesting sites.

How differences in reproductive success or survival among phenotypes might explain the supergene allelic frequencies stability in space and time is investigated using a set of analytical models by Giraldo-Deck et al. [61]. Assuming no differences in survival, no assortative mating, and by using the difference of reproductive success among female phenotypes in a captive population and data on lethality of inversion homozygous individuals, their model estimated that independent, satellite and sneaker males should fertilize 76%, 22% and 2% of the eggs, respectively (Giraldo-Deck et al. 2021) to maintain the stability of allelic frequencies. Given the differences in male phenotype frequencies in ruff populations, the *per capita* male fertilization success should therefore be 0.94, 1.20 and 2.94 for independent, satellite and sneaker males, respectively [61]. Assuming no difference in reproductive successs, sneaker males and females should survive 3.1 and 8.2 times longer than their independent counterparts to allow the inversion allelic frequencies being stable in time [61]. This theoretical study is a first step in the right direction to test for the existence of a frequency-dependent selection at work to maintain the coexistence of three fixed alternative male mating strategiesin male ruffs. However, there are some caveats to the modelling procedure. Firstly, individual fitness depends on both survival and reproductive success, and it should be more informative to combine these two traits into a single model to adequatly predict the under which conditions allelic frequencies relmain stable over time. Secondly, according to Thuman & Griffith [49], rather than assuming no assortative mating, the models should favor dissortative matings between mates on the basis of their genetic similarity. Thirdly, the availability of field data on survival and reproductive success for each of the male and female genotypes should increase the reliability of model predictions. The collection of such data may seem like wishful thinking now, but technological advances in remote data logging should meet this challenge in the near future.

 doi:10.20944/preprints202203.0388.v1

6. Conclusions

Our review of alternative male mating strategies in the ruff shows the immense interest of combining ecological, genetic and genomic data. Behavioral studies in the field started in the 1960s demonstrate the existence of different, well-defined male mating strategies. Genetic analyses in the 1980s using data issued from a careful breeding confirm that they are fixed and showed how they are transmitted from one generation to the next. Genomic studies initiated in 2016 provide insights on the molecular mechanisms involved in their origin, maintenance and evolution. The integration of data produced by these different approaches makes now the ruff an iconic model species for understanding the eco-evolutionary dynamics of phenotypic differences within natural populations. We suggest that unraveling the eco-evolutionary dynamics of fixed AMMS in the ruff should combine three different but converging approaches.

Firstly, the study of the molecular mechanisms that determine each of the strategies should be developed. Understanding the molecular, cellular, and physiological basis of cryptic female sexual selection is key, not only to understand what is happening in the ruff, but also to generalize across species. Another important point is to investigate signals of past or current selection on the loci involved in phenotypic differences among individuals carrying different alleles of the ruff supergene, and to what extent it might confirm the hypothesis of Loveland et al. [53] of hormonal profiles canalization into a restricted range that becomes associated with specific behaviors and morphological traits. This is a fascinating point which would solve a question akin to the chicken or the egg causality dilemna. Here the austosomal rearrangement is clearly the starting event of the appearance within populations of mating strategies that differ from that used by independent males homozygous for the ancestral allele. But remember that there seems to be cooperation between independent males and satellites on the leks. How such a degree of sophistication in the relationship between these two phenotypes could evolve and be achieved without selection after the chromosomal rearrangement is an open question that the search for selection signals at specific loci could help answer.

Secondly, it would be necessary to understand the variation in the cost-benefits of the life histories among the three phenotypes while providing them with an equal fitness. As previoulsy mentioned, careful studies of individuals in the field are essential to provide accurate estimates of the reproductive survival and success of males and females of each of the phenotypes. These studies should focus on what happens during the breeding season outside the leks. These studies should also cover all periods of the life history of individuals, including what happens during migration and wintering. We currently have only fragmentary elements, which often indicate differences between male phenotypes or between sexes.

