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Switch to diester preen waxes may reduce avian nest predation by mammalian olfactory cues

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Summary

It has long been recognised that nest depredation by olfactory-searching mammals greatly influences the reproductive success of ground-nesting birds. Yet adaptations of birds to diminish smell during nesting have rarely been investigated. Recently, a remarkable shift in the composition of uropygial gland secretions (preen waxes) was discovered in many ground-nesting shorebirds and ducks that begin incubation, during which the usual mixtures of monoester preen waxes are replaced by mixtures of less volatile diester waxes. In this study we show experimentally that an olfactory-searching dog had greater difficulty detecting mixtures of the less volatile diesters than mixtures of monoesters. This is consistent with the hypothesis that diester preen waxes reduce birds’ smell and thereby reduce predation risk.

Our first hypothesis, that diester waxes enhance plumage colouration and function as an individual quality signal during courtship (Piersma et al., 1999), is not the only explanation for the observed shift in preen wax composition, as spectral measurements of plumages of red knots before and after the shift to diester preen waxes showed no difference in colouration (Reneerkens and Korsten, 2004). Furthermore, the secretion of diester preen waxes continues during incubation, with a return to monoesters when the chicks hatch. A similar shift to diester preen waxes during incubation has already been found in wild-type and domesticated mallards Anas platyrhynchos (Jacob et al., 1979; Kolattukudy et al., 1987), which are also ground-breeders. For species whose males do not incubate, the shift to diester preen waxes is limited to the incubating females (Reneerkens et al., 2002). This indicates that the diester wax cocktail fulfils a specific function during incubation, but that function during this crucial phase is unknown (Reneerkens et al., 2002).

Ground-nesting birds are particularly vulnerable to loss of their clutch to predators (Whelan et al., 1994), which can greatly influence the population dynamics of ground-nesting birds (Blomqvist et al., 2002). Because of their high molecular mass, diesters are less volatile than monoesters and might thus be more difficult to detect by olfactory-searching predators. In this study we tested this hypothesis using a sniffer dog trained to locate different amounts of pure mono- or diester preen waxes.

Introduction

The application of secretions of the uropygial gland, also called preen waxes, is an important aspect of plumage maintenance in birds. Preen waxes repel water (Jacob and Ziswiler, 1982) and inhibit the growth of feather-degrading bacteria (Shawkey et al., 2003). In the European oystercatcher Haematopus ostralegus, six plover species (Charadriidae), and at least 19 sandpiper species, including the red knot Calidris canutus (Reneerkens et al., 2002, in press), preen wax composition changes over an annual cycle from lower-molecular-mass monoester waxes (total carbon number distribution in the range C24–C26 and C30–C38) to higher-molecular-mass diester waxes (total carbon number distribution in the range C32–C48; Sinninghe Damsté et al., 2000). The shift to diester preen waxes is completed when the birds are ready for a long northward flight to High Arctic breeding grounds (Reneerkens et al., 2002, in press), where courtship starts soon after arrival.

Red knots that departed on, or arrived after, the first part of a non-stop migratory flight of several thousand km did not secrete diester preen waxes. Birds of the same population, ready for the second part of the migratory journey to the High Arctic breeding grounds, did, however. Therefore, it was suggested that secretion of diester preen waxes was not related to long-distance flights per se but that the timing of the compositional preen wax shift was apparently related to breeding activities (Piersma et al., 1999).

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Key words: uropygial gland, preen wax, camouflage, olfaction, nest predation, sandpiper, Calidris canutus.
Materials and methods

Preen waxes

We collected pure preen wax biweekly from 14 red knots Calidris canutus L. kept in outdoor aviaries from 1 March 2002 to 24 June 2002 by softly massaging the papilla of the gland with a cotton bud. Waxes were extracted with ethyl acetate, weighed and dissolved in ethyl acetate (1 mg wax ml⁻¹). The solution was injected into a gas chromatograph (Hewlett-Packard 6890 Series II, Amstelveen, The Netherlands) using an on-column injector. Detection was accomplished using a flame-ionisation detector. Helium was the carrier gas. Separation of the chemical components was achieved using a fused-silica capillary column (Varian, Middelburg, The Netherlands; 25 m×0.32 mm i.d.) coated with CP-Sil 5CB (film thickness 0.12 μm). The samples were injected at 70°C, and the oven was subsequently heated to 130°C at 20°C min⁻¹ followed by 4°C min⁻¹ to 320°C, and held at this temperature for 35 min. From previous detailed molecular analysis of the intact monoester and diester preen waxes we learned that their gas chromatograms are characteristic for either monoester or diester preen waxes (see inset in Fig. 1A; Dekker et al., 2000; Sinninghe Damsté et al., 2000). For the purpose of this study, this enabled us to examine the gas chromatograms visually to determine which preen wax composition (mono- or diesters) was secreted at a given date. To characterise the relative abundance of mono- and diesters in the preen wax of an individual bird at a given date (Fig. 1A), appropriate peak areas in the gas chromatograms were integrated. The percentage of diesters in the wax mixture was estimated following the formula %diester = surface diester peaks / (surface diester peaks + surface monoester peaks) × 100. This relative abundance of diesters was averaged for all individual birds on a given day and the 95% confidence intervals, as shown in Fig. 1A, were calculated.

