COURTING MALE RUFF SANDPIPERS (*CALIDRIS PUGNAX*): DO MALES VARY THEIR TACTICS IN THE ABSENCE OF ONE MORPH?

by

Eden M. Stainback

A Signature Honors Project presented to the Honors College, East Carolina University in partial fulfillment of the requirements for Graduation with Honors

Approved by: Susan B. McRae, Ph.D.

East Carolina University

Department of Biology

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Courting male ruff sandpipers (*Calidris pugnax*): do males vary their tactics in the absence of one morph?

Eden M. Stainback

Department of Biology, East Carolina University, Greenville N.C. 27858

ABSTRACT-The ruff sandpiper (*Calidris pugnax*) is a medium sized wading bird native to Eurasia that displays strong sexual dimorphism in plumage. Unique among shorebirds, the ruff has a lek mating system where breeders gather on an arena and males display with the purpose of enticing females to mate. Within ruff populations there are three distinct genetic morphs that specify differences in male mating behavior and plumages. During migration, Independents and Satellites grow ornamental mating plumage, a head tuft and ruff around the neck, which is showcased during ritualized displays. Independents display 'Resident' behavior, each defending a small court on the lek, whereas Satellites move among courts and co-display with the Resident. Faeders, the third and rarest morph, are smaller in size and resemble females in appearance, lacking ornamental plumage. These are heritable, permanent genetic morphs: the Satellite and Faeder morphs are determined by variants of an autosomal chromosomal inversion. I observed behavior on the lek of captive bred ruff sandpipers at Sylvan Heights Bird Park (SHBP) in Scotland Neck, NC. This colony presently lacks Satellite males providing an opportunity to observe males for potentially compensatory behavior in the absence of this morph. Anecdotally, Faeder males have been observed to display in captivity under some circumstances, and an Independent male was observed co-displaying at SHBP in a previous year. To further explore this behavior, observations were made both in person

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and using video of ruffs before and during the mating season. Behaviors categorized as aggressive and display were recorded, as well as proximity to other males. These observations were then quantified using standardized methodologies to characterize the behavior of individual males and identify instances of co-display behavior by Independents and the Faeder. Observations of this colony from the 2020-2022 breeding seasons captured co-display behavior by the Faeder and at least one Independent. Observations from the 2023 breeding season did not. Failure to observe co-display behavior on 2023 may have been influenced by subdividing the males among two aviaries, thereby reducing the density of males. It is conceivable that previous peculiar observations of co-displaying by Independents and the Faeder were due to both the absence of Satellites in conjunction with limiting space for all males to have courts. Data from the 2023 season did reveal a significant relation between male level of aggression and rate of display. Neither of these parameters were significantly related to male mass, body condition (mass/wing chord), or age. Future studies should examine the possible role social environment plays in the behaviors of a species with a permanent genetic morphs. Such investigations are important for understanding the interplay between gene expression under different social contexts.

Acknowledgements

I would like to thank those that have made this study possible: my mentor, Dr. Susan B. McRae, who guided me through this study. My research partner, Amelia Moore, who assisted in study design, field data collection and video review. My director at Sylvan Heights Bird Park, Dr. Dustin Foote. My peers in the McRae lab, for their feedback and guidance through this process. The Department of Biology at East Carolina University for providing the opportunity to conduct research. My sincerest thanks to everyone mentioned.

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Introduction

The ruff sandpiper (*Calidris pugnax*) is a medium sized shorebird native to Eurasia notable for its intense sexual dimorphism in nuptial plumage. Unlike other shorebirds, the ruff exhibits a lek mating system, with male ruffs displaying on courts to entice their female counterparts (Van Rhijn, 1973). This species is further known for its three unique genetic morphs that cause differences in male size, courtship behavior and nuptial plumage: Independents, Satellites, and Faeders (Lank et al. 1995).

