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Sandpipers (Scolopacidae) switch from monoester to diester preen waxes during courtship and incubation, but why?

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Recently, a shift in preen wax composition, from lower molecular weight monoesters to higher molecular weight diesters, was described for individuals of a sandpiper species (red knot, *Calidris canutus*) that were about to leave for the tundra breeding grounds. The timing of the shift indicated that diester waxes served as a quality signal during mate choice. Here, this hypothesis is evaluated on the basis of a survey of preen wax composition in 19 sandpiper species. All of these species showed the same shift observed in the high-Arctic breeding red knots. As the shift also occurred in temperate breeding species, it is not specific to tundra-breeding sandpipers. Both sexes produced the diester waxes during the incubation period until hatching, in addition to the short period of courtship, indicating that diesters' functions extend beyond that of a sexually selected 'make-up'. The few non-incubating birds examined (males of curlew sandpipers (*C. ferruginea*) and ruffs (*Philomachus pugnax*)) had the lowest likelihood of secreting diesters, indicating a functional role for diester preen waxes during incubation. We propose that diester preen waxes enhance olfactory crypticism at the nest.

Keywords: uropygial gland; sandpipers; mate choice; mating system; chemical ecology

1. INTRODUCTION

A complete and abrupt shift has recently been discovered in the chemical composition of secretions from the uropygial gland (preen gland) in high-Arctic breeding red knots (*Calidris canutus*) (Piersma *et al.* 1999). Although these secretions were previously considered invariable and taxon-specific (Jacob & Ziswiler 1982), a rare class of diesters (Sinninghe Damsté *et al.* 2000) completely replaces the usual mixture of monoesters at the start of courtship in this species.

The uropygial gland secretions are preened into the plumage (hence the name 'preen waxes') and several functions have been proposed (Jacob & Ziswiler 1982). Preen waxes may delay feather wear, keep feathers flexible (Stettenheim 1972) and waterproof (Elder 1954; but see Fabricius 1959; Elowson 1984) and have anti-dermatophytic characteristics (Jacob *et al.* 1997). This compositional shift indicated an additional function for diester preen waxes during the period of courtship and mating (Piersma *et al.* 1999). Diester preen waxes are more viscous than monoester mixtures and they may be difficult to preen into the plumage under the prevailing cold temperatures during the summer season in the high Arctic. The change to a preen wax mixture that brings about additional costs led Piersma *et al.* (1999) to propose that diester preen waxes may function as a sexually selected quality signal, perhaps by enhancing the appearance or reflectance of the plumage.

In this study, we explore the idea that diester preen waxes function as a sexual signal. We do so in a comparative way by studying the chemical composition of preen gland secretions before, during and after the reproductive period in 19 closely related sandpiper species of the Charadriiform family Scolopacidae.

2. METHODS

(a) *Birds*

Sandpipers were caught at various stages of their annual cycle and preen wax samples were collected. All investigated species are migratory and use different areas for reproduction and wintering, often thousands of kilometres apart. Except for black-tailed godwits (*Limosa limosa*), redshanks (*Tringa totanus*), Asian dowitchers (*Limnodromus semipalmatus*) and some of the ruffs (*Philomachus pugnax*) that breed in temperate regions, all investigated sandpipers reproduce on the (sub-) Arctic tundra (table 1). They typically winter in (sub-) tropical or temperate coastal salt-water habitats (Piersma 1997). To reach the Arctic breeding areas between late May and early June, sandpipers make long-distance flights of thousands of kilometres, with one or two intermediate refuelling stops in wetland habitats (summarized in Piersma *et al.* 1996).

