Review

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

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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 results of exceptional quality 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 new, mechanistic light on the evolution and maintenance of the three fixed alternative male mating strategies. We then look if there is sufficient indication to support frequency-dependent selection as a key mechanism in maintaining these three strategies. Specifically, we search for evidence 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 phenotype 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]. 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 an individual's lifetime [7]. 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 their life, regardless of their condition. Theory suggests 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, 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 are 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 a negative frequency-dependent selection that provides each strategy with equal fitness [2]. However, only a few empirical examples document genetic-based, fixed alternative strategies, and the evidence of frequency-dependent selection and/or equal fitness between strategies remain elusive [2,5]. 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 [3]. 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 [3]. Sinervo and Lively [3] 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 questions about their origin, maintenance, and evolution. Austad, in 1984 [6], mentioned 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 [8,9]. However, it is a premonitory vision of the importance of the joint role of ecological (frequency-dependent selection) and evolutionary (complex genomic mechanisms) 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 new, mechanistic light on the origin and evolution of phenotypic polymorphism associated with the three alternative male mating strategies. We then examine whether currently available data do support frequency-dependent selection as a key mechanism in maintaining alternative male mating strategies. Specifically, we search for evidence 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.,[10–13]. 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 monotypic 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 [14,15] 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 [16–18].

2.2. Adult Description

The most conspicuous feature of ruffs is the polymorphism of male nuptial plumages. These plumages are progressively acquired by a prenuptial molt during the spring migration and lost during a post-breeding molt until mid-autumn [19]. 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 the bill and eyes [20]. The "whitish" males may wear the same ornamentation, but their color is here predominantly white [20]. Within these categories, the coloration patterns of individuals are highly variable, making reliable identification possible even with human eyes [19–22]. As male ruffs perform silent displays, which is unusual in birds, their voice cannot be used to signal individual identity. Lank and Dale [23] propose that the adaptive significance of this variation in ruff plumage is to signal the identity of each individual. The 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 [20,22] and then "faeder" by Jukema and 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). The posture adopted by the whitish male in the forefront is an artistic vision, as a satellite would probably never be seen facing off in this agonistic posture. Painting by Johann Friedrich Naumann (1780–1857), public domain.

Another characteristic of ruffs is sexual dimorphism in size and weight: males are larger and heavier (10% to 70% [26]) than females, which is unusual in shorebirds and especially in Calidridinae [27]. This sexual dimorphism is related to a polygynous 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 usually heavier with longer wings, tarsus, and overall body size than whitish males [31] even there is extensive overlap, whereas wing length and weight of naked-nape males are distinctly smaller and fall in-between those of females and darkish and whitish males [24].

2.3. Distribution and Habitats

The ruff breeds in lowlands of high and low Arctic and subarctic, in boreal and temperate zones in Europe and Siberia almost to the Pacific, towards the oceanic fringe of west Palearctic, and overlaps steppe zone in the continental interior (Figure 2) [26,32–34]. The main wintering area is in Africa, where most Fennoscandian breeders overwinter in the 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) [26,32–34]. Stable isotope measurements in different tissues have proven to be excellent tools for tracing ruff migration routes by identifying stopovers from overwintering sites to nesting sites [35]. 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 the earlier return of males to breeding grounds [36], which is a general pattern in birds in which male fitness depends on the number of matings (e.g., [37]). Migration departure of both sexes also differs in time; males leave their wintering areas earlier than females in March and their breeding areas in July several weeks before females. Juveniles fly northwards simultaneously or even later than females [36]. The ruff total world population is estimated at over two million individuals, with one million overwintering in western Africa and one million in the east and south Africa, and in Asia [26].
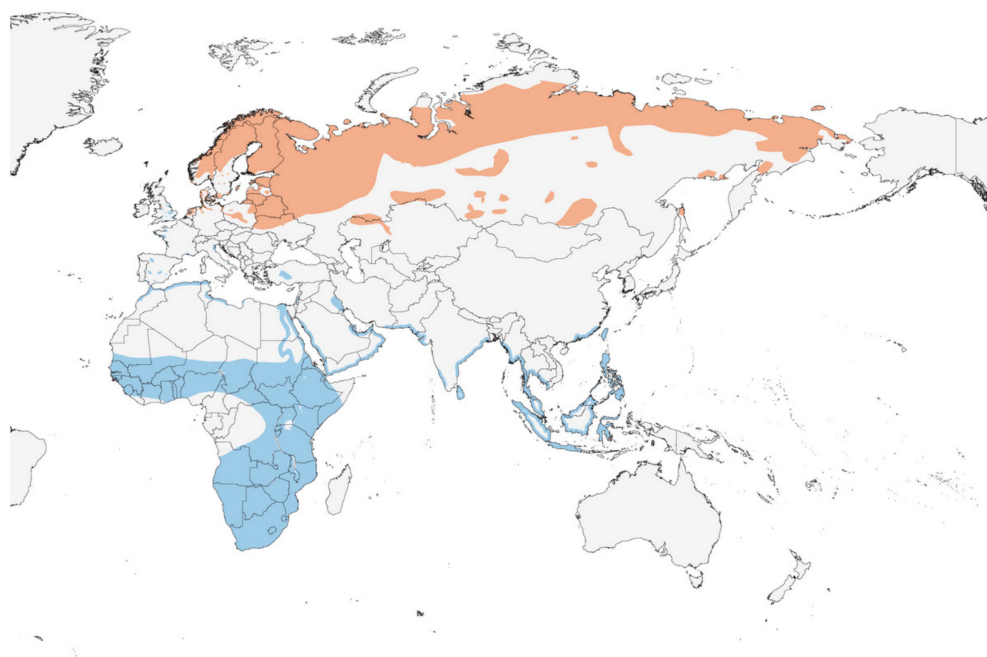
Figure 2. Distribution map of ruff (*Calidris pugnax*) breeding (orange) and wintering (blue) areas [14], © BirdLife International.