Variants of a chromosomal inversion on chromosome 11 (a supergene) have led to the emergence of the Faeder and Satellite morphs from the original Independent type (Farrell et al. 2013; Küpper et al., 2016; Lamichhaney et al., 2016). Among males, Independents account for 80-95% of the overall male population and are known for their territoriality on the lek and dominant display behavior on the lek and darker plumage (Van Rhijn, 1973). Independent males can be further categorized as either Residents, which account for ~20% of all Independents, or Marginals. Residents are Independent males that are actively seeking to mate at a given time. Residents are territorial and exclude other Independents from their courts, whereas Marginals are unsettled and non-breeding at a given time. Satellite males, accounting for 5-20% of the male population, known for their predominantly white ruff plumage, are submissive to the Independent males. This allows for co-displaying between Satellites and Independents, as Satellites do not defend their own courts and are instead tolerated on the courts of Independents. Lastly, the Faeder is a male morph that accounts for less than 1% of the male population, is smaller in size and lacks nuptial plumage, mimicking the female ruff (Lank et al. 2013). Their discrete appearance allows them to be tolerated on courts of other males and

attend to soliciting females (Küpper et al., 2016). The Faeder is the rarest amongst male morphs and was only discovered in 2006 (Jukema & Piersma, 2006) despite decades of field observations of the species by multiple research groups (Lamichhaney, et al., 2016).

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Satellite and Faeder morphs are all heterozygote carriers of the heritable autosomal chromosomal inversion (Küpper et al., 2016; Lamichhaney et al., 2016). Therefore, morphs created by the supergene are permanent: individuals do not change in the course of their lifetime. Courtship and display behavior is determined by the supergene variant, with previous studies suggesting environmental factors do not impact behavior (Lank et al. 1995). Ritualized, discrete courtship behaviors of displaying males are quantifiable (involving postures such as 'squat', 'half-squat', and 'oblique'), and are performed by Independents and Satellites to different degrees (Hogan-Warburg, 1966; Van Rhijn, 1973). Aggressive behaviors including 'bill thrust', 'bill point', and 'chase', are almost exclusively performed by Independents.

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Permanent genetic strategies are also found in species such as the side-blotched lizard (*Uta stansburiana*) which displays three heritable throat colors associated with alternative mating strategies in males (Hazard et al., 2019). The orange morph is known to defend large territories, the blue morph to guard a mate, and the yellow morph uses a sneak strategy to mate (Bleay et al., 2007). Previous studies have found that social environment have little influence on mating strategy in the side-blotched lizard, as each morph's repertoire remains consistent throughout changes in the male population demographic (Bleay et al., 2007). This differs from the conditional behavior strategies exhibited by animals such as the coho (*Oncorhynchus kisutch*), a species of salmon. In its juvenile state, the male coho adopt either a jack or hooknose physiology and maintain it

for the remainder of their life; this distinction is irreversible. The male coho display conditional behavioral tactics. However, they employ either sneak or fighting tactics based on situational needs regardless of physiology (Gross, 1991).

Data collection for this study occurred at Sylvan Heights Bird Park (36°08'42.8"N 77°24'50.5"W) in Scotland Neck, North Carolina. In addition to hundreds of birds from all around the world, Sylvan Heights is home to a colony of captive bred ruff sandpipers. The ruffs originated in Canada and were brought to Sylvan Heights in three shipments in 2010, 2014, and 2020 , respectively. Notably, none of these shipments included any Satellite males, only Independents and one Faeder. As of 2023, the colony was comprised of 30 individuals: 20 females, 9 Independent males, and 1 Faeder male.

