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EXPERIMENTAL TEST OF THE IMPORTANCE OF PREEN OIL IN ROCK DOVES (*COLUMBA LIVIA*)

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ABSTRACT.—Most species of birds have a uropygial gland, also known as a preen gland, which produces oil that birds spread through their plumage when preening. The plumage of waterfowl deprived of uropygial oil becomes brittle and is subject to breakage. For other groups of birds, however, the importance of preen oil remains unclear. Previous workers have argued that preen oil may serve little or no function in Columbiforms (pigeons and doves). We tested that assertion by removing uropygial glands from Rock Doves (*Columba livia*) and assessing their plumage condition after several months. The results of that experiment showed significant degradation of plumage in the absence of oil. Our results are the first rigorous demonstration that preen oil is important for plumage condition in nonwaterfowl.

We tested one possible function of preen oil—that it has insecticidal properties and that reduction in plumage condition on birds without glands is due to an increase in ectoparasites. We tested that hypothesis for feather-feeding lice (*Phthiraptera:Ischnocera*) using both *in vitro* and *in vivo* experiments. Lice raised in an incubator died more rapidly on feathers with preen oil than on feathers without oil, which suggests that preen oil may help combat lice. However, removal of the preen gland from captive birds had no significant effect on louse loads over the course of a four-month experiment. Although the results of our *in vivo* experiments suggest that preen oil may not be an important defense against lice, further experiments are needed. We also consider the possibility that preen oil may protect birds against other plumage-degrading organisms, such as bacteria and fungi. *Received 25 April 2002, 5 February 2003.*

RESUMEN.—La mayoría de las especies de aves tienen una glándula uropigial, la cual produce un aceite que las aves esparcen en su plumaje al acicalarse. Al privarse del aceite uropigial, el plumaje de las aves acuáticas se debilita, haciéndose quebradizo. Sin embargo, la importancia de la glándula uropigial en otros grupos de aves no es clara, e investigaciones previas han sugerido que el aceite podría ser poco o nada importante funcionalmente en Columbiformes. Pusimos a prueba esta aseveración removiendo la glándula uropigial de palomas *Columba livia*, y evaluando la condición de su plumaje luego de varios meses. Los resultados de este experimento mostraron una degradación significativa del plumaje en ausencia del aceite uropigial, lo que constituye la primera demostración rigurosa de que éste es importante para la condición del plumaje en aves no acuáticas.

Una posible función del aceite de acicalamiento es que tenga propiedades insecticidas y que el desmejoramiento de la condición del plumaje de aves sin glándulas se deba a un incremento de los ectoparásitos. Pusimos a prueba esta hipótesis en piojos que se alimentan de plumas (*Phthiraptera:Ischnocera*) mediante experimentos *in vitro* e *in vivo*. Piojos criados en incubadoras en plumas con aceite uropigial murieron más rápidamente que piojos criados en plumas sin aceite, lo que sugiere que éste podría ayudar a combatir los piojos. Sin embargo, la remoción de la glándula uropigial de aves en cautiverio no tuvo un efecto significativo sobre la carga de piojos a lo largo de un experimento de cuatro meses de duración. Aunque los resultados de nuestros experimentos *in vivo* sugieren que el aceite de acicalamiento podría no ser una defensa importante contra los piojos, es necesario hacer más experimentos. También consideramos la posibilidad de que el aceite proteja a las aves de otros organismos que degradan el plumaje, como bacterias y hongos.

MOST BIRDS HAVE a uropygial gland, also known as a preen gland, on their rump. The nipple-like protuberance of the gland exudes oil, which is spread throughout the plumage when a bird preens. Preen oil helps keep the plumage of waterfowl in good condition. The oil maintains the flexibility of feathers and keeps feather barbules from breaking (Jacob and Ziswiler 1982). The interlocking barbules, when in good condition, form a barrier that helps repel water. This waterproofing is lost on waterfowl deprived of

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oil, however, because their feather structure gradually deteriorates (Hou 1928, Elder 1954, Jacob and Ziswiler 1982).

The fact that many species of birds lack a preen gland suggests that preen oil is not universally important. Some or all species in at least nine families of birds, including ostriches (Struthionidae), parrots (Psittacidae), and pigeons and doves (Columbidae), have individuals that lack preen glands (Johnston 1988; see below for a complete listing). Johnston (1988) reported glandless individuals in 28 of 103 species of Columbiformes surveyed. Darwin (1896) himself noted that some Rock Doves (*Columba livia*) lack the gland. In our own work, we have occasionally caught Rock Doves that lack glands. Although we have not done a careful survey, we estimate that between 1 and 3% of Rock Doves in Utah and Illinois lack glands (B. R. Moyer unpubl. data).

