

Parental role division predicts avian preen wax cycles

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Previous studies have shown that preen wax composition in some sandpipers shifts from the usual monoesters to diesters during the breeding season, possibly to reduce the ability of mammalian predators to find nests using olfactory cues. To investigate further the relationship between incubation and wax secretion, we examined seven sandpiper species with different incubation patterns (species in which both sexes incubate, in which only males incubate and in which only females incubate). During the breeding period, diester preen wax was secreted almost exclusively by the incubating sex in species with uniparental incubation, and by both sexes in species with biparental incubation. These findings suggest that diester preen waxes have a function that is directly related to incubation. Unexpectedly, in female-incubating Curlew Sandpiper *Calidris ferruginea* and Buff-breasted Sandpiper *Tryngites subruficollis*, some males also secreted diester preen waxes during the breeding period. This suggests that some males may in fact incubate, that these waxes may be a remnant from their evolutionary past when both sexes incubated, or that males need to be olfactorily cryptic because they are involved in the making of nest scrapes. The seasonal pattern of preen wax composition was also studied in captive male, female and female-mimicking male ('faeder') Ruff *Philomachus pugnax*. Captive female Ruff changed preen wax composition from monoesters to diesters in the spring despite the fact that no incubation took place. This suggests that circannual rhythms rather than actual incubation behaviour may trigger the shift to diester waxes. All captive male Ruff, including the faeders, continued to secrete monoesters, supporting the hypothesis that only the incubating sex secretes diesters.

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Ducks and sandpipers show seasonal changes in the chemical composition of preen gland secretions (Jacob *et al.* 1979, Kolattukudy *et al.* 1987, Piersma *et al.* 1999, Reneerkens *et al.* 2002, 2007). Preen waxes are secreted by the uropygial gland ('preen gland'), and are applied to the feathers by the bill during daily maintenance activities. Waxes probably help keep the plumage waterproof, reduce feather abrasion (Jacob & Ziswiler 1982), and repel feather-degrading mites and bacteria (Moyer *et al.* 2003, Shawkey *et al.* 2003, J. Reneerkens *et al.* unpubl. data). Seasonal changes in preen wax composition, however, suggest that waxes serve different functions during different periods within an annual cycle.

The most striking shift in preen wax composition of sandpipers occurs just before the start of the breeding season when, within a few weeks, preen gland secretions consisting entirely of monoester waxes are replaced with waxes composed entirely of diesters (Piersma *et al.* 1999, Sinninghe Damsté *et al.* 2000, Reneerkens *et al.* 2002). This chemical shift is presumably endogenously triggered (Reneerkens *et al.* 2007). The secretion of diester preen waxes in sandpipers shows a clear temporal correlation with the breeding period (Reneerkens *et al.* 2002). Experimental evidence shows that the less volatile diester waxes are more difficult for olfactory-searching predators to detect, and their use may thus help prevent nest discovery in the wild (Reneerkens *et al.* 2005). An earlier hypothesis, that the shift to diester preen waxes might serve as an avian cosmetic (Piersma *et al.* 1999), has been rejected by Reneerkens and Korsten (2004). As diester waxes are not produced year-round, we infer that this potential advantage during incubation is outweighed by a different balance between costs and benefits at other times of the year. For example, it is not known if the physiological cost of producing these waxes differs, or if one of them is more effective in protecting feathers. To understand better the role that incubation plays in the production of diester waxes, we investigated the presence of diester preen wax secretion during the breeding season among seven species of sandpipers specifically chosen because of their contrasting male and female incubation patterns.

Sandpipers are an ideal group of birds to investigate preen wax secretion because they show great diversity in breeding systems, ranging from polyandrous species with sole male parental care, biparental monogamous species with shared incubation and chick care, uniparental care at different nests by both sexes, and lekking species, in which males play no

parental role beyond fertilization (Pitelka *et al.* 1974, Piersma *et al.* 1996). This variation within a group of closely related species can be used in a comparative manner to investigate functional aspects of physiological traits related to the period of reproduction. For example, across sandpiper species, the adrenocortical stress response during the breeding season in individuals that are most responsible for parental care is lower than that of individuals that are less responsible for parental care (O'Reilly & Wingfield 2001).

