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S16-1 Discerning adaptive value of seasonal variation in preen waxes: comparative and experimental approaches

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Abstract Birds possess a preen (or uropygial) gland on their rump that secretes substances which are preened into the plumage, and which are probably essential for plumage maintenance. Secretions of the uropygial gland consist predominantly of wax-esters: fatty acids esterified to alcohols. These wax components vary in chain length and in degree and location of branching of the carbon skeletons, resulting in complex mixtures of many different wax esters in preen gland secretions. We have found that shorebirds show pronounced seasonal changes in the composition of their preen waxes. Between arrival on and departure from breeding grounds, their usual monoester wax at winter quarters changes dramatically to a more complex diester-based wax, which is maintained throughout the breeding season. The diesters have higher molecular weights and probably different physical properties than monoesters, and the secretion and use of diesters rather than monoesters may entail specific costs and benefits. We discuss how natural and sexual selection could explain the evolution of compositional shifts in preen waxes and outline possible approaches for future research.

Key words Preen wax, Uropygial gland, Chemical signaling, Annual cycle, Sandpipers

1 Introduction

Almost all birds possess a uropygial or preen gland from which complex mixtures of waxes are secreted and smeared into plumage. The chemical composition of preen waxes have been the subject of many studies, resulting in detailed chemical characterization of the preen wax mixtures in a number of bird species — for review, see Jacob and Ziswiler (1982). The preen waxes in most species consist predominantly of wax esters (esterified alcohols and fatty acid moieties) in which the location and length of branching of the carbon skeleton varies. This results in complex mixtures of waxes (Dekker et al., 1999).

There has been much speculation about the function of uropygial gland secretions (e.g., Jacob, 1976, 1978a and references therein), but they are evidently essential for plumage maintenance. Waxes are hydrophobic and probably contribute to the waterproofing of feathers (Jacob and Ziswiler, 1982). Preen waxes may also protect plumage by delaying abrasion and keeping feathers flexible (Jacob and Ziswiler, 1982), and they can have antiparasitic effects (Jacob et al., 1997).

Recent research on a long distance-migrating, high arctic-breeding shorebird, the red knot (*Calidris canutus*), shows that the chemical composition of preen waxes shifts dramatically from the usual monoester mixture to a mixture consisting solely of diesters just before the breeding season (Piersma et al., 1999; Sinninghe Damsté et al., 2000). Piersma et al. (1999) proposed that the diester waxes could function as a sexually-selected quality signal. A recent comparison of the occurrence and timing of shifts in preen wax composition among 19 different but closely-related sandpiper species (Scolopacidae) showed that, in addition to the period of mate choice, sandpipers also secreted diester preen waxes throughout incubation (Reneerkens et al., 2002). Thus, there is more to the function of diesters than providing a quality signal during mate choice. Here we extend our dataset by providing information for six plover species (Charadriidae) and an oystercatcher (Haematopodidae) for the first time. We also discuss comparative and experimental tests of costs and benefits of mono- and diester- preen waxes that may help to unravel the functional aspects of such qualitative shifts.

2 Materials and methods

Preen wax was sampled from 25 shorebird species of plovers (Charadriidae), sandpipers (Scolopacidae) and oystercatchers (Haematopodidae) on spring and autumn migration, as well as during courtship, incubation and chick rearing. Preen gland secretions were collected on a cotton bud used to massage the preen gland “nipple”. Preen waxes were dissolved in ethyl acetate to a standard concentration of 1 mg/ml and analyzed by gas chromatography as described in Dekker et al. (2000). From visual examination of the gas chromatograms, their composition was then classified into mixtures of (1) monoesters, (2) diesters, and (3) mixtures of both mono- and di- esters (after Reneerkens et al., 2002).

3 Results

Gas chromatography of intact preen waxes revealed substantial changes in chemical composition over the annual cycle of the shorebirds. Shifts from mono- to di- ester preen waxes occurred in all species investigated at the start of courtship and mate choice; it was thus not limited to sandpipers (Fig. 1; Reneerkens et al., 2002). The secretion of diesters was maintained during the whole period of incubation and chick-rearing (Fig 1; Reneerkens et al., 2002).