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Many species that display permanent genetic strategies do not also exhibit conditional behavioral. Recent anecdotal observations at Sylvan Heights Bird Park (SHBP) in Scotland Neck, NC have suggested otherwise, however. In the 2022 breeding season, the sole Faeder male had been observed and was filmed co-displaying with Independents in the absence of a Satellite male. The Faeder was observed erecting his relatively short black mottled neck feathers alongside the Independent. The behavior of erecting neck feathers was documented once before by this morph when a Faeder male in captivity in Canada was housed during the breeding season in an aviary with only females (David Lank, pers. comm.). However, Faeders have not previously been observed to co-display. Typically, only Satellite males co-display with Independents. An Independent male was also anecdotally observed to co-display with another at Sylvan Heights Bird Park in 2021, exhibiting satellite-like behavior (A. Works and S. McRae,

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pers. comm.). In this study, I will seek to determine if the social environment (i.e., the lack of Satellite males) plays a role in behavioral flexibility in Independents and Faeder.

The exploration of the relationship between permanent genetic morphs and social behavior is important for the understanding of not only ruffs but similar species. Recent studies have shown a decline in ruff populations in their native range in relation to global warming (Zockler, 2002). Should populations of the ruff continue to decline, understanding their unique mating behaviors and how they relate to genetic morph will be important for conservation. Captive breeding would benefit from this understanding as well. Zoos in the U.S. have recently become interested in having ruff sandpiper exhibits for educational purposes (P. Dickson, pers. comm. to S. McRae). Furthermore, the observation of conditional behavior in species that previously displayed strict permanent genetic strategies could offer insight into the evolution of alternative mating strategies in polymorphic species in response to social and environmental variation.

Methods

Study site and ruff colony at Sylvan Heights Bird Park

Prior to beginning observations, morphometric data were collected for each ruff as they were sorted into breeding groups. Each bird was caught in their original aviary on March 4, 2023, and measurements were taken of wing chord (± 1 mm), tarsus (± 1 mm), bill length (± 1 mm), mass (± 2 g), and ruff length (± 5 mm) of males, if applicable. The birds were weighed in a bag that was tared before each use. The ruffs were then distributed between two aviaries, with 10 females and 5 males left in Wings of the Tropics (hereafter, WOT) and 10 females and 5 males moved to the Propagation Aviary (hereafter, Prop). The Faeder male was placed in Prop with four Resident males, but the oldest male (age 11 years) in Prop died prior to the beginning of the breeding season. The handling of the birds was done by trained professionals. All procedures described were previously approved by the ECU IACUC (Appendix 1).

To investigate male courtship behavior, observations were made both in person and via video camera. In-person observations took place at Sylvan Heights Bird Park in Scotland Neck, N.C., every week between April 29th, 2023, to July 15th, 2023. The observer stood outside of the aviary with recording tools (timer, clipboard, pen), and an observation sheet. Observations spanned one hour and scan sampling of male behavior was conducted at 2-minute intervals. That is, every two minutes the behavior of each male at that moment was recorded. Notable behaviors included those of display, such as squat, half-squat, and oblique, those of aggression, such as chase, fight, bill point, and bill thrust, and others such as walk up or proximity (Table 1). Periods lacking behavior were struck through. Additionally, female behaviors such as crouching, grass tossing, or belly

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scraping were recorded. Video observations were taken using an unattended Sony Handycam camera, which was placed on a tripod in each aviary following the completion of in person observations each day. Observations were completed 4 days per week between approximately 0800 h and 1200 h.

Table 1. Ethogram of male ruff sandpiper behavior on the lek

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Behavior	Category of Behavior	Description
Squat	Display	Still position with ruff and head tufts extended, bending his ankles fully with chest parallel to the ground and head pointing forward or downward. The body is typically in full contact with the ground. Maintains this position for at least 5 seconds.
Half Squat	Display	Still position with ruff and head tuft extended, bending his ankles partially without touching his breast to the ground and head pointing down or upward. Maintains this position for at least 5 seconds.
Oblique	Display	The male stands, legs straight or slightly bent with head and neck raised above his body and extends his ruff and head tufts.
Bill thrust	Aggressive	The male thrusts his head forward at another male within two bird lengths whilst standing still.
Bill point	Aggressive	The male stands still, extends neck and directs bill towards another bird within two bird lengths.
Chase	Aggressive	Male running in pursuit of another bird who is retreating.
Male Proximity	Proximity	Two or more males are standing within two bird lengths of each other for at least 2-3 seconds.
Female Proximity	Proximity	One or more female is standing or crouching within two bird lengths of a male for at least 2-3 seconds.
Walk-up	Solicitation	Male approaches a female and may attempt to mount. The male may peck the female's head.