Thirdly, another interesting research avenue would be to study if and how conditional strategies could be involved in the stabilization of the frequencies of each of the three fixed strategies.We have mentioned that the independent and satellite strategies are fixed, but also that the individuals using each of these strategies have access (independent residents and satellites residents) or not (marginal independents and peripheral satellites) to reproduction. Behavioral monitoring indicates possible shifts among these categories, *i.e.*, indeendent or satellite residents become marginals or peripherals and *vice-versa*, both within and between breeding seasons. This means that conditional strategies in space use on leks, and hence in social interaction and access to reproduction, are nested within fixed strategies. Besides, we repeatedly mention that mating off lek exists and is understudied. It might appear that lekking itself would be a conditional strategy [58] but long-term studies of individuals are required to validate this hypothesis. We believe that the ruf is a model of choice to assess the interactive role of fixed and conditional strategies in maintaining the phenotypic variation in AMMS, offering here again the possibility of generalization across species.

Author Contributions: All authors contributed equally to the manuscript. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Funding: MB and VMS are members of the LabEx (Laboratory of Excellence) TULIP. This research was thus funded by grant TULIP 10-LABX-0041 from the ANR (Agence National de la Recherche, French National Research Agency).

Acknowledgments: The authors acknowledge support from the CHANGE-EVOL team of the Theoretical and Experimental Ecology Station of the CNRS in Moulis (CNRS UAR 2029), and especially from Delphine Legrand and Hervé Philippe.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Waltz, E.C. Alternative mating tactics and the law of diminishing returns: the satellite threshold model. *Behav. Ecol. Sociobiol.* **1982**, *10*, 75-83.
2. Gross, M.R. Alternative reproductive strategies and tactics: diversity within sexes. *Trends Ecol. Evol.* 1996, 11, 92-98.
3. Sinervo, B.; Lively, C.M. The rock-paper-scissors game and the evolution of alternative male strategies. *Nature* **1996**, *380*, 240-243.
4. Shuster, S.M. Alternative mating strategies. In Evolutionary Behavioral Ecology; Fox, C.; Westneats D.F., Eds.; Cambridge University Press, Cambridge, 2010; pp. 434-450.
5. Taborsky, M.; Brockmann, H.J. Alternative reproductive tactics and life history phenotypes. In Animal Behaviour: Evolution and Mechanisms; Kappeler, P., Ed; Springer Verlag, Berlin, 2010; pp.537-586.
6. Austad S.N. Classification of alternative reproductive behaviors and methods for field-testing ESS models. *Am. Zool.* **1984**, *24*, 309-319.
7. Widemo, F. Alternative reproductive strategies in the ruff, *Philomachus pugnax*: a mixed ESS? *Anim. Behav.* **1998**, *56*, 329-336.
8. Plaistow, S.J.; Johnstone, R.JA.; Colegrave, N.; Spencer, M. Evolution of alternative mating tactics: conditional versus mixed strategies. *Behav. Ecol.* **2004**, *15*, 534-542.
