THE UNIVERSITY OF CHICAGO

DYNAMICS OF POLYANDRY IN SPOTTED SANDPIPERS (*ACTITIS MACULARIUS*): ORNAMENTATION, REPRODUCTIVE SUCCESS, AND TESTOSTERONE

A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES
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BY

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My dear Sir [Alfred Newton],

…Not long since I was speaking to Mr. Wallace, & I told him of the case of Rhynchœa [painted-snipe, *Rostratula*], of which the female is more beautiful than the male…He answered me…that you had simply explained it by the male being the incubator…The subject interests me greatly, as in the 4th Edit. of the Origin I gave the obvious explanation of female birds not being gaudily coloured &c on account of their incubating; I knew then of the Rhynchœa but passed over the case from not having space & from its appearing to me quite inexplicable…

Yours sincerely,
Charles Darwin[1]

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[1] Charles Darwin to Alfred Newton. January 19, 1867. In: *The Correspondence of Charles Darwin, Vol. 15: 1867* (Ed. by F. Burkhardt & J. V. Wyhe), p. 39. 2008. New York: Cambridge University Press.

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INTRODUCTION

Competition for mates, resulting in differential reproductive success, influences selection on male and female morphology, behavior, and physiology and can lead to both dimorphic or monomorphic traits. Based on theory, under intrasexual selection, the sex experiencing greater competition for mates develops ‘armaments,’ or traits to better defend territories and mates from individuals of the same sex, and under intersexual selection, the sex develops ‘ornaments,’ or traits meant to attract individuals of the opposite sex. Male reproductive success is typically limited by access to mates, and thus males experience a greater degree of reproductive skew than females, resulting in the evolution of male armaments or ornaments (e.g., Hill 1993; Møller 1993; Cuervo et al. 1996; Amundsen 2000). While female reproductive success is typically limited by the ability to produce offspring, females can also be under similar selection pressures as males as a consequence of competition for resources, territories, mates, and paternal care (Gwynne 1981; Berglund et al. 1996; Swenson 1997; Eens & Pinxten 2000; Ketterson et al. 2005; Steiger et al. 2006; Rosvall 2008; Tobias et al. 2012). Although relatively little is known about how sexual selection operates in females, these selection pressures may be greater in species in which female competition for mates exceeds that of males.

I investigated a species which exhibits this reversal in typical ‘sex roles’ to examine the influence of sexual selection on female morphology, physiology, and behavior. While sex-role reversal is unusual across vertebrates, is has been documented in fish, amphibians, and birds (Gwynne 1981; Berglund et al. 1996; Eens & Pinxten 2000). In sex-role reversed species, females can experience a greater degree of reproductive skew than males, potentially due to a female-biased operational sex ratio or male-biased parental investment. Compared to males, females are expected to display greater ornamentation in order to attract mates and may be more aggressive to defend territories and mates. Males, on the other hand, are expected to be more selective in mate choice and invest substantially in offspring care.

Sex-role reversal is intriguing in birds as both females and males have the potential to defend territories, mate with multiple individuals, and care for eggs and also hatched offspring. Although very rare in birds (~1%; Jenni 1974; Oring 1982, 1986), sex-role reversal has likely independently evolved several times (e.g., hawks (Accipitridae), shorebirds (Charadriidae, Jacanidae, Scolopacidae), buttonquail (Turnicidae), coucals (Cuculidae), and woodpeckers (Picidae); Vernon 1971; Andersson 1994, 2005; DeLay et al. 1996; Kotaka 1998; Ligon 1999). Furthermore, sex role reversal in birds is frequently associated with polyandrous mating systems, in which females pairbond with multiple males during the breeding season and males pairbond with only one female (Vernon 1971; Andersson 1994, 2005; DeLay et al. 1996; Ligon 1999). Shorebirds (Chardriiformes), particularly Scolopaci, are especially interesting due to the high percentage of polyandrous species in these taxa (Scolopaci: 10% - 15%; Chardriiformes: 5%; Table 1; Thomas et al. 2007), their diversity in mating and parental care systems, and their limited ability to increase clutch size.

While the proximate mechanisms, ecological correlates, and evolution of polyandry are not fully understood (e.g., Jenni 1974; Emlen & Oring 1977; Oring 1982; Clutton-Brock 1991; Valle 1994; Ligon 1999; Eens & Pinxten 2000; Owens 2002; Goymann et al. 2015), Andersson (2005) hypothesized that male-biased care of offspring is the first evolutionary step in polyandry in birds. With the reduced demand for maternal care, females could gain increased egg-laying ability, such that the number of eggs laid exceeds the number lost to predators, as well as the maximum capacity of a single nest. In turn, females compete for access to multiple mates, each male caring for one clutch of eggs. The proximate mechanisms regulating these competitive, and often aggressive, interactions across females are not well established. Testosterone is a candidate hormone as it strongly correlates with male aggressive behavior, sexually-selected display traits, and increased reproductive success (Raouf et al. 1997; De Ridder et al. 2000; McGlothlin et al. 2008; Laucht et al. 2010), but although females also produce testosterone, its influence on female behavior is less clear (French et al. 2013; Rosvall 2013b).

Table 1. Shorebird species (Chardriiformes) that exhibit well-documented sex-role reversal.

Family	Scientific name	Common name	References
Charadriidae	*Charadrius morinellus*	Eurasian dotterel	Owens et al. 1994
Jacanidae	*Jacana spinosa*	Northern jacana	Jenni & Collier 1972
	J. jacana	Wattled jacana	Osborne & Bourne 1977 Osborne 1982 Emlen et al. 1989
	Hydrophasianus chirurgus	Pheasant-tailed jacana	Hoffman 1949, 1950 Thong-aree et al. 1995
	Metopidius indicus	Bronze-winged jacana	Mathew 1964 Butchart 2000
	Irediparra gallinacea	Comb-crested jacana	Garnett 1985 Jenni 1996
	Actophilornis africana	African jacana	Tarboton 1992 Jenni 1996
	A. albinucha	Madagascan jacana	Jenni 1996
Scolopacidae	*Phalaropus lobatus*	Red-necked phalarope	Emlen & Oring 1977 Oring 1982 Reynolds 1987
	P. tricolor	Wilson's phalarope	Oring 1982, 1986 Colwell & Oring 1988
	P. fulicarius	Red phalarope	Schamel & Tracy 1977
	Actitis macularius	Spotted sandpiper	Hays 1972 Oring & Knudson 1972
Pedionomidae	*Pedionomus torquatus*	Plains-wanderer	Bennett 1983 Erckmann 1983
Rostratulidae	*Rostratula benghalensis*	Greater painted snipe	Erckmann 1983
Turnicidae	*Turnix suscitator*	Barred buttonquail	Ridley 1978 Starck 1991
	T. sylvaticus	Common buttonquail	Cramp & Simmons 1980

Polyandrous species are excellent systems for understanding the effects of sexual selection on females, such as the evolution of proximate mechanisms regulating female aggression and female-biased ornamentation as an honest signal to attract mates or display to competitors. In this thesis, I investigated possible outcomes of sexual selection pressures on female spotted sandpipers by examining sex differences in plumage ornamentation, breeding biology, and hormones potentially moderating female aggression. Specifically, I was interested in whether females have evolved female-biased ornamentation that could signal individual quality to potential mates or female competitors. Therefore, I examined relationships between degree of ornamentation, health metrics, reproductive success, offspring sex differences, and testosterone levels.

Of the sex-role reversed and polyandrous birds, spotted sandpipers (Scolopacidae: *Actitis macularius*), in particular, have been well-studied in terms of their natural history, behavior, and endocrinology. Females typically arrive at the breeding ground before males, and then compete for territories and mates (Maxson & Oring 1980). While females can lay a new clutch approximately a week after completing a previous clutch, the eggs need to be incubated for three weeks before hatching and then the offspring fledge two weeks later (Emlen & Oring 1977; Maxson & Oring 1980; Lank et al. 1985). By abandoning the nest to the male’s care, a female has the opportunity to seek additional mates and lay additional clutches during a single breeding season. As a result, female reproductive success depends on number of mates, and male reproductive success depends on the success of one clutch (Oring et al. 1983, 1991). However, there is the possibility that males could increase reproductive success through extra-pair copulations, although they would also risk decreased certainty of paternity as females can likely store sperm (Oring et al. 1992). Females are heavier, more aggressive, and more likely to initiate copulation than males (Maxson & Oring 1980). Although males have higher levels of testosterone than females, paired females have significantly higher levels than unpaired females (Fivizzani & Oring 1986). Because of the reversed reproductive skew between the sexes, it is likely that female spotted sandpipers are under significant sexual selection pressures to attract mates and defend territories.

In *Chapter 1: Plumage pattern dimorphism in a shorebird exhibiting sex-role reversal (Actitis macularius)*, I collaborated with Stephen Pruett-Jones to determine whether females are more ornamented than males in terms of their melanized plumage pattern, unique to breeding adults, and if so, whether health metrics could explain degree of ornamentation. We predicted that if plumage pattern was correlated with condition, an individual bird's pattern would vary across years. Using digital photographs taken in the field, we quantified spotted plumage pattern to determining spot size, shape, percent cover, and number of spots. We determined that while measures of 'spottiness' overlapped between the sexes, females had fewer, larger, and less regularly shaped spots than males, but that these spots covered a greater percentage of plumage. Female and males with greater ornamentation in terms of spots size had fewer ectoparasites, and females with greater spotted plumage cover were heavier and had higher hematocrit levels. The spotted plumage pattern appears to change across years, both in terms of the individual and the population. These results indicate that although the sexes both exhibit an ornamental trait, there may be stronger selection pressure on females to evolve an honest indicator of condition.

Stephen Pruett-Jones and I next investigated genetic paternity and maternity in *Chapter 2: Parentage, sex ratio, and reproductive success in the polyandrous spotted sandpiper (Actitis macularius)*. In polyandrous systems, females invest in acquiring mates and males invest in parental care. Accordingly, females are expected to display sexually-selected ornaments that correlate with reproductive success and males are expected to have high certainty of paternity (Trivers 1972; Whittingham et al. 1992). We predicted that the population would exhibit low rates of extra-pair paternity, female reproductive success would correlate with ornamentation, and female offspring would be at least as costly to produce as males in terms of mass and size. To establish rates of polyandry and extra-pair paternity in our population, we used seven microsatellite loci to determine genetic parentage of offspring. Approximately 18.2% - 33.3% of females were polyandrous, laying nests for multiple males in a single breeding seasons. Based on the genetic data, there were no extra-pair offspring in our population: the social father was the genetic father of every offspring in his clutch. Females that laid eggs had a greater percentage of spotted plumage cover than those that did not lay eggs or left the study site, but males exhibited no correlation between reproductive success and plumage pattern. Considering offspring sex differences, females had longer tarsi than males but were otherwise similar in mass, hatch date, and number. This suggests that female offspring are as costly as, or even costlier than, male offspring. Despite the possibility that adult female spotted sandpipers store sperm for a longer time interval than the interval between laying clutches (Oring et al. 1992), females were reproductively monogamous with their current mate, demonstrating that extra-pair young are rare in this polyandrous system. Furthermore, reversed sexually dimorphic plumage ornamentation in polyandrous systems can correlate with female reproductive success.

In *Chapter 3: Testosterone, reproductive success, and melanized plumage ornamentation in a polyandrous shorebird (Actitis macularius)*, I considered the function of testosterone in females in regard to reproductive stage, reproductive success, and plumage ornamentation. I expected that male, and to a lesser extent, female, testosterone levels would peak during courtship. I predicted that after this initial peak, female levels would remain relatively high throughout the breeding stages while male levels would fall significantly during incubation and care of offspring. I expected female testosterone levels would increase following a simulated competitive interaction and correlate with both reproductive success and melanized plumage ornamentation. I captured half of egg laying/incubating females on the nest and half immediately following a simulated territorial intrusion involving a mount and the call of an unknown spotted sandpiper. All courting adults were also caught following a simulated territorial intrusion, and remaining adults at other reproductive stages were caught without a stimulus. Circulating plasma testosterone levels were quantified using enzyme immunoassays. As with prior studies on spotted sandpipers (Rissman & Wingfield 1984; Fivizzani & Oring 1986), I found that males had higher levels of testosterone than females during courtship, and these levels dropped once incubation began. Female testosterone levels remained constant from courtship to incubation and were statistically similar to male levels during the incubation period. During egg laying/incubation, females captured following simulated territorial intrusions had higher testosterone levels than those without a stimulus. Both females and males showed some degree of positive covariation between plumage ornamentation and testosterone levels, but females challenged with the simulated territorial intrusion surprisingly displayed a negative relationship. Although there were no relationships in females or males between testosterone levels and reproductive success, the higher testosterone levels following a simulated territorial intrusion suggest that testosterone does play a functional role in regulated female aggression.

Considering the results of my dissertation research, it is likely that melanized plumage pattern is an honest signal in females, correlating with lower ectoparasite loads, higher mass, and higher hematocrit levels. Female plumage ornamentation may also signal reproductive success, in terms of whether eggs are laid during the breeding season, to potential male mates. In males, the evidence supporting the connections between ornamentation and condition is less convincing and there appears to be no connection between ornamentation and reproductive success. Although there was not a positive relationship between amount of melanized plumage and testosterone levels in females after territorial intrusions, the social costs involved in competitive female interactions could maintain the honesty of this plumage ornamentation.

As more studies address sexual selection known to occur in females, but largely overlooked historically, understanding female competition for mates and resources becomes even more relevant. Regarding the mechanisms of female aggression and other competitive behaviors, future studies should extend their focus from circulating testosterone levels to individual and sex differences in other hormone candidates, such as progesterone (e.g., Goymann & Wingfield 2004), as well as target tissues and resulting genomic expression (e.g., Rosvall 2013b). Taking a phylogenetic approach to the evolution of polyandry may reveal what factors determine the intensity of sexual selection across species or why, for instance, Charadriiformes express such diversity in mating systems. It is important to research non-model species, especially those expressing traits that are evident in both sexes. By investigating such traits, we can determine the different selective pressures each sex experiences and what factors affect the expression of ornamentation between and within the sexes. While female competition is especially prevalent in sex-role reversed species, it is undeniable that female vertebrates can experience intense competition over mates and resources (Lutnesky & Kosaki 1995; Woodley & Moore 1999; Amundsen 2000; Stockley & Bro-Jørgensen 2011). Rather than simply a correlate of selection on males as was once assumed, female ornamentation and testosterone levels are likely under direct selection in species exhibiting female competition.

CHAPTER 1: PLUMAGE PATTERN DIMORPHISM IN A SHOREBIRD EXHIBITING SEX-ROLE REVERSAL (*ACTITIS MACULARIUS*)[1]

ABSTRACT

In birds, both males and females can exhibit socially selected traits, but relatively few studies address the role of female ornaments despite their potential importance in competitive female-female interactions and male mate choice. We investigated the melanized plumage pattern of male and female spotted sandpipers (*Actitis macularius*), a species with sex-role reversal and a polyandrous mating system. While the sexes overlap in the spottiness metrics, females had fewer, but larger and more irregularly shaped spots that covered a greater percentage of their plumage than did males. Feather mite load best explained the first principal component of plumage pattern (i.e. spot size) in females as well as in males. Sandpipers with lower mite loads had larger spots, but this relationship was less strong in males. Considering the second principal component (i.e. spot shape and percent cover), mass, hematocrit levels, and day captured best explained variation across females. Heavier females with higher hematocrit levels were caught later in the season and had more irregular spots and a higher percentage of melanized plumage cover. Spot pattern in recaptured individuals changed with capture year, indicating that spottiness varies within an individual's life. Overall, these results show that although the differences between the sexes are subtle, spottiness in spotted sandpipers is a measurably sexually dimorphic trait with females as the more ornamented sex, and that melanized ornaments can be indicators of female, and possibly male, condition.

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[1] This manuscript is reprinted with permission: Blizard, M. & Pruett-Jones, S. 2017. *The Auk: Ornithological Advances*, 135, 363-376.

INTRODUCTION

From the tails of peacocks to the horns of rhinoceros beetles, sexually dimorphic traits have evolved repeatedly. Most frequently, it is the male that is ornamented (e.g., Hill 1993; Møller 1993; Cuervo et al. 1996; Amundsen 2000) due to intrasexual competition, mate choice, and/or mate stimulation (Andersson 1994; Servedio et al. 2013; Tazzyman et al. 2014). Selection can be equally important in driving the evolution of female ornaments (Amundsen 2000), and as females may also compete for mates (e.g., Gwynne 1981; Berglund et al. 1996; Swenson 1997; Eens & Pinxten 2000; Härdling et al. 2008), sexual selection pressures could influence exaggerated traits in females. Alternatively, females may exhibit an ornamented trait present in males, but in a less exaggerated form as a genetic by-product of selection on males (Lande 1980), a stimulant for male provisioning of offspring (e.g., Matessi et al. 2009), or mutual mate choice (e.g., Kraaijeveld et al. 2007).

In rare cases, females are the more ornamented sex (e.g., Johns 1964; Heinsohn et al. 2005; Muck & Goymann 2011; Clutton-Brock & Huchard 2013). The evolution of female-specific ornaments under sexual selection is expected in circumstances where females experience greater competition for mates (i.e. “sex-role reversal”) and therefore greater potential variance in reproductive success (Emlen & Oring 1977; Amundsen 2000). This reversal in typical sex roles is unusual across vertebrates and is usually associated with polyandrous mating systems (Andersson 2005), in which females pair with multiple males in a single breeding season. Polyandry, though uncommon (Andersson 2005), has evolved independently several times in birds (e.g., hawks (Accipitridae), shorebirds (Charadriidae, Jacanidae, Scolopacidae), buttonquail (Turnicidae), and coucals (Cuculidae); Vernon 1971; Andersson 1994; DeLay et al. 1996; Ligon 1999).

Plumage color is one way by which females may be ornamented, and the most abundant pigment in integument is melanin, which predominantly produces black, brown, and gray colors (Jawor & Breitwisch 2003; McGraw 2006). As melanin pigments also function in cryptic coloration (reviewed in Jawor & Breitwisch 2003), this can complicate understanding melanized ornaments. However, there is evidence in females that plumage melanization can positively correlate with greater antibody responses (tawny owls, *Strix aluco*; Gasparini et al. 2009), body condition (northern flickers, *Colaptes auratus*; Wiebe & Vitousek 2015), immunocompetence, and offspring ectoparasite resistance (barn owls, *Tyto albus*; Roulin et al. 2000, 2001).

In this study, we analyzed the spotted plumage of breeding spotted sandpipers (*Actitis macularius*). The spotted sandpiper is an ideal species in which to study female ornamentation as females are territorial, polyandrous, and exhibit sex-role reversal (Oring & Lank 1986; Fivizzani & Oring 1986). It is the most widespread sandpiper species breeding in North America and is a habitat generalist, living along lakes, streams, and wetlands (Oring et al. 1983). Females and males are the same size with respect to wing chord, tail, and tarsus length (Pyle 2008), but females are 20% - 25% heavier than males (Maxson & Oring 1980). Both sexes are philopatric, returning to the same breeding site every season from their wintering grounds (Oring et al. 1983), which stretch from northern Argentina to the southern United States (Nelson 1939; Hayes 1995).

Female spotted sandpipers arrive at the breeding ground first and compete for territories and males, once they arrive. Females that are unable to establish and maintain a territory may be excluded from the breeding ground, skewing the operational sex ratio (Maxson & Oring 1980). Both sexes display courtship behavior, but females are more likely to initiate copulation and spend significantly more time in agonistic interactions than males (Maxson & Oring 1980). A clutch of 4 eggs is typically incubated for 21 days by the male, who also cares for the offspring after hatching. After laying eggs in the nest of one male, a female can lay another clutch in the nest of second male in as few as 8 days (Emlen & Oring 1977; Maxson & Oring 1980; Lank et al. 1985). Female reproductive success increases with age and depends on number of clutches laid (i.e. number of mates; Oring et al. 1983, 1991).

When in breeding plumage, the white ventral surface of spotted sandpipers is covered in dark brown spots, each one localized to a single feather (Figure 1.1). These spots vary in number and size across individuals, and females have been reported as "spottier" than males (Mousley 1937; Hays 1972; Oring & Knudson 1972; Reed et al. 2013; see Dwight 1900 for observations of sexual dimorphism when the sexes were misidentified), but this sexual dimorphism has yet to be quantified. In aggressive and courtship interactions, the birds present to the conspecific their throat, chest, and abdomen, the precise areas where the feathers are spotted (Maxson & Oring 1980). Notably, the spotted pattern is entirely absent in juvenile and nonbreeding plumage,

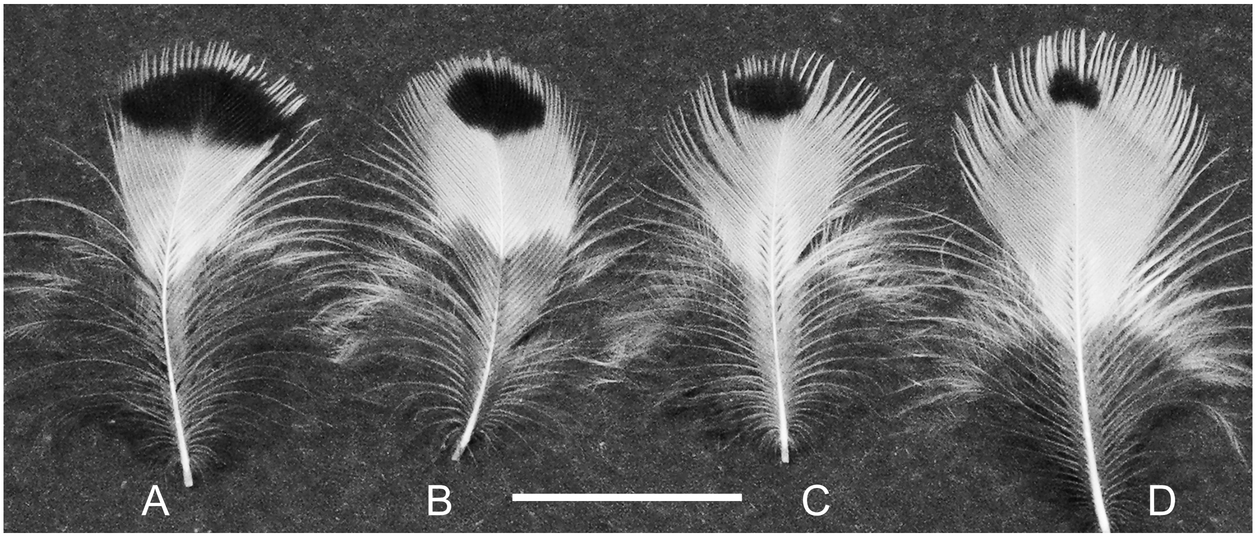

Figure 1.1. Examples of spotted breast feathers from (**A**, **B**) female and (**C**, **D**) male spotted sandpipers. Scale bar represents 1 cm.

suggesting the spots have a role in social interactions specifically during the breeding season. After quantifying the spotted plumage pattern, we considered spottiness relative to phenotypic metrics to explore the possible signaling roles of this plumage pattern and differences between the sexes.