Upon completion of data collection, in-person behavioral observations were summarized, and relative rates of behavior determined for each individual male. For each male and each observation period, the number of occurrences of each behavior was quantified. For each behavior, the number of time points observed was expressed as a proportion of the total observation period and entered into a spreadsheet (Microsoft Excel). Video data were reviewed 60 minutes at a time, with a two-minute habituation period allowed at the beginning of some videos to allow birds to settle after camera installation. Behaviors such as 'walk-up', copulation, and female 'crouch' (female solicitation of copulation) were recorded even when they occurred outside of the two-minute intervals. For each male, these behaviors were similarly quantified by number of occurrences over the total period of observation and entered as a proportion into the spreadsheet.

Raw data were then transferred to JMP Pro v. 17 where the distribution function was used to find the mean and standard deviation of each behavior. The mean and standard deviation were then used to calculate Z-scores. Behaviors were categorized as display, aggression, walk-up, or proximity behaviors (Table 1). For each observation period, the raw scores for squat, half squat, and oblique were summed and expressed per unit of time (observation periods) to produce rate of display. Average rate of display was determined for each male, and those averages were ten transformed to obtain Z-scores for each male. This process was repeated for each act of aggression (chase, bill point, bill thrust) to determine a level of aggression for each male. From these scores, each Resident male was ranked from 1 to 8 based on rate of behavior, with 1 being the most active. Graph builder was then used to make graphs identify trends in male behavior and female

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response as well as correlations between male behavior and morphometric data. Level of aggression and rate of display were plotted against male mass, body condition (mass /wing chord), and age by individual. Periods of female interest (Z-scores) were plotted against rate of display and level of aggression as well.

To determine whether male display rate and aggression in Independent males were related to male age or relative condition, Generalized Linear Models (linear regressions) were run with male mass, body condition (mass /wing chord), and age as predictors.

Results

The WOT aviary was enclosed with greenhouse panels, allowing minimal exposure to the elements, and the ruffs shared this pen with three white-rumped shamas (*Copsychus malabaricus*). The Prop aviary was against the propagation building but had three sides exposed to the elements. The ruffs shared this pen with a colony of zebra finches (*Taeniopygia guttata*). Prop males began courtship behavior approximately 4-6 weeks after the average onset of behavior in WOT males.

When partitioning the birds into either WOT or Prop the goal was to establish an equal number of males in each aviary, and a density that would allow each Independent to simultaneously have a court of a size similar to that observed seen in nature. Originally, each aviary contained five males; 5 Independents in WOT, and 4 Independents and the Faeder in Prop. Unfortunately, the eldest Independent male in Prop died before the mating season began. The remaining Independents, all under the age of 3, experienced delayed plumage growth and courtship behavior.

An average of 90 hours (range = x-y) of observations (in-person and video) was analyzed per male per aviary. In WOT, four Resident males were observed simultaneously, and in Prop, three Residents and 1 Faeder male were observed simultaneously. There was an additional 2-year-old Independent male in WOT, but he was excluded because his nuptial plumage peaked early and his peak in displaying was in March. His behavior had ceased by the time observations had begun. Of the four participating Residents in WOT, two were 9 years of age, one was 10 years of age, and one was 3 years of age. Of the males in Prop, two Residents were two years of age, one was three years of age, and the Faeder was 9 years of age (Table 2).