(b) *Sex and life-cycle stage*

The composition of preen wax secretions was studied in relation to sex and life-cycle stage. We determined the sex of the birds by examining sex-specific plumage traits, size differences and/or sex-specific behaviour (e.g. incubation in some of the species). The sexually monomorphic red knots were sexed using a standard and verified molecular technique (Baker *et al.* 1999). Most sanderlings (*C. alba*), semipalmated (*C. pusilla*), Baird's (*C. bairdii*) and white-rumped sandpipers (*C. fuscicollis*) were not sexed individually. Sexes of red phalaropes (*Phalaropus fulicarius*), Hudsonian godwits (*L. haemastica*) and bar-tailed

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Table 1. Frequencies of diester preen waxes that individuals of 19 sandpiper species secrete during spring migration, pre-breeding, incubation, chick guarding, autumn migration and during winter.
(Individuals that secreted mixtures of monoesters and diesters were scored as 0.5.)

	species		adults						
subfamily	common name (scientific name)	breeding range	spring migration	pre-breeding	incubation	chick guarding	autumn migration	winter	juveniles' breeding grounds
godwits	black-tailed godwit (*Limosa limosa*)	temperate	1/10		11/11	2/2			
	Hudsonian godwit (*Limosa haemastica*)	low Arctic	0/3		1/1				
	bar-tailed godwit (*Limosa lapponica*)	high Arctic	0.5/34	1/1			0/26	0/9	
shanks	redshank (*Tringa totanus*)	temperate	0/7		45/48	0.5/2	0/4		
turnstones	ruddy turnstone (*Arenaria interpres*)	high Arctic	0/40	10.5/12	15/15	0.5/2	0/19	0/3	0/6
phalaropes	red phalarope (*Phalaropus fulicarius*)	low and high Arctic			1.5/2		0/1		0/3
dowitchers	Asian dowitcher (*Limnodromus semipalmatus*)	temperate			1.5/2			0/4	
	short-billed dowitcher (*Limnodromus griseus*)	low Arctic	0/2		3/3				
sandpipers	red knot (*Calidris canutus*)	high Arctic	0.5/65	24/26	14.5/15	0/3	0/33	0/9	0/8
	sanderling (*Calidris alba*)	high Arctic	0.5/20	2/3	4/4		0/18		
	semipalmated sandpiper (*Calidris pusilla*)	low and high Arctic	0/6				0.5/1		
	western sandpiper (*Calidris mauri*)	high Arctic	0/4		32.5/33				
	little stint (*Calidris minuta*)	high Arctic	0/2	11/12	11/11	0.5/1	0.5/19	0/10	0/13
	Temminck's stint (*Calidris temminckii*)	low and high Arctic			2/2			0/2	
	white-rumped sandpiper (*Calidris fuscicollis*)	high Arctic	0/9		3/4				0/16
	Baird's sandpiper (*Calidris bairdii*)	high Arctic			16.5/17				0/2
	dunlin (*Calidris alpina*)	low and high Arctic	0.5/14	5/7	11/11	0.5/1	0/14	0/8	0/7
	curlew sandpiper (*Calidris ferruginea*)	high Arctic		11.5/20	6/6	0/2	0/20	0/5	0/8
	ruff (*Philomachus pugnax*)	temperate–high Arctic	10/92					0/9	
all species			13/308	65/81	177.5/185	4/13	1/155	0/59	0/63
percentage			4	80	96	13	0	0	0