Diester preen waxes have been described before in several bird species (e.g., Jacob, 1976, and references therein; Jacob 1982); but to our knowledge, comparable annual variation in preen wax composition has been described only for ducks (Jacob et al., 1979; Kolattukudy et al., 1987) and sandpipers (Reneerkens et al., 2002). Livezey et al. (1986) and Levy and Strain (1982) nevertheless noted that use of preen wax composition for phylogenetic reconstruction may be problematic because of intra-specific variation in preen wax composition due to differences between sexes and dietary factors. In red knots, however, there is no evidence for dietary effects on preen wax composition (our unpublished data). Sex differences in preen wax composition during the breeding period have been found in ruffs (*Philomachus pugnax*) and curlew sandpipers (*Calidris ferruginea*) (Reneerkens et al., 2002), and in mallards (*Anas platyrhynchos*).

4 Discussion

4.1 Costs and benefits of different preen wax mixtures

To understand the evolution of variation in preen wax

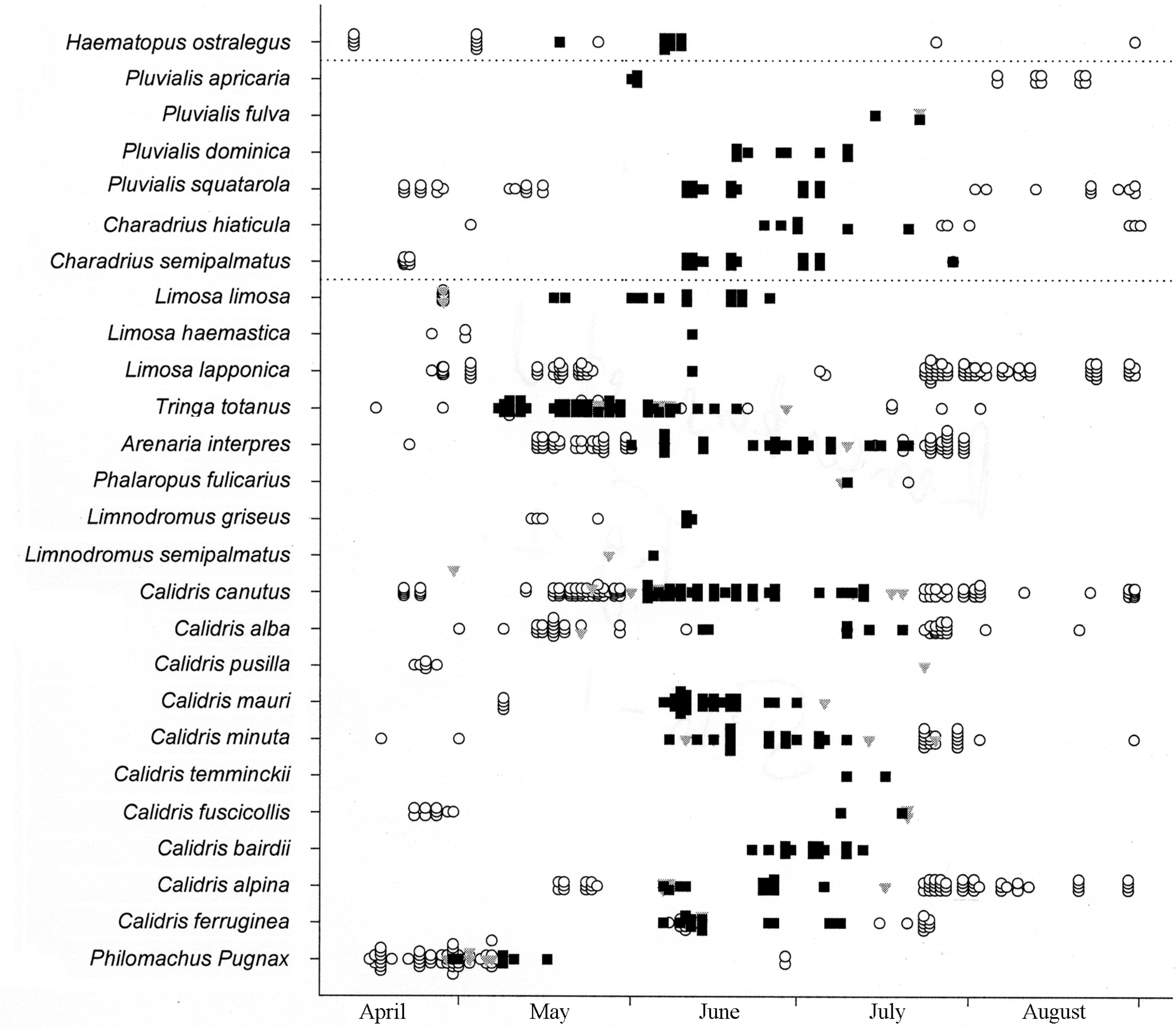

Fig. 1 Seasonal shifts from mono- (open circle) to di- ester (black square) preen waxes in adult shorebirds
Each symbol represents a single individual. Horizontal dotted lines divide from top to bottom: oystercatcher (Haematopodidae), plovers (Charadriidae) and sandpipers (Scolopacidae). Mixtures of mono- and di- esters are depicted as gray triangles. Diester secretion is maintained throughout the breeding period (mid May–early July) in all species investigated.

compounds, their functions need to established, i.e. the costs and benefits of different mixtures of preen waxes. Costs and benefits can be expressed in terms of energy, nutrition and time, or as reproductive currency. Natural selection is expected to select against costly traits if there are no benefits that outweigh their costs. For example, diester waxes could be functional in attracting mates but perform less well than monoesters in protecting plumage against ecto-parasites.