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., [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 night-time roosts on shallow water along lake edges (e.g., [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 also feeds on mollusks and earthworms. Outside of 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., [26,34]). Individuals forage for food by 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 [26].

2.5. Demography

The yearly survival is 0.52 ± 0.04 with a type II survivorship (constant over time), and no sex difference was reported [26,38]. The oldest ringed bird was 10 years 11 months old [26]. The age at first reproduction is 2 years, but probably older for males [26]. There is one brood per year. The mean clutch size is 3.72 eggs ± 0.31 ($n = 18$) [39]. 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 [40].

3. Behavioral Ecology of Breeding Ruffs

3.1. Male Strategies on Leks

Lekking behavior is the most striking feature of the ruff. Males aggregate in mating arenas ("leks") and display close together [41]. 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 attends to the young all by herself. Males provide no resources, except the sperm necessary to fertilize the egg, and no parental care [41]. Lekking is most prevalent in birds across the Animal kingdom, even if there is a strong taxonomic bias towards a few bird families [41]. Apart from the ruff, there are only a handful of Scolopacidae species that use this mating system (the Great Snipe (*Gallinago media*) [42], the Buff-breasted Sandpiper (*Calidris subruficollis*) [43], and the Pectoral Sandpiper (*Calidris melanotos*) [44]). However, ruff's lekking behavior is even more particular because the three plumage phenotypes of males described above (darkish, whitish, and naked-nape males) correspond each to different courtship behaviors on the lek [20,21,23,29,30]. We will therefore use from now on the term "phenotype" to designate both the plumage and the courtship behaviors subsuming the three fixed AMMS 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 the main arena. Those males are called *resident independents* [19,20]. The main arena (about 10 m in diameter) is located close (300–400 m) to suitable breeding sites [45] and may be used for several dozens of years [20,22]. Within the breeding habitat, ruffs form multiple smallish leks rather than aggregating into larger ones. Thus one finds clusters of leks [20,46]. Display territories are grassy areas that are trampled up to become bare ground at the end of the breeding season [20]. Each display territory has its own independent residence [20]. A peculiarity of displaying 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 [20]. The size of this space is variable (100–150 cm) and seems directly dependent on the size of the lek [20,45]. Resident males defend their display territories vigorously against territorially independent neighbors or any independent males moving in between territories. Residents return to their display territory after an absence from the lek; they arrive before dawn, even when completely dark, and stay until dusk, or even spend the night there ([20], overall spending most of their time (*ca.* 90% of daylight) during the 6–8 weeks of the lekking season in their display territories [19]. Residents may leave the lek to forage nearby around midday, though any conspecific interaction on the lek entails immediate back flight [20]. The number of attending residents can vary greatly in space (across arenas) and time (over

the season) but average between three and eight [20]. Many residents males remain faithful to the same lek for up to five breeding seasons [19].

Some, but not all, independent males succeed in establishing a display territory in 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 [20]. 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 to the lek but usually lose their fights with territory owners [47]. These floater individuals are usually young, inexperienced, and low-ranking males that may become territorial in subsequent years [19,20,47]. 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 [19]. More rarely, territory prospectors may oust resident males during the course of the breeding season [19,20]. Ousted resident males behave then as marginals and visit the lek even more rarely than other marginal males [20]. Whereas resident males rarely, if ever, visit another arena, they behave there as marginal males [20]. Marginal males are less faithful to the lek from one year to the next [48].