On 23 April 2002 all captive birds secreted pure monoesters whereas on 11 June 2002 only diesters were produced. We combined the 14 samples from each of these days (8.4 and 7.9 mg waxes, respectively) and dissolved them in ethyl acetate to an exact concentration of 1.0 mg wax ml⁻¹. These two samples were the basis for serial dilutions of preen wax with ethyl acetate. At each step the concentration was reduced by a factor of two.

Under a fume hood we pipetted 0.5 ml of the solution to square metal rods that were lying on clean aluminium foil. The solution was equally spread over two sides of the square rods using a pipet, such that the side with waxes never touched the aluminium foil. Control rods were applied with 0.5 ml pure ethyl acetate. After evaporating off the volatile solvent, the metal rods were kept in airtight glass jars. Rods and glass jars were boiled in water for 10 min and washed without detergent in a dishwashing machine before use. Metal rods and glass jars were never touched and always handled using a pair of metal pliers.

Sniffer dog

We trained a 6-year-old female German shepherd dog to locate different amounts of both mono- and diester waxes. The dog had positive health certificates on stamina and had been recommended for breeding. Initially, the dog was taught to sniff systematically a row of six plastic tubes mounted 1 m apart on a wooden board, to locate the metal rod applied with smell of the dogs owner at a randomly chosen position and be rewarded for it by being allowed to play with the rod for some time and by compliments from the trainer. The dog trainer applied his own smell to the rod by touching it and keeping it in his pocket. Control rods remained untouched and were placed in the remaining locations. After the dog had located the rod with the smell of the dog trainer convincingly several times, human smell was replaced by 1 mg mono- or diester preen waxes. To get an idea of the amounts at which the dog started to fail locating the preen waxes, the amounts of preen waxes on the rod were gradually decreased during the training procedure. Training took place from January 2003 to February 2004, and the actual experiments on four different days during the period February to July 2004 in familiar surroundings, in the garage of the dog-owner.

The experiment was performed with different amounts of mono- and diester preen waxes, between 0.24 and 15.6 μg. The intention of the experiment was to examine whether detection probabilities are equal for the same amounts of preen wax. Under natural conditions, the quantity of wax molecules in the air will depend on the distance from the source. The amounts of preen wax used in this qualitative experiment, in which the dog sniffed the rods at a distance of only a few cm, therefore do not need to reflect the natural amounts expressed by birds. The order of sessions with respect to composition (mono- or diesters) and amount of preen wax was randomised. Dog and trainer were unaware of the location of the treated rod. If it had smelled the preen wax, the dog would take the metal rod (see supplementary material). On failing to locate the rod with wax, the dog continued systematically searching the row of tubes, sometimes up to 30 times before giving up. On giving up, the dog often started searching elsewhere in the room where the experiments were carried out. The dog never indicated a finding of preen wax on control rods, i.e. never made a mistake.

The success with which the dog located the wax was scored for wax composition and amount. Each combination of wax composition and amount was tested 20 times (280 experiments in total) over 4 days, on each of which all combinations of wax amounts and composition were tested five times. Detection chance (\(P_{\text{detection}}\)) was analysed using a logistic model \(\ln(P_{\text{detection}}/1-P_{\text{detection}})=a+b\times\text{amount}\), which was fitted to the data by iteration (Crawley, 1993). Factors (amount of wax, wax composition and their interaction) were added separately to the model and a \(\chi^2\) test was used to estimate whether the addition of factors caused significant reductions of deviance.

Results

The complete shift (from mono- to diesters) in the preen wax composition of individual red knots took place within a month (Fig. 1A). Diesters are less volatile than monoesters, as indicated by their gas chromatograms (inset in Fig. 1A). With
Diester waxes reduce smell of nesting birds

Fig. 1. Red knots shift from mono- to diester preen waxes, the latter being more difficult to detect by a sniffer dog. (A) The shift from mono- to diester preen waxes (see inset) in spring takes place within 1 month in individual captive red knots (N=14 individuals; 95% confidence intervals around mean values of percentage of diesters are indicated by dots). (B) The likelihood of successful detection is a function of the type and amount of preen wax. Each data point represents detection success during 20 sessions (monoesters: black circles, diesters: open circles). Fits from the used logistic model (see Materials and methods) are depicted in the graph as lines (solid, monoesters: \( \ln(P_{\text{detection}}/1-P_{\text{detection}}) = 1.4519 + 0.6602 \times \text{amount} \); broken, diesters: \( \ln(P_{\text{detection}}/1-P_{\text{detection}}) = 0.1786 + 0.6602 \times \text{amount} \)).

Discussion

The results of our experiment using the single sniffer dog are consistent with the idea that diesters are more difficult to detect by the dog increasingly failed to locate them (Fig 1B). The model that included both main factors (amount and composition of preen wax) significantly contributed to the fit compared with models that included only a single main factor (from the model with amount as a factor only: \( \chi^2=10.5, \text{d.f.}=1, P<0.005 \); from the model with composition only: \( \chi^2=40.1, \text{d.f.}=1, P<0.001 \)). This tells us that the decline in detection success with lowering amounts of preen waxes is steeper for diester waxes than for monoester waxes.

This experiment would not have been possible without the intensive help of Ton van der Heide, who took care of the dog training. Adee Schoon gave useful advice on dog training. Jaap van der Meer helped with the statistical analysis. Bird handling was carried out under the auspices of the Animal Experiment Committee (DEC) of the Dutch Royal Academy of Sciences (KNAW). Our work is financially supported by ALW grant 810.34.003 of the Netherlands Organisation for Scientific Research (NWO) to T.P. and J.S.S.D.

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