We performed an experiment with captive red knots in which, in addition to annual cycles in body mass and molt (Piersma and Ramenofsky, 1998), annual changes in preen wax composition followed the pattern in free-living conspecifics. The birds were provided with daily food just sufficient to maintain stable body mass. This allowed us to compare the presence and annual timing of shifts in preen wax composition in them with controls offered *ad libitum* food. Significantly, fewer food-restricted birds switched to diester waxes during the breeding period, indicating that there are energetic or nutritional costs involved in the shift to diester preen waxes (J. Reneerkens, unpubl. data).

4.2 Do preen waxes provide a quality signal?

Piersma et al. (1999) postulated that diester preen waxes could alter the appearance of plumage and act as a sexually selected quality signal during mate choice. The honesty of the signal would be guaranteed by the energetic and/or time costs associated with the shift to more viscous diester waxes that would be more difficult to apply under the low temperatures that prevail in the High Arctic. By assessing the time that captive birds spent preening before and after a switch from mono- to di- ester waxes under different ambient temperatures, one could evaluate such time costs. Birds would be expected to spend more time preening diester waxes on to their plumage than monoester waxes at similar ambient temperatures.

A prerequisite for a function such as quality signal is that different preen wax cocktails can be distinguished visibly by conspecifics. The eyes of birds eyes are designed differently from those of humans (Hart et al., 2000), and can detect wavelengths of light invisible to the human eye (e.g., Maier, 1994; Cuthill et al., 2000). We used photospectrometry to obtain objective measures of light reflection (i.e., color) of breeding plumage in red knots. The reflectance of feathers of the same individual, both with a coat of mono- and diester waxes and after removal of the waxes with a solvent, were measured. The results showed that: (1) the removal of preen waxes did not change the intensity of reflection of the light spectrum presumed to be visible for birds (300–750 nm), and (2) a coat of diester waxes did not alter the reflectance of plumage from that with a coat of monoester waxes (Reneerkens and Korsten, unpubl. data). As diesters could alter plumage shine or gloss, rather than color, the quality signal hypothesis cannot be rejected as yet. Mate choice experiments with preen wax-manipulated birds would provide the best tests of the hypothesis.