The *satellite strategy* is used by whitish males. Satellites are slightly smaller on average 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 [19]. However, satellites behave opportunistically: they follow females visiting the arena, and in doing so, they can move among territories [20]. Satellites spend less time on the lek than residents [19,20]. Resident males have variable reactions towards satellites: they can attack and try to expel them, or on the contrary, they may allow the resident to come into their display territories and to stay there in their company [20]. 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) [19]. Different satellites may have different preferences for display territories on the lek [19] 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 recognition made possible by the unique plumage of each individual [23].

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 a 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 [20,48] and seem less faithful to the lek from one year to the next [48].

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 ornamentation and do not perform display behavior [8,24,34]. They wander stealthily through the lek, where their female-like phenotype inhibits aggressive interactions with other males. Sneaker males rapidly sneak copulations when females solicit matings from ornamented displaying males [8,34]. However, precise and detailed quantitative data on the behaviors of sneaker males are still missing (see [48]).

The frequency of these three male phenotypes is rather constant over time and across space [19,20,22], 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., [23,25,34,49]). The relative constancy of these

phenotypes does not support cyclic changes in phenotype proportions within populations as predicted by the rock-paper-scissor model for maintaining alternative male-mating strategies (e.g., [3,50].

3.2. Male-Female Interactions on Leks

Females return from migration and arrive later than males on breeding grounds [20]. They form mixed flocks with marginal and satellite males that forage on meadows and visit lekking areas. While males progressively install on leks, females begin their solitary life in the surroundings [20]. Males on leks have to incite flying flocks or single females to land. Usually, most ornamented males on leks, whatever their phenotype, perform a *reception ceremony* [19,20], in which they flap both wings and display their white underside, which results in flash-like signals showing the location of the lek unambiguously. 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 [19,20]. Females observe male displays, walking on the leks and either fly away or solicit copulation by crouching while installed on a display territory [20]. The installation of a female on a display territory is favored when the resident interrupts his squat posture. Van Rhijn [19] showed that this interruption and the subsequent series of short movements are higher when the resident male is accompanied by one or more satellites.

The advantage for a resident of having a satellite is thus the rise of his activity level that incites a female visit on his territory. In that sense, satellite males can be considered "kingmakers" [34]. However, Van Rhijn [19] 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 [19]. 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 visits their territory. During the interruption of the squat posture, these males harass their satellites by turning around them, pecking at their wattles, and even actually attacking them. Some satellites manage to resist such harassment and hinder resident copulation, even to the extent of mounting themselves on the crouching female [19,20]. The behavioral interactions between residents and satellites on leks include thus cooperative and competitive elements [50]. Hugie and Lank [50] emphasize that residents do not merely tolerate satellites but sometimes appear to actively recruit them onto their courts. Furthermore, most intolerant behavior appears to be an attempt to control rather than 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 [50]. 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 [8,19,20,24,34].

The comparison of independent-satellite copulation success on leks shows that males copulating with a female are mostly resident independents (61%–80% in [21], 83–92 in [23], 76% in [51], 82%–95% in the extensive study of Vervoort and Kempenaers [48]). The latter study reveals that among satellites, only central males manage to copulate on leks. Vervoort and Kempenaers mention that the rarity of sneakers has so far precluded a quantitative assessment of the mating success of this phenotype [48], but these authors make an excellent start doing this and report that sneaker males perform 7% of the copulations they observed

on their lek clusters. 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 [22,52].

Widemo [22] mentions that satellites are remarkably quick to mount a female soliciting an independent male. Satellites complete 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 Rhijn [19] reports that the mating duration of satellite males is shorter than that of residents, and Küpper et al. [8] makes the same observation for sneakers. However, 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 and Piersma [24] mention that sneakers had testes 2.5 times the size of "normal" males in April. Küpper et al. [8] showed that testes of satellites could be even larger than those of sneakers. Loveland et al. [53] examined the difference in testes' weight among phenotypes. Their conclusion from very direct data, albeit from captive birds, is that gonadal masses are similar among phenotypes, which represents a greater relative investment by sneakers and possibly as suggested in [8] by satellites as well. This conclusion differs from that of [8], which was measured with ultrasound and involved controlling for seasonal timing via regression analysis. So there is some difference here. Captivity could play a role, but the data from [53] are much more real. Taken altogether, these results suggest that these differences in testes' weight relative to body 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 [22,48]. Ruffs have the longest sperm of any shorebird yet measured, which supports an evolutionary history of sperm competition [54,55]. 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 [56]. This observation leads Lank et al. to suggest that female ruffs actively genetically diversify their offspring [56]. 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 [57]. Thuman and Griffith [57] speculate that their results provide support for the 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's genetic diversification and the ultimate mechanism of the maintenance of stable genetic polymorphism for alternative reproductive strategies [8,9].