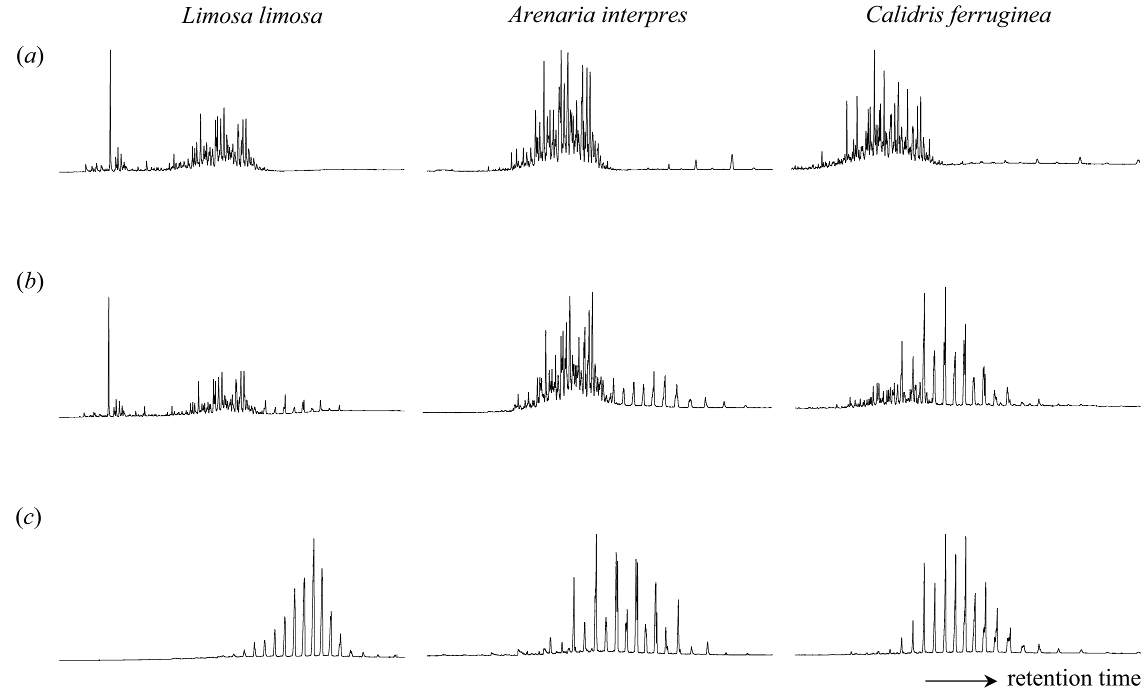

Figure 1. Gas chromatograms of typical (*a*) monoester, (*b*) mono/diester, and (*c*) diester secretions of black-tailed godwit (*Limosa limosa*), ruddy turnstone (*Arenaria interpres*) and curlew sandpiper (*Calidris ferruginea*).

godwits (*L. lapponica*) could not be compared, as a single individual or only one of the sexes was caught during courtship and incubation.

Birds caught shortly after arrival on the Arctic breeding grounds were considered to be in the period of mate choice and courtship. High-Arctic breeding sandpipers start courtship displays within a few days of arrival (e.g. Reneerkens *et al.* 2002). Sometimes they arrive already paired-up, as observed in some of the curlew sandpipers (H. Schekkerman and I. Tulp, personal communication). Birds on the breeding grounds with fully developed brood-patches were considered to be incubating even if not caught on the nest.

In redshanks and western sandpipers (*C. mauri*), it was possible to relate the composition of preen-gland secretions to the number of days before hatching. Hatching dates were calculated from known laying dates, using the incubation lengths (24 days and 19 days, respectively) measured at the study sites (A. Niehaus and W. Tijssen, personal communication).

(c) *Sample processing*

By softly massaging the nipple of the preen gland, a tiny sample of preen wax can be obtained on a cotton bud. The waxes were dissolved in ethyl acetate. We then evaporated the solvent with a gentle flow of nitrogen gas and weighed the waxes (ranging from 0.1–4.3 mg). Subsequently, the waxes were redissolved in ethyl acetate to a concentration of $1\ \mathrm{mg\ ml^{-1}}$ and injected into a gas chromatograph (GC). Details of the analytic procedures are described elsewhere (Dekker *et al.* 2000). Gas chromatograms of the wax mixtures are characteristic for either mono- and diesters (Piersma *et al.* 1999; Sinninghe Damsté *et al.* 2000) enabling easy classification of samples into three groups: (i) monoesters, (ii) diesters and (iii) a mixture of mono- and diesters (figure 1). This classification was confirmed by GC and by GC followed by mass spectrometry analysis of hydrolysed waxes (cf. Dekker *et al.* 2000). We scored the fraction of diesters in the preen waxes as: 0, only monoesters; 0.5, mixture of mono- and diesters; and 1, predominantly (more than 95%) diesters.

