university of groningen

umcg

University of Groningen

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

Reneerkens, J; Piersma, T; Sinninghe Damsté, Jaap S.

Published in:
Journal of Experimental Biology

DOI:
10.1242/jeb.01872

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2005

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Reneerkens, J., Piersma, T., & Sinninghe Damsté, J. S. (2005). Switch to diester preen waxes may reduce avian nest predation by mammalian predators using olfactory cues. *Journal of Experimental Biology*, *208*(22), 4199-4202. https://doi.org/10.1242/jeb.01872

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 10-11-2026

The Journal of Experimental Biology 208, 4199-4202
Published by The Company of Biologists 2005
doi:10.1242/jeb.01872

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

Jeroen Reneerkens[1,*], Theunis Piersma[1,2] and Jaap S. Sinninghe Damsté[1]

[1]*Royal Netherlands Institute for Sea Research (NIOZ), PO Box 59, 1790 AB Den Burg, Texel, The Netherlands* and [2]*Centre for Ecological and Evolutionary Studies, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands*

*Author for correspondence (e-mail: reneer@nioz.nl)

Accepted 6 September 2005

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.

Supplementary material available online at
http://jeb.biologists.org/cgi/content/full/208/22/4199/DC1

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

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 C_{24}–C_{26} and C_{30}–C_{38}) to higher-molecular-mass diester waxes (total carbon number distribution in the range C_{32}–C_{48}; 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), 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).

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.

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 χ^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

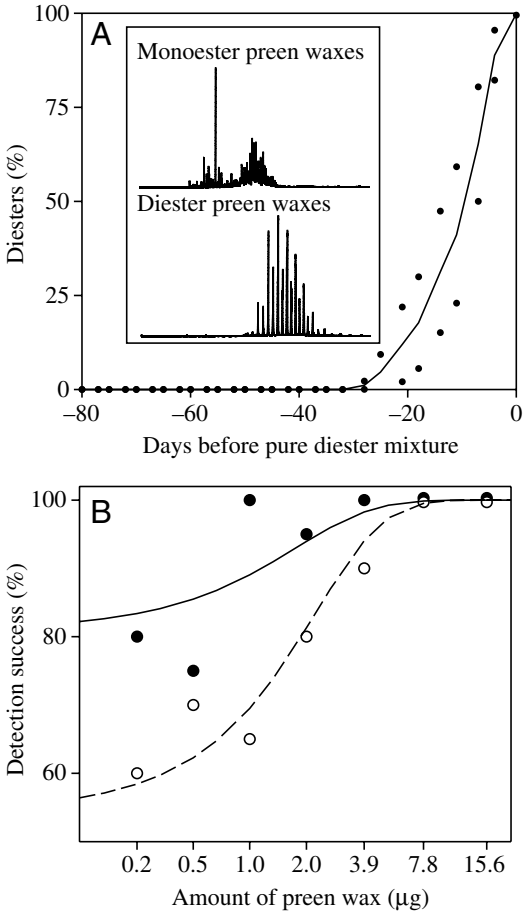

Fig. 1. Red knots shift from mono- to diester preen waxes, the latter being more difficult to detect by a sniffer dog. (A) The shift from mono- to diester preen waxes (see inset) in spring takes place within 1 month in individual captive red knots (N=14 individuals; 95% confidence intervals around mean 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: $\ln(P_{detection}/1–P_{detection})=1.4519+0.6602\times$amount; broken, diesters: $\ln(P_{detection}/1–P_{detection})=0.1786+0.6602\times$amount).

decreasing amounts of preen wax 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: χ^2=10.5, d.f.=1, P<0.005; from the model with composition only: χ^2=40.1, 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.

Discussion

The results of our experiment using the single sniffer dog are consistent with the idea that diesters are more difficult to smell than monoesters. This suggests that the use of diesters during incubation would improve ‘olfactory crypsis’. Although it is unknown how much preen wax is expressed by incubating birds, the smell of preen waxes, and hence detection chance, will decrease with distance from the bird. At a certain distance from the source the smell of preen wax will reach critical levels at which predators might not detect them. The results of our experiment suggest that this maximum detection distance is smaller for diester than for monoester preen waxes.