METHODS

Breeding system of study species

The incidence of diester preen wax secretion was compared between males and females in sandpiper species with different mating systems. We selected two species with biparental incubation, Red Knot *Calidris canutus* and Western Sandpiper *Calidris mauri*. Red Knots are monogamous, and equally share incubation duties in shifts of 15–20 h (Tulp *et al.* 1998); females usually depart soon after hatching, leaving the males to care for the brood (Whitfield & Brade 1991, Harrington 2001). Western Sandpipers are also monogamous and both parents incubate, but unlike Red Knots, males spend an increasing proportion of time incubating as the hatching date approaches (Erckmann 1981). After hatching, males also usually remain with the young longer than females (Holmes 1971). In Temminck's Stint *Calidris temminckii*, both sexes incubate and rear young, but do so independently (uniparental care with different clutches and broods). First clutches are incubated by the female's first mate, and the second clutch is incubated by the females themselves (Hildén 1975, Breieghagen 1988).

We also studied five species with uniparental care. In Curlew Sandpipers *Calidris ferruginea*, pair bonds form, but incubation and parental care is solely by females (Holmes & Pitelka 1964, Tomkovich 1988), although males make scrapes which might form the future nest used by the female (Holmes & Pitelka 1964, Pitelka *et al.* 1974). In lekking Buff-breasted Sandpipers *Tryngites subruficollis* and Ruffs *Philomachus pugnax*, only females incubate and tend chicks (van Rhijn 1991, Lancot & Laredo 1994), and males are not known to be involved in nest construction. Finally, in Red Phalaropes *Phalaropus fulicarius*, sex roles are reversed and males provide all care during incubation and chick guarding (Tracy *et al.* 2002).

Sampling birds in the field and in captivity

All selected species breed in either the Arctic or sub-Arctic tundra, except for Ruff, which also breeds in temperate climate zones (Piersma *et al.* 1996). Birds were typically caught during territory establishment and incubation (May to early July), although Ruffs were caught during migration *en route* to breeding areas located further north.

Birds were captured during staging and pre-nesting mainly by using wind-assisted clap-nets, and during incubation with small spring-triggered bow-nets placed over nests. Individuals that do not incubate (male Buff-breasted Sandpipers and Curlew Sandpipers and female Red Phalaropes) were captured prior to the start of incubation. This was necessary because these individuals typically leave the breeding grounds soon after females are fertilized or have laid eggs (van de Kam *et al.* 2004) and would be impossible to capture otherwise. Capturing birds at this time should not bias the detection of diesters, as previous studies indicate that most (*c.* 80%) sandpipers that do incubate have completed the shift into diester preen waxes during the courtship period (Reneerens *et al.* 2002). Birds guarding chicks were excluded from the analysis, as they are known to secrete monoester preen waxes only (Reneerens *et al.* 2002).

To provide a detailed profile of seasonal change in preen wax composition and its potential relationship with incubation behaviour, we sampled five male and five female captive Ruff on a weekly basis between 4 April and 11 July 2001. Most of these females laid eggs, but these were removed immediately and the females did not incubate. Two experimental males died before the end of the season, reducing the number of male samples for the latter part of the season. In addition to these ten captive birds, we collected preen wax samples from two captive 'faeder' Ruffs; faeders are rare males that permanently mimic females as part of their mating strategy (Jukema & Piersma 2006). The sexually active faeders were sampled on 5 June 2006, at the height of the breeding season.

Seasonal shifts in preen wax composition have not previously been described for Buff-breasted Sandpipers. Therefore, in addition to samples from breeding grounds in Alaska, preen wax samples were also collected from a wintering location in Brazil, in December 2001. More details on the locations of the study sites are given in Table 1.

Sexing methods

For all individuals, we measured bill length, wing length (maximum chord, stretched and flattened),

Table 1. Percentage of individuals that secreted diester waxes for seven sandpiper species with different parental care systems. Sample size for each sex and the average fraction of the preen wax sample of all individuals that was composed of diesters is given in parentheses.