9. Küpper, C.; Stocks, M.; Risse, J. E.; dos Remedios, N.; Farrell, L. L.; McRae, S. B.; Morgan, T.C.; Karlionova, N.; Pinchuk; P., Verkuil, Y.I.; Kitaysky, A.S.; Wingfield, J.C.; Piersma, T.; Zeng, K.; Slate, J.; Blaxter, M.; Lank, D.B.; Burke, T. A supergene determines highly divergent male reproductive morphs in the ruff. *Nat. Genet.* **2016**, *48*, 79.
10. Lamichhaney, S.; Fan, G.; Widemo, F.; Gunnarsson, U.; Thalmann, D. S.; Hoeppner, M. P.; Kerje, S.: Gustafson, U.; Shi, C.; Zhang, H.; Chen, W.; Liang, X.; Huang, L.; Wang, J.; Liang, E.; Wu, Q.; Lee, S.M.-Y.; Xu, X.; Höglund J.; Liu, X.; Andersson, L. Structural genomic changes underlie alternative reproductive strategies in the ruff (Philomachus pugnax). *Nat. Genet.* **2016**, *48*, 84.
11. Piersma, T.; van Gils, J.; Wiersma, P. Family Scolopacidae (sandpipers, snipes and phalaropes). In Handbook of the Birds of the World, Vol. 3. Hoatzin to Auks; del Hoyo, J.; Elliott, A; Sargatal, J., Eds; Lynx Edicions, Barcelona, 1996; pp. 444-533.
12. Colwell,M.A. *Shorebirds Ecology, Conservation and Management*. University of California Press, Berkeley and Los Angeles, 2010, 328p.
13. Jehl, J.R., Jr. It's Calidridine. *Wader Study Group Bulletin* **2010**, *117*, 195.
14. Van Gils, J., P. Wiersma, and G. M. Kirwan. Ruff (*Calidris pugnax*), version 1.0. In Birds of the World. del Hoyo, J.; Elliott, A.; Sargatal, J.; Christie, D.A.; de Juana A., Eds. Cornell Lab of Ornithology, Ithaca, 2020.
15. Gibson, R.; Baker A. Multiple gene sequences resolve phylogenetic relationships in the shorebird suborder Scolopaci (Aves: Charadriiformes). *Mol. Phyl. Evol.* **2012**, *64*, 66–72.
16. Chen, T.-Y.; Zhang, F.; Wang, G.-H.; Zhang, M.-Y. ; Liang T. & Lu C.-H. Complete mitochondrial genome of the Ruff, *Calidris pugnax* (Aves, Scolopacidae). *Mitochondrial DNA B Resour* **2020**, *5*, 1246-1247, DOI: 10.1080/23802359.2020.1731356.
17. Banks, R. C. Classification and nomenclature of the sandpipers (Aves: Arenariinae). *Zootaxa* **2012**, *3513*, 86–88.
18. Chesser, R.T.; Banks, R.C.: Barker, F.K.; Cicero, C.; Dunn, J.L.; Kratter A.W.; Lovette, I.J.; Rasmussen, P.C.; Remsen J.V. Jr.: Rising, J.D.; Stotz, D.F.; Winker, K. Fifty-Fourth Supplement to the American Ornithologists' Union Check-list of North American Birds, *The Auk* **2013**, *130*, 558–571.
19. Crochet, P.-A.; Barthel, P.H.; Bauer, H.-G.; van den Berg, A.B.; Bezzel, E.; Collinson, J.M.; Dubois,
P.J.; Fromholtz, J.; Helbig; A.J.; Jiguet, F.; Jirle, E.; Knox, A.G.; Kirwan, G.; Lagerqvist, M.; Le
Maréchal, P.; van Loon, A.J.; Päckert, M.; Parkin, D.T.; Pons, J.-M.; Raty, L.; Roselaar, C.S.; Sangster, G.; Steinheimer, F.D.; Svensson, L.; Tyrberg, T.; Votier, S.C.; Yésou, P. AERC TAC's
taxonomic recommendations: 2015 report. **2015,** http://www.aerc.eu/tac.html.
20. Van Rhijn, J. G. *The Ruff*. T. & A.D. Poyser, London, 1991, 209p.
21. Hogan-Warburg A.J. Social behavior of the Ruff (*Philomachus pugnax* (L.). *Ardea* **1966,** *54*, 109–229.
22. Andersen, F. Contributions to the breeding biology of the Ruff (*Philomachus pugnax* (L.)): II. *Dansk OrnithoL Foren. Tidsskr*. **1948**, *42*,125-148.