3. RESULTS

During migration and in winter, all 19 investigated sandpiper species secreted mixtures of monoester preen waxes. As in red knots (Piersma *et al.* 1999), shifts from mono- to diester waxes only occurred at the start of courtship and mating (table 1; figure 2).

Shortly before departure to the breeding grounds (usually late May), diester waxes were produced by a few individuals. By contrast, shortly after arrival on the breeding grounds, the majority of individuals (80%) secreted diesters (table 1). This indicates that the shift in preen wax composition occurs around arrival on the Arctic tundra. Almost all individuals (96%) produced diesters during incubation (table 1). Only a few incubating redshanks and western sandpipers secreted (some) monoester compounds five days or less before hatching. However, most adults with chicks secreted monoester preen waxes (table 1), indicating a sharp shift from diesters to monoesters at hatching. During autumn migration and winter preen-gland secretions never consisted of diesters (table 1). Recently fledged juveniles (63 individuals of eight species) only secreted monoester waxes (table 1).

Redshanks showed more overlap in the temporal pattern of mono- and diester secretion than other species (figure 2). This is caused by the temporal overlap of life-cycle stages (table 1). Where verifiable, diester secretion during courtship and incubation occurred evenly in both sexes. However, curlew sandpipers and ruffs were different. In curlew sandpipers, only two of the 10 male curlew sandpipers secreted complete diester mixtures shortly after arrival on the breeding grounds. By contrast, eight of the 10 females from this period secreted diester waxes only