METHODS

Study Site

We worked along 20 km of shoreline on the southern and southeastern coast of Beaver Island, Michigan (45.5928 N, 85.5188 W). This 144.5 km^2 island is one of several islands in the Beaver Island Archipelago and is the largest island in Lake Michigan. spotted sandpipers breed along the shoreline in areas where there is a mixture of rocky coasts, sand dunes, wetlands, and semi-open shrubby vegetation. Much of the land is privately owned, but human presence and activity is seasonal, peaking during the months of July and August. spotted sandpipers are the most abundant shorebird nesting at the field site, and 2 other shorebird species, killdeer (*Charadrius vociferus*) and Wilson's snipe (*Gallinago delicata*), also nest in the area at lower densities. An estimated 90 adult spotted sandpipers breed along the coast of Beaver Island each season, and our study site encompassed the territories of ~45 adults (17 females, 28 males, and 25 - 35 nests). Nesting density varied along the shoreline from 20 m to 1,875 m between nests.

Fieldwork

Fieldwork was conducted from early May through late July for 3 consecutive seasons from 2013 to 2015.We captured adult sandpipers during territory establishment and courtship with mist nets and playback calls. Once adults began incubating eggs, birds were caught with a nest trap at least 5 days after nest discovery. In total, we captured 87 adults (32 females, 55 males), 16 of which were recaptured across more than one breeding season (3 females, 13 males). For individual identification in the field, we banded each bird with a permanent metal band and a unique combination of 3 colored plastic bands. We recorded the following phenotypic measures: mass, tarsus length, and feather mite load on the primary and secondary wing feathers using a standard scale of 0 to 4 (Thompson et al. 1997) and took a blood sample from the brachial vein (Owen 2011) to determine sex and hematocrit level. We also recorded probable sex of each adult based on behavioral observations. Genetic analysis (see below) of 11 females and 42 males confirmed our observations. For analysis of the plumage pattern on the chest and abdomen, each bird was held with the wings gently restrained against the body and the ventral surface was photographed from ~135 cm above the bird (Figure 1.2A), using the same camera each field season (Nikon D70 with an AF-S Nikkor 55 - 200 mm telephoto lens; Nikon, Tokyo, Japan). A scale bar, present in every photo, was used to normalize any differences in distance. Between photographs, each bird was adjusted in the hand to smooth the feathers.

Labwork and Pattern Analysis

To calculate hematocrit level, blood samples were centrifuged in heparanized microhematocrit capillary tubes and then the length (i.e. volume) of the red blood cells relative to the total amount of blood was measured to the nearest 0.01 mm using calipers. Sex of individuals was verified by PCR amplification of the CHD gene using the P2 and P8 microsatellite primers (Griffiths et al. 1998) after DNA extraction, which followed standard techniques (DNAeasy; Qiagen, Venlo, Netherlands).

We used the following method to quantify spotted plumage pattern so that individual birds could be objectively compared to one another, under the reasonable assumption that our methods correlate with the birds' visual perception. The spotted patterns, photographed in the field, were saved in the raw file format on the camera to maximize information captured and preserve image quality and then converted to uncompressed TIFF files on a computer. We selected one photo of each sandpiper each year based on image clarity, position of the bird, and visibility of the scale bar. We cropped the images first (Photoshop CS6; Adobe Systems, San Jose, California, USA) to remove any identifying marks (i.e. leg bands) and then renamed the files using random numbers to prevent observer bias. Using ImageJ (Rasband 2015), we scaled and rotated the images so that the bird's left and right wrists (i.e. the forearm joint consisting of the ulna, radius, ulnare, and carpometacarpus bones) were aligned horizontally. The images were cropped to a final size of 2.7 × 2.7 cm to maximize the area of plumage analyzed relative to individual size (Figure 1.2A, B). The cropped square was centered on the chest and only encompassed ventral plumage, with the top edge level with the wrists. We transformed all images to grayscale and applied a Gaussian blur to smooth the edges and reduce noise. We then adjusted the threshold to convert the image into an 8-bit binary image necessary for particle measurement on ImageJ (Figure 1.2C; Schneider et al. 2012). Lastly, we used the watershed algorithm on ImageJ to break apart overlapping spots (Figure 1.2D). Although this method is not guaranteed to separate all spots whose perimeters intersect, it is a precise mathematical method that first calculates the Euclidean distance map, determines ultimate eroded points, and then expands the points outward (Leymarie & Levine 1992; Schneider et al. 2012). All outline drawings produced by ImageJ were overlaid onto the original 2.7 × 2.7 cm photograph to check for accuracy (Figure 1.2E).

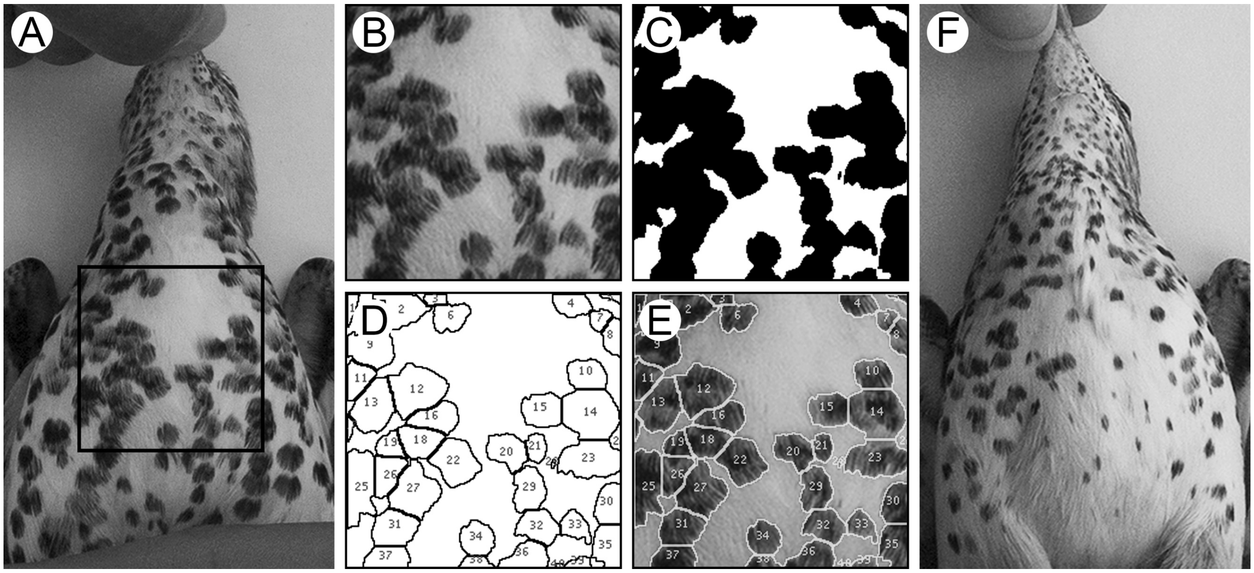

Figure 1.2. Our method of analyzing the ventral plumage pattern of spotted sandpipers using ImageJ (Rasband 2015). (**A, F**) Photographs of each adult were taken in the field, scaled, and cropped to 2.7 × 2.7 cm, represented in (**A**) by a black outline and also (**B**). These images were converted into (**C**) binary files, to which we applied (**D**) the watershed algorithm to separate overlapping spots. (**E**) The outline drawings were overlaid onto the original photograph to check for accuracy, and then analyzed using particle analysis. (**A**) is a female spotted sandpiper and (**F**) is a male.

Lastly, we quantified spottiness of the plumage pattern outline drawings on ImageJ by using particle analysis to determine spot count, size, orientation, shape, and percent cover of spotted plumage. Spot size was measured in terms of area, perimeter, and major and minor axes (i.e. the axes of the fit ellipse, which has the same area, centroid, and orientation as the spot). The major and minor axes were used in lieu of the spots' absolute maximum and minimum diameters, as the former are more robust measures, not influenced by irregularities in the spot's perimeter. However, these variables were strongly correlated with each other: the average major and minor axes correlated, respectively, to the average spot's true maximum (females: $r = 0.99$, df = 30, $p < 0.001$; males: $r = 0.99$, df = 53, $p < 0.001$) and minimum diameters (females: $r = 0.99$, $p < 0.001$; males: $r = 0.99$, $p < 0.001$). Spot shape was measured in terms of aspect ratio, solidity, roundness, and circularity. As calculated by ImageJ, aspect ratio is a measure of elongation (major axis/minor axis), solidity is a measure of compactness (area/convex area), roundness is a measure of similarity to a circle based on major axis ((4 × area)/(π × major axis^2)), and circularity is also a measure of similarity to a circle, but based on perimeter ((4 × area)/perimeter^2; Rasband 2015). To ascertain the accuracy of the watershed algorithm, one of us (M.A.B.) counted the number of spots in the 2.7 × 2.7 cm square on the chests of 27 females and 27 males. As the count data were not normal, we used Spearman's rank correlation to verify that spot count by ImageJ significantly correlated with spot count by human eye ($S = 6{,}018.3$, $r_S = 0.77$, $p < 0.001$). ImageJ tended to underestimate the number of spots (slope of linear regression = 0.79 with count by ImageJ as a function of count by human eye). We also compared our method of quantifying spotted pattern from the binary images with granularity analysis (Stoddard & Stevens 2010), which we ran in MATLAB 7.14 (MathWorks 2012).

Statistics

We ran all statistical tests in R 3.3.1 (R Development Core Team 2015). We used a two-way ANOVA to determine differences between the sexes as a fixed effect and across the years as a random effect, with all replicate individuals removed from the data set. We used Mann-Whitney-Wilcoxon tests to verify the results of our particle analysis methods with the results of the granularity analysis. To further examine the differences between the sexes, we used linear discriminant analyses to determine whether individuals could be correctly assigned to their sex and/or year class based on their spottiness metrics. To quantify spottiness and reduce the number of intercorrelated variables (Tables 1.A.5 and 1.A.6), we conducted principal component analyses including all plumage metrics on both sexes combined and independently.

We used forward and backward stepwise multiple regression models (‘stats’ R package; R Development Core Team 2015) to determine whether the following were explanatory variables for the observed variation in spottiness: log of mass, tarsus length, hematocrit levels, mite score, and day of the season (see Table 1.A.7 for explanatory variable correlations). Day of the season was normalized by counting up from “day 1,” the first day a spotted sandpiper was observed on the breeding grounds each year. All variables were centered to 0 in the model using the scale function. We used AICc (Cavanaugh 1997) to assess alternative models (‘AICcmodavg’ R package; Mazerolle 2016) and ran the regression models on each sex independently. To evaluate how an individual changes among years captured, we used linear mixed-effects models (‘nlme’ R package; Pinheiro et al. 2017) to test whether the variation in PC scores across recaptured individuals could be explained by sex, capture year (i.e. first, second, or third as a categorical variable), or the 2 variables’ interaction. Bird identity was included as a random variable (3 females, 13 males), and to account for changes in the sex ratio of captured adults year to year, we used the PC scores calculated separately by sex.

RESULTS

Quantifying Spottiness

Females had a greater percentage of plumage covered by spots, but had fewer total spots than males (Tables 1.1 and 1.2, Figure 1.3). Thus, female spots were larger than those of males, as measured by average spot area and perimeter, as well as the major and minor axes. Females had more elongated spots (i.e. larger aspect ratios) that were less round and tended to be less solid than those of males. However, circularity of spots did not differ significantly between the sexes. The interaction between sex and year was not significant for any of the metrics (Table

Table 1.1. Average body metrics and spottiness variables of spotted sandpipers, not including replicates captured across years. Body metrics were measured in the field over three years, and spottiness variables were calculated using particle analysis on ImageJ (Rasband 2015).

		Females (*n* = 32)		Males (*n* = 55)	
		$\bar{x}$	SE	$\bar{x}$	SE
Body metrics	Mass (g)	50.28	1.31	39.46	0.38
	Tarsus (mm)	28.12	0.19	27.96	0.13
	Mites (scale)	1.11	0.14	1.19	0.11
	Hematocrit (%)	47.71	0.85	48.63	0.48
Spot metrics	Area (cm^2)	0.07	0.004	0.04	0.002
	Perimeter (cm)	1.05	0.03	0.75	0.02
	Major axis (cm)	0.34	0.01	0.25	0.005
	Minor axis (cm)	0.23	0.008	0.16	0.004
	Percent cover	38.42	1.88	22.90	1.01
	Spot count	42.56	1.56	48.49	1.80
	Circularity	0.72	0.008	0.74	0.008
	Aspect ratio	1.60	0.03	1.70	0.03
	Roundness	0.67	0.008	0.64	0.007
	Solidity	0.89	0.004	0.89	0.003
	Angle (˚)	93.15	1.82	87.49	1.33

Table 1.2. Analysis of variance of spottiness metrics of spotted sandpipers (*n* = 87) between the sexes (fixed effect) and across years (random effect), including interactions. Asterisks indicate significance with Bonferroni corrections applied to the table (*$p < 0.005$, **$p < 9.1\text{x}10^{-5}$).

	Sex		Sex:Year	
	F	*p*	*F*	*p*
Area	87.19	<0.001 **	0.006	0.94
Perimeter	91.51	<0.001 **	0.07	0.80
Major axis	85.77	<0.001 **	0.002	0.97
Minor axis	74.92	<0.001 **	0.04	0.84
Percent cover	60.14	<0.001 **	0.07	0.79
Spot count	7.01	0.01	0.23	0.64
Circularity	0.34	0.56	0.25	0.62
Aspect ratio	8.49	0.005	0.75	0.39
Roundness	8.74	0.004 *	0.64	0.42
Solidity	3.36	0.07	0.08	0.78
Angle	5.71	0.02	0.18	0.67

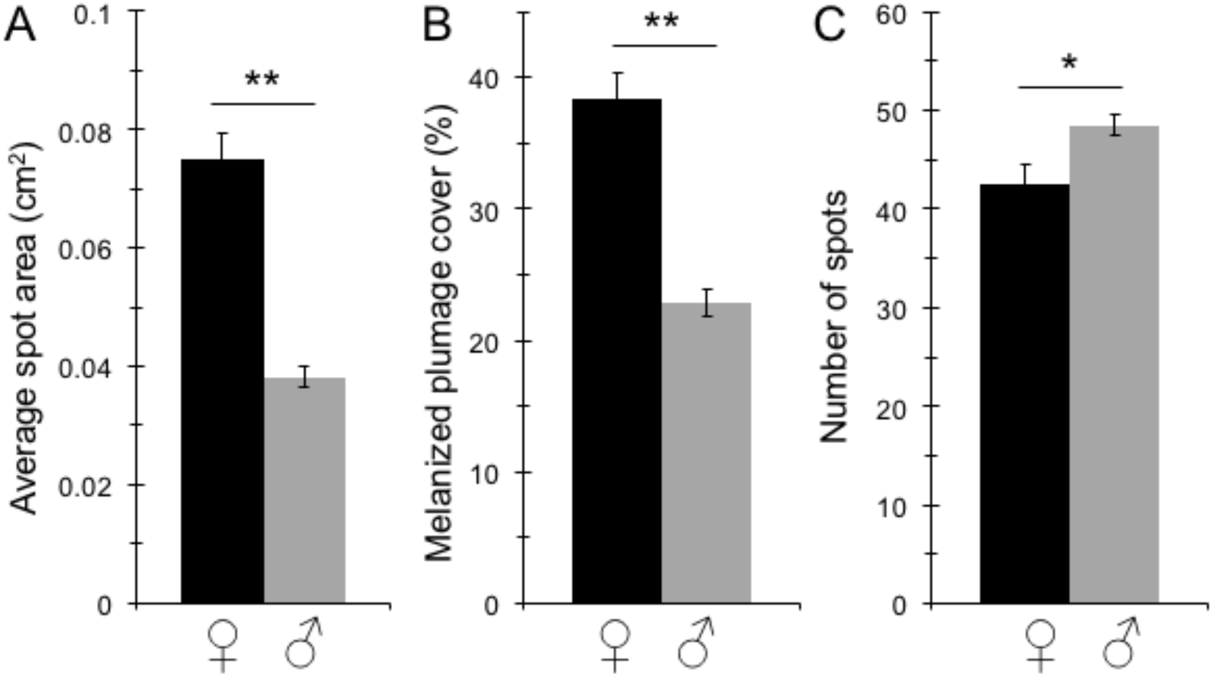

Figure 1.3. Sex differences in (**A**) spot area, (**B**) percentage of melanized plumage, and (**C**) number of spots (error bar: mean ± SE). Females: black, males: gray (ANOVA: $*p < 0.05$, $**p < 0.001$).

1.2). Our particle analysis metrics were correlated to the granularity analysis results (spot area vs. maximum energy peak in the spectrum: $U = 2173$, $p < 0.001$; spot cover vs. total energy in the spectrum: $U = 2258$, $p < 0.001$).

Based on the 11 spottiness metrics, 82% of adult spotted sandpipers (69% of females and 89% of males) were correctly assigned to their true sex by linear discriminant analyses (Wilks' $\lambda = 0.41$, $p < 0.001$). Focusing on year, but with the sexes separated, 66% of females were correctly matched to their year of capture (2013: 89%; 2014: 50%; 2015: 62%; Wilks' $\lambda = 0.29$, $p < 0.002$) and 89% of males were correctly matched to their year of capture (2013: 96%; 2014: 73%; 2015: 92%; Wilks' $\lambda = 0.32$, $p < 0.001$). Furthermore, with the sexes combined, the linear discriminant analysis properly matched 68% of individuals to their combined sex and year class

(2013: females = 56%, males = 93%; 2014: females = 20%, males = 73%; 2015: females = 38%, males = 85%; Wilks' λ = 0.03, $p < 0.001$).

For females and males combined, variables associated primarily with spot size (area, perimeter, major axis, and minor axis), and also shape (aspect ratio and roundness) and percent cover loaded most heavily on PC 1 (Table 1.3). PC 2 was most heavily loaded by spot shape metrics (circularity and solidity) and percent cover. Individuals with larger, rounder, and less elongated spots had greater percent cover and lower PC 1 scores; individuals with more irregularly shaped spots based on circularity and solidity and greater percent cover had higher PC 2 scores (Figure 1.4). PC 3 was most heavily weighted by angle, but neither sex clustered within the range of scores.

Table 1.3. Variable loadings of the first three principal components (PC) calculated from spottiness metrics of spotted sandpipers, with both sexes combined. Text in bold indicates the most heavily loaded metrics for each corresponding PC ($r_s < -0.55$ or $r_s > 0.55$).

	PC 1		PC 2		PC 3	
	Loading	r_s	Loading	r_s	Loading	r_s
Minor axis	**0.410**	**0.98**	0.091	0.10	-0.074	-0.07
Area	**0.392**	**0.94**	0.174	0.21	-0.083	-0.09
Major axis	**0.386**	**0.91**	0.174	0.26	-0.140	-0.11
Perimeter	**0.380**	**0.88**	0.232	0.36	-0.102	-0.09
Aspect ratio	**-0.312**	**-0.77**	0.313	0.46	-0.124	-0.08
Roundness	**0.312**	**0.75**	-0.268	-0.42	0.146	0.12
Percent cover	**0.311**	**0.68**	**0.342**	**0.57**	-0.046	-0.05
Spot count	-0.196	-0.45	0.313	0.47	0.073	0.12
Solidity	0.150	0.36	**-0.450**	**-0.72**	-0.115	-0.12
Circularity	0.133	0.30	**-0.528**	**-0.86**	0.029	-0.05
Angle	0.119	0.25	0.109	0.19	**0.949**	**0.91**
Variance explained	52%		25%		8%	

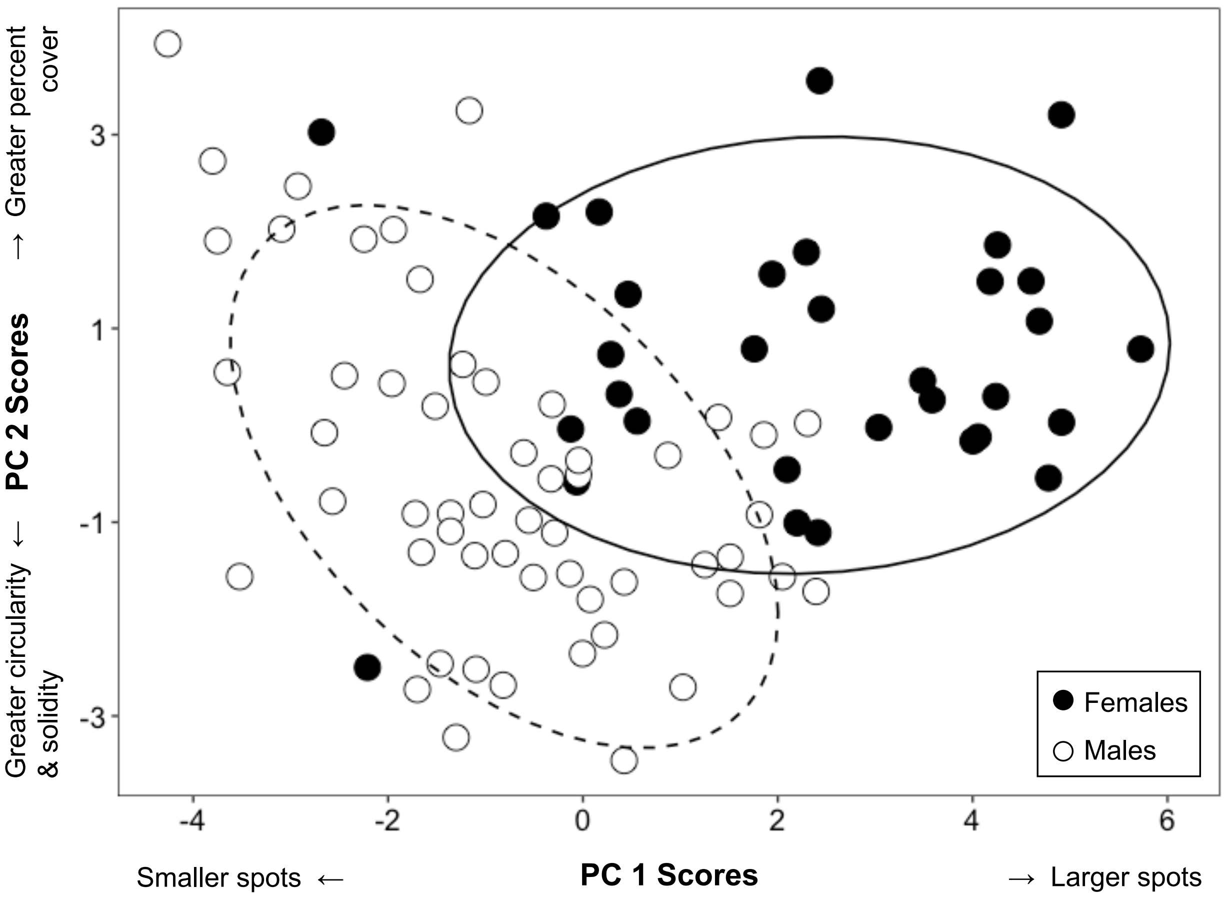

Figure 1.4. The first and second principal components of spottiness calculated by combining the 11 ImageJ spottiness metrics. The first principal component accounts for 52% of the variance among individuals and the second, 25% of variance. Each female is represented by a black circle and each male by an open circle. Ellipses are drawn around 75% confidence intervals for each sex; females: solid line, males: dashed line.