Species	Role division during incubation	Site*	Sample period	Life-cycle stage	Diester secretion†	
					Males	Females
Red Knot <i>Calidris canutus</i>	biparental	A,B,E	1 June–14 July	pre-nesting and incubation	100 (24, 0.94)	100 (16, 1.00)
Western Sandpiper <i>Calidris mauri</i>	biparental	C	7 June–6 July	incubation	100 (17, 1.00)	100 (18, 0.96)
Temminck's Stint <i>Calidris temminckii</i>	uniparental (by either parent)	D	2 June–7 July	pre-nesting and incubation	100 (27, 1.00)	100 (29, 1.00)
Curlew Sandpiper <i>Calidris ferruginea</i>	female-only	E	7 June–16 July	pre-nesting and incubation	46.2 (13, 0.43)	97.0 (33, 0.94)
Ruff <i>Philomachus pugnax</i>	female-only	F	14 March–17 May	spring migration	0 (47, 0.00)	51.6 (31, 0.28)
Buff-breasted Sandpiper <i>Tryngites subruficollis</i>	female-only	G	5–7 June	pre-nesting	85.7 (14, 0.07)	100 (5, 1.00)
		H	12–23 December	wintering	0 (15, 0.00)	0 (14, 0.00)
Red Phalarope <i>Phalaropus fulicarius</i>	male-only	E	24 June–21 July	pre-nesting and incubation	85.7 (21, 0.70)	0 (12, 0.00)

*Letter refers to the following study sites: A = Alert, Ellesmere Island, Canada; B = Zackenberg, northeast Greenland; C = Kanagayak, Yukon-Kuskowin Delta, western Alaska; D = Enontekiö and Oulu, Finland; E = various locations in Siberia, Russia (Medusa Bay, Taimyr Peninsula and Chukotka, east Siberia); F = Friesland, The Netherlands; G = Prudhoe Bay, Alaska; H = Estação Ecológica do Taim, Rio Grande, Brazil.

†See text for the method used to estimate the fraction of diesters in the preen gland secretions of individual birds.

tarsus and total head (head and bill) length to the nearest millimetre, and body mass to the nearest gram. We used these measurements either in univariate or multivariate (discriminant function analysis) analyses to distinguish the sexes of Ruffs (Jukema & Piersma 2006), Buff-breasted Sandpipers (R. Lanctot unpubl. data), Curlew Sandpipers (Prater *et al.* 1977) and Western Sandpipers (Page & Fearis 1971). Red Phalaropes could be sexed reliably on the basis of their plumage (Prater *et al.* 1977), and Red Knot and Temminck's Stint were sexed using molecular techniques (modified protocol after Griffiths *et al.* 1998, Baker *et al.* 1999). Additionally, Buff-breasted Sandpipers were caught in leks and behaviour of the individuals before being caught confirmed sex assignment. We also used molecular methods to confirm the sexes of 13 of the 46 Curlew Sandpipers; all assignments were consistent with those made in the field based on size and plumage only.

Preen wax collection and analysis

Preen wax was sampled by carefully making a smear of the papilla of the preen gland with a cotton bud. The cotton buds with collected waxes were wrapped in aluminium foil to avoid contamination and stored at room temperature or kept refrigerated before shipment to the laboratory of the Royal Netherlands Institute for Sea Research for chemical analysis. The composition of the preen wax secretions was determined on the basis of the characteristic gas chromatogram patterns. These patterns were verified by gas chromatography/mass spectrometry of complete and hydrolysed waxes (cf. Dekker *et al.* 2000, Sinninghe Damsté *et al.* 2000) secreted by both sexes of each species. The fraction of diesters in the secretions was estimated by measuring the area (by integration of the peak area with integration software) of the typical diester peaks in the gas chromatograms of diesters divided by the sum of the integrated area of all peaks (monoesters and diesters).

In cases when, as described for Red Knots, two distinct monoester wax mixtures were identified – 'monoesters A' and 'monoesters B' – (Sinninghe Damsté *et al.* 2000, Reneerckens *et al.* 2007), we determined the relative abundance in the secretions by measuring the surface of the highest peaks of the two monoester mixtures in the gas chromatograms only (cf. Reneerckens *et al.* 2007). In contrast to peaks of diester waxes, the peaks in the gas chromatogram of monoesters A and B overlap and are usually difficult to discriminate visually.

Statistical analysis

We used a generalized linear mixed model with the fraction of diesters in individual preen wax samples as the response variable, 'species' as a random variable, and parental care system (both sexes incubate, whether at the same or different nests; and male-only or female-only incubation) and sex as fixed variables. We used a logit link function in view of the binomial distribution of the data. Wald tests were used to test for the significance of fixed effects at the level of 5% and all two-way interaction terms were tested. The samples of wintering Buff-breasted Sandpipers were excluded from this analysis because incubation does not occur in winter. We did not lump species together within a breeding system and did not use any phylogenetic correction in our statistical tests because the number of species and mating systems did not allow such analysis.