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. Investigating if there is a genetic determinism in the transmission of these morphological and behavioral phenotypes would help to gain insights into the evolution and maintenance of the alternative mating strategies. Surprisingly, 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 [25,57]. 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 [58]. Pedigree analyses reveal that the three male phenotypes are controlled by a single Mendelian locus with three alleles. Two alleles, *Faeder* (corresponding to the sneaker strategy of naked-nape males) and *Satellite* (corresponding to the independent strategy of whitish males), are dominant to *Independent* (corresponding to the independent strategy of blackish males), but the precise genetic architecture of phenotype determination remained unclear until the use of genomic methods [8,9,25,57,58].

4.1. Structural Genomics

Single loci controlling the polymorphism of complex phenotypes (known as supergenes) are indeed a target of choice for genomic studies [59]. Two independent studies published in 2016 in *Nature Genetics* managed to unravel the location and the structure of the ruff supergene that directs the three male phenotypes [8,9]. It consists of a block of ca. 90–125 genes located on a single chromosome (*ca.* 20% of chromosome 11) inherited together, mostly without recombination [8,9,34,59]. The structure of the block differs between the three alleles. The *Faeder* allele derives from the *Independent* allele by an inversion of the supergene [8,9] that probably happened ca. 3.8 million years ago [9] (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 [9] (Figure 3). Satellite males have indeed intermediate phenotypic characteristics between the independent males, such as a ruff, and the sneaker males, such as a nonagonistic behavior. Satellite males also have their own characteristics, like the whitish color of the ruff [34,59]. Importantly, these two studies report that the inversion disrupts the gene coding for a protein playing an essential role in the formation of centromeres (the *CENPN* gene encoding centromere protein N) [8,9]. Homozygosity for the inversion is lethal ([8]), which means that there is no carrier of two *Satellite/Satellite* alleles, of two *Faeder/Faeder* alleles, but also of the *Faeder/Satellite* alleles. The results of Loveland et al. [53] suggest that the two inversion alleles, incomplete *CENPN* transcripts or proteins would make cell divisions impossible.
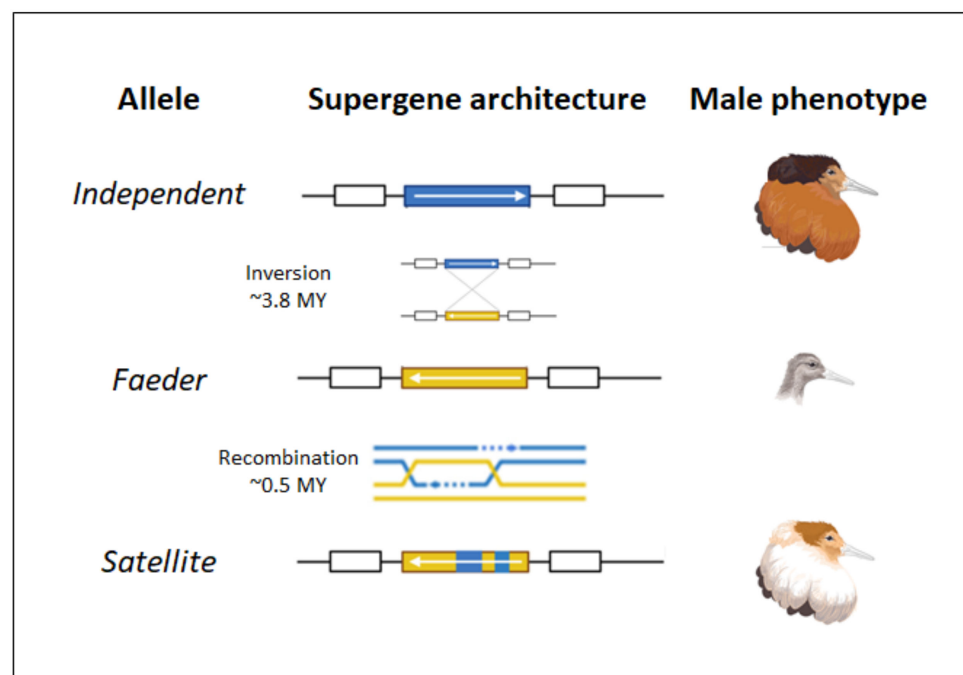
Figure 3. Schematic overview of the evolution of the ruff supergene that regulates the male phenotypes. The inversion of the ruff supergene that occurred ca. 3.8 million years ago on chromosome 11 gave rise to the sneaker phenotype. An exceptional viable recombination event between the *Independent* and *Faeder* allele (*ca.* 500.000 years ago) produced the *Satellite* allele that gives rise to the satellite phenotype. Modified from [53]. Male ruff head profiles from [60], with permission.