Explanatory Variables of Spottiness

When the sexes were analyzed separately, the first and second PC scores loaded the 11 variables similarly for females and males (Pearson correlation: PC 1: $r = 0.99$, df = 9, $p < 0.001$; PC 2: $r = 0.98$, df = 9, $p < 0.001$; PC 3: $r = 0.04$, df = 9, $p = 0.92$; Table 1.A.8). Analyzing female PC 1, the best linear regression model included mites as an explanatory variable (Table 1.A.9). Females with smaller mite loads had larger spots (Figure 1.5A). For PC 2, the best model

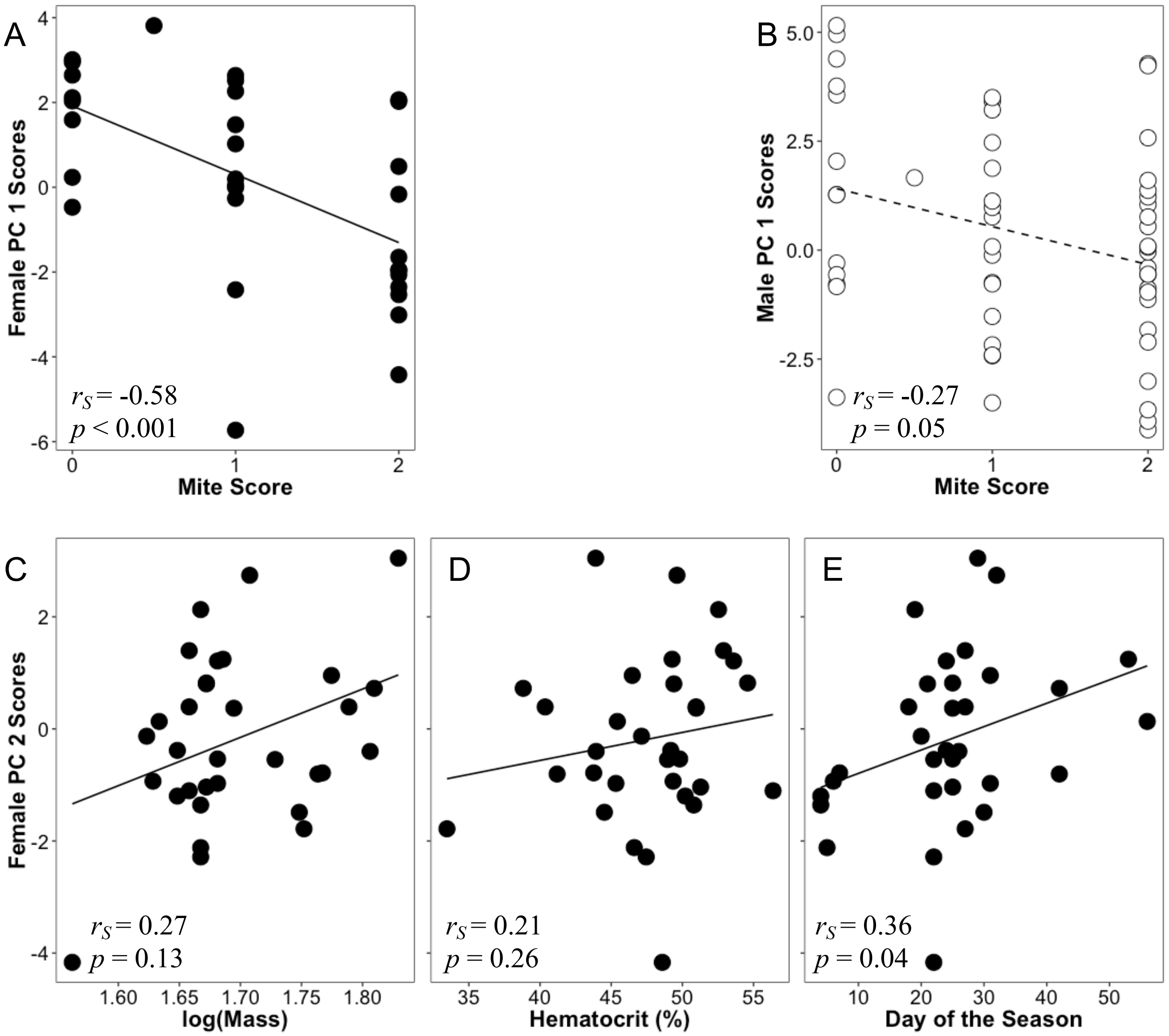

Figure 1.5. Relationships among variables that remained significant in explaining spottiness after stepwise multiple regression. Data from (**A**, **C** - **E**) females (*n* = 32) and (**B**) males (n = 55) were analyzed separately. Lines indicate linear associations between the variables, and as the data were not normal, we used Spearman rank correlations.

for females included mass, hematocrit levels, and day of the season captured (Figure 1.5C - E and Table 1.A.9). Heavier females with higher hematocrit levels (i.e. more red blood cells relative to total blood volume) had more irregular spots in terms of circularity and solidity, greater percent cover, and tended to be caught later in the breeding season.

For males, the best model explaining variation in PC 1 included mites (Table 1.A.9). As with females, males with lower mite loads had larger spots (Figure 1.5B). For PC 2, the model including tarsus and day of the season had the lowest AICc score, but day of season had a weak effect within the model. Additionally, ΔAICc is <2 when comparing the null model to the model including tarsus and day of the season or the model including tarsus alone, indicating that the null model also has substantial support in describing the data (Burnham & Anderson 2002). Thus, although males caught earlier in the breeding season and with longer tarsi tended to have greater percent cover and less circular spots, the null model was the most parsimonious model.

In the analysis of individual variation among years, variation in spottiness of recaptured birds was explained by capture year (Table 1.4). Sex and the interaction between sex and capture year did not significantly explain variation within the models. With the first capture year as the

Table 1.4. Results of linear mixed-effects models explaining variation across the first and second principal components of plumage spottiness of adults (*n* = 16) captured across multiple breeding seasons. Sex, capture year, and their interaction were included as explanatory variables and identity was included as a random factor (**p* < 0.05).

		Estimate	SE	df	*t*	*p*	
PC 1:	(Intercept)	1.23	1.31	17	0.93	0.36	
	Sex Male	-0.56	1.46	14	-0.38	0.71	
	Capture Year Second	-5.69	2.63	17	-2.17	0.04	*
	Capture Year Third	-1.06	2.08	17	-0.51	0.62	
	Sex Male:Capture Year Second	4.07	2.79	17	1.46	0.16	
	Sex Male:Capture Year Third	-1.04	2.33	17	-0.45	0.66	
PC 2:	(Intercept)	-0.70	0.88	17	-0.79	0.44	
	Sex Male	0.21	0.98	14	0.22	0.83	
	Capture Year Second	2.73	1.41	17	1.94	0.07	
	Capture Year Third	2.52	1.05	17	2.41	0.03	*
	Sex Male:Capture Year Second	-2.49	1.48	17	-1.68	0.11	
	Sex Male:Capture Year Third	-2.00	1.18	17	-1.70	0.11	

reference, PC 1 significantly decreased in the second capture year and there was a nonsignificant decrease in the third capture year; PC 2 tended to increase in the second capture year and increased significantly in the third capture year (Table 1.4). In other words, spot size decreased from the first to the second capture. Spot circularity and solidity decreased and percent cover increased from the first to the third capture, with the same trend from the first to the second capture (Figure 1.6).

DISCUSSION

The spots of female spotted sandpipers were larger, covered a greater percentage of plumage, but were less numerous and less regularly shaped than the spots of males. The sexes overlapped in the spottiness metrics, but the dimorphism was great enough that 82% of individuals could be correctly assigned to their sex by linear discriminant analyses. Analysis of spotted pattern by image granularity (Stoddard & Stevens 2010) was equivalent to the corresponding metrics in our method of pattern quantification. Mixed factors explained male and female patterns of spottiness. Considering both sexes, larger spots correlated with fewer mites, and this relationship was stronger in females than in males. Since mite load was defined by mites located on the wing feathers, these mites may not directly cause damage to neck or chest plumage, but mites on the wings may correlate with the bird's total ectoparasite load and, more generally, overall body condition. As spotted sandpipers molt their breast feathers twice per year and their wing feathers only once, after the breeding season (Pyle 2008), feather mites have accumulated for many months before the next breeding season (Haribal et al. 2011). Consequently, the mites on the wing may affect not only the current health of the sandpiper, but

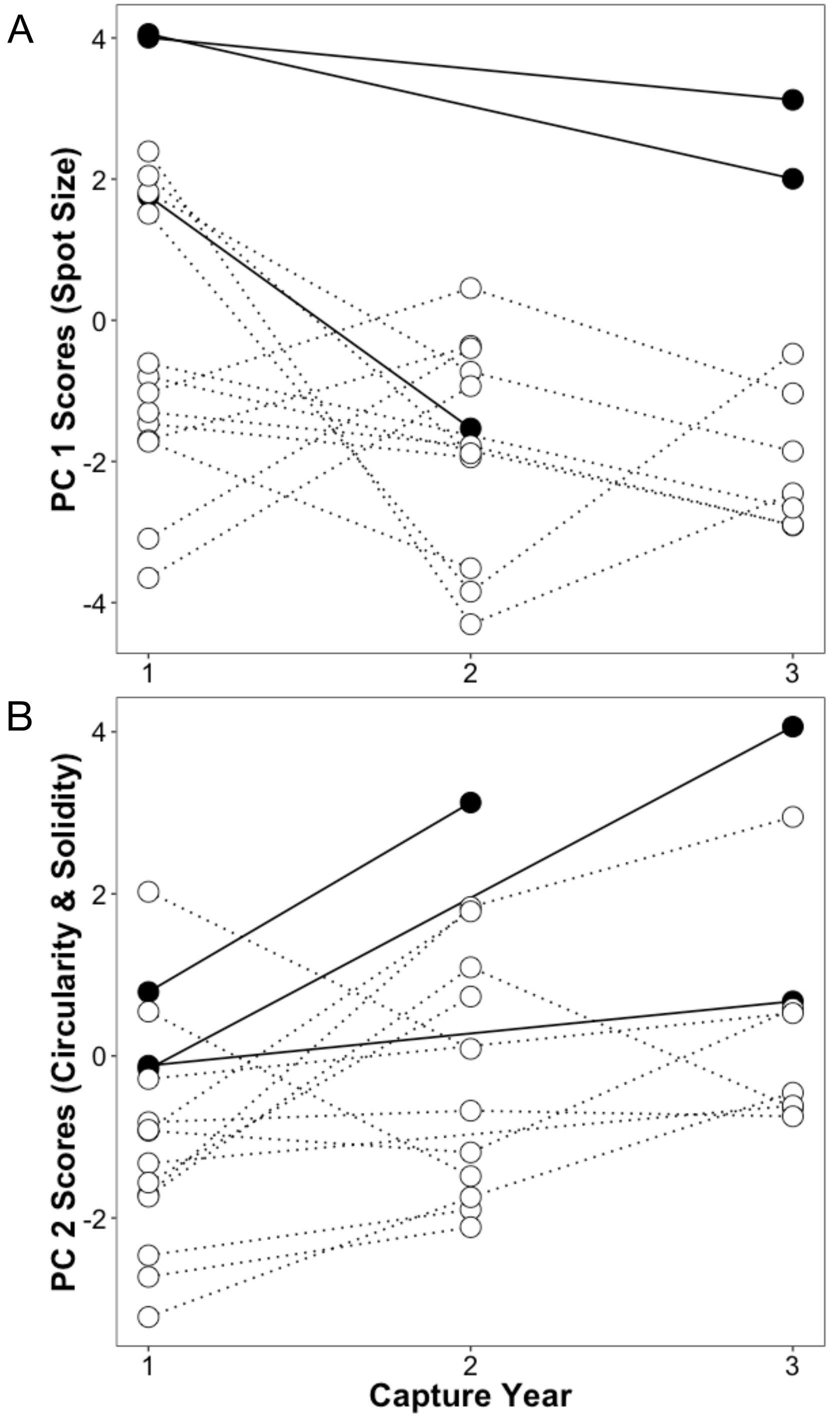

Figure 1.6. Individual changes in the (**A**) first and (B) second principal components for adult Spotted Sandpipers (*n* = 16) captured across multiple years with the sexes analyzed together. Lines connect PC scores of the same individual from year to year. Females: black circles/solid lines, males: open circles/dashed lines.

our limited sample size does not allow us to test this prediction, melanized plumage ornamentation may thus be indicators of an individual's condition the prior year (e.g., Johnsen et al. 1996).

Melanized feathers are more resistant to abrasion (Barrowclough & Sibley 1980; Burtt 1986; Bonser 1995), and while the spots do not cover the entirety of a feather, the largest of the spots approach the feather's edges (Figure 1.1A). Melanized feathers are also more resistant to bacterial damage (e.g., due to *Bacillus licheniformis*; Goldstein et al. 2004; Gunderson et al. 2008), and ectoparasite damage (e.g., Møller 1994). In great tits (*Parus major*), for instance, flea infestations one year resulted in decreased melanin-based ornament size the following year in both sexes (Fitze & Richner 2002). In penduline tits (*Remiz pendulinus*), nest mites were negatively associated with the size of males' black facial ornaments (Darolová et al. 1997). Therefore, it is possible that the larger spots of spotted sandpipers are less likely to be damaged by ectoparasites than smaller spots (but see Bush et al. 2006 regarding feather lice).

In females, spot shape and percent cover were best explained by mass, hematocrit, and day of the season captured, with greater percent cover and shape irregularity correlating with greater mass, higher hematocrit levels, and capture later in the breeding season. In birds, low hematocrit levels indicate anemia, potentially due to bacterial infections, internal parasites (Dein 1986), or mineral deficiencies (Sturkie & Griminger 1986). It is likely that the variable date captured indirectly relates to spot shape and percent cover via weight as spotted sandpipers are a migratory species and arrive at the breeding ground with lower body mass (Reed et al. 2013). Pattern regularity, not irregularity, is expected to positively correlate with condition (e.g., Gluckman & Cardoso 2009). However, our measures of spot shape are dependent on the watershed technique, especially for individuals with great spot overlap, and may not reflect the shape of individual spots if analyzed on each feather separately.

Our data indicate that healthier females, as defined by fewer feather mites, greater mass, and higher hematocrit levels, were more spotted than less-healthy females. In males, however, no explanatory variable was significant from the null model in regard to spot shape and percent cover. The question remains why both sexes exhibit a conspicuous spotted pattern in this polyandrous species, especially as the relationships between ornamentation and individual phenotypic metrics differ between the sexes. Although sexual selection models allow for an exaggeration as well as a reduction in traits with respect to mate preference, there is often an inherent bias towards trait exaggeration (Tazzyman et al. 2014). Furthermore, as male spotted sandpipers are responsible for almost all incubation and parental care (Maxson & Oring 1980), natural selection is likely selecting for less conspicuous males (see Martin & Badyaev 1996; Götmark et al. 1997 for natural selection selecting for crypsis of the parenting sex). Males' spots may be a genetic by-product of selection on females, or females may be choosing males signaling quality of parental care. Additionally, the spotted patterns could potentially be used for individual identity within a breeding season, such as the facial markings of ruddy turnstones (*Arenaria interpres*), another shorebird species (Whitfield 1986).

From the data on recaptured birds, changes in an individual's spot pattern among years related to capture year. These results suggest that pattern varies within an individual's life: spot size tended to decrease and spot shape became more irregular with age (Figure 1.6). Additionally, as this analysis was limited in sample size, especially for females, we were unable to draw strong conclusions about female consistency among years. That being said, the population as a whole noticeably changed in spottiness among years based on the 11 spottiness metrics alone. Linear discriminant analyses correctly assigned a significant proportion of individuals to the correct capture year, as well as the correct year and sex class simultaneously.

Factors such as wintering ground conditions could influence the development of the spot-bearing breeding plumage, resulting in the changes observed at the breeding ground across years at both the population and the individual level. For instance, in migratory species (e.g., American redstarts, *Setophaga ruticilla*), temperature and humidity during the winter affect feather chroma and brightness of breeding plumage (Reudink et al. 2015) and regrown feathers lost during the winter have lower chroma (Tonra et al. 2014). Molting into breeding plumage farther from the wintering grounds can result in lighter melanin-based plumage (barn swallows, *Hirundo rustica*; Norris et al. 2009). Also, the speed of feather growth, a potential proxy for body condition, can cause varying effects on the size of plumage ornaments (e.g., barn swallows and house sparrows, *Passer domesticus*; Vágási et al. 2012; Saino et al. 2015).

It is likely that the spotted plumage pattern in spotted sandpipers is under sexual selection given the sexual dimorphism in spot size, percent cover, and spot shape. Since the spotted pattern is absent in the sister species (the socially monogamous common sandpiper, *A. hypoleucos*) and in all related species in the Scolopaci suborder (see Gibson & Baker 2012 for phylogeny), it is likely that the plumage pattern is relatively recent in the evolutionary history of sandpipers. Furthermore, evolutionary increases in melanization across Charadriiformes (i.e. shorebirds) strongly correlate with increases in testosterone and sexual dimorphism of plumage, although species exhibiting sex-role reversal were removed and female testosterone levels were not included (Bókony et al. 2008).

As more studies address mutual mate choice and male mate choice, the importance of female ornamentation is being recognized (Amundsen 2000; LeBas 2006; Kraaijeveld et al.

2007; Clutton-Brock 2009; Rubenstein & Lovette 2009; Edward & Chapman 2011; Muck & Goymann 2011; Wiebe & Vitousek 2015; Argüelles-Ticó et al. 2016). In systems with sex-role reversal, female competition for mates outweighs that of males, and one would expect a corresponding reversal in the more ornamented sex. By investigating a morphological trait that is evident in both sexes and varies across individuals, we can determine what factors may be affecting the expression between and within the sexes. In the case of spotted sandpipers, their spotted pattern is likely a recently evolved trait that is more strongly expressed in females in terms of percent cover and spot size. Beyond the sexual dimorphism in plumage pattern, female spottiness positively correlates with several metrics associated with health and male spottiness positively correlates with a subset of those health metrics. Determining the predictive variables for expression not only indicates its role as a potential signal to conspecifics, whether male or female, but may also reveal the different selective pressures each sex experiences.

APPENDIX

Table 1.A.5. Pairwise Spearman rank correlations of the plumage spottiness metrics with the sexes combined. Correlations in bold indicate $p < 0.05$ with Bonferroni corrections applied.

	Area	Perimeter	Major axis	Minor axis	Percent cover	Count	Circularity	Aspect ratio	Roundness	Solidity
Perimeter	**0.98**									
Major axis	**0.98**	**0.98**								
Minor axis	**0.98**	**0.95**	**0.96**							
Percent cover	**0.80**	**0.85**	**0.78**	**0.76**						
Count	**-0.40**	**-0.31**	**-0.41**	**-0.43**	0.12					
Circularity	0.03	-0.13	<0.001	0.11	**-0.30**	**-0.45**				
Aspect ratio	**-0.58**	**-0.50**	**-0.53**	**-0.68**	**-0.33**	**0.40**	**-0.57**			
Roundness	**0.56**	**0.49**	**0.50**	**0.67**	**0.35**	**-0.35**	**0.50**	**-0.97**		
Solidity	0.15	0.04	0.13	**0.23**	-0.09	**-0.39**	**0.70**	**-0.54**	**0.50**	
Angle	**0.26**	**0.26**	**0.24**	**0.25**	0.26	0.04	-0.14	-0.14	0.17	-0.05

Table 1.A.6. Pairwise Spearman rank correlations of the plumage spottiness metrics, analyzed with the sexes separated. Correlations in bold indicate $p < 0.05$ with Bonferroni corrections applied.

	Area	Perimeter	Major axis	Minor axis	Percent cover	Spot count	Circularity	Aspect ratio	Roundness	Solidity
Females										
Perimeter	**0.97**									
Major axis	**0.96**	**0.96**								
Minor axis	**0.97**	**0.95**	**0.96**							
Percent cover	**0.75**	**0.73**	**0.67**	**0.72**						
Spot count	**-0.35**	-0.33	**-0.40**	-0.32	0.22					
Circularity	0.16	0.01	0.16	0.22	-0.04	-0.24				
Aspect ratio	**-0.54**	**-0.49**	**-0.49**	**-0.66**	-0.31	0.16	**-0.58**			
Roundness	**0.56**	**0.51**	**0.49**	**0.68**	0.35	-0.15	**0.53**	**-0.97**		
Solidity	0.21	0.10	0.22	0.26	-0.02	-0.34	**0.86**	**-0.51**	**0.45**	
Angle	0.24	0.23	0.12	0.21	0.32	0.05	-0.02	-0.16	0.25	-0.02
Males										
Perimeter	**0.95**									
Major axis	**0.97**	**0.96**								
Minor axis	**0.96**	**0.88**	**0.91**							
Percent cover	**0.59**	**0.69**	**0.57**	**0.49**						
Spot count	**-0.33**	-0.17	**-0.34**	**-0.41**	**0.47**					
Circularity	**0.28**	0.04	0.22	**0.41**	**-0.27**	**-0.65**				
Aspect ratio	**-0.57**	**-0.41**	**-0.47**	**-0.72**	-0.15	**0.44**	**-0.73**			
Roundness	**0.54**	**0.40**	**0.42**	**0.71**	0.18	**-0.37**	**0.65**	**-0.97**		
Solidity	0.26	0.10	0.21	**0.39**	-0.12	**-0.42**	**0.68**	**-0.61**	**0.57**	
Angle	0.09	0.08	0.10	0.07	0.11	0.12	-0.14	-0.01	0.02	-0.05

Table 1.A.7. Spearman rank correlation matrix of predictor variables used in the linear regression models. Correlations in bold indicate $p < 0.05$ with Bonferroni corrections applied.

	Mass (log)	Tarsus	Mites	Hematocrit
Tarsus	0.19			
Mites	-0.06	0.27		
Hematocrit	-0.12	-0.14	-0.13	
Day of Season	**-0.40**	0.24	**0.33**	-0.28

Table 1.A.8. Variable loadings of the first 3 principal components (PC) calculated from spottiness metrics of each sex separately. Text in bold indicates the most heavily loaded metrics for each corresponding PC ($r_s < -0.55$ or $r_s > 0.55$).

	PC 1		PC 2		PC 3	
Females	Loading	r_s	Loading	r_s	Loading	r_s
Minor axis	**0.41**	**0.97**	0.11	0.15	-0.07	-0.07
Area	**0.38**	**0.91**	0.21	0.28	-0.10	-0.10
Major axis	**0.37**	**0.89**	0.20	0.27	-0.22	-0.24
Perimeter	**0.37**	**0.87**	0.27	0.38	-0.15	-0.14
Aspect ratio	**-0.33**	**-0.77**	0.29	0.48	-0.24	-0.19
Roundness	**0.32**	**0.76**	-0.25	-0.41	0.31	0.27
Percent cover	**0.26**	**0.62**	**0.39**	**0.55**	0.17	0.24
Spot count	-0.22	-0.39	0.27	0.31	0.42	0.47
Solidity	0.18	0.42	**-0.47**	**-0.75**	-0.04	-0.10
Circularity	0.22	0.43	**-0.47**	**-0.80**	0.05	0.05
Angle	0.10	0.26	0.12	0.23	**0.74**	**0.75**
Variance explained	52%		24%		10%	
Males						
Minor axis	**0.41**	**0.98**	-0.11	0.16	0.03	0.02
Area	**0.39**	**0.92**	-0.20	0.31	0.11	0.10
Major axis	**0.37**	**0.85**	-0.22	0.36	0.21	0.19
Perimeter	**0.35**	**0.80**	-0.32	0.51	0.12	0.12
Aspect ratio	**-0.34**	**-0.81**	-0.24	0.31	0.24	0.23
Roundness	**0.33**	**0.77**	0.19	-0.28	-0.29	-0.26
Percent cover	0.19	0.41	**-0.49**	**0.80**	-0.25	-0.21
Spot count	-0.20	-0.44	**-0.37**	**0.60**	-0.46	-0.35
Solidity	0.24	0.54	0.35	-0.53	-0.20	-0.23
Circularity	0.25	0.59	**0.44**	**-0.65**	0.01	0.16
Angle	0.02	0.00	0.00	0.03	**0.69**	**0.76**
Variance explained	51%		24%		10%	

Table 1.A.9. Results of stepwise linear regression models for predicting the first and second principal components of plumage spottiness. The sexes were analyzed separately, and all variables were scaled in the models. For each PC model, every step is additive to the one immediately above it, indicated by (+) and the subsequent changes in ΔAICc, but the significance of each explanatory factor is analyzed independently from the others in the same model. *K* is the number of parameters in the model. Models listed in bold are those that fit the data best.