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Table 2. Summary of Male Ruff Histories

Male	Band No.	Band Scheme	Age (years)	Year Hatched	Location of Birth	Imported or hatched at Sylvan Heights?	Aviary
Independent 1	1661	SYR_ABB_B	10	2013	Burnaby, B.C., Canada	Imported	WOT
Independent 2	db602	dB_CdB_D	10	2013	Burnaby, B.C., Canada	Imported	WOT
Independent 3	1313	SGB_APz_C	9	2014	Burnaby, B.C., Canada	Imported	WOT
Independent 4	1098	S_BR_C	3	2020	Burnaby, B.C., Canada	Imported	WOT
Independent 5	1099	S_AGz_C	3	2020	Scotland Neck	Sylvan Heights Hatch	Prop
Independent 6	G01	G_A	2	2021	Scotland Neck	Sylvan Heights Hatch	Prop
Independent 7	Go5.502	Go_A	2	2021	Scotland Neck	Sylvan Heights Hatch	Prop
Faeder	1698	GB_AdB_B	9	2014	Burnaby, B.C., Canada	Imported	Prop

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Although there were no instances of co-display observed this year, I was able to characterize courtship behavior of each of the 7 surviving Independents and the Faeder. Level of aggression was significantly related to rate of display (t=3.1, p=0.021, r^2=0.62) (Fig. 1).

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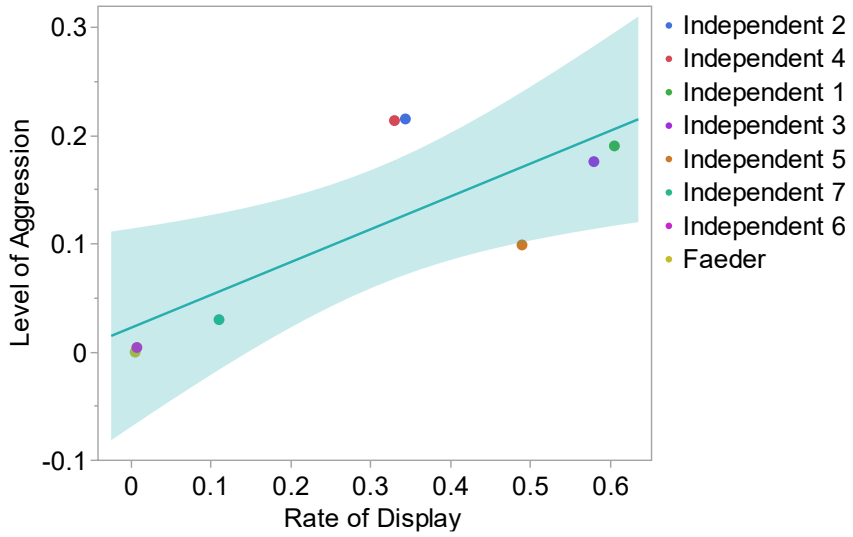

Figure 1. Level of aggression plotted against rate of display by male. Each male is represented by a different color.

Males were ranked in terms of their levels of display activity and aggression, and these were related to their age and condition based on morphometrics (Fig. 2). As might be expected, older males trended toward exhibiting higher levels of aggression and rates of display (Fig. 2). Mass and body condition also showed a nonsignificant positive trend, with the two outliers being Independent 6 and the Faeder. The Faeder morph is not known for their aggression or display behavior. However, the Faeder was subject to aggression from Independent 5. Throughout the observed season Independent 5 aggressed the Faeder approximately 15 times. Faeders are generally tolerated on Resident's courts, but this Faeder does sport a darker plumage than usual, possibly making him less effective as a female mimic. Independent 6 was a younger male (age = 2

years) that experienced delayed nuptial plumage growth and onset of behavior, so he had only just begun displaying as the observation period ended.