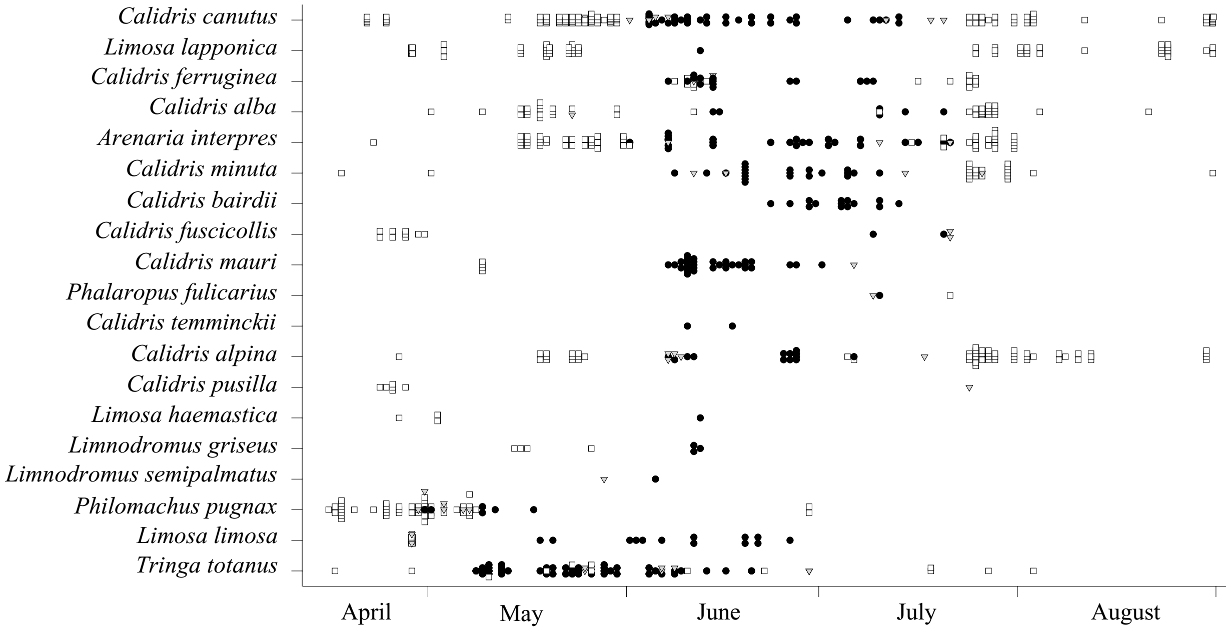

Figure 2. Seasonal changes in chemical composition of preen waxes of adult birds in 19 sandpiper species. Species are ordered from top to bottom on the basis of median lattitude of their breeding range, with the northernmost breeding species first. Squares, monoesters; triangles, mixture of mono- and diesters; filled circles, diesters.

(Mann–Whitney *U*-test with tied ranks, $U_{1,20} = 82.0$, $p = 0.008$). Of the 49 male and 43 female ruffs caught during spring migration (table 1), none of the males and 14 of the females produced (mixtures with) diesters (figure 2). All captive female (and no male) ruffs sampled during the period of incubation secreted diester preen waxes (J. Reneerkens and D. B. Lank, unpublished data).

4. DISCUSSION

(a) *How common are changes from mono- to diester preen waxes?*

Jacob & Poltz (1973) and Jacob (1978) characterized preen wax components of seven shorebird species, including three of the species that we investigated (redshank, red knot and dunlin) and found monoesters with some traces of diesters. It is not known when their samples were taken. In this study, we demonstrated that in all 19 species examined, during the short period of mate choice and incubation, wax composition shifted from mono- to diesters. As temperate breeding species start their reproductive activities earlier than high-Arctic breeding species, diesters are secreted earlier in temperate breeding species (black-tailed godwit, redshank and ruff; figure 2).

(b) *What function(s) do diester preen waxes serve?*

In all 19 sandpiper species, diester waxes are secreted during the relatively brief periods of courtship and incubation, indicating a common function shaped by the specific demands during these life-cycle stages. Because adults (as well as juveniles) of Arctic breeding species excrete monoesters after hatching, Arctic conditions (e.g. low temperatures, strong winds and high ultraviolet radiation) are unlikely to have selectively favoured diester preen waxes as a way of plumage protection. As they also occur in redshanks, black-tailed godwits and Asian dowitchers, shifts to diesters are not restricted to Arctic breeders. The more distantly related oystercatcher (*Haematopus ostralegus*, Haematopodidae) breeds and overwinters in western Europe and also shifts to diester preen waxes during the breeding season (J. Reneerkens, unpublished data).

Ruffs and curlew sandpipers are, to our knowledge, the only two investigated sandpipers in which incubation is completely or largely restricted to females. Secretion of diester waxes is also restricted to female ruffs and occurs significantly more often in female than in male curlew sandpipers. In wild-type and domesticated mallards (*Anas platyrhynchos*), females, but not males, show similar qualitative shifts from mono- to diester preen waxes during courtship and incubation (Jacob *et al.* 1979; Kolattukudy *et al.* 1987). In mallards, incubation is also restricted to females. The change to diester wax secretion in incubating individuals indicates that diesters are important for birds on the nest. Diester preen waxes have higher molecular weights than monoesters and consequently are less volatile. Thus, they may reduce the smell and enhance olfactory crypticism. If diesters make it more difficult for mammalian predators, such as Arctic foxes (*Alopex lagopus*), to smell out the bird on the nest, a shift from mono- to diester preen waxes during incubation would have a large selective advantage.

Piersma *et al.* (1999) proposed that diesters enhance sexually selected quality signals that make the plumage brighter or shinier, enabling visual discrimination of fit mates during mate choice. The present study does not falsify this hypothesis, but indicates that it is not the whole story because diester preen waxes are also secreted during incubation, that is, after fertilization. Small differences in the smell or in the visibility of plumage due to different wax compositions may not be detectable by the human senses (e.g. Viitala *et al.* 1995). However, if different preen wax compositions can be distinguished by conspecifics, they could potentially play a part during mate choice before becoming fully functional during incubation.