		Step	Estimate	SE	*t*	*p*		*K*	ΔAICc
Females	PC 1:	Full						7	8.12
(*n* = 32)		Null	0.13	0.36	0.36	0.76		2	8.76
		+ Mites	**-1.29**	**0.36**	**-3.55**	**0.001**	**	**3**	**0**[a]
	PC 2:	Full						7	5.64
		Null	-0.18	0.20	-0.87	0.39		2	12.85
		+ Mass	**1.05**	**0.26**	**3.95**	**<0.001**	***	3	10.95
		+ Hematocrit	**1.04**	**0.27**	**3.91**	**<0.001**	***	4	3.46
		+ Day	**0.53**	**0.22**	**2.47**	**0.02**	*	**5**	**0**[b]
Males	PC 1:	Full						7	8.62
(*n* = 55)		Null	0.37	0.31	1.20	0.24		2	2.74
		+ Mites	**-0.70**	**0.31**	**-2.24**	**0.03**	*	**3**	**0**[c]
	PC 2:	Full						7	4.92
		Null	**0.04**	**0.22**	**0.17**	**0.86**		**2**	**1.83**
		+ Tarsus	0.54	0.23	2.33	0.02	*	3	0.33
		+ Day	-0.37	0.23	-1.60	0.11		4	0[d]

Lowest AICc value for models: (a) 140.72, (b) 107.65, (c) 252.50, and (d) 215.09.

CHAPTER 2: PARENTAGE, SEX RATIO, AND REPRODUCTIVE SUCCESS IN THE POLYANDROUS SPOTTED SANDPIPER (*ACTITIS MACULARIUS*)

ABSTRACT

Polyandry may evolve when males invest a substantial amount of time and energy in parental care. Females are then able to invest time and energy in seeking additional mates and laying additional eggs. Accordingly, certainty of paternity by males is expected to be high in polyandrous systems, and females are expected to display sexually-selected ornaments. We investigated genetic parentage in spotted sandpipers (*Actitis macularius*), a sequentially polyandrous shorebird species, to determine rates of extra-pair paternity, intraspecific nest parasitism, and polyandry. We also examined plumage ornamentation as it relates to female and male reproductive success, offspring sex ratio, and nesting biology. Based on seven microsatellite loci, for every offspring in a clutch, the social father was the most probable genetic father. On two occasions, males abandoned clutches to have a second clutch with a different female, and in at least one of these cases, the female incubated and cared for the offspring. There were no confirmed cases of multiple genetic mothers per clutch, but due to unbanded breeding females in the population, we could not rule out the possibility of intraspecific nest parasitism. These results suggest that, despite the potential for mixed paternity within a clutch, adults in this population were reproductively monogamous with their current mate. Based on genetic and observational data from nest attempts, 18.2% - 33.3% of females were polyandrous. Females that completed a clutch had greater plumage cover than those that did not lay eggs or left the study site. Secondary sex ratio was unbiased, and there were no sex differences in egg mass, offspring mass, or day of the season hatched. However, female offspring were larger than males as

measured by tarsus length, even though breeding adults in the population did not exhibit this sexual dimorphism. This study suggests that, in a polyandrous system, extra-pair young are rare, female offspring may be costlier than males, and reversed sexually dimorphic plumage pattern correlates with reproductive success in females.

INTRODUCTION

The ultimate causes and proximate mechanisms favoring the evolution of polyandry are still debated (e.g., Jenni 1974; Emlen & Oring 1977; Oring 1982; Clutton-Brock 1991; Valle 1994; Ligon 1999; Eens & Pinxten 2000; Owens 2002; Goymann et al. 2015). However, male-biased care of offspring is proposed as the first evolutionary step in polyandry in birds (Andersson 2005), as this would allow females to increase investment in eggs, self-maintenance and repair, or time spent seeking additional mates. Since males provide all, or almost all, parental care in polyandrous species, cuckoldry rates are expected to be low (Trivers 1972; Whittingham et al. 1992). In other words, the forfeiture of a male's unrealized offspring with additional mates can be offset by increased certainty of paternity in the current offspring. Previous research suggests that rates of extra-pair paternity are, in fact, low in polyandrous birds: 0% in Wilson's phalaropes (*Phalaropus tricolor*; Delehanty et al. 1998), 1.7% in red-necked phalaropes (*Phalaropus lobatus*; Schamel et al. 2004), 2.8% in comb-crested jacanas (*Irediparra gallinacea*; Haig et al. 2003), 4.6% in Eurasian dotterel (*Charadrius morinellus*; Owens et al. 1995), 6.5% in red phalaropes (*Phalaropus fulicarius*; Dale et al. 1999), and 7.5% in wattled jacanas (*Jacana jacana*; Emlen et al. 1998), but increasing to 14.2% in black coucals (*Centropus grillii*; Muck et al. 2009). Overall, these rates of extra-pair paternity are notably lower than those of socially monogamous birds (Griffith et al. 2002), but clearly some extra-pair paternity is present in polyandrous mating systems.

In polyandrous species, the cost of caring for extra-pair young can be especially significant for secondary males. Unlike biparental systems in which a low certainty of paternity may result in reduced paternal care or nest abandonment (reviewed in Møller 2000), reduced paternal care in polyandrous species would not be compensated by increased maternal care, and thus any genetic offspring of the male may not survive. As such, males of polyandrous species may display specialized behaviors that increase their certainty of paternity. For example, males may selectively pair with females who have not previously laid nests that season (red-necked phalarope, *Phalaropus lobatus*; Whitfield 1990) or destroy the first several eggs in a clutch and then resume typical paternal care once the female has laid replacement eggs (bronze-winged jacanas, *Metopidius indicus* and pheasant-tailed jacanas, *Hydrophasianus chirurgus*; Butchart 1999; Chen et al. 2008). Spotted sandpipers are known to have extra-pair copulations, potentially as a method by which females attract new mates (Colwell & Oring 1989). In a paternity study of polyandrous spotted sandpipers using DNA fingerprinting, only one of 34 chicks was the result of extra-pair fertilization during one breeding season (Oring et al. 1992). However, when eggs were collected in the following season to experimentally increase re-nesting by females (i.e. mimicking high nest predation), extra-pair fertilization increased from 2.9% to 14.3%.

Since male reproductive success in polyandrous systems is generally limited by clutch size and female reproductive success is limited by number of mates (e.g., Oring et al. 1983, 1991), male mate choice and female-female competition are common, leading to the evolution of female-specific sexually-selected traits (Emlen & Oring 1977; Amundsen 2000). Little is known in regard to female plumage ornamentation and reproductive success in polyandrous species.

However, in spotted sandpipers, females are more ornamented than males in terms of spotted plumage pattern and, in females, increasing spot size and percent cover is associated with increasing health (i.e. decreased mite loads, greater body mass, and higher hematocrit levels; Blizard & Pruett-Jones 2017). In barn owls (*Tyto albus*), a socially monogamous species with substantial paternal care of offspring and female-biased plumage ornamentation, males reduce offspring provisioning rate when the ornamentation of their mate is reduced (Roulin 1999a,b).

In this research, we investigated genetic parentage in a wild population of spotted sandpipers over the course of three breeding seasons to determine rates of extra-pair paternity, intraspecific nest parasitism, differences between male and female offspring, and relationships between breeding plumage ornamentation and reproductive success. Based on theory (Andersson 2005) and previous genetic work of extra-pair offspring in polyandrous species (e.g., Oring et al. 1992; Owens et al. 1995; Delehanty et al. 1998; Emlen et al. 1998; Dale et al. 1999; Haig et al. 2003; Schamel et al. 2004), we expected that there would be a low rate of extra-pair paternity in spotted sandpipers. Furthermore, we predicted that any extra-pair offspring would be sired by previous mates, as female spotted sandpipers can likely store sperm (Oring et al. 1992). We also expected that more ornamented females would have greater reproductive success than less ornamented females.

Study system

Spotted sandpipers are territorial, sex-role reversed, and sequentially polyandrous (Fivizzani & Oring 1986; Oring & Lank 1986). Females arrive at the breeding grounds first, compete for territories to attract males, and are more likely to initiate copulation than males (Maxson & Oring 1980). Copulation rates peak one day prior to laying the first egg, and copulation continues for several days afterwards (Oring et al. 1994). Females can lay a second clutch of four eggs as few as eight days after completing a previous clutch (Emlen & Oring 1977; Maxson & Oring 1980; Lank et al. 1985). After a clutch is completed, females may help incubate the eggs for the first several days, but the male incubates the eggs until they hatch, approximately 21 days, and then cares for the precocial offspring for approximately 15 days. Based on observational data, female reproductive success increases with age and depends on number of clutches laid (i.e. number of mates), whereas male reproductive success depends on successfully raising a clutch of chicks (Oring et al. 1983, 1991).

METHODS

Study Site and Fieldwork

We carried out this research along 20 km of shoreline on the southern and southeastern side of Beaver Island, Michigan (45˚36'N, 85˚31'W) over the course of three consecutive breeding seasons from 2013 to 2015. The study area encompassed the territories of approximately 12 - 22 female spotted sandpipers, with a total of 45 - 50 adults (Table 2.1). Distance between nests varied from 20 m to 1,875 m, but in areas of the highest nest density across multiple female territories, at most three nests were within 20 m of each other. Further details on the study site are in Blizard & Pruett Jones (2017).

Each year, we captured adult spotted sandpipers using mist nests and nest traps. We banded each bird with a permanent metal band and a unique combination of three colored, plastic bands for identification in the field. We took morphological measurements after capture and collected blood samples from the brachial vein. All blood samples were stored on ice for no more than 3 hours before being stored in buffer solution (DNAeasy; Qiagen, Venlo, Netherlands) and refrigerated.

Interactions between adults (displays, copulations, agonistic interactions, etc.) were noted during fieldwork, as were territory boundaries, which were mapped with portable, handheld GPS units (eTrex Vista HCx, Garmin, Olathe, KS, USA). Nests were monitored every four to five days until hatched, depredated, or abandoned. After the offspring hatched, they were captured by hand, banded with a permanent metal band and a two-color band combination unique to the clutch, and a blood sample was collected. We continued to monitor the chicks until they were capable of sustained flight. In total, our banded adult population consisted of 32 females and 55 males, 24 of which were recaptured or observed across multiple breeding season (9 females, 15

Table 2.1. Number of breeding adult spotted sandpipers, nests, and chicks on the field site each year. In comparison, totals are included from the population breeding on Little Pelican Island (LPI), Minnesota between 1974 - 1981 (Maxson & Oring 1980).

		Beaver Island field site				LPI
		2013	2014	2015	Total	Total
Adults	Females[a]	12 - 16	14 - 19	20 - 22	~42	6 - 20[b]
	Males[a]	30	28	27 - 29	~55	12 - 32[b]
Nests	Total[c]	39	30	28	97	317
	Hatched	16 - 17	13 - 15	10 - 13	39 - 45 (40% - 46%)	140 (44%)
	Sampled	14	13	9	36 (37%)	-
	Fledged[d]	7	11	6	24 (25%)	99 (31%)
Chicks	Total[e]	45	39	30	114	-
	Sampled	41	38	28	107	-

[a] The number of adults captured, sightings of previously banded individuals, and observations of unbanded individuals.

[b] Typically 14 - 20 females (8 of the 9 years) and 21 - 24 males (7 of the 9 years)

[c] Total number of nests includes all nests, whether successful, depredated, abandoned, or renesting attempts.

[d] Number of nests fledged indicate the minimum number as we could not continue to observe clutches that hatched at the end of the breeding season.

[e] Number of offspring includes all observed chicks per breeding season.

males). Additionally, three spotted sandpipers born on the field site returned as breeding adults in subsequent years. See Table 2.1 for annual adult population estimates and number of offspring.

Genetic Analyses

DNA was extracted from the blood samples using standard procedures (DNAeasy; Qiagen, Venlo, Netherlands). The sex of all birds was determined by PCR amplification of the CHD gene using the P2 and P8 microsatellite primers (Griffiths et al. 1998). Behavioral observations of 11 females and 42 males in the field confirmed the results of the sexing PCRs.

We used previously developed sandpiper microsatellite markers from the ruff (*Philomachus pugnax*; Thuman et al. 2002) and pectoral sandpiper (*Calidris melanotos*; Carter & Kempenaers 2007) in our parentage analyses. Seven microsatellite primers (*Ruff 1*, *Ruff 6*, *Ruff 10*, *Cme 1*, *Cme 6*, *Cme 7*, and *Cme 9*) were ultimately chosen based on conformity to Hardy-Weinberg equilibrium and polymorphism in their respective species, as well as from preliminary tests of spotted sandpiper DNA amplification. We used 5'-fluorescent-labeled microsatellite primers (PET, 6-FAM, NED, and VIC; Applied Biosystems) and followed the specified thermocycler protocols and annealing temperatures (Thuman et al. 2002; Carter & Kempenaers 2007) using a PCR reaction mix of 19.75 µl water, 10 µl buffer, 4 µl $MgCl_2$, 1 µl dNTPs, 1.5 µl forward primer, 1.5 µl reverse primer, and 0.25 µl Taq per well (GoTaq G2 Flexi DNA Polymerase, Promega, Madison, WI, USA). A random subset of products, approximately 20%, were checked for DNA product using gel electrophoresis prior to fragment length analysis at the University of Chicago Comprehensive Cancer Center DNA Sequencing and Genotyping Facility (Chicago, Illinois, USA).

Determination of Genetic Parentage

We used Peak Scanner Software v1.0 (Applied Biosystems, Life Technologies Corporation) to genotype individuals. The observed fragment lengths and repeat motif size corresponded to those previously reported (Thuman et al. 2002; Carter & Kempenaers 2007). Allele lengths of each locus were then binned, considering all individuals across all years. We used *CERVUS* v3.0.7 (Marshall et al. 1998; Kalinowski et al. 2007) to calculate allele frequency in the population of prospective breeders (all adults captured or sighted), test for Hardy-Weinberg equilibrium, and determine frequency of null alleles. The data from each year were analyzed separately. We then determined average non-exclusion probability of the true parent pair on *CERVUS* using simulations of 100,000 offspring drawn from each year's adult genotype data set. Lastly, we ran the parentage analyses on *CERVUS* using the allele frequency output, simulation output, and genotype data. As females are not often sighted at a nest, we ran the analyses to search for the best offspring-mother-father trio without specifying any known mother or father for the chicks. All banded females sighted or captured in the breeding season were considered possible mothers to the offspring that year and we treated the males likewise. For each offspring, *CERVUS* assigned the best parent pair using the likelihood (LOD) score based on number of mismatched loci within potential offspring-mother-father trios, taking into account loci scoring errors.

CERVUS ranked probability of all possible parent pairs of each chick by the trio's LOD scores, which was the primary method by which we assigned parentage. However, in cases with low trio confidence (i.e. <80%) or more than three loci mismatches within a trio, we applied similar steps as Webster et al. (2004) to assign parentage: we considered the parents' individual number of mismatching alleles with the offspring, whether the mismatching loci were those with high null allele frequencies, and other biological limitations (i.e. timing of nest completion and observations of incubating parents). Given that mothers were not frequently observed at the nest, the number of inconclusive genetic mothers, and to account for any banded females that may have been present but not banded in prior years, we also ran parentage analyses on *CERVUS* including the genotypes of all adult females. After determining mating status of females as no mate, monogamous, or polyandrous using the genetic and observational data, we compared female reproductive investment relative to mating status using a Wilcoxon rank sum test as the data were not normal.

Offspring Sex Differences

To determine whether offspring sex ratio differed from expected (50% females, 50% males), we used χ^2 test across years. To examine whether there were sex differences in egg mass, we tested whether variance in egg mass (log mass) within a clutch decreased as offspring sex-bias increased using a Spearman rank correlation. We also tested whether overall mass of a clutch of eggs (log mass) increased with an increasing or a decreasing number of female offspring using Pearson correlations. For these two above-mentioned analyses, we only included complete clutches, i.e. those that had all four chicks sampled ($n = 14$). After normalizing the day of season across years by counting up from ‘day 1’ (i.e. the first day a spotted sandpiper was observed on the breeding grounds each year), we compared the hatch date of females and males using a Wilcoxon rank sum test as the data were not normal. We used a two-way ANOVA to compare offspring size (i.e. mass (log) and tarsus length) between the sexes and across clutches, with age as a covariate.

Plumage Ornamentation and Reproductive Success

We examined measures of the adults' spotted plumage pattern in our analysis of reproductive success. Specifically, we focused on percent of plumage covered in spots, as this variable was one of the best predictors of plumage pattern variation within and between the sexes (Blizard & Pruett-Jones 2017). Detailed descriptions of our methods in quantifying plumage pattern are presented in Blizard & Pruett-Jones (2017). To categorize female and male reproductive success, we used a measure of 'furthest reproductive stage reached' each season (i.e. in ascending order: no known nest attempt, nest, and offspring fledged) to account for migrants and multiple nest attempts.

Examining the sexes separately, we analyzed furthest reproductive stage reached in relation to percent plumage cover using a directional one-way ANOVA with year as a random effect (Horváth & Rice 2015). Our null hypothesis was that there would be no difference in percent cover across the furthest reproductive stage reached, while the alternative hypothesis was that percent cover would increase as reproductive stage increased. Using *t*-tests, we also considered several binary levels of reproductive success: presence or absence of a nest, whether or not the nest(s) hatched, and whether or not the offspring fledged in relation to female and male percent cover. We also tested whether percent plumage cover correlated with the date of season that the first nest hatched or the proportion of female offspring of that first clutch. For the later tests, we only considered completely or nearly completely sampled clutches (i.e. 3 or 4 offspring out of a total of 4 offspring; 13 adult females and 14 adult males). Lastly, we tested whether the percent cover of mates was correlated, and if this correlation differed between successful and unsuccessful broods, which would suggest mutual mate choice in this species.

Other than the genetic parentage analyses run on *CERVUS*, we used R 3.3.1 (R Development Core Team 2015) for all statistical analyses.

RESULTS

Each year we studied 16 - 22 territorial females and 28 - 30 males, and we found between 28 - 39 active nests (Table 2.1). We sampled a total of 87 adults (32 females, 55 males) and 107 chicks from 36 hatched clutches. Our annual population size was comparable to the Little Pelican Island population of spotted sandpipers, although our population had a lower percentage of clutches with offspring that fledged (Table 2.1; Oring et al. 1983). Arrival of breeding adults (beginning on May 2nd - 6th) coincided with similar dates as the Little Pelican Island population (typically May 10th), which is 1˚31' to the north (Oring & Lank 1982). The adults breeding on our field site were lower in density, spread along ~20 km of shoreline compared to <1 km of shoreline on Little Pelican Island (1.6 ha). Additionally, Maxson and Oring (1978) trapped and removed mammalian nest and chick predators (rodents: *Microtus pennsylvanicus*, *Peromyscus maniculatus*, *Clethrionomys gapperi* and mink: *Mustela vison*) from Little Pelican Island, which increased spotted sandpiper reproductive success. Overall, 34.1% of banded females returned to the field site the next year (range: 26.3% - 40.9%) and 52.2% of males returned (range: 35.7% - 44.8%), which is lower than average Little Pelican Island return rates (females: 54.8%, ranging from 42.1% - 66.7% (excluding 1 year of 9 with 6 of 6 females returning); males: 52.2%, ranging from 38.1% - 76.2%; Oring et al. 1983).

Parentage Analyses

With the exception of *Cme 1* and *Cme 7*, the microsatellites had high heterozygosity

Table 2.2. Summary of 2013 - 2015 adult spotted sandpiper allele frequency analysis determined from *CERVUS*. Deviations from Hardy-Weinberg equilibrium were calculated using χ^2 goodness-of-fit test with Yates' correction when df = 1 and a minimum expected frequency of an allele of 1. For each locus, n = 87, except n = 86 for *Ruff 6*. Abbreviations: k = number of alleles, H_{obs} = observed heterozygosity, H_{exp} = expected heterozygosity, PIC = polymorphic information content (Botstein et al. 1980), F_{null} = null allele frequency, ND = Hardy-Weinberg analysis not done, * $p < 0.001$ with Bonferroni corrections applied. Microsatellite primers for *Cme 1*, *6*, *7*, and *9* are from Carter and Kempenaers (2007) and *Ruff 1*, *6*, and *10* are from Thuman et al. (2002).

						Non-exclusion probability			
Locus	Allele size (bp)	k	H_{obs}	H_{exp}	PIC	1st parent	2nd parent	Parent pair	F_{null}
Cme 1	66 - 78	7	0.678	0.740	0.704	0.654	0.471	0.274	0.036
Cme 6	188 - 214	14	0.805	0.899	0.884	0.354	0.214	0.072	0.054
Cme 7	66 - 78	6	0.414*	0.683	0.614	0.755	0.599	0.437	0.252
Cme 9	123 - 163	18	0.782	0.861	0.842	0.437	0.278	0.110	0.044
Ruff 1	182 - 242	15	0.828	0.888	0.872	0.381	0.234	0.084	0.035
Ruff 6	122 - 222	19	0.942	0.899	0.885	0.352	0.213	0.071	-0.028
Ruff 10	270 - 382	23	0.609^{ND}	0.950	0.941	0.204	0.113	0.022	0.215
Combined						$2.09\text{x}10^{-3}$	$9.51\text{x}10^{-5}$	$1.30\text{x}10^{-7}$	

with up to 23 alleles (Table 2.2). We had very low rates of non-exclusion probabilities of the parent pair. Loci mismatches within offspring-mother-father trios were likely due to mislabeling alleles, mutations, or problems arising from null alleles, especially those at *Cme 7*, which had significantly lower observed heterozygosity than expected and corresponding null allele frequencies greater than 0.05 (Table 2.2). *CERVUS* was unable to test *Ruff 10*, another locus with greater than 0.05 null allele frequency, for Hardy-Weinberg equilibrium as the program cannot carry out the test on any locus for which $N \times q^2 < 1$ (where N = number of genotypes, q = the frequency of the rarest allele, and 1 = minimum expected frequency used). Heterozygosity of the remaining loci did not differ significantly from that expected under Hardy-Weinberg equilibrium.