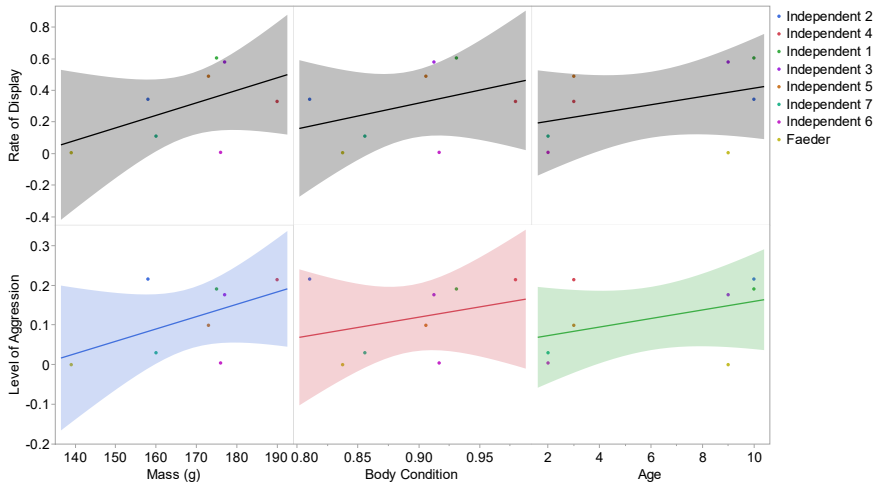

Figure 2. Level of aggression and rate of display plotted against morphometric variables of mass (g), body condition (mass (g)/wing chord (mm)), and age by male. Each male is represented by a different color.

Rate of display and aggression showed trends of positive relations to male mass, body condition (mass/tarsus), and age in years (Fig. 1). However, level of aggression was not significantly related to male mass, body condition, or age. Similarly, rate of display was not significantly related to male mass, body condition, or age (Table 3).

Table 3. Linear regression statistics relating male behavior and individual characteristics

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Predictor	Estimate	Standard Error	t-ratio	p-value
Level of Aggression vs. Rate of Display	0.30	0.097	3.1	0.021
Level of Aggression vs. Male Mass	0.003	0.0021	1.5	0.19
Level of Aggression vs. Male Body Condition (mass/tarsus)	0.52	0.66	0.78	0.47
Level of Aggression vs. Male age (years)	0.011	0.0092	1.2	0.29
Rate of Display vs. Male Mass	0.008	0.0055	1.4	0.20
Rate of Display vs. Male Body Condition (mass/tarsus)	1.6	1.7	0.97	0.37
Rate of Display vs. Male Age (years)	0.026	0.024	1.1	0.32

Periods of female interest seemed to increase with level of male aggression and rate of display (Fig. 3). The percent of periods of female interest was not found to be significantly linearly related to male level of aggression (t=0.82, p=0.44). Female interest seemed to decrease at the highest levels of male aggression (Fig. 3). Nor was female interest significantly related to rate of male display (t=1.8, p=0.12). Periods of female interest were then plotted against male morphometrics (Fig. 4). Periods of female interest were not significantly related to male mass, body condition, or age. (Table 4).

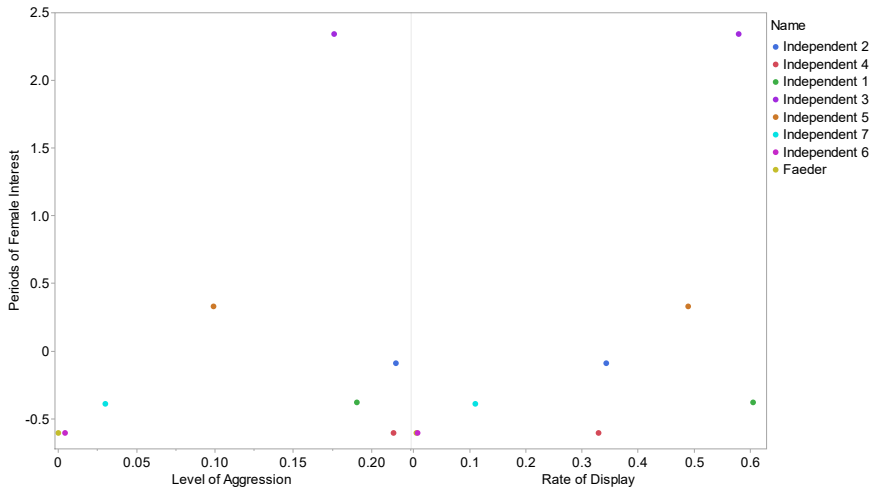

Figure 3. Periods of female interest plotted against male level of aggression and rate of display. Periods of female interest are determined by the number of 2-minute intervals in which a female was showing interest in a male (crouching within two bird lengths of him).