4.2. Functional Genomics

The two studies that document the inversion and the recombination of the ruff supergene provide a list of candidate genes located in this genomic region that could explain differences in male phenotypes [8,9]. 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 [8,9]. Altogether, the functions allocated to the genes linked to the supergene and their molecular variation resulting from the inversion and recombination events are coherent with the variation of the ruff male phenotypes [8,9]. The analyses of nucleotide sequence divergence of candidate genes provided new insights into the evolution of the three phenotypes. Many gene sequences involved in steroid metabolism located on the inversion are more divergent between independents and the two other phenotypes. However, in the recombined areas of the inversion, gene sequences are more divergent between the sneaker and the two other phenotypes. A gene involved in sperm motility (*GAS8*) was more divergent between independent and the two other phenotypes than between satellite and sneaker males [8]. A candidate gene 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 [9]. These 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 [9].

Recent studies go further than gene function assignment by showing changes in the expression of genes located in the inversion [53]. The key point is to investigate if gene expression is altered on genes located on the supergene and on other chromosomes [53]. As previously mentioned, one of the inversion breakpoints is located within the *CENPN* gene. In independent males carrying no inversion, the expression of exons belonging to the *CENPN* transcripts is broadly similar upstream and downstream of the inversion breakpoint, whereas, in both sneaker and satellite males, a *CENPN* exon that is downstream from the inversion breakpoint has at least twofold lower expression than that of an exon upstream from the inversion breakpoint [53]. Loveland et al. [53] detail allelic imbalance in recently recombined areas, as well as a correlation of expression between the inversion gene *SDR42E1* and aromatase that is shared by independents and satellites but completely lost in sneakers. Besides, Loveland et al. [53] show that divergence in gene sequence does not predict gene expression patterns.

The identification of the very molecular processes responsible for male morphological and behavioral differences is a complex task, as exemplified by a recent experimental study [61]. The role of steroid hormones, especially testosterone, in eliciting morphological and behavioral changes among the three phenotypes has been suggested by three lines of evidence: (1) castration of males prevents the molt of the nuptial plumage in independent, and satellite males [62], (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 a higher physiological concentration of testosterone than satellite or sneaker males ([9,53]). 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 [53,61]. In particular, *HSD17B2* encodes the enzyme that converts testosterone back to its precursor, androstenedione. *HSD17B2* is thus a 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 [8]. 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 intensifies [63]. *HSD17B2* contains several deletions in the *Faeder and Satellite* alleles of the supergene and in their immediate surrounding [8,9]. 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 [61]. A simple explanation could be higher levels of *HSD17B2* enzymes in these males. However, gonadal *HSD17B2* levels are similar between independent and inversion phenotypes [61,63]. This result suggests thus the existence of impairment in androstenedione to testosterone conversion in males carrying the *Faeder and Satellite* alleles [61]. Based on several similar differences in hormonal produc-

tion 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 [61,64,65]. Loveland et al. [61] went further by proposing that "over time, sequence evolution combined with selection on certain inversion haplotypes may further canalize hormonal profiles into a restricted range that becomes associated with specific behaviors and morphological traits" [61].

5. Evolution and Maintenance of Male Alternative Strategies in Ruffs

The evolution of fixed alternative male mating strategies in ruffs relies thus on genomic rearrangements that give 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, [9]) 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., [23,25,34,49]), which remain stable across space and over time. Game theory and population genetics predict that the spread and the long-term maintenance of these strategies from the event of genomic rearrangement are in agreement with the prediction of an equal fitness of the three phenotypes provided by frequency-dependent selection [2,4–7]. However, a detailed understanding of how frequency-dependent selection works should be based on a thorough analysis of the fitness costs-benefits associated with each phenotype. We summarize here the available information for such an analysis. We will see to what extent 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 in 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 higher fitness than those individuals that are homozygous for the ancestral *Independent* allele [8,9]. For Lamichhaney et al. [9], 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 [9]. Küpper et al. [8] propose that the costs related to the lethality of inversion homozygous carriers and to the alleged low survival of heterozygous individuals at the supergene they observed in experimental crossings might be balanced by a higher reproductive success of sneaker and satellite males [8].

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, many important data are still missing. However, some elements might help us to understand how this frequency-dependent selection is likely to occur.