Of the total 107 offspring, parent pairs were matched to 60 offspring (56.07%) with 95% confidence, 28 (26.17%) with 80% confidence, 19 (17.76%) with <80% confidence. These latter offspring with <80% confidence were assigned parentage as discussed above in the methods section. In cases of parents assigned by *CERVUS* with 80% - 95% certainty, the number of loci mismatches within the offspring-mother-father trio were typically 0 to 1 (44 offspring: 53.0%) or 2 (27 offspring: 32.5%). Given our result (below) that social fathers were the same as the genetic fathers, we re-ran *CERVUS* with the fathers assigned to determine maternity.

Paternity

For all offspring, the genetic father matched the observed social father. In other words, for every clutch, the eggs laid in the nest were sired by the male incubating the eggs and caring for the offspring. Even in cases when a male was known to be the second mate of a female whose previous nest hatched ($n \geq 5$), no males were cuckolded. Furthermore, as no paternity analysis had inconclusive results, we determined that there were no unbanded males in the population that had offspring. In 2013, 13 males (48.5% of males) incubated clutches that hatched, 13 males (52.0%) in 2014, and 9 males (36.0%) in 2015. Because the social father was the genetic father for every nest, we expanded analysis to include observational data as well: in 2013, 18 males (66.7% of males) incubated clutches that hatched, 14 males (56.0%) in 2014, and 10 males (40.0%) in 2015. Nests failed due to predation (e.g., raccoons, *Procyon lotor*; blue jays, *Cyanocitta cristata*) or were abandoned, possibly due to death of the male or high human or predator disturbance in the area. Over the three years combined, 0.59 ± 0.065 SE clutches hatched. Based on the genetic results and observational data, one captured male (3.7%) in 2013, two males (8.0%) in 2014, and eight (32.0%) in 2015 did not have nests or were never seen at a nest.

Males were not fully faithful to their mate's nest. In 2013, one male abandoned his offspring and had another nest with a second female after the offspring of the first nest hatched. Additionally, in 2015, two males abandoned their first nest before the eggs hatched to incubate a second clutch with a new mate. One of these abandoned nests was incubated and the offspring later cared for by the male's first mate. The other nest was likely abandoned due to human disturbance. In the second case, the male's mate was unavailable to re-nest as she was relaying a clutch for her first male, whose clutch had been depredated.

Maternity

Although we had no definitive case of intraspecific nest parasitism (i.e. a clutch with multiple genetic mothers), we could not rule out the possibility due to unbanded and unknown females that laid clutches. There was one clutch in which maternity may have been due to two females; the offspring all shared the same genetic father, and maternity results indicate that either intraspecific parasitism occurred or the genetic mother was unbanded but related to a banded female in the population. In our analyses, this clutch was assigned to the latter, more conservative, scenario. Based on chicks with inconclusive maternal results, there were at least five unbanded or unrecorded females who laid clutches that hatched in 2013, four in 2014, and one in 2015.

We defined polyandrous females as those which had more than one mate per breeding season. Based on the maternity data, there were two polyandrous females in 2013 (13.3% of breeding females), one in 2014 (5.6%), and two in 2015 (9.1%). Each of these females laid two clutches, each cared for by a different male. However, these data on polyandry were determined from sampled chicks from clutches that hatched, so it underestimates the true number of breeding females in the population and likely the rate of polyandry as well. Including behavioral observations of nests and copulations, there were a minimum of five polyandrous females in 2013 (33.3% of females), five in 2014 (27.8%), and four in 2015 (18.2%). In our population, females had at most three mates per year, averaging 1.25 mates in 2013, 1.11 in 2014, and 0.81 in 2015. There was no difference in average egg mass between polyandrous females (n = 14) and monogamous females (n = 19; W = 93, p = 0.73).

Females displayed no consistency in mate choice across years. Every female we established as a genetic mother across multiple years mated with a different male(s) each breeding season, even when previous or future mates were present (n = 5 females with two to four mates across years, n = 11 nests). Observational data including nest attempts support these results (n = 6 females with two to five mates across years, n = 18 nests).

Offspring Sex Differences

In 2013, 21 of the 41 sampled offspring were male (51.22%), in 2014, 20 of 38 offspring were male (52.63%), and in 2015, 16 of 28 offspring were male (57.14%). Sex ratio was not significantly different than expected across years (χ^2_2 = 0.24, p = 0.89) or with all years combined (χ^2 = 0.46, df = 1, p = 0.50). There was no significant correlation between the sex bias in a clutch and its variance in egg mass (S = 284.42, r_S = 0.37, p = 0.09), nor was there any correlation between total clutch mass and number of female or male offspring (t = -0.40, df = 12, r = -0.12, p = 0.69). Female offspring were not born any earlier or later in the breeding season than were males (W = 1493.5, p = 0.67). Variation in offspring mass (log) was significantly

Table 2.3. Analysis of variance of spotted sandpiper offspring mass and size (tarsus length) between the sexes and across the clutches, with offspring age as a random effect. * $p < 0.001$

		df	Sum Sq	Mean Sq	*F*	*p*	
log(Mass (g))	Sex	1	0.000	<0.001	0.003	0.958	
(*n* = 107)	Clutch	35	1.264	0.036	4.769	<0.001	*
	Residuals	69	0.523	0.008			
Tarsus (mm)	Sex	1	4.960	4.965	12.94	<0.001	*
(*n* = 106)	Clutch	35	55.590	1.588	4.140	<0.001	*
	Residuals	68	26.090	0.384			

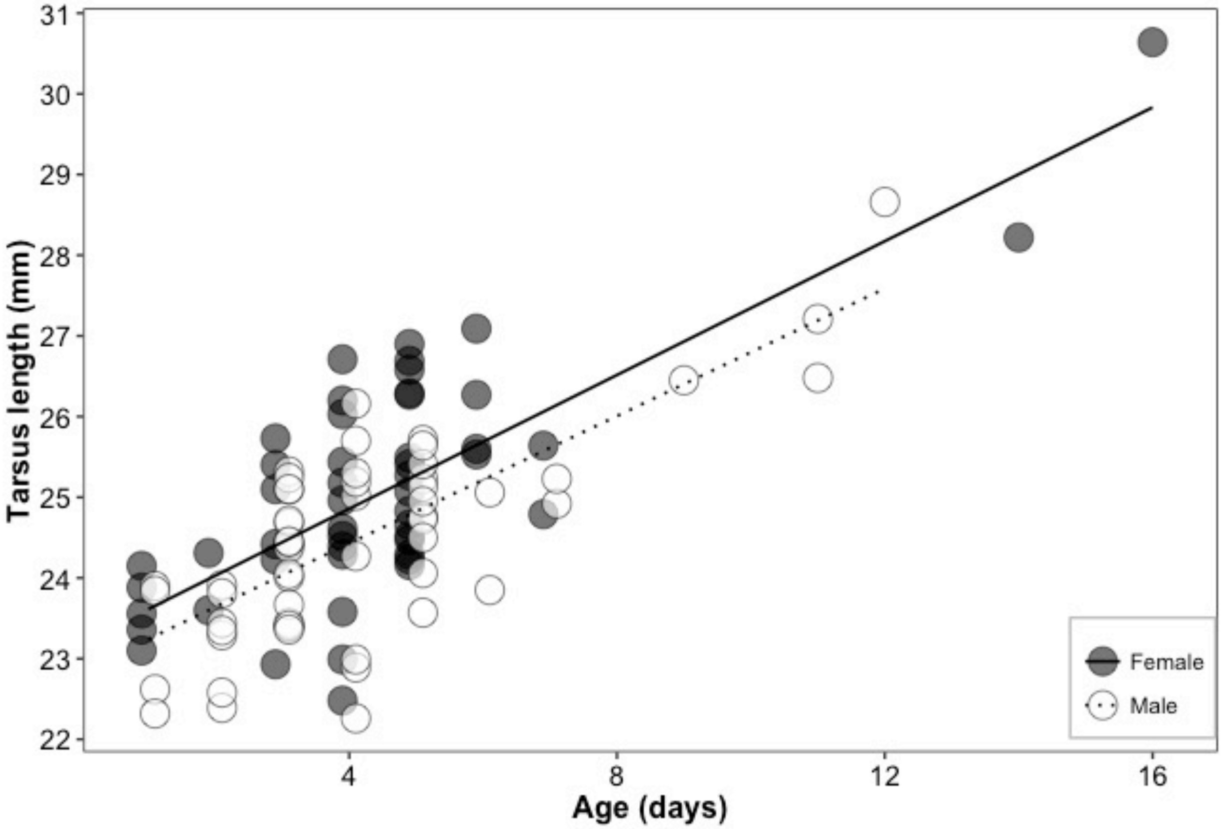

Figure 2.1. Offspring size, measured by tarsus length (mm), as a function of age. Lines indicate linear regressions, calculated separately by sex. Females: gray circles/solid line, males: open circles/dotted line.

explained by differences across clutches, but not between the sexes (Table 2.3). Variation in offspring tarsus length was significantly explained by clutch and sex differences, with females having longer tarsi than males (Figure 2.1).

Reproductive success

Spotted plumage cover variation in females was significantly explained by variation in furthest reproductive stage reached, specifically matching our prediction of a positive relationship (Table 2.4, Figure 2.2). There was no such relationship across male reproductive stage and percent cover. In particular, females that laid clutches had greater percent cover than did females with no nests ($t = 2.15$, df = 32, $p = 0.04$), but males incubating nests did not have greater percent cover than those without a nest ($t = 0.23$, df = 71, $p = 0.82$). There was no significance in regard to percent cover in females or males for whether the nest hatched or not (females: $t = 1.29$, df = 33, $p = 0.21$; males: $t = 0.29$, df = 70, $p = 0.77$) or for whether the offspring fledged or not (females: $t = 1.02$, df = 30, $p = 0.31$; males: $t = -0.45$, df = 60, $p = 0.65$). There was no significant correlation in females (two-sided Spearman rank correlation: $S = 495.41$, $p = 0.12$, $r_S = 0.39$) or males (two-sided Spearman rank correlation: $S = 5515.9$, $p = 0.67$,

Table 2.4. Directional analysis of variance of female and male spotted sandpiper plumage cover in relation to furthest reproductive stage reached, with year included as a random effect. Asterisks indicate significance at adjusted directional *p* values (* $p < 0.01$).

		df	Sum Sq	Mean Sq	*F*	*p*	adjusted *p*	
Females	Reproductive Stage	2	685.40	342.70	3.70	0.04	0.01	*
(*n* = 35)	Residuals	30	2782.00	92.70				
Males	Reproductive Stage	2	14.00	7.19	0.13	0.88	0.15	
(*n* = 73)	Residuals	68	3731.00	54.86				

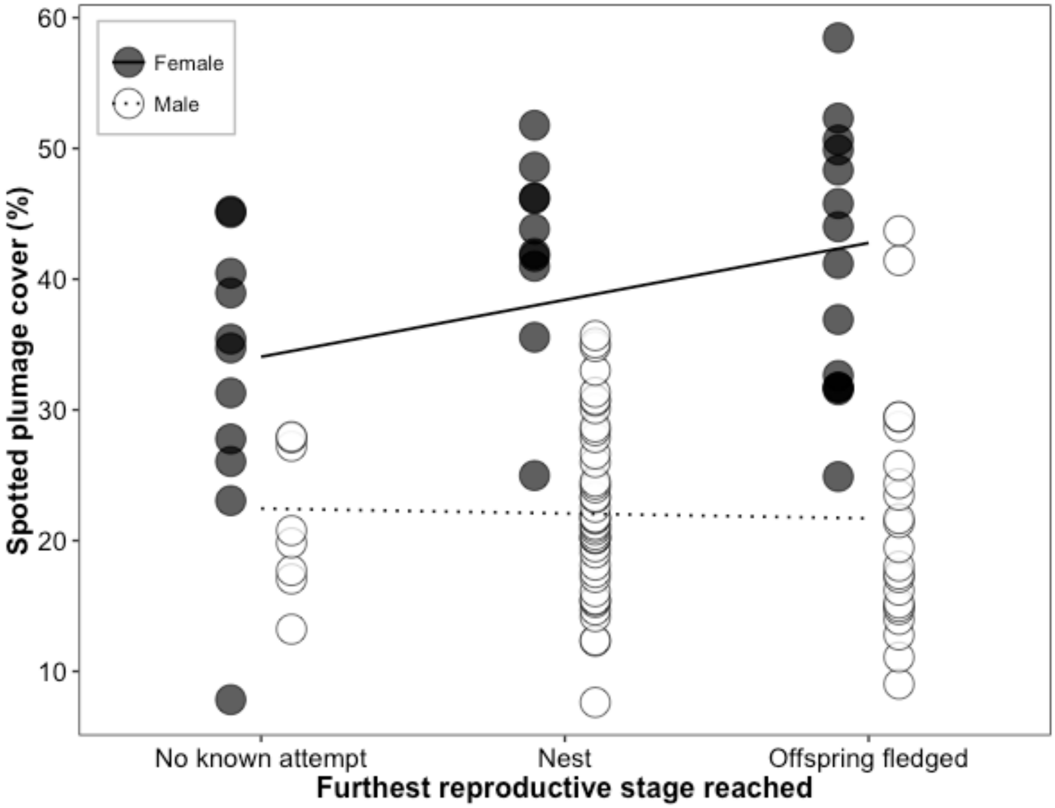

Figure 2.2. Furthest reproductive stage reached of adult spotted sandpipers and plumage ornamentation, in terms of percent cover of spotted plumage, during the corresponding breeding season. Lines indicate linear regressions. Females: gray circles/solid line, males: open circles/dotted line.

$r_S = 0.08$) with percent plumage cover and the day of season that the first nest hatched. There was also no significant relationship between percent plumage cover and proportion of female offspring for females (two-sided Pearson correlation df = 11, $p = 0.82$, $r = 0.07$) or males (two-sided Spearman rank correlation: $S = 2177.3$, $p = 0.80$, $r_S = 0.05$). Although we predicted that the plumage patterns of females and males in mated pairs would correlate with each other (i.e. more spotted females would choose more spotted males as mates), there was no correlation within pairs (one-sided Pearson correlation: df = 19, $p = 0.51$, $r = -0.01$) or within pairs that had offspring fledge successfully (one-sided Pearson correlation: df = 12, $p = 0.28$, $r = 0.17$).

DISCUSSION

We found no evidence of multiple females laying eggs in the same clutch, but could not conclusively rule out intraspecific nest parasitism. Over the course of three breeding seasons, no clutches contained extra-pair young, which supports the theory of polyandry evolving in systems with high certainty of paternity (Trivers 1972; Whittingham et al. 1992). Additionally, the absence of cuckoldry was similar to another polyandrous bird, Wilson's phalaropes (Delehanty et al. 1998). Since we did not manipulate clutch loss to maintain high numbers of sexually receptive adults (i.e. increase the likelihood of extra-pair copulations), our data reflect normal levels of paternity certainty in this population. Oring et al. (1992) suggest that female spotted sandpipers can store sperm for up to 31 days. Even at a low population density, in which search times for eligible mates may be high, females in our population laid successful nests just 10 to 19 days after previously completing a nest, well within the estimates of sperm storage. This genetic monogamy on the females' side may be the results of frequent copulations with the current mate as observed in the Little Pelican Island, Minnesota spotted sandpiper population (Oring et al. 1994) and perhaps also due to the chance of nest abandonment by the male. Although we were generally unable to determine the cause of nest failure, our data suggest that there are a small number of males who abandon nests, mate with another female, and care for the resulting offspring. In one case, the first female undertook all nest incubation and care of the offspring after being abandoned, and as such, she had no opportunity to seek and court additional mates. Interestingly enough, this female was an experienced breeder who previously laid nests in 2013 and 2014.

Across the three breeding seasons, rates of polyandrous females varied from 5.6% - 13.3%, but the number of polyandrous females generated from the genetic analyses possibly underestimates the total number of polyandrous females. Observational data of nest attempts, combined with genetic information, indicate the percentage of polyandrous females is closer to 18.2% - 33.3%. A female's average number of mates, 0.81 - 1.25 per season, was lower than in the Little Pelican Island population, averaging 1.35 - 2.06 per season (Oring et al. 1983). Since polyandrous females invested equally in egg mass as monogamous females, females do not appear to be limited in terms reproductive effort based on the lower number of mates per season ascertained in our population. As the percentage of nests hatched in our population is similar to the Little Pelican Island population (Maxson & Oring 1980), the lower number of mates per season is likely not driven by higher re-nesting attempts following nest predation or different arrival times of breeding adults, but rather our lower population density. We were able to determine that several nests had mothers who were neither banded nor sampled during present or subsequent breeding seasons, and we could not rule out the possibility of intraspecific nest parasitism in one clutch.

The sex ratio of spotted sandpiper offspring indicates equivalent numbers of females and males at hatching. Similar to the lack of relationship in spotted sandpiper offspring sex ratio and nest completion date discovered by Andersson et al. (2003), female offspring in our population did not hatch at different times during the season than male offspring did. Interestingly, in the sister species of spotted sandpipers, the socially monogamous common sandpiper (*A. hypoleucos*), the sex ratio of nests at the beginning of the season are male-biased and nests at the end of the season are female-biased (Andersson et al. 2003). In our population, offspring tarsus length varied significantly between the sexes, with females having longer legs. Since there is no statistically significant dimorphism in tarsus length of adult spotted sandpipers (Pyle 2008; ad hoc analysis: 32 females and 55 males: $W = 957.5$, $p = 0.25$), this implies that females may reach a similar adult body size more quickly than males and female offspring may be costlier than males. Although egg and chick mass did not differ between the sexes, longer legs may indicate that females deposit more calcium in these eggs.

As adults, females are more ornamented than males in terms of spotted plumage cover, and increased ornamentation in females is associated with lower mite load, higher hematocrit levels, and greater body mass (Blizard & Pruett-Jones 2017). Examining reproductive success in this study, females with greater plumage cover reached further reproductive stages than those with less percent cover, a relationship not observed across males. In particular, females with greater percent cover were more likely to lay eggs than those with less percent cover. Some of these latter females with lesser plumage cover may have been migrants passing through at the beginning of the season, which suggests that the plumage pattern may differ across populations. The connection between female reproductive success and a reversed sexually dimorphic plumage ornament, coupled with the correlation with mites, mass, and hematocrit levels (Blizard & Pruett-Jones 2017), strongly suggests that plumage pattern is under sexual selection and is an honest signal.

This study provides additional evidence that in a polyandrous species, extra-pair young are rare, even when females mate with multiple males and can store sperm. Our results indicate that females and males in this population were reproductively monogamous with their current mate. Furthermore, in this sex-role reversed system, female offspring are potentially as costly as males or costlier, as measured by size and as suggested by the lack of change in offspring sex ratio across the breeding season. In addition, reproductive success across females, but not males, is related to increased ornamentation of a reversed sexually dimorphic plumage pattern, supporting the likelihood that the plumage pattern is an honest signal of female quality.

CHAPTER 3: TESTOSTERONE, REPRODUCTIVE SUCCESS, AND MELANIZED PLUMAGE ORNAMENTATION IN A POLYANDROUS SHOREBIRD (*ACTITIS MACULARIUS*)

ABSTRACT

Despite abundant evidence of testosterone's function in male aggression and sexually-selected traits, the role of this steroid hormone in females is unclear. To examine the role of testosterone in females, I investigated spotted sandpipers (*Actitis macularius*), an avian species exhibiting sex-role reversal and a polyandrous mating system. Female spotted sandpipers are territorial, more aggressive than males, experience greater competition for mates than males, and can have multiple mates in one season. Accordingly, I expected that female plasma testosterone levels would remain high when defending territories during the beginning of the breeding season and acquiring mates, which may occur several times throughout the season. I also expected that testosterone in adults, particularly females, would correlate with reproductive success and percent cover of melanized plumage ornamentation. Females had significantly less testosterone (log) than males, but only during the courtship stage, after which the sexes had comparable levels. While female levels remained similar from courtship to the beginning of egg laying and incubation, male levels dropped. These patterns are likely due to female competition for additional mates throughout the season and male-biased parental care of eggs and offspring. Females in the egg laying/early incubation stage which were captured following a simulated territorial intrusion had higher testosterone levels than those captured on the nest. This elevation in female testosterone, as well as the maintenance of testosterone levels from courtship through incubation suggest that females may use testosterone to mediate aggressive behavior during

competitive interactions. Both male and female plumage ornamentation could be explained by testosterone, but males exhibited more consistent positive correlations at reproductive stages when aggressive behaviors may be more relevant. Reproductive success did not correlate with testosterone levels in females or males.

INTRODUCTION

Across vertebrates, testosterone is often associated with aggressive behavior, sexually-selected display traits, and increased reproductive success in males (e.g., Raouf et al. 1997; De Ridder et al. 2000; Sinervo et al. 2000; McGlothlin et al. 2008; Setchell et al. 2008; Malo et al. 2009; Laucht et al. 2010). Females produce testosterone as well, but its influence on female behavior is less clear (e.g., French et al. 2013; Rosvall 2013b). In this study, I investigated whether there are similar relationships among testosterone, sexual signals, and reproductive success in females that experience strong intrasexual competition for territories and mates. I focused on spotted sandpipers (*Actitis macularius*), a shorebird species exhibiting sex-role reversal and a polyandrous mating system, to determine 1) whether there are temporal patterns in male and female testosterone levels across the breeding season that are unique to polyandrous systems, 2) whether competitive interactions increase circulating testosterone levels in females, and 3) whether female testosterone levels correlate positively with reproductive success and melanized plumage ornamentation.

In males, during the breeding season, increasing testosterone levels increase courtship behavior and male-male competition for territories and mates (Fusani 2008). Decreasing testosterone levels allow for the expression of paternal behaviors such as incubation, brooding, and feeding offspring, modulated by other hormones (Wingfield et al. 1990). For seasonal breeders, testosterone usually peaks during the start of the breeding season, which reduces the negative effects that high levels of testosterone have on the body at times when testosterone-mediated behaviors are not necessary (i.e. the challenge hypothesis; Wingfield et al. 1990). There is also a positive correlation between testosterone and melanin-based plumage in males (Jawor & Breitwisch 2003). This correlation is stronger in more sexually dimorphic Chardriifomes and Corvida, taxa in which plumage development is thought to rely upon androgens (Bókony et al. 2008). Melanin is one of the most common pigments found in the skin and feathers of birds (McGraw 2006), and there is evidence that melanized ornaments are honest signals (reviewed in Jawor & Breitwisch 2003). Thus, as testosterone increases, we can expect size of melanin-based ornaments to increase as well, especially in species with high sexual dimorphism (i.e. higher degree of reproductive skew). Even though the proximate mechanisms linking testosterone to melanization are not known, melanocytes, the cells that add melanin to feathers and skin, have receptors for two other steroid hormones: estrogen and progesterone (Hearing 1998).

Female testosterone data have conventionally been used as a control for organizational effects of testosterone to which males are compared, and studies exploring the relationship between aggression and testosterone in females have been inconclusive (Eens & Pinxten 2000). With experimentally increased testosterone levels, females may display more male-like behaviors or morphology (e.g., Nottebohm 1980; Wingfield 1984; Lank et al. 1999; Sandell 2007) or the effects within a species may be contradictory (e.g., European robins, *Erithacus rubecula*; Kriner & Schwabl 1991). There are also species in which aggressive behavior in females is associated with decreased testosterone (song sparrows, *Melospiza melodia*; Elekonich

& Wingfield 2000) or no change in testosterone (European stonechat, *Saxicola torquata rubicola*; Canoine & Gwinner 2005).