RESULTS

Six of the seven sandpiper species produced both monoesters and diesters in their preen wax (Table 1). The exception was Temminck's Stint, in which both males and females secreted only pure diester preen waxes (Table 1). Both male and female Buff-breasted Sandpiper secreted pure monoester waxes in winter, whereas diester preen waxes were secreted during the breeding season (Table 1). In all species investigated, the total carbon number distribution of the secreted diester waxes ranged between C_{34} and C_{50} , but in a few species (Red Knot, Ruff, Curlew Sandpiper) small amounts of C_{30} – C_{32} diesters were also found, and Temminck's Stint also secreted C_{52} diesters. The majority of the diesters comprise 1,2-diols esterified with straight-chain fatty acids at both positions, but (part of) the shorter chained diesters comprise β -hydroxy fatty acids esterified with a fatty acid at one and an alcohol at the other position (C_{32} – C_{39} diesters in Curlew Sandpipers, C_{34} – C_{37} diesters in Western Sandpipers, C_{32} – C_{40} diesters in Ruffs and C_{35} – C_{43} diesters in Red Phalaropes). Temminck's Stints secreted diesters based on 1,2-diols only.

The secretion of diester preen waxes during the breeding season varied significantly with the sex of the birds within a given breeding system (generalized linear mixed model; Wald statistic = 7.18, $df = 2$, $P = 0.028$). Diester secretion occurred equally in both males and females of species where both sexes incubate (Red Knot, Western Sandpiper and Temminck's Stint; Table 1). In the single species with male-only incubation, the Red Phalarope, only males

secreted diester preen waxes, although three of the 21 incubating males did not (Table 1).

In species with female-only incubation (Ruff, Curlew Sandpiper and Buff-breasted Sandpiper), nearly all females secreted diester preen wax during the breeding season, although at varying concentrations. In Buff-breasted Sandpiper, all females switched entirely to (diol-based) diesters during the breeding season. Male Ruffs never produced diesters (Table 1). Perhaps unexpectedly, diesters were secreted by some male Curlew Sandpipers and Buff-breasted Sandpipers (Table 1). Six of 13 Curlew Sandpiper males secreted diesters during the breeding season (average fraction in all males was 43%). Both male and female Buff-breasted Sandpipers secreted the same monoester preen wax during winter. Male Buff-breasted Sandpipers continued to secrete mainly monoesters during the breeding season, but of a different composition and with a small percentage of diesters (Fig. 1, Table 1). The diesters produced by male Buff-breasted Sandpipers had carbon chain lengths of C_{36} – C_{50} with an even-over-odd dominance, as in Red Knots (Sinninghe Damsté *et al.* 2000). Females also secreted shorter diesters with carbon chain lengths between C_{24} and C_{50} .

Shifts from mono- to diester preen waxes occurred prior to the actual start of incubation, during the period of courtship and mate choice on the breeding grounds in all species investigated here (cf. Reneerkens *et al.* 2002). In Ruffs, several females secreted diesters during migratory refuelling on a stopover site in the Netherlands (Table 1, Fig. 2), but none of the males did.

All five captive female Ruffs that were repeatedly sampled exhibited a change in preen wax composition from monoesters to diesters at the beginning of the breeding season, and then reverted to monoesters following breeding (Fig. 2). As in the free-living Ruffs, the monoester preen wax in the captive females changed from one distinct monoester mixture in winter (monoesters A) to another (monoesters B; Reneerkens *et al.* 2007; Fig. 2) before wax composition shifted to diesters. By contrast, both free-living and captive male Ruffs secreted only monoester A preen waxes throughout the spring and summer. In addition, the two captive faeders (male Ruffs that mimic females in plumage; Jukema & Piersma 2006) secreted only monoesters A in early June, at the height of their breeding season.