Firstly, the copulation success of ruff males on leks is very unevenly distributed both among phenotypes and among males, which corresponds to the theoretical prediction of Plainstow et al. [7] 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 previously mentioned, resident independent males obtain the vast majority of copulations. Besides, there is a high level of mating skew among males of similar phenotype, with some males mating with many females and most mating with none [22,48,66,67]. Should we conclude that this success is responsible for the greater frequency of the independent phenotype in ruff populations? The answer could be negative for practical reasons. Copulation success is monitored on leks for practical facilities, but copulations can occur outside leks on foraging sites. These observations remain still anecdotal, however. Lank and Smith [52], in a rare study following individual

behavior on and off leks, mention that over 90% of the social displays they observed occurred away from leks. However, females very rarely mate with males off leks (D. Lank, pers. communication). So it should be useful to compare the frequencies of the copulations by phenotypes outside leks. It is possible that these might be biased towards satellites or sneakers more than those observed at leks because these birds spend more time with females. However, so do the many marginal independents, which also come and go from leks with females. Besides, more detailed monitoring of sneaker males' reproductive success on leks 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 and Griffith [56] provides evidence of disassortative 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 [56]. Female cryptic choice by sperm competition, therefore, could play a role in maintaining the polymorphism of fixed alternative male mating strategies in the ruff. Disassortative mating by post-copulatory sperm competition [56] is supported by indirect evidence. Ruff males have large testes, which is unusual in lekking bird species [68], and both sneaker and satellite males have testes that are even 2,5 larger than those of independents [8]. Intraspecific variation in testis size in birds is positively related to variation in the number of sperm per ejaculate [68], and the chances of fertilization for a given male are proportional to the relative number of sperm simultaneously inseminated in a sperm competition situation [68]. A 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 [68]. It should be remembered here that among the coding sequences located on the ruff supergene, there is a gene involved in sperm motility that is more divergent between independent males and the two other phenotypes than between satellite and sneaker males [8], which might result in a difference in sperm motility between phenotypes. As mentioned earlier, female ruffs hold the record of registered polyandry in lekking bird species, with more than 50% of polyandrous females [55]. Females visit leks about one week before laying their first egg and do not visit leks during incubation 55]. So there are many mating possibilities and time before egg-laying for cryptic mate choice by sperm competition. Finally, individual recognition plays an important role in relationships between independent and satellite males on leks. A model predicts that traits selected to signal individual identity are neither associated with fitness differences nor condition-dependent [69]. Accordingly, female ruffs should not have a visual indicator of the quality of the males with whom they wish to mate, except for the position and the display of residents on the lek. We suggest that cryptic female choice by sperm competition may have evolved because the plumage of the male is not an honest signal of its quality.

In the light of all these elements, we anticipate that every component of the life history of the three ruff phenotypes that can affect disassortative mating by post-copulatory sperm competition is likely to be involved in frequency-dependent selection. Besides, as the supergene is located on an autosomal chromosome, females carry the same alleles as males. As previously mentioned, testosterone implants on females generate the appearance of both independent and satellite male plumages and behaviors [58]. However, little is known about the allelic frequencies of the ruff supergene in females. How females carrying the inversion do differ from females carrying two ancestral alleles in reproductive success is a key question. Size differences similar to that of males also occur among female genotypes [57,70]. 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 a lower laying rate, smaller egg size, and lower offspring survival than females with two ancestral alleles [70]. From these data, the lower reproductive success of females carrying the *Faeder* allele calls further into question the maintenance of these phenotypes 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 females carrying the *Faeder* allele or even an earlier return to 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. [70]. 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 the lethality of inversion homozygous individuals, their model estimated that independent, satellite and sneaker males should fertilize 76%, 22% and 2% of the eggs, respectively [70] 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 [70]. Assuming no difference in reproductive success, sneaker males and females should survive 3.1 and 8.2 times longer than their independent counterparts to allow the allelic inversion frequencies to be stable in time [70]. This modeling exercise 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 strategies in male ruffs. However, there are some caveats to the modeling 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 adequately predict under which conditions allelic frequencies remain stable over time. Secondly, according to Thuman and Griffith [56], rather than assuming no assortative mating, the models should also consider the possibility of disassortative 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.

6. Conclusions

Our review of alternative male mating strategies in the ruff shows the immense interest in 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 careful breeding confirm that they are fixed and show how they are transmitted from one generation to the next. Genomic studies initiated in 2016 provide insights into 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. Investigating how molecular, cellular, and physiological mechanisms could result in 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 that would solve a question akin to the chicken or the egg causality dilemma. Here the autosomal 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. However, 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 previously 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 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., independent 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 [66]. Besides, mating off lek exists and is understudied. It might appear that lekking itself would be a conditional strategy [52], but long-term studies of individuals are required to validate this hypothesis. We believe that the ruff 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.