In species with both male and female territorial defense, females may exhibit an increase in testosterone at the start of the breeding season similar to that observed in males, but to a lesser extent (e.g., Schoech et al. 1991; Ketterson et al. 2005). This peak in female testosterone levels correlates with female aggressive behaviors (e.g., Searcy 1988; Rosvall 2013a). Across species, these results imply that female testosterone levels should be higher in species in which females help their mates defend territories or directly compete amongst one another for limited resources, such as nesting sites, territories, or paternal care of offspring. Indeed, females of socially monogamous species tend to have higher testosterone levels than females of polygynous species (Ketterson et al. 2005; Steiger et al. 2006; Rosvall 2008).

Few studies have addressed how testosterone levels during courtship relate to female reproductive success. In dark-eyed juncos (*Junco hyemalis*), female testosterone levels positively correlate with reproductive success: larger females produce more testosterone, are more aggressive, and have greater reproductive success than smaller females (Cain & Ketterson 2012). While there may be reproductive benefits of testosterone in aggressive interactions, high concentrations can also be harmful. Exogenously elevating testosterone levels in females after courtship, but remaining within physiologically relevant limits, can result in negative effects on female reproductive success, including reduced numbers of offspring (Veiga & Polo 2008; Rosvall 2013a; De Jong et al. 2016). Therefore, it is important to determine how testosterone's role may differ at different reproductive stages.

For species in which the 'sex-roles' are reversed (i.e. females experience greater competition for mates than males do and males generally care for offspring), one might expect females to have greater testosterone than males, particularly in polyandrous species in which females pair with several males during a single breeding season (Andersson 2005). Nevertheless, this expected reversal between the sexes in testosterone levels has not been demonstrated (Fivizzani et al. 1986; Fivizzani & Oring 1986; Gratto-Trevor et al. 1990; Ball 1991; Eens & Pinxten 2000; Goymann & Wingfield 2004). As in typical sex-role species, testosterone levels in sex-role reversed species are lower in females than males, although male testosterone levels may drop more closely to female levels, especially once incubation begins. In common moorhens (*Gallinule chloropus*), females who win intrasexual competitions have greater testosterone levels than losing females (Petrie 1983). In another sex-role reversed species (black coucals, *Centropus grillii*), it is progesterone, a metabolic precursor of testosterone, that seems to modulate aggressive interactions (Goymann & Wingfield 2004).

Considering female ornamentation, one would expect the evolution of female-specific ornaments in sex-role reversed species (Emlen & Oring 1977; Amundsen 2000). In barred buttonquails (*Turnix suscitator*), a polyandrous species in which females defend territories aggressively against one another, female testosterone levels correlate with body condition and the size and blackness of female-specific melanized ornaments in a captive study (Muck & Goymann 2011). In another sex-role reversed species (Eurasian dotterel, *Charadrius morinellus*), females who are more ornamented based on percentage of melanized plumage, initiated and won more female-female fights than dull females (Owens et al. 1994).

To elucidate testosterone's function in females, I studied spotted sandpipers over three breeding seasons to determine temporal patterns in testosterone levels and whether testosterone in females increases following simulated territorial intrusions and correlates with reproductive success and plumage ornamentation. In particular, I expected male testosterone levels to drop significantly after courtship, but remain higher than female levels, to allow for paternal incubating and brooding behaviors, while I expected female testosterone levels to be higher than those of monogamous species and drop only slightly after courtship. If testosterone does play a role in regulating female aggressive behaviors, I predicted that females exposed to a simulated territorial intrusion would have higher levels of circulating testosterone than females captured at the same reproductive stage without a stimulus and that polyandrous females would have higher testosterone levels than monogamous females. Further, I expected that female, and potentially male, testosterone levels would correlate positively with reproductive success and amount of melanized plumage ornamentation.

Study System

Female spotted sandpipers arrive on the breeding grounds before males and compete for both territories and mates (Maxson & Oring 1980). Although males typically pair with one mate in a breeding season, females pair with up to 5 males in a breeding season, averaging 1.2 - 1.9 mates. Consequently, male reproductive success depends on number of offspring that survive from his clutch of four eggs, but female reproductive success depends on the number of clutches laid (i.e. number of mates; Oring et al. 1983, 1991; Chapter 2). While claiming territories and mates, females spend significantly more time in aggressive interactions (i.e. chasing and fighting) than males. As in other sex-role reversed species, male spotted sandpipers have higher testosterone levels than females (Rissman & Wingfield 1984; Fivizzani & Oring 1986). However, previous research by Fivizzani & Oring (1986) has shown that females with mates have almost seven times more testosterone than those without, a result not observed in typical sex-role species. In males, once incubation starts, testosterone decreases from levels six times higher than females to levels similar to those measured in females during incubation, but in females, testosterone remains relatively constant throughout the breeding season (Fivizzani & Oring 1986; Oring et al. 1989).

Body length measurements are similar between females and males (Pyle 2008), but females are approximately 20% - 25% heavier than males (Maxson & Oring 1980). During the winter, both sexes have white chests and abdomens, but molt into white feathers with dark brown spots for the breeding season. Although the sexes overlap in terms of spots size and shape, females have larger spots which cover a greater percentage of their plumage than males (Blizard & Pruett-Jones 2017). During intrasexual competition and courtship, the birds display this spotted plumage to conspecifics (Maxson & Oring 1980). Prior research suggests that this melanin-based pattern is an honest signal in females: increased ornamentation in females correlates with decreased ectoparasite load, increased mass, and increased hematocrit levels (Blizard & Pruett-Jones 2017). Furthermore, females who are more ornamented are more likely to lay eggs (Chapter 2).

METHODS

Fieldwork

I studied a population of spotted sandpipers on the southern and southeastern coast of Beaver Island, Michigan (45˚36’N, 85˚31’W) over three breeding seasons (2013 - 2015). This 20 km stretch of shoreline included the breeding territories of 12 - 22 female spotted sandpipers each year and 27 - 30 males (Blizard & Pruett-Jones (2017) give more details of the population and the study site). From May through the end of July, I used mist nets and nest traps to capture adults. All adults were banded with a permanent numbered metal band and a unique color combination of three plastic bands so that individuals could be identified within and across seasons. I captured 87 adults in total (32 females, 55 males), 16 of which were recaptured across multiple breeding seasons (3 females, 13 males).

I took a blood sample from the brachial vein (Owen 2011) to determine genetic reproductive success, quantify plasma hormone levels, and verify sex (see below). I also took morphological measurements (i.e. weight and tarsus length) and digital photographs of the ventral plumage pattern (for further details on plumage pattern analysis, see Blizard & Pruett-Jones 2017). Blood samples were stored on ice for no more than three h, at which time I centrifuged the samples to separate the red blood cells from plasma and used a Hamilton syringe to draw the plasma out of the microcapillary tubes. The plasma was immediately frozen, and the red blood was stored in a buffer solution (DNAeasy, Qiagen, Venlo, Netherlands) and refrigerated.

Nests were monitored until the eggs hatched, were abandoned, or were depredated. I determined the date of the first egg and nest completion date using observational data and backtracking from the hatch date (Maxson & Oring 1980; Lank et al. 1985). I categorized reproductive stages of adults as the following: courtship, egg laying/incubation, caring for offspring, and transitional. Adults captured at the beginning of the season, but prior to the presence of nests on their territory, were considered to be in the 'courtship' stage. Adults captured on the nest were considered to 'incubating,' and females in the process of laying eggs were also considered in this category. Any adults captured with hatched chicks were 'caring for offspring,' but this category only included males as it is very unusual for females to remain with the nest or exhibit parental behaviors other than incubating the eggs for a few days (Maxson &

Oring 1980; Lank et al. 1985). Adults captured toward the end of the season, when they leave their territories and no longer respond to territorial intrusions, were considered ‘transitional’.

All birds captured in the courtship stage and approximately half of the females in the egg laying/incubation stage were captured immediately following a simulated territorial intrusion, which included both a recorded call of an unfamiliar adult and a mounted wooden model of a spotted sandpiper. To capture birds with playback, I used a randomly selected call from a selection of three files, non-repeating across years (Macaulay Library, The Cornell Lab of Ornithology, University of Cornell, Ithaca, NY, USA). I repeated the call for 10-min intervals from a MP3 player (iPod Nano, Apple, Cupertino, CA, USA) and speaker (Ecoxgear Eco Extreme, Grace Digital, Inc., San Diego, CA, USA), with 5 minutes of silence between intervals, for a total of no more than 60 minutes. The mount was the same size as a female spotted sandpiper in terms of leg, wing, and body lengths, and I painted it to resemble a breeding adult Figure 3.1). Birds were captured in mist nets placed in the vicinity of the speaker and mount. To

Figure 3.1. Mounted wooden model of a spotted sandpiper used during simulated territorial intrusions prior to capture. Scale bar centered on the bottom of the image represents 1 cm.

capture birds without the use of a stimulus (i.e. playbacks and mounts), I used mist nests placed in the natural flight paths of adults or a nest trap if the bird was incubating eggs.

I captured chicks 3 - 5 days after hatching to band with a permanent numbered metal band and a clutch-specific two-color combination of plastic bands. As with the adults, I took morphological measurements and a small blood sample (5 - 15 μL) from the brachial vein to resolve sex and genetic parentage. Chicks were then monitored to determine clutch success, which was defined as successful fledging of at least one chick (i.e. capable of sustained flight). In total, I captured 107 chicks (41 in 2013, 38 in 2014, and 28 in 2015).

Genetic and Hormone Analyses

I extracted DNA from the blood samples collected from adults and chicks following standard techniques (DNAeasy; Qiagen, Venlo, Netherlands). The sex of each bird was verified by amplification of the CHD gene using the P2 and P8 microsatellite primers (Griffiths et al. 1998), and for adults, always concordant with my field assignations. To determine reproductive success of all sampled adults in the population, I used seven previously developed sandpiper microsatellite markers: three developed from the ruff (*Philomachus pugnax*; Thuman et al. 2002) and four from the pectoral sandpiper (*Calidris melanotos*; Carter & Kempenaers 2007; see Chapter 2 for further details on the genetic parentage analyses). I determined reproductive success using the results of the genetic analyses and observational data on nest attempts. For each adult, I quantified reproductive success as 1) binary levels of reproductive success: presence or absence of a nest, whether or not the nest(s) hatched, and whether or not the offspring fledged and 2) an ordered rank of furthest reproductive stage reached across the season: no nest, eggs laid, eggs hatched, and offspring fledged.

Plasma samples were processed at the Brookfield Zoo's Endocrinology Service Lab (Brookfield, Illinois, USA) for testosterone analysis using enzyme immunoassays (ELISA kit: ADI-901-065; Enzo Life Sciences, Farmingdale, New York, USA). Methodology followed suggested guidelines in the manual. Plasma was diluted to 1:40 due to limited plasma volumes (samples: 38 from 32 females, 81 from 55 males). The testosterone analysis had an average recovery of 93.4% with an intra-assay coefficient of variation of 5.6% at 51.4% binding and an inter-assay coefficient of variation of 9.2% at 62.9% binding. Plasma samples collected from birds 15 or more minutes after capture were excluded from the analysis, as plasma testosterone levels begin to drop after 15 minutes (Deviche et al. 2010; but see Rissman & Wingfield (1984) for plasma samples taken within 30 minutes of capture).

Statistics

All statistical tests were run in R 3.3.1 (R Development Core Team 2015). Testosterone levels were log transformed to normalize the data, verified using Shapiro-Wilk tests. The testosterone levels of females and males were compared across the reproductive stages allowing for interactions between reproductive stage at time of capture and sex using linear mixed-effects regression models ('lme4' R package; Bates et al. 2015), with year and individual identity included as categorical random effects (recaptured individuals: 1 female across years, 1 female within years, 7 males across years, and 8 males within years). For this analysis, the reproductive stages were limited to two: courtship and egg laying/incubation, as no males were captured in the transition stage and no females were captured while caring for offspring. I also excluded all females in the egg laying/incubation stage which were captured following a simulated territorial intrusion as males could only be captured with nest traps at this stage. The results of the linear regression model were further examined using non-parametric pair-wise tests. I used a one-way analysis of variance to determine whether there was a difference in testosterone level between egg laying/incubating females captured after a simulated territorial intrusion (*n* = 8) and those captured with a nest trap (*n* = 7), with year included as a categorical random effect. I also used analyses of variance to consider testosterone levels relative to the mating success of females (i.e. 0, 1, or 2 mates) with reproductive stage at time of capture included as a random effect.

To determine whether there was a relationship between extent of melanized plumage ornamentation and testosterone, I focused on the amount of ventral surface covered by spots (i.e. percent plumage cover). This metric of spotted plumage pattern was one of the best predictors of plumage pattern variation within and between the sexes (Blizard & Pruett-Jones 2017). For each sex separately (25 females, 69 males), I used linear mixed-effect regression models to compare variation in percent plumage cover relative to testosterone level, reproductive stage at time of capture, and the interaction between these two variables, with individual identity included as a random effect (‘lme4’ R package; Bates et al. 2015). I also compared female percent plumage cover to her mate’s testosterone levels (log) using linear regression models (‘stats’ R package; R Development Core Team 2015) with reproductive stage at time of male capture included as variable interacting with testosterone.

Within each sex, binary measurements of reproductive success were compared to testosterone levels using two-tailed *t*-tests. To consider furthest reproductive success as a cumulative variable (e.g., given a bird has offspring that fledge, the bird must have had a nest with eggs that hatched), I used cumulative proportion modeling, tested both forwards and backwards (‘ordinal’ R package; Christensen 2015) on each sex separately (25 females, 69 males). The following explanatory variables were considered in the models: percent plumage cover, tarsus length, mass with an interaction with day of season captured, and testosterone level with an interaction with reproductive stage when captured. All numerical variables were centered to 0 using the scale function. Given the sample sizes, I used AICc scores (Cavanaugh 1997) to assess the alternative models (‘AICcmodavg’ R package; Mazerolle 2016). Lastly, I considered the proportion of female offspring in a clutch and the testosterone levels of the parent collected during the courtship or during egg laying/incubation when caught after a simulated territorial intrusion. Only complete or nearly complete clutches (i.e. 3 or 4 offspring sampled out of 4 total offspring) were included in the analyses.

RESULTS

After excluding plasma samples collected more than 15 minutes following capture (Deviche et al. 2010), I had a total of 25 samples from 21 females and 69 samples from 47 males. Two females and 10 males had multiple samples from two or more years. Two females and 8 males had multiple samples taken within the same breeding season, but at different reproductive

Table 3.1. Testosterone levels for female and male spotted sandpipers captured at different reproductive stages across the breeding season. Averages were calculated from back-transformations after data were log transformed.

	Females			Males		
	n	Average (ng/mL)	Range (ng/mL)	*n*	Average (ng/mL)	Range (ng/mL)
Courtship*	8	0.84	0.37 - 3.33	17	4.71	0.16 - 15.69
Egg laying/incubation*	8	1.09	0.15 - 3.03	-	-	-
Egg laying/incubation	7	0.37	0.06 - 3.63	46	0.52	0.01 - 13.77
Caring for offspring	-	-	-	6	0.23	0.05 - 1.50
Transition	2	0.41	0.12 - 1.40		-	-

*Caught after simulated territorial intrusion

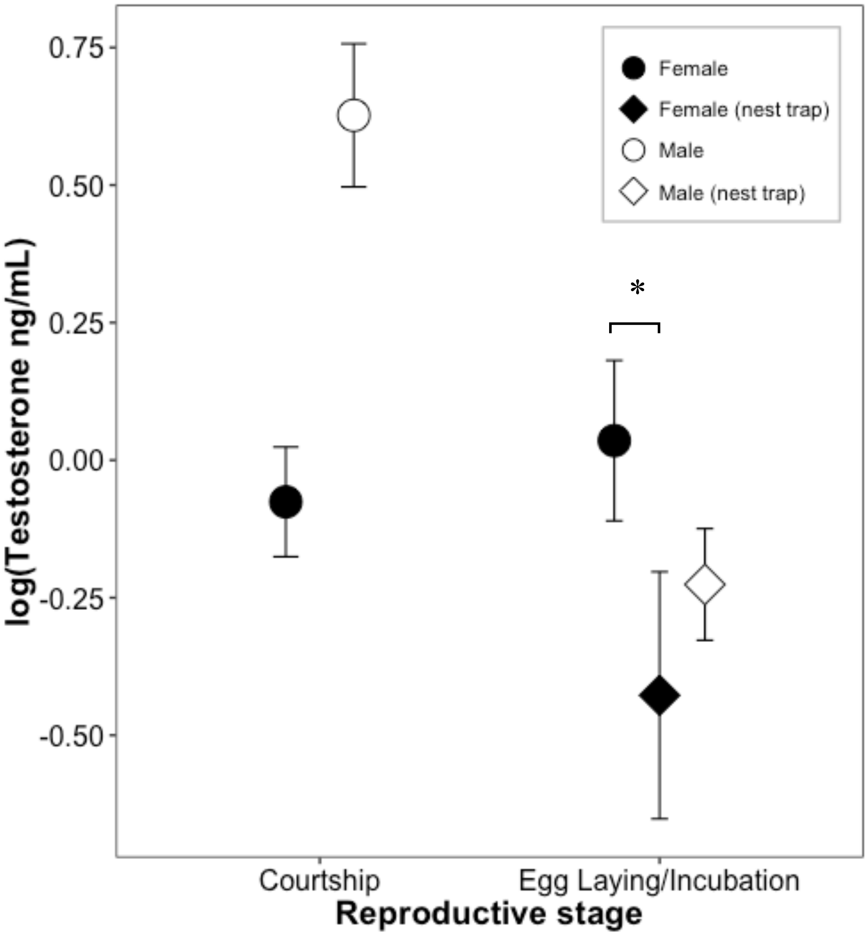

Figure 3.2. Average plasma testosterone levels (log) sampled from females and males during different reproductive stages. Females: filled shapes, males: open shapes, birds captured after a simulated territorial intrusion: circles, birds captured without a stimulus (i.e. trapped at the nest): diamonds. Error bars indicate standard error of the mean, and the asterisk indicates the statistically significant difference (by ANOVA) between females caught with and without a stimulus during the egg laying/incubation stage.

stages. Females had an average of 1.10 ± 0.21 SE ng/mL of testosterone (*n* = 25), ranging from 0.01 - 3.63 ng/mL, and males had an average of 2.57 ± 0.44 SE ng/mL (*n* = 69), ranging from 0.01 - 15.69 ng/mL (Table 3.1). Considering variation in testosterone levels across adults, excluding females caught after simulated territorial intrusions during the egg-laying/incubation period, reproductive stage and sex, but not their interaction, best explained variation in testosterone levels (log; Table 3.2, Figure 3.2). In post-hoc pair-wise analyses with Bonferroni corrections applied, males in the courting stage had greater testosterone than females ($W = 13$, $p = 0.003$) and males in the egg-laying/incubation stage had similar levels of testosterone as females ($W = 138$, $p \approx 1$). Within the sexes, courting males had greater testosterone levels than incubating males ($W = 696$, $p < 0.001$), but courting females had similar levels as egg-laying/incubating females ($W = 41$, $p = 0.61$).

Table 3.2. Results of linear regression models for predicting testosterone levels across adults ($n = 78$) with sex, reproductive stage (courtship or egg laying/incubation), and their interaction as fixed effects and individual identity and years included as random effects. *K* is the number of parameters in the model. The model listed in bold fit the testosterone data best, and each explanatory factor for that model, reproductive stage and sex, was analyzed independently.

Model	Estimate	SE	*t*	*K*	ΔAICc
Null	0.11	0.20	0.54	4	15.30
Reproductive Stage Egg/Incubation	**-0.75**	**0.15**	**-5.06**	**6**	0*
+ Sex Male	**0.41**	**0.17**	**2.38**		
Full				7	1.23
Reproductive stage				5	1.38
Sex				5	17.79

*Lowest AICc value: 157.55

Table 3.3. Analyses of variance of testosterone levels (log) in 1) females captured during the egg laying/incubation stage with or without a stimulus (i.e. simulated territorial intrusion) with year was included as a random effect and 2) female mating success (i.e. number of mates) with reproductive stage included as a random effect (*$p < 0.05$).

		df	Sum Sq	Mean Sq	*F*	*p*	
Simulated territorial intrusions ($n = 15$)	Stimulus	1	0.68	0.68	5.26	0.04	*
	Residuals	12	1.42	0.13			
Number of mates ($n = 21$)	Polyandrous	1	0.29	0.29	1.42	0.25	
	Residuals	20	4.13	0.21			

Females captured during egg-laying or incubation had higher testosterone levels when caught after exposure to a simulated territorial intrusion than those females caught without a stimulus (i.e. trapped at the nest; Table 3.3, Figure 3.2). There was no significant relationship between female testosterone level and mating success (i.e. number of mates; Table 3.3).

For both females and males, testosterone, reproductive stage at time of capture, and the interaction between the variables best explained variation in plumage ornamentation (Table 3.4). For courting and egg laying/incubating females, percent plumage cover tended to increase with increasing testosterone levels (Figure 3.3). However, for egg laying/incubating females captured following simulated territorial intrusions, there was a negative relationship between plumage cover and testosterone. For courting and incubating males, there was also a tendency of increasing percent plumage cover with increasing testosterone, and over males caring for offspring, there was a negative relationship between plumage cover and testosterone. Variation in percent plumage cover of females (n = 17) was better explained by the null model (null AICc = 130.58, AICc of alternative model = 136.23) than by their mate's testosterone level (log), interacting with reproductive stage at time of capture.

For both females and males, testosterone did not significantly differ across any measure of binary reproductive success: presence or absence of a nest (females: t = 1.48, df = 6, p = 0.19; males: t = -0.08, df = 15, p = 0.94), whether or not the nest(s) hatched (females: t = 1.17, df = 6, p = 0.29; males: t = 1.07, df = 15, p = 0.30), and whether or not the offspring fledged (females: t = 1.44, df = 6, p = 0.20; males: t = 0.85, df = 14, p = 0.41). The null model better explained variation in furthest reproductive stage reached (females: null AICc = 70.17, males: null AICc = 179.33) than any of the following explanatory variables: percent plumage cover, tarsus length, mass with an interaction with day of season captured, and testosterone level with an interaction with reproductive stage when captured. For adult females, there was no significant correlation between proportion of female offspring and testosterone levels (df = 4, *r* = -0.11, *p* = 0.84), and for adult males there was a strong trend for more female offspring with higher testosterone levels, despite the dataset including only 4 males (df = 2, *p* = 0.06, *r* = 0.94).

Table 3.4. Results of linear mixed-effects models for predicting plumage cover of females (*n* = 25) and males (*n* = 69) with testosterone level (log), reproductive stage, and their interaction included as explanatory variables. Individual identity was included as a random factor. *K* is the number of parameters in the model. Models listed in bold are those that fit the data best.