DISCUSSION

In seven sandpiper species with varying parental care systems, diester preen wax secretion during the

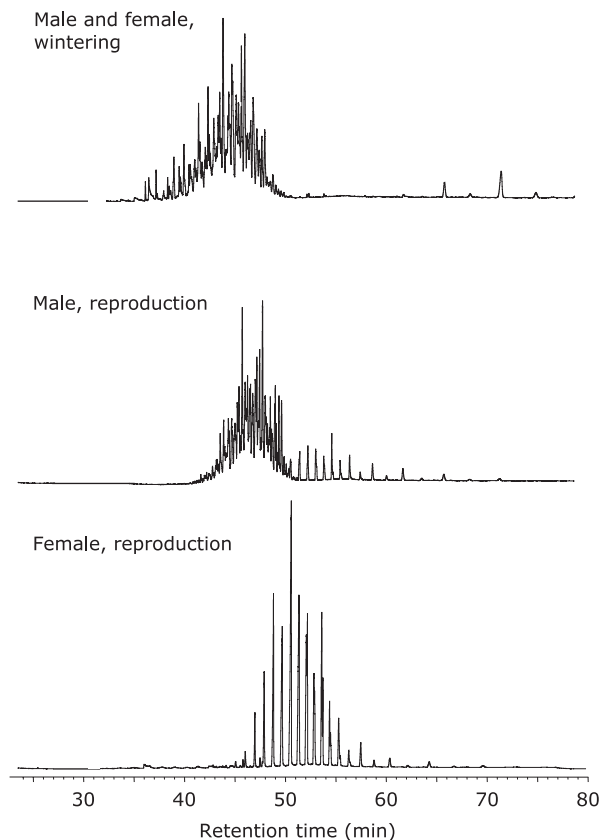


Figure 1. Typical gas chromatograms of preen wax of female and male Buff-breasted Sandpiper in winter (top) and in the Arctic during the pre-breeding period of males (middle) and females (bottom). Note that the typical diester peaks in the gas chromatogram of females in the reproductive period are also visible (right) in the predominantly monoester-based gas chromatograms of males during reproduction.

pre-incubation period of post-migratory arrival, courtship and egg-laying, as well as during incubation (cf. Reneerkens *et al.* 2002), occurred primarily in the incubating sex or sexes. In species where both sexes incubate, diester preen waxes were secreted by both males and females during incubation. In species in which only one of the two sexes incubates, diester preen wax secretion occurred only (or mainly) in the incubating sex. The proportion of diesters in preen waxes of the incubating sex was only less than 90% for Ruffs (Table 1), possibly because estimates were based on samples from individuals caught during spring migration. In a captive population of Ruffs, all females shifted from monoesters to pure diester preen waxes shortly before the start of the breeding season of wild Ruffs (Fig. 2). Although a single female Curlew Sandpiper and three male Red Phalaropes did not