23. Widemo, F. Alternative reproductive strategies in the ruff, *Philomachus pugnax*: a mixed ESS? Anim. Behav. **1998**, *56*, 329–336.
24. Jukema, J.; Piersma, T. Permanent female mimics in a lekking shorebird. *Biol. Lett*. **2006**, *2*, 161-164.
25. Lank, D. B.; Smith, C. M.; Hanotte, O.; Burke, T.; Cooke, F. Genetic polymorphism for alternative mating behaviour in lekking male ruff *Philomachus pugnax*. *Nature* **1995**, *378*, 59-62.
26. Cramp, S.; Simmons, K.E.L. *The Birds of the Western Palearctic*. Volume III. Waders to Gulls. Oxford University Press, Oxford and New York, 1983, 913 p.
27. Jehl, J. R., & Murray, B. G. The evolution of normal and reverse sexual size dimorphism in shorebirds and other birds. In Current Ornithology. Johnston, R.F., Ed., Plenum Press, New York, 1986, pp. 1-86.
28. Tinbergen, N. Ruff. *Brit. Birds* **1959**, *52*, 302-306.
29. Van Rhijn, J. G. Behavioural dimorphism in male ruffs, *Philomachus pugnax* (L.). *Behaviour* **1973**, *47*, 153-227.
30. Van Rhijn, J. G. A scenario for the evolution of social organization in ruffs *Philomachus pugnax* and other Charadriiform species. *Ardea* **1985**, *73*, 25-37.
31. Höglund, J.; Lundberg, A. Plumage color correlates with body size in the ruff (*Philomachus pugnax*). *The Auk* **1989**, *106*, 336-338.
32. Zwarts, L.; Bijlsma, R.; van der Kamp, J; Wymenga, E. *Living on the edge. Wetlands and birds in a changing Sahel*. KNNV Publishing, Zeist, 2009, 564p.
33. Verkuil, Y. I. (2010). *The ephemeral shorebird. Population history of ruffs*. PhD Dissertation, University of Groningen, Groningen, 2010, 192p.
34. Küpper C. (2016) *Ruff Shorebird, The*. In Encyclopedia of Evolutionary Psychological Science; Shackelford, T.K.; Weekes-Shackelford, V.A., Eds; Springer International Publishing, Cham. 2016; pp.1-4.
35. Gill, J.A.; Clark, J.; Clark, N.; Sutherland, W.J. Sex differences in the migration, moult and wintering areas of British-ringed Ruff. *Ringing and Migration* **1995**, *16*, 159-167.
36. Kokko, H.; Gunnarsson, T.G.; Morrell, L.J.; Gill, J.A. Why do female migratory birds arrive later than males? J. Anim. Ecol. **2006**, *75*, 1293-1303.
37. Andersen, F. Contributions to the breeding biology of the Ruff (*Philomachus pugnax*). *Dansk OrnithoL Foren. Tidsskr*. **1944**, *38*, 26-30.
38. Jaatinen, K.; Lehikoinen, A.; Lank, D.B. Female-biased sex ratios and the proportion of cryptic male morphs of migrant juvenile ruffs (*Philomachus pugnax*) in Finland. *Ornis Fennica* **2010**, *87*, 125-134.
39. Höglund, J.; Alatalo, R.V. *Leks*. Princeton University Press, Princeton, 1995, 248p.
40. Höglund, J.; Lundberg, A. Sexual selection in a monomorphic lek-breeding bird: correlates of male mating success in the great snipe *Gallinago media*. *Behav. Ecol. Sociobiol.* **1987**, *21*, 211-216.
41. Lanctot, R. B.; Scribner, K. T.; Kempenaers, B.; Weatherhead, P. J. Lekking without a paradox in the Buff-Breasted Sandpiper. *Am. Nat.* **1997**, *149*, 1051–1070.
42. Kempenaers, B.; Valcu, M. Breeding site sampling across the Arctic by individual males of a polygynous shorebird. *Nature* **2017**, *541*, 528-531.
43. Widemo, F. Competition for females on leks when male competitive abilities differ: empirical test of a model. *Behav. Ecol.* **1998**, *9*,427-430.