Predation of eggs by mammalian, olfactory-searching predators largely determines the reproductive output of young sandpipers (Blomqvist et al., 2002). This severe natural selection has led to the evolution of cryptic plumage and egg coloration to conceal nests and incubating birds from visually searching egg predators (Solís and De Lope, 1995; Jukema et al., 2003). It has long been known that many mammalian predators rely on smell to locate prey (Whelan et al., 1994), and although folk wisdom relates that snipe and quail are impossible to detect by hunting dogs as long as they are on their nest, to the best of our knowledge, this is the first experimental test of olfactory crypsis as a potential complementary anti-predation strategy. Future research should reveal if our findings using a single dog can be generalised to natural predators in a field situation where detection probabilities also depend on distance from the incubating bird, wind conditions and habitat characteristics.

Seasonal shifts in preen wax composition are presumably the result of a changing balance between costs and benefits of the production, and use of diester rather than monoester preen waxes. In the non-breeding season sandpipers live in large flocks and can fly away from mammalian predators. Their reliance on monoesters during times when olfactory crypsis is unimportant suggests that the production or use of diesters carries a cost. A greater understanding of the energetic costs and of functional properties, such as anti-parasitic aspects or waterproofing, of mono- and diester preen waxes is necessary to better understand seasonal shifts in preen wax composition.

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.

References

Blomqvist, S., Holmgren, N., Åkesson, S., Hedenström, A. and Pettersson, J. (2002). Indirect effects of lemming cycles on sandpiper dynamics: 50 years of counts from southern Sweden. *Oecologia* **133**, 146-158.

Crawley, M. J. (1993). *GLIM for Ecologists*. Oxford: Blackwell Science.

Dekker, M. H. A., Piersma, T. and 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.

Jacob, J. and Ziswiler, V. (1982). The uropygial gland. In *Avian Biology* (ed. D. S. Farner, J. R. King and K. C. Parkes), pp. 199-314. New York: Academic Press.

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

Jukema, J., Tulp, I. and Bruinzeel, L. (2003). Differential moult patterns in relation to anti-predator behaviour during incubation in four tundra plovers. *Ibis* **145**, 270-276.

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

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

Reneerkens, J. and Korsten, P. (2004). Plumage reflectance is not affected by preen wax composition in red knots *Calidris canutus*. *J. Avian Biol.* **35**, 405-409.

Reneerkens, J., Piersma, T. and Sinninghe Damsté, J. S. (2002). Sandpipers (Scolopacidae) switch from monoester to diester preen waxes during courtship and incubation, but why? *Proc. R. Soc. Lond, B* **269**, 2135-2139.

Reneerkens, J., Piersma, T. and Sinninghe Damsté, J. S. (in press). Discerning adaptive values of seasonal variation in preen waxes: comparative and experimental approaches. *Proc. 23th Int. Ornith. Con.*

Shawkey, M. D., Pillai, S. R. and Hill, G. E. (2003). Chemical warfare? Effects of uropygial oil on feather-degrading bacteria. *J. Avian Biol.* **34**, 345-349.

Sinninghe Damsté, J. S., Dekker, M., Van Dongen, B. E., Schouten, S. and Piersma, T. (2000). Structural identification of the diester preen-gland waxes of the red knot (*Calidris canutus*). *J. Nat. Prod.* **63**, 381-384.

Solís, J. C. and De Lope, F. (1995). Nest and egg crypsis in the ground-nesting Stone Curlew (*Burhinus oedicnemus*). *J. Avian Biol.* **26**, 135-138.

Whelan, C. J., Dilger, M. L., Robson, D., Hallyn, N. and Dilger, S. (1994). Effects of olfactory cues on artificial nest experiments. *Auk* **111**, 945-952.