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. [CrossRef]
2. Gross, M.R. Alternative reproductive strategies and tactics: Diversity within sexes. *Trends Ecol. Evol.* **1996**, *11*, 92–98. [CrossRef]
3. Sinervo, B.; Lively, C.M. The rock-paper-scissors game and the evolution of alternative male strategies. *Nature* **1996**, *380*, 240–243. [CrossRef]
4. Shuster, S.M. Alternative mating strategies. In *Evolutionary Behavioral Ecology*; Fox, C., Westneats, D.F., Eds.; Cambridge University Press: Cambridge, UK, 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: Berlin/Heidelberg, Germany, 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. [CrossRef]

7.   Plaistow, S.J.; Johnstone, R.J.A.; Colegrave, N.; Spencer, M. Evolution of alternative mating tactics: Conditional versus mixed strategies. *Behav. Ecol.* **2004**, *15*, 534–542. [CrossRef]

8.   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.; et al. A supergene determines highly divergent male reproductive morphs in the ruff. *Nat. Genet.* **2016**, *48*, 79. [CrossRef]

9.   Lamichhaney, S.; Fan, G.; Widemo, F.; Gunnarsson, U.; Thalmann, D.S.; Hoeppner, M.P.; Kerje, S.; Gustafson, U.; Shi, C.; Zhang, H.; et al. Structural genomic changes underlie alternative reproductive strategies in the ruff (*Philomachus pugnax*). *Nat. Genet.* **2016**, *48*, 84. [CrossRef]

10.   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, Spain, 1996; pp. 444–533.

11.   Colwell, M.A. *Shorebirds Ecology, Conservation and Management*; University of California Press: Berkeley, CA, USA; Los Angeles, CA, USA, 2010; 328p.

12.   Jehl, J.R., Jr. It's Calidridine. *Wader Study Group Bull.* **2010**, *117*, 195.

13.   Van Gils, J.P.; Wiersma, 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, NY, USA, 2020.

14.   Gibson, R.; Baker, A. Multiple gene sequences resolve phylogenetic relationships in the shorebird suborder Scolopaci (Aves: Charadriiformes). *Mol. Phyl. Evol.* **2012**, *64*, 66–72. [CrossRef]

15.   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 Part B* **2020**, *5*, 1246–1247. [CrossRef]

16.   Banks, R.C. Classification and nomenclature of the sandpipers (Aves: Arenariinae). *Zootaxa* **2012**, *3513*, 86–88. [CrossRef]

17.   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.; et al. Fifty-Fourth Supplement to the American Ornithologists' Union Check-list of North American Birds. *The Auk* **2013**, *130*, 558–571. [CrossRef]

18.   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.; et al. AERC TAC's Taxonomic Recommendations: 2015 Report. 2015. Available online: http://www.aerc.eu/tac.html (accessed on 21 January 2022).

19.   Van Rhijn, J.G. *The Ruff*; T. & A.D. Poyser: London, UK, 1991; 209p.

20.   Hogan-Warburg, A.J. Social behavior of the Ruff (*Philomachus pugnax* (L.). *Ardea* **1966**, *54*, 109–229. [CrossRef]

21.   Andersen, F. Contributions to the breeding biology of the Ruff (*Philomachus pugnax* (L.)): II. *Dan. Ornithol Tidssk* **1948**, *42*, 125–148.

22.   Widemo, F. Alternative reproductive strategies in the ruff, *Philomachus pugnax*: A mixed ESS? *Anim. Behav.* **1998**, *56*, 329–336. [CrossRef]

23.   Lank, D.B.; Dale, J. Visual signals for individual identification. The silent "song" of ruffs. *Auk* **2001**, *118*, 759–765.

24.   Jukema, J.; Piersma, T. Permanent female mimics in a lekking shorebird. *Biol. Lett.* **2006**, *2*, 161–164. [CrossRef]

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. [CrossRef]

26.   Cramp, S.; Simmons, K.E.L. *The Birds of the Western Palearctic*; Volume III. Waders to Gulls; Oxford University Press: Oxford, UK; New York, NY, USA, 1983; 913p.

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, NY, USA, 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. [CrossRef]

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*). *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, The Netherlands, 2009; 564p.

33.   Verkuil, Y.I. The Ephemeral Shorebird. Population History of Ruffs. Ph.D. Thesis, University of Groningen, Groningen, The Netherlands, 2010; 192p.

34.   Shorebird, R. *Encyclopedia of Evolutionary Psychological Science*; Shackelford, T.K., Weekes-Shackelford, V.A., Eds.; Springer International Publishing: Cham, Switzerland, 2016; pp. 1–4.

35.   Schmaltz, L.E.; Loonstra, A.H.J.; Wymenga, E.; Hobson, K.A.; Piersma, T. Quantifying the non-breeding provenance of staging Ruffs, Philomachus pugnax, using stable isotope analysis of different tissues. *J. Ornithol.* **2018**, *159*, 191–203. [CrossRef]

36.   Gill, J.A.; Clark, J.; Clark, N.; Sutherland, W.J. Sex differences in the migration, moult and wintering areas of British-ringed Ruff. *Ringing Migr.* **1995**, *16*, 159–167. [CrossRef]

37.   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. [CrossRef] [PubMed]

38. Schmaltz, L.E.; Juillet, C.; Tinbergen, J.M.; Verkuil, Y.I.; Hooijmeijer, J.C.E.W.; Piersma, T. Apparent annual survival of staging ruffs during a period of population decline: Insights from sex and site-use related differences. *Popul. Ecol.* **2015**, *57*, 613–624. [CrossRef]

39. Andersen, F. Contributions to the breeding biology of the Ruff (*Philomachus pugnax*). *Dan. Ornithol Tidssk* **1944**, *38*, 26–30.