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REFERENCES

Baker, A. J., Piersma, T. & Greenslade, A. D. 1999 Molecular versus phenotypic sexing in red knots. *Condor* **101**, 887–893.

Dekker, M. H. A., Piersma, T. & Sinninghe Damsté, J. S. 2000 Molecular analysis of intact preen waxes of *Calidris canutus* (Aves: Scolopacidae) by gas chromatography/mass spectrometry. *Lipids* **35**, 533–541.

Elder, W. H. 1954 The oil gland of birds. *Wilson Bull.* **66**, 6–31.

Elowson, A. M. 1984 Spread-wing postures and the water repellency of feathers: a test of Rijke's hypothesis. *Auk* **101**, 371–383.

Fabricius, E. 1959 What makes plumage waterproof? *Wildfowl Trust Rep.* **10**, 105–113.

Jacob, J. 1978 Chemotaxonomic relationships within the order Charadriiformes. *Biochem. Syst. Ecol.* **6**, 347–350.

Jacob, J. & Poltz, J. 1973 Chemotaxonomische Untersuchungen an Limikolen. Die Zusammensetzung des Bürzeldrüsen Sekretes von Austernfischer, Rotschenkel, Knutt und Alpenstrandläufer. *Biochem. Syst.* **1**, 169–172.

Jacob, J. & Ziswiler, V. 1982 The uropygial gland. In *Avian biology*, vol. 4 (ed. D. S. Farner, J. R. King & K. C. Parkes), pp. 199–324. New York: Academic.

Jacob, J., Balthazart, J. & Schoffeniels, E. 1979 Sex differences in the chemical composition of uropygial gland waxes in domestic ducks. *Biochem. Syst. Ecol.* **7**, 149–153.

Jacob, J., Eigener, U. & Hoppe, U. 1997 The structure of preen gland waxes from pelecaniform birds containing 3,7-dimethyloctan-1-ol: an active ingredient against dermatophytes. *Zeitschrift für Naturforschung* **52**, 114–123.

Kolattukudy, P. E., Bohnet, S. & Rogers, L. 1987 Diesters of 3-hydroxy fatty acids produced by the uropygial glands of female mallards uniquely during the mating season. *J. Lipid Res.* **28**, 582–588.

Piersma, T. 1997 Do global patterns of habitat use and migration strategies coevolve with relative investments in immunocompetence due to spatial variation in parasite pressure? *Oikos* **80**, 623–631.

Piersma, T., van Gils, J. & Wiersma, P. 1996 Family Scolopacidae (sandpipers, snipes and phalaropes). In *Handbook of the birds of the world,* vol. 3. *Hoatzin to Auks* (ed. J. del Hoyo, A. Elliott & J. Sargatal), pp. 444–533. Barcelona, Spain: Lynx Edicions.

Piersma, T., Dekker, M. & Sinninghe Damsté, J. S. 1999 An avian equivalent of make-up? *Ecol. Lett.* **2**, 201–203.

Reneerkens, J., Morrison, R. I. G., Ramenofsky, M., Piersma, T. & Wingfield, J. C. 2002 Baseline and stress-induced levels of corticosterone during different life-cycle sub-stages in a shorebird on the High Arctic breeding grounds. *Physiol. Biochem. Zool.* **75**, 200–208.

Sinninghe Damsté, J. S., Dekker, M., van Dongen, B., Schouten, S. & Piersma, T. 2000 Structural identification of the diester preen gland wax in the red knot (*Calidris canutus*). *J. Nat. Prod.* **63**, 381–384.

Stettenheim, P. 1972 The integument of birds. *Avian biology,* vol. II (D. S. Farner & J. R. King), pp. 1–63. New York: Academic.

Viitala, J., Korpimäki, E., Palokangas, P. & Koivula, M. 1995 Attraction of kestrels to vole scent marks visible in ultraviolet light. *Nature* **373**, 425–427.