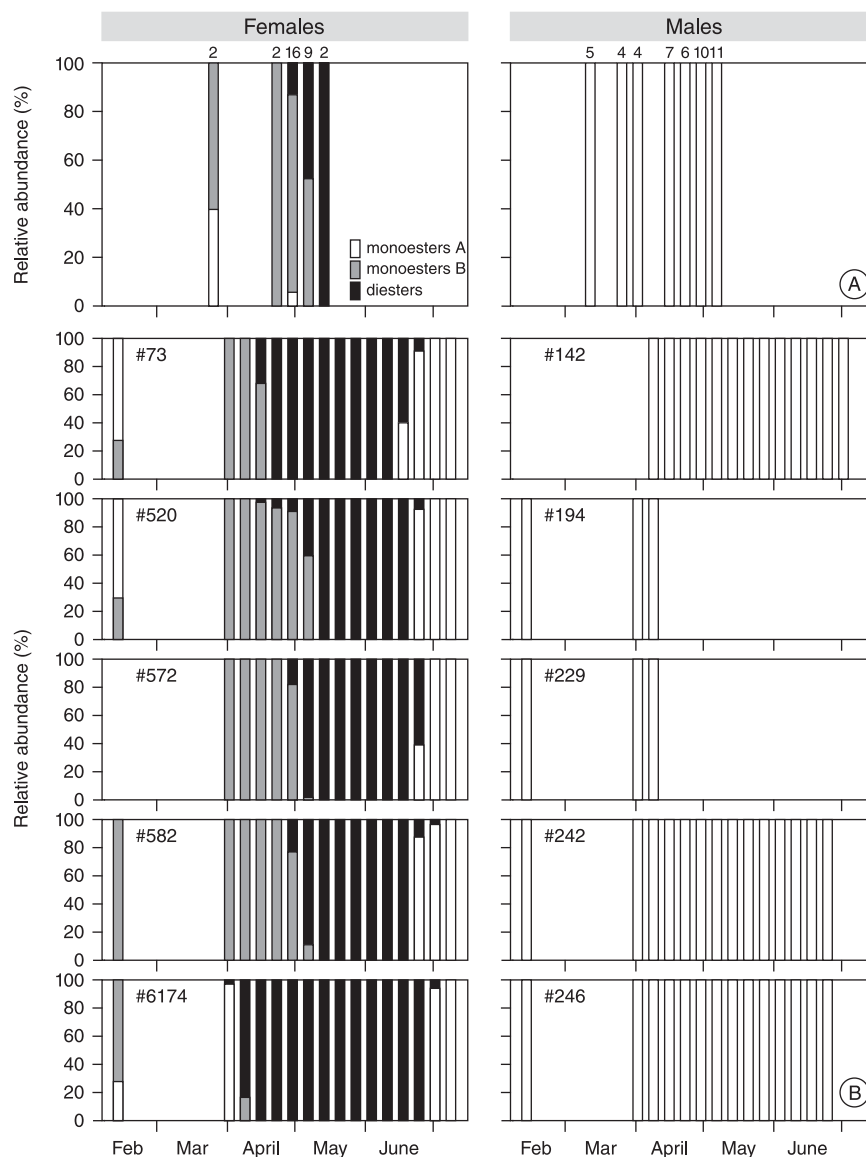


Figure 2. Seasonal changes in preen wax composition of free-living (A) and captive (B) female (left panel) and male (right panel) Ruffs in spring. Average preen wax composition per week is shown with monoester A in white, monoesters B shown in grey, and diesters in black. Numbers in parentheses in (A) indicate sample sizes in each week. Each block in (B) represents individual birds, which are denoted by their ring numbers (#).

secrete (pure) diesters during incubation, the overall pattern strongly suggests that diester preen waxes are most important for individual sandpipers that incubate. That only incubating adults secrete diester preen waxes is supported by the observations of Reneerkens *et al.* (2005), who showed experimentally that the less volatile diester preen waxes were more difficult to detect by a sniffer dog than were monoester waxes. The secretion of diester preen waxes appears to make incubating birds and their

nests more cryptic and thus less detectable to mammalian predators using olfactory cues.

If shifts to diester preen waxes occur only in individuals of the sex that usually incubates, why then do birds secrete diesters prior to incubation, such as during courtship and egg-laying (Reneerkens *et al.* 2002) and spring migration in Red Knots (Piersma *et al.* 1999) and Ruffs (Fig. 2)? Like the captive Ruffs in this study, Red Knots in captivity switched from monoester to diester preen waxes at the same time

as their free-living conspecifics, even though they did not actually incubate. Preen wax shifts from monoesters to diesters in Red Knots appear to be under endogenous control (Reneerkens *et al.* 2007), which enables feathers to be coated with diesters in time for the onset of incubation; this may explain why diester preen waxes are secreted and presumably accumulate on feathers for several weeks before the potential onset of incubation in most individuals.

Diester preen waxes are unlikely to play a role as a visual quality signal or 'avian make-up' (Reneerkens & Korsten 2004) during the pre-incubation period. The increased difficulty for predators to smell diester preen waxes demonstrated by Reneerkens *et al.* (2005) may, however, already be important before the actual start of incubation. Arctic sandpipers create nest cups by scraping their breast on the ground and may thereby unintentionally transfer preen waxes from their feathers into the nest cups. These potential olfactory traces could make the nest less liable to detection by ground predators using olfactory cues during the egg-laying period. This may have particular selective consequences in the early High Arctic breeding season, when in some years snow cover could conceivably narrow the search area that predators would have to cover to find sandpiper nests.

The importance of secretion of less volatile diester waxes during nest building may also explain why the dichotomy in diester preen wax secretion between sexes is not absolute in two of the three species with female-only incubation. Six of the 13 male Curlew Sandpipers secreted diesters and the preen wax of 12 of the 14 male Buff-breasted Sandpipers also contained diesters during the pre-nesting period, although only very small amounts (an average of 7% compared with the 43% in male Curlew Sandpipers; Table 1). These differences in diester secretions in males of sandpipers with female-only incubation could be explained by species differences in the contribution of males in nest construction. Even though male Curlew Sandpipers are thought not to take part in incubation, they do assist with nest scraping (Holmes & Pitelka 1964). This has, as far as we know, never been described for males of Ruff and Buff-breasted Sandpiper. It is possible that nest scraping behaviour has selectively favoured evolution of seasonal preen wax shifts in male Curlew Sandpipers. This hypothesis, however, cannot explain why only some of the male Curlew Sandpipers shifted to diesters. Neither can it explain why most male Buff-breasted Sandpipers secrete small amounts of diesters during the pre-incubation period. The latter might indicate

a greater involvement of males in the nest-building process or another aspect of Buff-breasted Sandpiper biology that is not presently appreciated, but it is unclear why they do not secrete pure diesters.