40. 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 Fenn.* **2010**, *87*, 125–134.

41. Höglund, J.; Alatalo, R.V. *Leks*; Princeton University Press: Princeton, NJ, USA, 1995; 248p.

42. 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. [CrossRef]

43. 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. [CrossRef]

44. Kempenaers, B.; Valcu, M. Breeding site sampling across the Arctic by individual males of a polygynous shorebird. *Nature* **2017**, *541*, 528–531. [CrossRef] [PubMed]

45. Höglund, J.; Widemo, F.; Sutherland, W.J.; Nordenfors, H. Ruffs, *Philomachus pugnax*, and distribution models: Can leks be regarded as patches? *Oikos* **1998**, *82*, 370–376. [CrossRef]

46. Höglund, J.; Montgomerie, R.; Widemo, F. Costs and consequences of variation in the size of ruff leks. *Behav. Ecol. Sociobiol.* **1993**, *32*, 31–39. [CrossRef]

47. Widemo, F. Competition for females on leks when male competitive abilities differ: Empirical test of a model. *Behav. Ecol.* **1998**, *9*, 427–430. [CrossRef]

48. Vervoort, R.; Kempenaers, B. Variation in lek attendance and copulation success of independent and satellite male ruffs *Calidris pugnax*. *Ardea* **2019**, *107*, 303–320. [CrossRef]

49. Verkuil, Y.I.; Jukema, J.; Gill, J.A.; Karlionova, N.; Melter, J.; Hooijmeijer, J.C.E.W.; Piesma, T. Non-breeding fæder ruffs *Philomachus pugnax* associate according to sex, not morphology. *Bird Study* **2008**, *55*, 241–246. [CrossRef]

50. 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. [CrossRef]

51. Mustonen, J. Influence of Intraspecific Relationships on the Allocation of Temporal Resources in the Lekking Ruff, Calidris pugnax. Master's Thesis, University of Oulu, Oulu, Finland, 2020; 52p.

52. Lank, D.B.; Smith, C.M. Conditional lekking in ruff (*Philomachus pugnax*). *Behav. Ecol. Sociobiol.* **1987**, *20*, 137–145. [CrossRef]

53. 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. [CrossRef]

54. Johnson, D.D.P.; Briskie, J.V. Sperm competition and sperm length in shorebirds. *Condor* **1999**, *101*, 848–854.

55. 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. [CrossRef]

56. 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. [CrossRef]

57. 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. [CrossRef] [PubMed]

58. 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. Lond. Ser. B* **1999**, *266*, 2323–2330. [CrossRef]

59. Jiggins, C.D. A flamboyant behavioral polymorphism is controlled by a lethal supergene. *Nat. Gen.* **2016**, *48*, 7–8. [CrossRef] [PubMed]

60. Taylor, S.; Campagna, L. Avian supergenes. *Science* **2016**, *351*, 446–447. [CrossRef]

61. 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. [CrossRef]

62. 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. [CrossRef]

63. Morgan, T. Hormonal Regulation of Alternative Reproductive Strategies. Master's Thesis, University of Alaska, Fairbanks, AK, USA, 2010; 76p.

64. 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. R. Soc. B* **2020**, *287*, 20200196. [CrossRef]

65. 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. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 2011347117. [CrossRef]

66. Hill, W.L. Correlates of male mating success in the ruff *Philomachus pugnax*, a lekking shorebird. *Behav. Ecol. Sociobiol.* **1991**, *29*, 367–372. [CrossRef]

67. Widemo, F.; Owens, I.P.F. Lek size, male mating skew and the evolution of lekking. *Nature* **1995**, *373*, 148–151. [CrossRef]

68. Möller, A.P. Sperm competition, sperm depletion, paternal care, and relative testis size in birds. *Am. Nat.* **1991**, *137*, 882–906. [CrossRef]

69. Dale, J.; Lank, D.B.; Reeve, H.K. Signaling individual identity versus quality: A model and case studies with ruffs, queleas, and house finches. *Am. Nat.* **2001**, *158*, 75–86. [CrossRef] [PubMed]

70. Giraldo-Deck, L.M.; Loveland, J.L.; Goymann, W.; Tschirren, B.; Burke, T.; Kempenaers, B.; Lank, D.B.; Küpper, C. Intralocus conflicts associated with a supergene. *Nat. Commun.* **2022**, *13*, 1384. [CrossRef] [PubMed]