Montalti et al. (2000) recently argued that preen oil is not necessary for Rock Doves to maintain their plumage in good condition. Goodwin (1983) went so far as to claim that columbiform preen glands may generally be nonfunctional. He argued that the fine powder down produced by pigeons and doves functions in lieu of preen oil. The purpose of the current study was to test the importance of preen oil in Rock Doves by removing the uropygial gland and quantifying plumage condition over a period of several months.

One way in which preen oil might help maintain plumage in good condition is by acting as a defense against feather-degrading ectoparasites, such as feather lice (Phthiraptera: *Ischnocera*) (Jacob and Ziswiler 1982, Poulsen 1994, Dumbacher and Pruett-Jones 1996). Interestingly, feather lice rapidly die when anointed with a tiny drop of preen oil (B. R. Moyer pers. obs.). We tested the effect of preen oil on feather lice in two ways. First, we compared survival of lice raised in an incubator on feathers with and without preen oil. Next, we conducted a longitudinal study of louse loads on Rock Doves from which glands were experimentally removed.

Methods

Plumage condition.—We investigated effects of preen oil on the plumage condition of 15 feral Rock Doves. We captured 15 birds using walk-in traps. The birds were housed individually in wire mesh cages (30 × 30 × 30 cm) and provided *ad libitum* grain, grit, and water. After an acclimation period of one month, we randomly assigned eight (experimental) birds to a gland removal treatment and seven (control) birds to a sham removal treatment.

We anesthetized each bird with Nebutal (sodium pentobarbital), then made a short incision and excised the preen gland from birds assigned to the removal treatment. We made an incision adjacent to the gland on birds assigned to the sham removal treatment. For all birds, incisions were sewn shut with silk thread and birds were given water with aspirin (1 mg mL^{-1}) for several days following surgery. To prevent birds from pulling out their stitches, we temporarily blocked preening in all birds by fitting them with bits immediately after surgery. Bits were small, C-shaped pieces of metal placed between the mandibles and crimped partly shut in the nares. In addition to preventing birds from removing their stitches, bits also prevented efficient preening that birds need for controlling lice (e.g. see Clayton et al. 1999). We therefore removed bits from all birds two weeks following surgery. We also removed stitches at that time.

At the end of the experiment, which lasted four months, we assessed the effect of gland removal on plumage condition. We used categorical scores of 1 to 3: 1 = poor condition (severe damage to barbules), 2 = fair (moderate damage), 3 = good (little or no damage). These categories correspond roughly to the three categories depicted in Figure 2 of Clayton (1990). Plumage condition was scored by one of us (D.H.C.) who was blind to the treatment identity of the birds when scoring.

Differences in feather molt could conceivably influence plumage condition. If removing the preen gland reduced the intensity of feather molt for some reason, glandectomized birds would have fewer new feathers by the end of the experiment, causing their plumage to be in poorer overall condition. We therefore also assessed molt over the course of the experiment using categorical scores of 0 to 3, which corresponded to the extent of pinfeather growth on the rump and back of each bird (0 = none, 1 = few, 2 = moderate, 3 = many).

We also compared preening rates of birds with and without glands for two months following surgery. Absence of preen oil could conceivably lead to dry, itchy skin and a consequent increase in preening by birds from which glands were removed. Increased plumage abrasion caused by a higher rate of preening could break feather barbules, leading to a reduction in plumage condition. We compared preening rates using bouts of scan sampling (Altmann 1974). Scan sampling observations were made immediately before surgery and one and two months following surgery. There were five bouts of scan sampling on each of those three occasions. Bouts were conducted at different times of day. During each bout an observer seated

inside a blind in the animal room serially scanned the cages, noting the instantaneous preening behavior of a different bird every 9 s; the behavior of a given bird was noted 10 times over the course of the scan-sampling period. We stopped collecting data on preening after two months because there were no differences.

Feather lice.—We conducted *in vitro* and *in vivo* tests of the effect of preen oil on feather-feeding lice. We used two species of lice (*Columbicola columbae* and *Campanulotes bidentatus compar*) that are specific to Rock Doves (Nelson and Murray 1971, Clayton et al. 1999). Feather damage caused by those lice interferes with thermoregulation, leading to a compensatory increase in metabolic rate (Booth et al. 1993), and ultimately a reduction in overwinter survival (Clayton et al. 1999).