44. Vervoort, R.; Kempenaers, B. Variation in lek attendance and copulation success of independent and satellite male ruffs *Calidris pugnax*. *Ardea* **2019**, *107*, 303-320.
45. Hugie, D.M.; Lank, D.B. The resident's dilemma: a female choice model for the evolution of alternative mating strategies in lekking male ruffs (*Philomachus pugnax*). *Behav. Ecol.* **1997**, *8*, 218-225.
46. Lank, D.B.; Farrell, L.L.; Burke, T.; Piersma, T.; McRae, S.B. A dominant allele controls development into female mimic male and diminutive female ruffs. *Biol. Lett.* **2013**, *9*, 20130653.
47. Johnson, D.D.P.; Briskie, J.V. Sperm competition and sperm length in shorebirds. *The Condor* **1999**, *101*, 848-854.
48. Lank, D.B.; Smith, C.M.; Hanotte, O.; Ohtonen, A.; Bailey, S.; Burke, T. High frequency of polyandry in a lek mating system. *Behav. Ecol.* **2002**, *13*, 209-215.
49. Thuman, K.A.; Griffith, S.C. Genetic similarity and the nonrandom distribution of paternity in a genetically highly polyandrous shorebird. *Anim. Behav.* **2005**, *69*, 765-770.
50. Lank, D.B.; Coupe, M.; Wynne-Edwards, K. Testosterone-induced male traits in female ruffs (Philomachus pugnax): autosomal inheritance and gender differentiation. *Proc. R. Soc. London, Ser. B* **1999**, *266*, 2323-2330.
51. Jiggins, C.D. A flamboyant behavioral polymorphism is controlled by a lethal supergene. *Nat. Gen.* **2016**, *48*, 7-8.
52. Loveland, J.L.; Lank, D.B.; Küpper, C. Gene expression modification by an autosomal inversion associated with three male mating morphs. *Front. Genet.* **2021**, *12*, 641620.
53. Loveland, J.L.; Giraldo-Deck, L.M.; Lank, D.B., Goymann, W.; Gahr, M.; Küpper, C. Functional differences in the hypothalamic-pituitary-gonadal axis are associated with alternative reproductive tactics based on an inversion polymorphism. *Horm. Behav.* **2021**, *127*, 104877.
54. van Oordt, G.J.; Junge G.C.A. Die hormonal Wirkung der Gonaden auf Sommer- und Prachtkleid III. Der Einfluss der Kastration auf männliche Kampfläufer (*Philomachus pugnax*). *Wilhelm Roux' Arch. Entwicklungsmech. Org*. **1936**, *134*, 112-121.
55. Morgan, T. *Hormonal regulation of alternative reproductive strategies*. MSc Thesis, University of Alaska, Fairbanks, 2010, 76p.
56. Horton, B.M., Michael, C.M., Prichard, M.R., Maney, D.L. Vasoactive intestinal peptide as a mediator of the effects of a supergene on social behaviour. *Proc. Royal Soc. B* **2020**, *287*, 20200196.

57. Merritt, J.R.; Grogan, K.E.; Zinzow-Kramer, W.M.; Sun, D.; Ortlund, E.A.; Yi, S.V.; Maney, D.L. A supergene-linked estrogen receptor drives alternative phenotypes in a polymorphic songbird. *PNAS*. **2020**, *117*, 2011347117.
58. Lank, D.B.; Smith C.M. Conditional lekking in ruff (*Philomachus pugnax*). *Behav. Ecol. Sociobiol.* **1987**, *20*, 137 145.
59. Möller, A. P. Sperm competition, sperm depletion, paternal care, and relative testis size in birds. *Am. Nat.* **1991**, *137*, 882-906.
60. Mustonen, J. *Influence of Intraspecific Relationships on the Allocation of Temporal Resources in the Lekking Ruff*, Calidris pugnax. MSc Thesis, University of Oulu, Oulu, 2020, 52p.
61. Giraldo-Deck, L.; Loveland, J.; Goymann, W.; Tschirren, B.; Burke, T.; Kempenaers, B., Lank, D.B., Küpper, C. Influence of intraspecific relationships on the allocation of temporal resources in the lekking ruff, *Calidris pugnax*. Preprint, Research Square, 2020, 19p.