	Model	Estimate	SE	*t*	*K*	ΔAICc
Females	(Intercept)				3	17.12
	Testosterone*Reproductive Stage				**10**	**0**[a]
	(Intercept)	41.27	2.58	16.00		
	log(T)	-6.29	0.02	-293		
	Egg Laying/Incubation (nest)	3.74	0.01	597.3		
	Egg Laying/Incubation (T)	-3.31	4.08	-0.8		
	RS Transition	-0.64	8.01	-0.1		
	log(T):Egg Laying/Incubation (nest)	9.38	0.02	428.6		
	log(T):Egg Laying/Incubation (T)	-5.89	8.18	-0.7		
	log(T):Transition	15.46	8.79	1.8		
	Testosterone + Reproductive Stage				7	8.45
	Reproductive stage				6	9.16
	Testosterone				4	15.95
Males	Null				3	13.47
	Testosterone*Reproductive Stage				**8**	**0**[b]
	(Intercept)	13.79	4.74	2.91		
	log(T)	-7.96	5.84	-1.36		
	Courtship	9.66	5.59	1.73		
	Egg Laying/Incubation (nest)	9.96	4.86	2.05		
	log(T):Courtship	7.56	6.87	1.10		
	log(T): Egg Laying/Incubation (nest)	10.58	6.12	1.73		
	Testosterone + Reproductive Stage					8.28
	Reproductive stage					9.47
	Testosterone					12.61

Lowest AICc values for models: (a) 162.83 and (b) 456.29

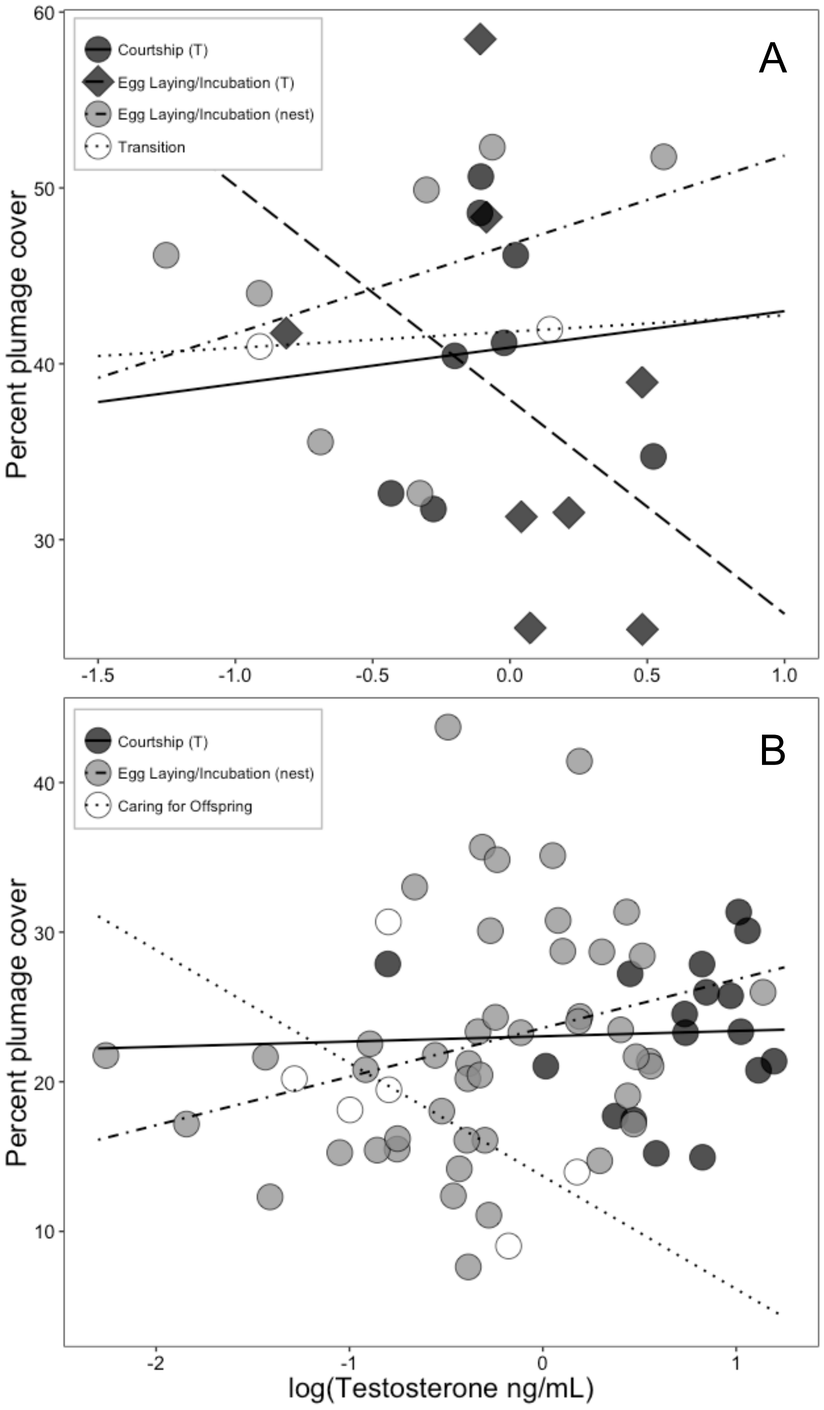

Figure 3.3. (**A**) Female and (**B**) male ornamentation, as measured by percent plumage cover, relative to testosterone levels (log). Lines indicate linear regressions on each reproductive stage. Courting adults caught by stimulus: dark gray circles/solid line, egg laying/incubating females caught after territorial intrusions: dark gray diamonds/dashed line, egg laying/incubating females or incubating males caught on the nest: light gray circles/dot-dashed line, females caught at the end of the breeding season or males caught with hatched offspring: open circles/dotted line.

DISCUSSION

During courtship, males had testosterone levels 5.6 times higher on average than female levels based on back-transformed data, and then male testosterone levels (log) dropped to the statistically the same levels as females during incubation (1.4 times higher than female levels). Overall, these patterns of testosterone levels between the sexes and across the breeding stages are concordant with results from other populations of spotted sandpipers (Rissman & Wingfield 1984; Fivizzani & Oring 1986), as well as studies of other polyandrous bird species (Gratto-Trevor et al. 1990; Goymann & Wingfield 2004). Although female testosterone levels (log) remained statistically constant from courtship through egg laying/incubation, testosterone levels were on average 2.25 times higher during courtship than during egg laying/incubation (back-transformed data), which may be indicative of the competitive female-female interactions to gain territories and mates during the beginning of the breeding season. Levels of female testosterone were similar to those also quantified using enzyme immunoassays in monogamous species with aggressive females (juncos; Jawor et al. 2007) However, there was no relationship between female mating success (i.e. unpaired, monogamous, or polyandrous) and testosterone levels.

In the test on females captured during the egg laying/incubation stage using simulating competitive interactions, females captured following a stimulus had significantly higher testosterone levels than those captured on the nest. This elevation in testosterone level, as well as the maintenance of testosterone levels from courtship to incubation suggest that females use testosterone to mediate aggressive behavior during competitive interactions. If female spotted sandpipers were to have testosterone levels as high as those observed in males while courting, this may result in negative effects on them. For instance, experimentally maintained or increased levels of testosterone in females of typical sex role species can lead to delayed egg laying (red-winged blackbirds, *Agelaius phoeniceus*; Searcy 1988), lower incubation temperature (tree swallows, *Tachycineta bicolor*; great tits, *Parus major*; Rosvall 2013a; De Jong et al. 2016), lower feeding rates (spotless starlings, *Sturnus unicolor*; Veiga & Polo 2008), reduced brooding behaviors (dark-eyed junco; O'Neal et al. 2008), and decreased brood success (Veiga & Polo 2008; Rosvall 2013a; De Jong et al. 2016). All above-mentioned studies, other than Searcy (1988), used testosterone implants that increased plasma testosterone to levels within physiologically relevant bounds in females.

In conjuncture with reproductive stage when captured, testosterone levels explained variation in melanized spotted plumage cover for both females and males better than the null model. However, in females, there was an unexpected negative relationship between the steroid hormone and plumage cover for females challenged with a simulated territorial intrusion while egg laying and incubating. This result was unexpected especially as females respond aggressively towards territorial intrusions at this reproductive stage, while males do not (Blizard, personal observation), so the circulating testosterone levels should represent an induced release of testosterone in the body. Additionally, prior research in a captive population of sex-role reversed barred buttonquail also suggested that there would be a positive correlation between the steroid hormone and size of melanized ornaments in females (Muck & Goymann 2011). However, this study on spotted sandpipers considered only percent cover of spotted plumage, not ornament contrast against background plumage or ornament hue, saturation, and brightness, which indicates melanin content (i.e. degree of melanization and amount of eumelanin relative to phaeomelanin; McGraw et al. 2005). Within the courtship and incubation stages, the positive relationship between testosterone and male plumage cover was expected given what is currently known about plumage melanization and testosterone in males (Jawor & Breitwisch 2003). Male spotted sandpipers may be aggressive, especially to defend their territories, a subset of the female's territory, or towards other males that may be competing for their mate (Maxson & Oring 1980). However, male testosterone levels in this study did not vary with their mate's spotted ornamentation, which correlates with female condition and reproductive success (Blizard & Pruett-Jones 2017; Chapter 2). This suggests that male-male competition for territories and mates is low in this population.

There was no significant relationship observed between testosterone and any measure male or female reproductive success. Bird mass and size also did not significantly explain variation in reproductive success. Given the limited sample size, it could be that there was not enough power to detect significance. Additionally, plasma levels alone cannot portray a complete story of the role testosterone may play in female aggression and ornamentation. Location, sensitivity, and density of testosterone receptors, the rate at which other molecules are synthesized from testosterone (Oring & Fivizzani 1991; see Hutchison 1971 for data from males), and the genomic effect of testosterone on target tissues (Rosvall 2013b) could differ between individuals and between the sexes. As such, we may not be able to assume that testosterone acts in a similar manner in females as in males (Goymann & Wingfield 2014).

Although testosterone had no significant relationship with reproductive success in female or male spotted sandpipers and testosterone positively correlated with melanized plumage more consistently in males, the plumage ornamentation of spotted sandpipers is likely a biologically relevant signal in competitive female interactions (Blizard & Pruett-Jones 2017; Chapter 2). Furthermore, females exhibited increased testosterone levels when exposed to a potential conspecific threat to their territory, which suggests that testosterone is involved in regulating female aggression in this species. Potentially, differences in female testosterone levels are in part an evolutionary covariate of direct selection on male testosterone levels and the associated male behaviors and sexual signals. While female competition is especially apparent in sex-role reversed species, it is undeniable that across vertebrate species, females can experience intense competition over mates or resources as males do (Lutnesky & Kosaki 1995; Woodley & Moore 1999; Amundsen 2000; Stockley & Bro-Jørgensen 2011). While outwardly similar to competition in males, the effects of aggression and its underlying mechanisms may differ in females. As our understanding of competitive female behavior and the associated traits is increasing, it is increasingly likely female aggression is also under selection pressures.

LITERATURE CITED

Amundsen, T. 2000. Why are female birds ornamented? *Trends in Ecology & Evolution*, 15, 149-155.

Andersson, M. 1994. *Sexual Selection*. Princeton: Princeton University Press.

Andersson, M. 2005. Evolution of classical polyandry: Three steps to female emancipation. *Ethology*, 111, 1-23.

Andersson, M., Wallander, J., Oring, L. W., Akst, E., Reed, J. M. & Fleischer, R. C. 2003. Adaptive seasonal trend in brood sex ratio: Test in two sister species with contrasting breeding systems. *Journal of Evolutionary Biology*, 16, 510-515.

Argüelles-Ticó, A., Küpper, C., Kelsh, R. N., Kosztolányi, A., Székely, T. & van Dijk, R. E. 2016. Geographic variation in breeding system and environment predicts melanin-based plumage ornamentation of male and female Kentish plovers. *Behavioral Ecology and Sociobiology*, 70, 49-60.

Ball, G. F. 1991. Endocrine mechanisms and the evolution of avian parental care. In: *Acta XX Congressus Internationalis Ornithologici 20*, pp. 984-991. Wellington: Ornithological Trust Board.

Barrowclough, G. F. & Sibley, F. C. 1980. Feather pigmentation and abrasion: Test of a hypothesis. *The Auk*, 97, 881-883.

Bates, D., Maechler, M., Bolker, B. & Walker, S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1-48.

Bennett, S. 1983. A review of the distribution, status and biology of the plains-wanderer *Pedionomus torquatus*, Gould. *Emu*, 83, 1-11.

Berglund, A., Bisazza, A. & Pilastro, A. 1996. Armaments and ornaments: An evolutionary explanation of traits of dual utility. *Biological Journal of the Linnean Society*, 58, 385-399.

Blizard, M. & Pruett-Jones, S. 2017. Plumage pattern dimorphism in a shorebird exhibiting sex-role reversal (*Actitis macularius*). *The Auk: Ornithological Advances*, 135, 363-376.

Bókony, V., Garamszegi, L. Z., Hirschenhauser, K. & Liker, A. 2008. Testosterone and melanin-based black plumage coloration: A comparative study. *Behavioral Ecology and Sociobiology*, 62, 1229-1238.

Bonser, R. H. C. 1995. Melanin and the abrasion resistance of feathers. *The Condor*, 97, 590-591.

Botstein, D., White, R. L., Skolnick, M. & Davis, R. W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, 32, 314-331.

Burnham, K. P. & Anderson, D. R. 2002. *Model Selection and Multimodel Inference: A Practical Information-theoretic Approach.* 2nd edn. New York: Springer.

Burtt, E. H., Jr. 1986. An analysis of physical, physiological, and optical aspects of avian coloration with emphasis on wood-warblers. *Ornithological Monographs*, 38, iii-126.

Bush, S. E., Kim, D., Moyer, B. R., Lever, J. & Clayton, D. H. 2006. Is melanin a defense against feather-feeding lice? *The Auk*, 123, 153-161.

Butchart, S. H. 1999. Sexual conflicts and copulation patterns in polyandrous bronze-winged jacanas *Metopidius indicus*. *Behaviour*, 136, 443-468.

Butchart, S. H. 2000. Population structure and breeding system of the sex-role reversed, polyandrous bronze-winged jacana *Metopidius indicus*. *Ibis*, 142, 93-102.

Cain, K. E. & Ketterson, E. D. 2012. Competitive females are successful females; phenotype, mechanism, and selection in a common songbird. *Behavioral Ecology and Sociobiology*, 66, 241-252.

Canoine, V. & Gwinner, E. 2005. The hormonal response of female European stonechats to a territorial intrusion: The role of the male partner. *Hormones and Behavior*, 47, 563-568.

Carter, K. L. & Kempenaers, B. 2007. Eleven polymorphic microsatellite markers for paternity analysis in the pectoral sandpiper, *Calidris melanotos*. *Molecular Ecology Notes*, 7, 658-660.

Cavanaugh, J. E. 1997. Unifying the derivations of the Akaike and corrected Akaike information criteria. *Statistics & Probability Letters*, 33, 201-208.

Chen, T. C., Lin, Y. S., Deng, P. L. & Ding, T. S. 2008. Male pheasant-tailed jacanas commit infanticides to avoid cuckoldry when paternity of eggs is doubtful. *Journal of Natural History*, 42, 2991-3000.

Christensen, R. H. B. 2015. ordinal: Regression Models for Ordinal Data. R package version 2015.6-28. http://www.cran.r-project.org/package=ordinal.

Clutton-Brock, T. H. 1991. *The Evolution of Parental Care*. Princeton: Princeton University Press.

Clutton-Brock, T. H. 2009. Sexual selection in females. *Animal Behaviour*, 77, 3-11.

Clutton-Brock, T. H. & Huchard, E. 2013. Social competition and selection in males and females. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368, 20130074.

Colwell, M. A. & Oring, L. W. 1988. Breeding biology of Wilson's phalarope in southcentral Saskatchewan. *The Wilson Bulletin*, 1, 567-582.

Colwell, M. A. & Oring, L. W. 1989. Extra-pair mating in the spotted sandpiper: A female mate acquisition tactic. *Animal Behaviour*, 38, 675-684.

Cramp, S. & Simmons, K. E. L. 1980. *The Birds of the Western Palearctic, Volume II*. Oxford: Oxford University Press.

Cuervo, J. J., de Lope, F. & Møller, A. P. 1996. The function of long tails in female barn swallows (*Hirundo rustica*): An experimental study. *Behavioral Ecology*, 7, 132-136.

Dale, J., Montgomerie, R., Michaud, D. & Boag, P. 1999. Frequency and timing of extrapair fertilisation in the polyandrous red phalarope (*Phalaropus fulicarius*). *Behavioral Ecology and Sociobiology*, 46, 50-56.

Darolová, A., Hoi, H. & Schleicher, B. 1997. The effect of ectoparasite nest load on the breeding biology of the penduline tit *Remiz pendulinus*. *Ibis*, 139, 115-120.

De Jong, B., Lens L., Amininasab, S. M., van Oers, K., Darras, V. M., Eens, M., Pinxten, R., Komdeur, J. & Groothuis, T. G. 2016. Effects of experimentally sustained elevated testosterone on incubation behaviour and reproductive success in female great tits (*Parus major*). *General and Comparative Endocrinology*, 230, 38-47.

De Ridder, E., Pinxten, R. & Eens, M. 2000. Experimental evidence of a testosterone-induced shift from paternal to mating behaviour in a facultatively polygynous songbird. *Behavioral Ecology and Sociobiology*, 49, 24-30.

Dein, J. 1986. Hematology. In: *Clinical Avian Medicine* (Ed. by G. J. Harrison & W. R. Harrison), pp. 174-191. London: Saunders.

DeLay, L. S., Faaborg, J., Naranjo, J., Paz, S. M., de Vries, T. & Parker, P. G. 1996. Paternal care in the cooperatively polyandrous Galapagos hawk. *The Condor*, 98, 300-311.

Delehanty, D. J., Fleischer, R. C., Colwell, M. A. & Oring, L. W. 1998. Sex-role reversal and the absence of extra-pair fertilization in Wilson's phalaropes. *Animal Behaviour*, 55, 995-1002.

Deviche, P. J., Hurley, L. L., Fokidis, H. B., Lerbour, B., Silverin, B., Silverin, B., Sabo, J. & Sharp, P. J. 2010. Acute stress rapidly decreases plasma testosterone in a free-ranging male songbird: Potential site of action and mechanism. *General and Comparative Endocrinology*, 169, 82-90.

Dwight, J. 1900. The moult of the North American shore birds (Limicolæ). *The Auk*, 17, 368-385.

Edward, D. A. & Chapman T. 2011. The evolution and significance of male mate choice. *Trends in Ecology & Evolution*, 26, 647-654.

Eens, M. & Pinxten, R. 2000. Sex-role reversal in vertebrates: Behavioural and endocrinological accounts. *Behavioural Processes*, 51, 135-147.

Elekonich, M. M. & Wingfield, J. S. 2000. Seasonality and hormonal control of territorial aggression in female song sparrows (Passeriformes: Emberizidae: *Melospiza melodia*). *Ethology*, 106, 493-510.

Emlen, S. T., Demong, N. J. & Emlen, D. 1989. Experimental induction of infanticide in female wattled jacanas. *The Auk*, 106, 1-7.

Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science*, 197, 215-223.

Emlen, S. T., Wrege, P. H. & Webster, M. S. 1998. Cuckoldry as a cost of polyandry in the sex-role reversed jacana, *Jacana jacana*. *Proceedings of the Royal Society of London B: Biological Sciences*, 265, 2359-2364.

Erckmann, W. J. 1983. The evolution of polyandry in shorebirds: An evaluation of hypotheses. In: *Social Behaviour of Female Vertebrates* (Ed. by S. K. Wasser), pp. 113-168. New York: Academic Press.

Fitze, P. S. & Richner, H. 2002. Differential effects of a parasite on ornamental structures based on melanins and carotenoids. *Behavioral Ecology*, 13, 401-407.

Fivizzani, A. J., Colwell, M. A. & Oring, L. W. 1986. Plasma steroid hormone levels in free-living Wilson's phalaropes, *Phalaropus tricolor*. *General and Comparative Endocrinology*, 62, 137-144.

Fivizzani, A. J. & Oring, L. W. 1986. Plasma steroid hormones in relation to behavioral sex role reversal in the spotted sandpiper, *Actitis macularia*. *Biology of Reproduction*, 35, 1195-1201.

French, J. A., Mustoe, A. C., Cavanaugh, J. & Birnie, A. K. 2013. The influence of androgenic steroid hormones on female aggression in 'atypical' mammals. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 368, 20130084.

Fusani, L. 2008. Testosterone control of male courtship in birds. *Hormones and Behavior*, 54, 227-233.

Garnett, S. 1985. Evidence for polyandry in the comb-crested jacana. *The Stilt*, 7, 24.

Gasparini, J., Bize, P., Piault, R., Wakamatsu, K., Blount, J. D., Ducrest, A. L. & Roulin, A. 2009. Strength and cost of an induced immune response are associated with a heritable melanin-based colour trait in female tawny owls. *Journal of Animal Ecology*, 78, 608-616.

Gibson, R. & Baker, A. 2012. Multiple gene sequences resolve phylogenetic relationships in the shorebird suborder Scolopaci (Aves: Charadriiformes). *Molecular Phylogenetics and Evolution*, 64, 66-72.

Gluckman, T. L. & Cardoso, G. C. 2009. A method to quantify the regularity of barred plumage patterns. *Behavioral Ecology and Sociobiology*, 63, 1837-1844.

Goldstein, G., Flory, K. R., Browne, B. A., Majid, S., Ichida, J. M. & Burtt, E. H., Jr. 2004. Bacterial degradation of black and white feathers. *The Auk*, 121, 656-659.

Götmark, F., Post, P., Olsson, J. & Himmelmann, D. 1997. Natural selection and sexual dimorphism: Sex-biased sparrowhawk predation favours crypsis in female chaffinches. *Oikos*, 80, 540-548.

Goymann, W., Makomba, M., Urasa, F. & Schwabl, I. 2015. Social monogamy vs. polyandry: Ecological factors associated with sex roles in two closely related birds within the same habitat. *Journal of Evolutionary Biology*, 28, 1335-1353.

Goymann, W. & Wingfield, J. C. 2004. Competing females and caring males. Sex steroids in African black coucals, *Centropus grillii*. *Animal Behaviour*, 68, 733-740.

Goymann, W. & Wingfield, J. C. 2014. Male-to-female testosterone ratios, dimorphism, and life history – what does it really tell us? *Behavioral Ecology*, 25, 685-699.

Gratto-Trevor, C. L., Fivizzani, A. J., Oring, L. W. & Cooke, F. 1990. Seasonal changes in gonadal steroids of a monogamous versus a polyandrous shorebird. *General and Comparative Endocrinology*, 80, 407-418.

Griffith, S. C., Owens, I. P. F. & Thuman, K. A. 2002. Extra pair paternity in birds: A review of interspecific variation and adaptive function. *Molecular Ecology*, 11, 2195-2212.

Griffiths, R., Double, M. C., Orr, K. & Dawson, R. J. G. 1998. A DNA test to sex most birds. *Molecular Ecology*, 7, 1071-1075.

Gunderson, A. R., Frame, A. M., Swaddle, J. P. & Forsyth, M. H. 2008. Resistance of melanized feathers to bacterial degradation: Is it really so black and white? *Journal of Avian Biology*, 39, 539-545.

Gwynne, D. T. 1981. Sexual difference theory: Mormon crickets show role reversal in mate choice. *Science*, 213, 779-780.

Haig, S. M., Mace, T. R. & Mullins, T. D. 2003. Parentage and relatedness in polyandrous comb-crested jacanas using ISSRs. *Journal of Heredity*, 94, 302-309.