For the *in vitro* experiment, we used oil-free feathers removed from two wild-caught, glandless Rock Doves. We placed five feathers (two rump, two breast, and one neck) in each of 12 culture jars (32 mm in diameter × 83 mm tall glass jars lined with paper and with ventilation holes in their plastic lids). Jars were randomly assigned to an experimental treatment in which preen oil was present, or to a control treatment without oil. We harvested oil by gently squeezing the preen gland of several wild caught Rock Doves. We anointed one breast feather and one rump feather in each experimental jar with a small drop of oil (approximately 25–50 µL), which was spread evenly over the feather. That quantity of oil approximated the amount we expected to be on the feather of a typical bird. We placed 10 male and 10 female *C. b. compar* lice in each jar. Jars were kept in a stainless steel lined incubator (model I-36VL, Percival Scientific, Perry, Iowa) on a 12 h photoperiod at 37°C and a relative humidity of 75% (Nelson and Murray 1971). Condition of the lice (dead or alive) was checked after one week.

For the *in vivo* experiment, we monitored natural populations of both species of lice on glandectomized and control birds over the course of the four month study. Feather lice are "permanent" ectoparasites that carry out their entire life cycle (about one month) on the body of the host. Feather lice require direct contact between host individuals to transfer to a new host (Clayton and Tompkins 1994). It was not possible for lice to move among birds in our *in vivo* experiment because we housed each bird in a separate cage. We quantified number of lice on all birds before and after surgery using a visual examination method (Clayton and Drown 2001), which estimates the total number of lice on a bird from a subsample observed during timed examinations of specific body regions ($r^2 \geq 0.73$, $P < 0.0001$, using the "complete data" multivariate equations from Clayton and Drown 2001). The louse load data were normalized prior to analysis using $\log_{10}$ transformations. At the beginning of the experiment all birds had lice, with means (±1 SE) of 669 ± 179 (experimentals) and 679 ± 191 (controls) lice per bird. There was no significant difference in louse load between the two treatments at the start of the experiment ($t = 0.38$, df = 13, $P = 0.71$).

RESULTS

Plumage condition.—Preen oil had a significant effect on plumage condition. The plumage of birds without glands was in significantly poorer condition than that of control birds after four months (Wilcoxon test, $Z = 2.0$, $P < 0.05$; Fig. 1). The plumage of all birds with preen glands was in good condition. In contrast, half of the birds without glands suffered a reduction in plumage condition, with many feathers missing barbules, causing them to lose their normal fluffy appearance.

The difference in plumage condition between treatments was not confounded by differences in body molt. Experimental birds had a mean (±1 SE) molt score of 1.3 ± 0.38 compared to 1.1 ± 0.24 in controls, which was a nonsignificant difference ($t = 0.37$, df = 13, $P = 0.72$).

Differences in plumage condition were not confounded by differences in preening rate. Experimental birds spent a mean (±1 SE) of 20.7 ± 3.1% of their time preening, compared to 22.1 ± 1.5% by controls. A repeated-measures ANOVA revealed no effect of gland removal on preening rate ($F = 0.06$, df = 1 and 12, $P = 0.80$). There was an effect of time on preening ($F = 7.3$, df = 2 and 24, $P < 0.005$). Preening rates of birds in both treatments increased over the course of the experiment, perhaps because the birds spent

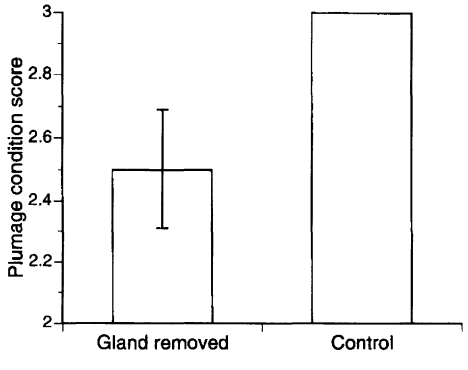

FIG. 1. Mean (±1 SE) plumage condition score of eight birds with preen glands removed, compared to seven control birds with glands intact: 1 = poor, 2 = fair, 3 = good.

less time in nervous vigilant behavior as they became acclimated to captivity. There was no treatment × time interaction ($F = 1.1$, df = 2 and 24, $P = 0.35$). Preening rates in the two groups were similar to those in other studies of captive Rock Doves (B. R. Moyer unpubl. data).