Jacob (1978b) suggested that the preen wax products of female mallards showed pheromonal activity. However, preen wax compounds are not particularly volatile, a property that would not be enhanced under prevailing ambient temperatures on the arctic tundra where many shorebirds reproduce. Rather, because temperatures there are substantially lower than on wintering grounds, a shift to a lower rather than a higher molecular weight of wax mixture would be expected. Jacob et al. (1979) then suggested that products resulting from hydrolysis of preen waxes (alcohols and fatty acids) were more likely to function as pheromones. Yet we have never detected alcohols, diols or fatty acids, the hydrolysed products of wax esters, in the plumage of red knots.

As diester preen waxes continue to be secreted during incubation, they are likely to fulfill functions other than signaling individual quality. In sandpiper species in which only one sex incubates, diesters were only secreted by the incubating sex (Reneerkens et al., 2002), suggesting that diester preen waxes are more likely to play a role during incubation.

4.3 Naturally selected functions

Shorebirds typically nest on open ground and hence are vulnerable to mammalian predation. A shift to less volatile diester preen waxes could reduce the smell of an incubating bird and diminish the chance of being detected by predators that use the sense of smell to detect prey. Such "olfactory crypticism" would have a large selective advantage. Comparisons of the chemical composition of preen waxes in closely related species that have or have not been exposed to predators (e.g., island populations that always have been free of mammalian predators) could test this hypothesis. The hypothesis could also be tested experimentally with dogs or rats that are trained to smell out hidden objects coated with either mono- or di- esters.

If feather abrasion is more serious during incubation than at other stages of the annual cycle, e.g., because of repeated contact with the ground, then wear and tear could selectively favor preen waxes that protect feathers better against abrasion. Abrasion of feathers impregnated with different preen wax mixtures can be studied in laboratory experiments.

Preen waxes may also be important in regulating the growth and ecology of bacteria, fungi and feather mites hosted by birds. Both inhibition and stimulation of growth of bacteria and fungi, which occur naturally on skin and feathers (Burtt and Ichida, 1999), have been reported (Bandyopadhyay and Bhattacharyya, 1996, 1999; Jacob et al., 1997; Law-Brown, 2001). Alcohols and fatty acids, which are hydrolysed products of preen waxes, have been shown to affect the growth of dermatophytes (Jacob et al., 1997; Bandyopadhyay and Bhattacharyya, 1996, 1999). Fatty acids may inhibit growth of ectosymbionts by lowering pH, but it needs to be questioned how common the hydrolysis of wax esters is under natural conditions. Diester preen waxes in chickens were found to inhibit the growth of five species of fungal dermatophytes yet promote the growth of another (Bandyopadhyay and

Bhattacharyya, 1999). The bacterial flora of chicken skin also depends largely on the presence of (diester) preen waxes (Bandyopadhyay and Bhattacharyya, 1996). Seven of the 17 wax compounds in red-billed wood hoopoes (*Phoeniculus purpureus*) showed inhibitory action against 13 species of pathogenic bacteria and one parasitic bacterium (Law-Brown, 2001). Preen waxes possibly also play a role in protecting eggs against fungal infection if they are smeared onto the eggs, either directly or via plumage. As birds have been suggested to ingest preen waxes (Elder, 1954), the secretions could also offer protection against ingested (pathogenic) bacteria.

Feather mites appear not to be affected by the preen wax secretions of wood hoopoes (Law-Brown, 2001). Indeed, feather mites may feed on preen waxes and material trapped in them (Blanco et al., 2001). Removal of preen waxes by mites probably entails only small costs for birds and could even be beneficial, as microorganisms, including pathogens, may proliferate if preen waxes are not replaced regularly (Blanco et al., 2001). Seasonal variation in ectosymbionts may lead to the evolution of qualitative variation in preen waxes if different preen wax mixtures have different effects on them. We need to find out whether mono- or di- ester secretions of shorebirds have different effects on ectosymbionts if their role in the evolution of shifts in preen wax composition is to be understood. Microbiological tests with different ectosymbionts grown on culture media with both mono- and di- ester wax supplements (cf., Jacob et al., 1997; Law-Brown, 2001) could show whether ectosymbionts selectively favor different preen wax secretions.

Interesting patterns in intra-individual variation of preen waxes have been discovered only recently. Descriptive and experimental research is needed to gain insight into the exciting adaptive interaction in their variation.

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