An alternative explanation of why male Buff-breasted Sandpipers and Curlew Sandpipers sometimes secrete diester waxes, although only in small amounts in the former species, is that the diester secretion is a remnant of an evolutionary past when both males and females shared incubation. Based on phylogenetic patterns of parental care, Borowik and McLennan (1999) suggested that biparental incubation is ancestral in calidridine sandpipers and that Curlew Sandpipers lost male care, as did Buff-breasted Sandpipers and Ruffs. Male Curlew Sandpipers sometimes develop (incomplete) brood patches (Tomkovich 1988, Tomkovich & Soloviev 2006), which is consistent with this hypothesis. Considering this scenario, the (partial) shifts to diester preen waxes in male Curlew Sandpipers and male Buff-breasted Sandpipers might be a remnant of their past. Ruff would be the only species of the three with female-only incubation in which males have completely lost the ability to produce diester preen waxes. A recent phylogenetic reconstruction of the sandpiper family shows that the Ruff divergence is very ancient and occurred at about the same time as that of Curlew Sandpipers (A.J. Baker unpubl. data). This phylogenetic reconstruction shows that the divergence of Buff-breasted Sandpipers is rather old, too. Therefore, we suggest that diester preen wax secretion by male Buff-breasted Sandpipers and male Curlew Sandpipers might indicate that the loss of male incubation in these species has occurred more recently, or that these species have been subjected to different selection pressures.

Faeders, male Ruffs that mimic females in plumage and 'sneak' copulations at leks, secrete monoesters like other males. Faeders have been proposed as the ancestral male type of Ruffs (Jukema & Piersma 2006), which presumably participated in incubation (van Rhijn 1985). Given the strong correlation between incubation and diester preen wax secretion, the lack of diester preen waxes suggests that faeders are unlikely to participate in any incubation duties at the present time. Consistent with this, behavioural observations of faeders in captive breeding situations have shown no indication that faeders participate in nesting or incubation (D.B. Lank *et al.* unpubl. obs.). Faeders and 'normal' male Ruffs might have been subjected to strong selection pressures to eliminate diester production in their evolutionary past and

consequently have lost this physiological characteristic related to incubation.

The function of the shift from monoesters A to monoesters B, which occurs in female Ruffs (Fig. 2) and in both sexes of Red Knot (Reneerkens *et al.* 2007), remains unclear. The fact that only female Ruffs shift to producing monoesters B suggests that this wax is a discrete transient mixture that is secreted when biosynthesis of monoesters A is changing to diesters. However, because many of the fatty acids that compose monoesters B in Red Knots have branched (methyl-substituted) carbon chains, whereas those that compose diester preen waxes are unbranched (J. Reneerkens *et al.* unpubl. data), we believe that different types of fatty acids have to be synthesized for each preen wax mixture. Male Buff-breasted Sandpipers produce different monoesters in winter and summer, suggesting that they are producing monoesters A and B, as opposed to male Ruffs, which only produce monoester A. The occurrence of a monoester A (during winter) and monoester B (spring) type is now known to occur in many more sandpiper species (J. Reneerkens *et al.* unpubl. data).

In summary, this comparative study on sandpipers revealed that seasonal shifts in preen wax composition from monoester to diester preen waxes are largely restricted to incubating birds, but also occur (facultatively or in small concentrations) in some males of species that presumably have lost paternal care. In these males, increasing olfactory crypsis by seasonal preen wax shifts may also be involved because they make nest scrapes, but this remains to be investigated. While we suggest that diester preen waxes are useful during nest construction and incubation for birds to become more cryptic from mammalian predators, we do not yet understand what their drawbacks are for use under other conditions. Possibilities include higher production costs and/or lower effectiveness with respect to the alternative functions of preen waxes. We suggest that future studies should experimentally address the premises of differences between the monoester and diester preen waxes relative to cost in syntheses and efficiency in protecting feathers.

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