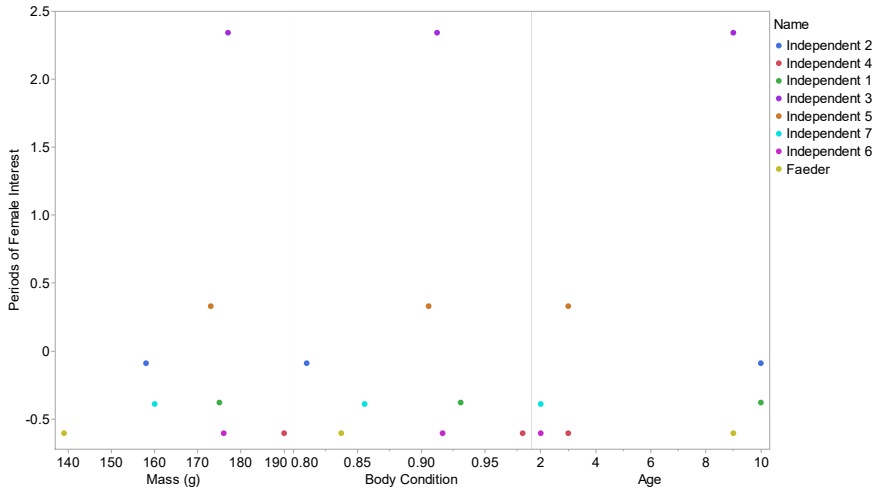

Figure 4. Periods of female interest plotted against male morphometrics.

Table 4. Summary of statistical results relating periods of female interest to male behavior and individual characteristics

Predictor	Estimate	Standard Error	t-ratio	p-value
Level of Aggression	3.4	54.0	0.82	0.44
Rate of Display	2.4	17.0	1.8	0.12
Male Mass	0.014	0.35	0.54	0.61
Male Body Condition (mass/tarsus)	1.1	100.0	0.15	0.88
Male Age (years)	0.08	1.3	0.79	0.46

Independent 3 from WOT was an outlier in terms of female interest (Fig. 3). This male exhibited a similar rate of display as Independent 1 yet solicited far more female interest. Independent 1 was 10 years of age and displayed a dark brown ruff and head tuft with underlayment of light brown feathers. Independent 3 was 9 years of age and displayed a dark brown head tuft but a predominantly white ruff accented by rufus feathers.

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Discussion

The original goal of this study was to observe changes in Independent and Faeder behavior in the absence of Satellite males to explore the possible flexibility between permanent genetic morphs and alternate reproductive tactics. This study was inspired by anecdotal observations of co-display behavior at Sylvan Heights Bird Park in the absence of Satellite males. This included co-display behavior between two Resident males from observations and still photographs taken by a previous graduate student, Anna Works, in 2021. Additionally, there is video footage of the Faeder co-displaying with a Resident male in the 2022 season (taken by S. McRae). Independent 1 was previously observed co-displaying with a fellow Independent not represented in this study. Independent 1 did not initiate co-display behavior this season, rather he was the most aggressive male observed.

A possible explanation for the lack of co-display behavior is the early segregation of the birds into two aviaries. By splitting the colony in half, we reduced male density and crowding in each pen, leaving more room for independents to establish courts on the lek, and possibly reducing the probability of co-display. While neither the Independents nor the Faeder were observed co-displaying this season, interesting insights were still gained through my observations. Moreover, since reducing the density of males coincided with the first year in three that the behavior was not observed, male morph distribution and density both seem to be important factors.