Härdling, R., Gosden, T. & Aguilée, R. 2008. Male mating constraints affect mutual mate choice: Prudent male courting and sperm-limited females. *The American Naturalist*, 172, 259-271.

Haribal, M., Proctor, H., Dhondt, A. A. & Rodriquez, E. 2011. Biology of house finch feather mites, *Proctophyllodes pinnatus* (Acari: Proctophyllodidae), parallels variation in preen gland secretions. *International Journal of Acarology*, 37, 75-90.

Hayes, F. E. 1995. Status, distribution and biogeography of the birds of Paraguay. *Monographs in Field Ornithology*, 1, 1-230.

Hays, H. 1972. Polyandry in the spotted sandpiper. *The Living Bird*, 11, 43-57.

Hearing, V. J. 1998. Regulation of melanin formation. In: *The Pigmentary System: Physiology and Pathophysiology* (Ed. by J. J. Nordlund, R. E. Boissy, V. J. Hearing, R. A. King & J. P. Ortonne), pp. 423-438. New York: Oxford University Press.

Heinsohn, R., Legge, S. & Endler, J. A. 2005. Extreme reversed sexual dichromatism in a bird without sex role reversal. *Science*, 309, 617-619.

Hill, G. E. 1993. Male mate choice and the evolution of female plumage coloration in the house finch. *Evolution*, 47, 1515-1525.

Hoffman, A. 1949. Über die brutpflege des polyandrischen Wasserfasans *Hydrophasianus chirurgus* (Scop.). *Zoologische Jahrbücher*, 78, 367-403.

Hoffman, A. 1950. Zur Brutbiologie des polyandrischen Wasserfasans *Hydrophasianus chirurgus* (Scop.) *Berliner Ornithologische,* 2, 119-126.

Horváth, L. & Rice, G. 2015. Testing equality of means when the observations are from functional time series. *Journal of Time Series Analysis*, 36, 84-108.

Hutchison, J. B. 1971. Effects of hypothalamic implants of gonadal steroids on courtship behaviour in Barbary doves (*Streptopelia risoria*). *Journal of Endocrinology*, 50, 97-113.

Jawor, J. M. & Breitwisch, R. 2003. Melanin ornaments, honesty, and sexual selection. *The Auk*, 120, 249-265.

Jawor, J. M., McGlothlin, J. W., Casto, J. M., Greives, T. J., Snajdr, E. A., Bentley, G. E. & Ketterson, E. D. 2007. Testosterone response to GnRH in a female songbird varies with stage of reproduction: Implications for adult behaviour and maternal effects. *Functional Ecology*, 21, 767-775.

Jenni, D. A. 1974. Evolution of polyandry in birds. *American Zoologist*, 14, 129-144.

Jenni, D. A. 1996. Family Jacanaidae (Jacanas). In: *Handbook of the Birds of the World* (Ed by: J. del Hoyo, A. Elliott & J. Sargatal), pp. 276-291. Barcelona: Lynx Edicions.

Jenni, D. A. & Collier, G. 1972. Polyandry in the American jacana (*Jacana spinosa*). *The Auk*, 1, 743-765.

Johns, J. E. 1964. Testosterone-induced nuptial feathers in phalaropes. *The Condor*, 66, 449-455.

Johnsen, T. S., Hengeveld, J. D., Blank, J. L., Yasukawa, K. & Nolan, V., Jr. 1996. Epaulet brightness and condition in female red-winged blackbirds. *The Auk*, 113, 356-362.

Kalinowski, S. T., Taper, M. L. & Marshall, T. C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, 16, 1099-1106.

Ketterson, E. D., Nolan, V., Jr. & Sandell, M. 2005. Testosterone in females: Mediator of adaptive traits, constraint on sexual dimorphism, or both? *The American Naturalist*, 166, S85-98.

Kotaka, N. 1998. Classical polyandry in the great spotted woodpecker *Dendrocopos major*. *Ibis*, 140, 335-336.

Kraaijeveld, K., Kraaijeveld-Smit, F. J. L. & Komdeur, J. 2007. The evolution of mutual ornamentation. *Animal Behaviour*, 74, 657-677.

Kriner, E. & Schwabl, H. 1991. Control of winter song and territorial aggression of female robins (*Erithacus rubecula*) by testosterone. *Ethology*, 87, 37-44.

Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution*, 34, 292-305.

Lank, D. B., Coupe, M. & Wynne-Edwards, K. E. 1999. Testosterone-induced male traits in female ruffs (*Philomachus pugnax*): Autosomal inheritance and gender differentiation. *Proceedings of the Royal Society of London B: Biological Sciences*, 266, 2323-2330.

Lank, D. B., Oring, L. W. & Maxson, S. J. 1985. Mate and nutrient limitation of egg-laying in a polyandrous shorebird. *Ecology*, 66, 1513-1524.

Laucht, S., Kempenaers, B. & Dale, J. 2010. Bill color, not badge size, indicates testosterone-related information in house sparrows. *Behavioral Ecology and Sociobiology*, 64, 1461-1471.

LeBas, N. R. 2006. Female finery is not for males. *Trends in Ecology & Evolution*, 21, 170-173.

Leymarie, F. & Levine, M. D. 1992. Fast raster scan distance propagation on the discrete rectangular lattice. *CVGIP: Image Understanding*, 55, 84-94.

Ligon, D. J. 1999. *The Evolution of Avian Breeding Systems*. New York: Oxford University Press.

Lutnesky, M. M. & Kosaki, R. K. 1995. Female-female competition in a coral reef fish and a test of the temporal threshold model of polygynous mating. *The American Naturalist*, 146, 832-847.

Malo, A. F., Roldan, E. R., Garde, J. J., Soler, A. J., Vicente, J., Gortazar, C. & Gomendio, M. 2009. What does testosterone do for red deer males? *Proceedings of the Royal Society of London B: Biological Sciences*, 276, 971-980.

Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, 7, 639-655.

Martin, T. E., & Badyaev, A. V. 1996. Sexual dichromatism in birds: Importance of nest predation and nest location for females versus males. *Evolution,* 50, 2454-2460.

Mathew, D. N. 1964. Observations on the breeding habits of the bronze-winged jacana (*Metopidius indicus* (Latham)). *Journal of the Bombay Natural History Society*, 61, 295-302.

Matessi, G., Carmagnani, C., Griggio, M. & Pilastro, A. 2009. Male rock sparrows differentially allocate nest defence but not food provisioning to offspring. *Behaviour*, 146, 209-223.

MATLAB and Statistics Toolbox Release. 2012. The MathWorks, Inc., Natick, Massachusetts.

Maxson, S. J. & Oring, L. W. 1978. Mice as a source of egg loss among ground-nesting birds. *The Auk*, 95, 582-584.

Maxson, S. J. & Oring, L. W. 1980. Breeding season time and energy budgets of the polyandrous spotted sandpiper. *Behaviour*, 74, 200-263.

Mazerolle, M. J. 2016. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.1-0. https://cran.r-project.org/package=AICcmodavg.

McGlothlin, J. W., Jawor, J. M., Greives, T. J., Castro, J. M., Phillips, J. L. & Ketterson, E. D. 2008. Hormones and honest signals: Males with larger ornaments elevate testosterone more when challenged. *Journal of Evolutionary Biology*, 21, 39-48.

McGraw, K. J. 2006. Mechanics of melanin-based coloration. In: *Bird Coloration Vol. I: Mechanisms and Measurements* (Ed. by G. E. Hill & K. J. McGraw), pp. 243-294. Cambridge: Harvard University Press.

McGraw, K. J., Safran, R. J. & Wakamatsu, K. 2005. How feather colour reflects its melanin content. *Functional Ecology*, 19, 816-821.

Møller, A. P. 1993. Sexual selection in the barn swallow *Hirundo rustica*. III. Female tail ornaments. *Evolution*, 47, 417-431.

Møller, A. P. 1994. *Sexual Selection and the Barn Swallow*. Oxford: Oxford University Press.

Møller, A. P. 2000. Male parental care, female reproductive success, and extrapair paternity. *Behavioral Ecology*, 11, 161-168.

Mousley, H. 1937. Nesting habits of the spotted sandpiper. *The Auk*, 54, 445-451.

Muck, C. & Goymann, W. 2011. Throat patch size and darkness covaries with testosterone in females of a sex-role reversed species. *Behavioral Ecology*, 22, 1312-1319.

Muck, C., Kempenaers, B., Kuhn, S., Valcu, M. & Goymann, W. 2009. Paternity in the classical polyandrous black coucal (*Centropus grillii*) – a cuckoo accepting cuckoldry? *Behavioral Ecology*, 20, 1185-1193.

Nelson, M. M. 1939. The biology of the spotted sandpiper (*Actitis macularia, Lynn*). Ph.D. thesis, University of Michigan.

Norris, D. R., Kleven, O., Johnsen, A. & Kyser, T. K. 2009. Melanin-based feather colour and moulting latitude in a migratory songbird. *Ethology*, 115, 1009-1014.

Nottebohm, F. 1980. Testosterone triggers growth of brain vocal control nuclei in adult female canaries. *Brain Research*, 189, 429-436.

O'Neal, D. M., Reichard, D. G., Pavilis, K. & Ketterson, E. D. 2008. Experimentally-elevated testosterone, female parental care, and reproductive success in a songbird, the dark-eyed junco (*Junco hyemalis*). *Hormones and Behavior*, 54, 571-578.

Oring, L. W. 1982. Avian mating systems. In: *Avian Biology Volume 6* (Ed. by D. S. Farner, J. R. King & K. C. Parkes), pp. 1-92. New York: Academic Press.

Oring, L. W. 1986. Avian polyandry. In: *Current Ornithology* (Ed. by R. F. Johnston), pp. 309-351. New York: Plenum Press.

Oring, L. W., Colwell, M. A. & Reed, J. M. 1991. Lifetime reproductive success in the spotted sandpiper (*Actitis macularia*): Sex differences and variance components. *Behavioral Ecology and Sociobiology*, 28, 425-432.

Oring, L. W. & Fivizzani, A. J. 1991. Reproductive endocrinology of sex-role reversal. *Acta XX Congressus Internationalis Ornithologici*, 20, 2072-2080.

Oring, L. W., Fivizzani, A. J. & El Halawani, M. E. 1989. Testosterone-induced inhibition of incubation in the spotted sandpiper (*Actitis mecularia*). *Hormones and Behavior*, 23, 412-423.

Oring, L. W., Fleischer, R. C., Reed, J. M. & Marsden, K. E. 1992. Cuckoldry through stored sperm in the sequentially polyandrous spotted sandpiper. *Nature*, 359, 631-633.

Oring, L. W. & Knudson, M. L. 1972. Monogamy and polyandry in the spotted sandpiper. *The Living Bird*, 11, 59-73.

Oring, L. W. & Lank, D. B. 1982. Sexual selection, arrival times, philopatry and site fidelity in the polyandrous spotted sandpiper. *Behavioral Ecology and Sociobiology*, 10, 185-191.

Oring, L. W. & Lank, D. B. 1986. Polyandry in spotted sandpipers: The impact of environment and experience. In: *Environmental Aspects of Social Evolution: Birds and Mammals* (Ed. by D. I. Rubenstein & P. Wrangham), pp. 21-42. Princeton: Princeton University Press.

Oring, L. W., Lank, D. B. & Maxson, S. J. 1983. Population studies of the polyandrous spotted sandpiper. *The Auk*, 100, 272-285.

Oring, L. W., Reed, J. M. & Maxson, S. J. 1994. Copulation patterns and mate guarding in the sex-role reversed, polyandrous spotted sandpiper, *Actitis macularia*. *Animal Behaviour*, 47, 1065-1072.

Osborne, D. R. 1982. Replacement nesting and polyandry in the wattled jacana. *The Wilson Bulletin*, 94, 206-208.

Osborne, D. R. & Bourne, G. R. 1977. Breeding behavior and food habits of the wattled jacana. *The Condor*, 79, 98-105.

Owen, J. C. 2011. Collecting, processing, and storing avian blood: A review. *Journal of Field Ornithology*, 84, 339-354.

Owens, I. P. F. 2002. Male-only care and classical polyandry in birds: Phylogeny, ecology and sex differences in remating opportunities. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 357, 283-293.

Owens, I. P. F., Burke, T. & Thompson, D. B. A. 1994. Extraordinary sex roles in the Eurasian dotterel: Female mating arenas, female-female competition, and female mate choice. *The American Naturalist*, 144, 76-100.

Owens, I. P. F., Dixon, A., Burke, T. & Thompson, D. B. A. 1995. Strategic paternity assurance in the sex-role reversed Eurasian dotterel (*Charadrius morinellus*): Behavioral and genetic evidence. *Behavioral Ecology*, 6, 14-21.

Petrie, M. 1983. Female moorhens compete for small fat males. *Science*, 220, 413-414.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team. 2017. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-131, https://CRAN.R-project.org/package=nlme.

Pyle, P. 2008. *Identification guide to North American birds: Part II.* Point Reyes Station: Slate Creek Press.

R Development Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org.

Raouf, S. A., Parker, P. G., Ketterson, E. D., Nolan, V. & Ziegenfus, C. 1997. Testosterone affects reproductive success by influencing extra-pair fertilizations in male dark-eyed juncos (Aves: *Junco hyemalis*). *Proceedings of the Royal Society of London B: Biological Sciences*, 264, 1599-1603.

Rasband, W. S. 2015. ImageJ. U. S. National Institutes of Health, Bethesda, Maryland, http://imagej.nih.gov/ij/1997-2015.

Reed, J. M., Oring, L. W. & Gray, E. M. 2013. Spotted sandpiper (*Actitis macularius*). *In: The Birds of North America Online* (Ed. by P. G. Rodewald). Ithaca: Cornell Lab of Ornithology.

Reudink, M. W., McKellar, A. E., Marini, K. L. D., McArthur, S. L., Marra, P. P. & Ratcliffe, L. M. 2015. Inter-annual variation in American redstart (*Setophaga ruticilla*) plumage colour is associated with rainfall and temperature during moult: An 11-year study. *Oecologia*, 178, 161-173.

Reynolds, J. D. 1987. Mating system and nesting biology of the red-necked phalarope *Phalaropus lobatus*: What constrains polyandry? *Ibis*, 129, 225-242.

Ridley, M. 1978. Paternal care. *Animal Behaviour*, 26, 904-932.

Rissman, E. F. & Wingfield, J. C. 1984. Hormonal correlates of polyandry in the spotted sandpiper, *Actitis macularia*. *General and Comparative Endocrinology*, 56, 401-405.

Rosvall, K. A. 2008. Sexual selection on aggressiveness in females: Evidence from an experimental test with tree swallows. *Animal Behaviour*, 75, 1603-1610.

Rosvall, K. A. 2013a. Life history trade-offs and behavioral sensitivity to testosterone: An experimental test when female aggression and maternal care co-occur. *PLoS ONE*, 8, e54120.

Rosvall, K. A. 2013b. Proximate perspectives on the evolution of female aggression: Good for the gander, good for the goose? *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 368, 20130083.

Roulin, A. 1999a. Delayed maturation of plumage coloration and plumage spottiness in the barn owl *Tyto alba*. *Journal für Ornithologie*, 140, 193-197.

Roulin, A. 1999b. Nonrandom pairing by male barn owls (*Tyto alba*) with respect to a female plumage trait. *Behavioral Ecology*, 10, 688-695.

Roulin, A., Jungi, T. W., Pfister, H. & Dijkstra, C. 2000. Female barn owls (*Tyto alba*) advertise good genes. *Proceedings of the Royal Society of London B: Biological Sciences*, 267, 937-941.

Roulin, A., Riols, C., Dijkstra, C. & Ducrest, A. L. 2001. Female plumage spottiness signals parasite resistance in the barn owl (*Tyto alba*). *Behavioral Ecology*, 12, 103-110.

Rubenstein, D. R. & Lovette, I. J. 2009. Reproductive skew and selection on female ornamentation in social species. *Nature*, 462, 786-789.

Saino, N., Romano, M., Romano, A., Rubolini, D., Ambrosini, R., Caprioli, M., Parolini, M., Scandolara, C., Bazzi, G. & Costanzo, A. 2015. White tail spots in breeding barn swallows *Hirundo rustica* signal body condition during winter moult. *Ibis*, 157, 722-730.

Sandell, M. I. 2007. Exogenous testosterone increases female aggression in the European starling (*Sturnus vulgaris*). *Behavioral Ecology and Sociobiology*, 62, 255-262.

Schamel, D. & Tracy, D. M. 1977. Polyandry, replacement clutches, and site tenacity in the red phalarope (*Phalaropus fulicarius*) at Barrow, Alaska. *Bird-Banding*, 48, 314-324.

Schamel, D., Tracy, D. M., Lank, D. B. & Westneat, D. F. 2004. Mate guarding, copulation strategies and paternity in the sex-role reversed, socially polyandrous red-necked phalarope *Phalaropus lobatus*. *Behavioral Ecology & Sociobiology*, 57, 110-118.

Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9, 671-675.

Schoech, S. J., Mumme, R. L. & Moore, M. C., 1991. Reproductive endocrinology and mechanisms of breeding inhibition in cooperatively breeding Florida scrub jays (*Aphelocoma c. coerulescens*). *Condor*, 93, 354-364.

Searcy, W. A. 1988. Do female red-winged blackbirds limit their own breeding densities? *Ecology*, 69, 85-95.

Servedio, M. R., Price, T. D. & Lande, R. 2013. Evolution of displays within the pair bond. *Proceedings of the Royal Society of London B: Biological Sciences*, 280, 20123020.

Setchell, J. M., Smith, T., Wickings, E. J. & Knapp, L. A. 2008. Social correlates of testosterone and ornamentation in male mandrills. *Hormones and Behavior*, 54, 365-372.

Sinervo, B., Miles, D. B., Frankino, W. A., Klukowski, M. & DeNardo, D. F. 2000. Testosterone, endurance, and Darwinian fitness: Natural and sexual selection on the physiological bases of alternative male behaviors in side-blotched lizards. *Hormones and Behavior*, 38, 222-233.

Starck, J. M. 1991. Biogeography and life history of *Turnix suscitator*. *Journal of Zoological Systematics and Evolutionary Research*, 29, 213-237.

Steiger, S. S., Goymann, W. & Kempenaers, B. 2006. Plasma steroid hormones in two Arctic-breeding shorebirds: Monogamy versus polygyny. *General and Comparative Endocrinology*, 147, 133-140.

Stockley, P. & Bro-Jørgensen, J. 2011. Female competition and its evolutionary consequences in mammals. *Biological Reviews*, 86, 341-366.

Stoddard, M. C. & Stevens, M. 2010. Pattern mimicry of host eggs by the common cuckoo, as seen through a bird’s eye. *Proceedings of the Royal Society of London B: Biological Sciences*, 277, 1387-93.

Sturkie, P. D. & Griminger, P. 1986. Body fluids: Blood. In: *Avian Physiology*. 4th edn. (Ed. by P. D. Sturkie), pp. 103-129. New York: Springer-Verlag.

Swenson, R. O. 1997. Sex-role reversal in the tidewater goby, *Eucyclogobius newberryi*. *Environmental Biology of Fishes*, 50, 27-40.

Tarboton, W. R. 1992. Aspects of the breeding biology of the African jacana. *Ostrich*, 63, 158-164.

Tazzyman, S. J., Iwasa, Y. & Pomiankowski, A. 2014. The handicap process favors exaggerated, rather than reduced, sexual ornaments. *Evolution*, 68, 2534-2549.

Thomas, G. H., Szekely, T. & Reynolds, J. D. 2007. Sexual conflict and the evolution of breeding systems in shorebirds. In: *Advances in the Study of Behavior, Volume 37* (Ed. by H. J. Brockmann, T. J. Roper, M. Naguib, K. E. WynneEdwards, C. Barnard & J. Mitani), pp. 279-342. Amsterdam: Academic Press.

Thompson, C. W., Hillgarth, N., Leu, M. & McClure, H. E. 1997. High parasite load in house finches (*Carpodacus mexicanus*) is correlated with reduced expression of a sexually selected trait. *The American Naturalist*, 149, 270-294.

Thong-aree, S., Khobkhet, O., Lauhachinda, V. & Pong-Umpai, S. 1995. Breeding biology of pheasant-tailed jacana *Hydrophasianus chirurgus* in central Thailand. *Natural History Bulletin of the Siam Society*, 43, 289-302.

Thuman, K. A., Widemo, F. & Piertney, S. B. 2002. Characterization of polymorphic microsatellite DNA markers in the ruff (*Philomachus pugnax*). *Molecular Ecology Notes*, 2, 276-277.

Tobias, J. A., Montgomerie, R. & Lyon, B. E. 2012. The evolution of female ornaments and weaponry: Social selection, sexual selection and ecological competition. *Philosophical Transactions of the Royal Society B*, 367, 2274-2293.

Tonra, C. M., Marini, K. L. D., Marra, P. P., Germain, R. R., Holberton, R. L. & Reudink, M. W. 2014. Color expression in experimentally regrown feathers of an overwintering migratory bird: Implications for signaling and seasonal interactions. *Ecology and Evolution*, 4, 1222-1232.

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man* (Ed. by B. Campbell), pp. 136-179. Chicago: Aldine.

Vágási, C. I., Pap, P. L., Vincze, O., Benkö, Z., Marton, A. & Barta, Z. 2012. Haste makes waste but condition matters: Molt rate-feather quality trade-off in a sedentary songbird. *PLoS ONE*, 7, e40651.

Valle, C. A. 1994. Parental role-reversed polyandry and paternity. *The Auk*, 111, 476-478.

Veiga, J. P. & Polo, V. 2008. Fitness consequences of increased testosterone levels in female spotless starlings. *The American Naturalist*, 172, 42-53.

Vernon, C. J. 1971. Notes on the biology of the black coucal. *Ostrich*, 42, 242-258.

Webster, M. S., Tarvin, K. A., Tuttle, E. M. & Pruett-Jones, S. 2004. Reproductive promiscuity in the splendid fairy-wren: Effects of group size and auxiliary reproduction. *Behavioral Ecology*, 15, 907-915.

Whitfield, D. P. 1986. Plumage variability and territoriality in breeding turnstone *Arenaria interpres*: Status signaling or individual recognition? *Animal Behaviour*, 34, 1471-1482.

Whitfield, D. P. 1990. Male choice and sperm competition as constraints on polyandry in the red-necked phalarope *Phalaropus lobatus*. *Behavioral Ecology and Sociobiology*, 27, 247-254.

Whittingham, L. A., Taylor, P. D. & Robertson, R. J. 1992. Confidence of paternity and male parental care. *American Naturalist*, 139, 1115-1125.

Wiebe, K. L. & Vitousek, M. N. 2015. Melanin plumage ornaments in both sexes of Northern flicker are associated with body condition and predict reproductive output independent of age. *The Auk: Ornithological Advances*, 132, 507-517.

Wingfield, J. C. 1984. Androgens and mating systems: Testosterone-induced polygyny in normally monogamous birds. *The Auk*, 101, 665-671.

Wingfield, J. C., Hegner, R. E., Dufty, A. M., Jr. & Ball, G. F. 1990. The “challenge hypothesis”: Theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *The American Naturalist*, 136, 829-846.

Woodley, S. K. & Moore, M. C. 1999. Female territorial aggression and steroid hormones in mountain spiny lizards. *Animal Behaviour*, 57, 1083-1089.