The timing of onset of molt and courtship behavior amongst the seven Residents was inconsistent. The Residents in WOT, except for one, all began displaying at relatively similar times. This was vastly different from the Prop males, whose onset of molt and courtship behavior was gradual over several weeks. One reason for this

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difference may be the age gap between the WOT Residents and Prop Residents. All of the Residents in WOT, with the exception of one, were aged 9 or 10, whereas all of the Residents in Prop were aged 2 to 3. The Resident male that came into plumage early in WOT was also 2 years old. Though there was not a significant relation between age and behavior, I would be interested to see if this trend continued into a larger sample size.

I speculate that the irregularity in courtship behavior may have been caused in part by the lack of an elder, experienced male to stimulate competition and lead by example. As the remaining males were so young they had little to no courtship experience prior to this season, and it is possible that having an experienced male would have changed the courtship behavior of the younger males. To test this hypothesis in future studies I would redistribute the Resident males (to provide two elders (males aged <9) and two youth (males aged >4). Alternatively, light availability and exposure to other environmental factors could also have impacted onset of behavior. Studies have found that environmental factors such as weather, temperature, and light availability impact courtship onset in avian species (Gwinner, 1996), so difference in light availability and exposure to elements may have played a role in the delay of behavior onset in Prop males.

Another limitation of the results was a small sample size. Though there were nine males total, only eight were used for data collection and observation. This is a small sample size which causes statistical limitations in analysis. A larger sample size could yield better results, either confirming these findings or otherwise finding significant relations.

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Possible density effect on lack of co-display - we possibly gave them too much space…

relate female interest results to lit. on Hot shot versus Hot spot hypotheses for lek formation
Relate also to lit. on lek size (females prefer larger leks)

Independent 3 garnered more female attention though he maintained a similar rate of display as Independent 1. One possible explanation for this is the 'hot spot' vs. 'hot shot' hypothesis. The 'hot spot' hypothesis posits that males are more likely to establish leks in areas of high female density to better their chances of mating (Bradbury et al., 1983). Alternatively, the 'hot shot' hypothesis states that female will prefer one more socially dominant male, and that subordinate males will gather around him to intercept females seeking to mate (Beehler & Foster, 1988). A third alternative is the female preference hypothesis, which proposes that males cluster because females prefer to mate with clustered males (Bradbury et al., 1981). Independent 3 was not the most aggressive male nor did he show the highest rate of display, so he was not the most dominant male. This coupled with his lack of overall proximity to other males makes the 'hot shot' and female preference hypotheses unlikely.

The 'hot spot' hypothesis may have merit in this situation. Previous studies have proposed that males will seek to establish leks in areas of high female density, and that females will show preference towards specific lek sites and male phenotypes. Gibson, Bradbury, and Vehrencamp (1991) studied mate choice in lekking of the sage grouse. They were able to conclude that females not only prefer certain males due to phenotypic traits or past mating success, but they are likely to copy the interest of their female peers. Additionally, they found that females show preference to lek sites in which they have successfully mated in the past. In theory, Independent 3 could have been more successful in soliciting attention because he 1) sported a ruff color that was completely different from others, 2) occupied a territory that had been successful in previous years, or 3) was preferred by one female who was then copied by others. To test this theory, future studies

would need to track court locations over multiple breeding seasons, as well as document female copulations by male by territory over time.

Overall, though I did not observe the behavior I was seeking, this study still provided valuable insights into the courtship behaviors of the male ruff sandpiper. Future research of this colony could benefit from restructuring the male demographics between aviaries and the continued breeding of more males into the colony.

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Appendix 1

Animal Care and Use Committee
003 Ed Warren Life Sciences Building | East Carolina University | Greenville NC 27834 – 4354
252-744-2436 office | 252-744-2355 fax

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