Feather lice.—Preen oil doubled the mortality of lice *in vitro* ($\chi^2 = 21.3$, $P < 0.0001$; Fig. 2). However, oil had no effect on lice *in vivo* (Fig. 3). Over the course of the four-month experiment, louse loads decreased on both experimental and control birds, presumably as a result of removal of bits after two weeks and increasing rates of preening by captive birds with plenty of time to preen over the course of the experiment. Populations of lice on experimental and control birds were strikingly similar throughout the experiment. No bird developed a louse load that exceeded levels found on wild birds. The largest infestation in our experiment was 4,509 on one individual at month 1; Rock Doves in the wild can have over 11,000 lice (Clayton et al. 1999). At the end of the experiment all birds still had lice. A repeated-measures ANOVA revealed no effect of gland removal on louse load ($F = 0.01$, df = 1 and 13, $P = 0.94$). There was an effect of time on louse load ($F = 58.4$, df = 3 and 39, $P < 0.001$), but there was no treatment × time interaction ($F = 0.20$, df = 3 and 39, $P = 0.90$).

Discussion

We removed preen glands from Rock Doves and found a significant negative effect on plumage condition after four months. Plumage of glandless birds was in significantly poorer condition, with more missing barbules, than the plumage of control birds with glands. Those results are consistent with those of previous studies of waterfowl showing that preen oil is important for maintaining plumage in good condition and for preventing breakage of barbules (Hou 1928, Elder 1954). Our results provide the first rigorous evidence that the preen gland is important for plumage condition in birds other than waterfowl. These results run counter to the conclusions of Goodwin (1983) and Montalti et al. (2000), who suggested that the preen gland has little or no function in Columbiformes, in general, or Rock Doves, in particular.

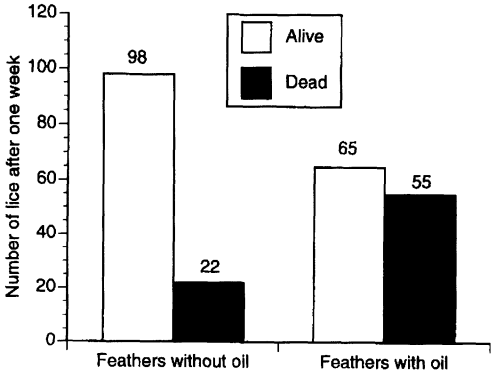

FIG. 2. Survival of lice *in vitro* on feathers with and without preen oil ($n = 120$ lice per treatment). Numbers above bars indicate number of lice in each category.

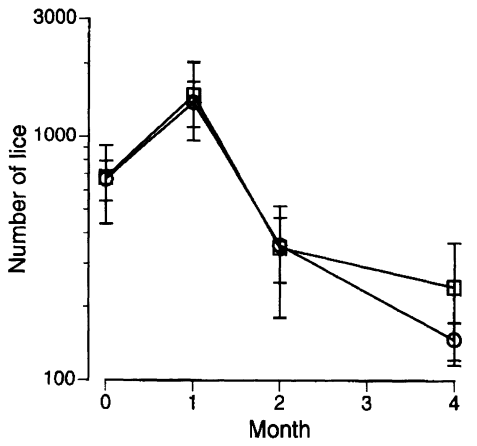

FIG. 3. Change over time in mean (±1 SE) number of lice on eight birds without glands (circles) compared to seven birds with glands (squares).

Like us, Montalti et al. (2000) removed preen glands from Rock Doves, but they observed no reduction in plumage condition. Why did the results of Montalti et al. (2000) differ from ours? A possible explanation stems from the fact that Montalti et al. (2000) assessed plumage condition for only two months following gland removal, whereas we followed birds for four months. Elder (1954) showed that plumage degradation takes several months to show up on ducks with surgically removed glands. Perhaps the same is true for other birds.

What causes the plumage of birds without glands to degrade more rapidly than that of birds with glands? We found no significant differences between treatments in molt intensity or preening rates. However, perhaps the plumage

of birds with preen glands is more resilient to abrasion from preening. Preen oil helps keep feathers strong and flexible (Jacob and Ziswiler 1982). Accordingly, even though birds without uropygial glands preened at the same rate as birds with glands, the former may have suffered more breakage of feather barbules.

Why was there a significant effect of preen oil on lice *in vitro*, but not *in vivo*? We consider four factors that may be relevant. First, it is conceivable that large fluctuations in louse load over the course of the *in vivo* experiment (Fig. 3) obscured any small effect that preen oil may have had on lice. Although bits were in place for only two weeks, they undoubtedly contributed to the increase in louse load on birds early in the experiment. Soon after bits were removed, birds brought their lice under control. Indeed, they reduced their loads to a level below those on birds when they were first captured. That was probably due to the fact that captive birds spend more time preening than free-ranging birds (B. R. Moyer unpubl. data).

Another factor to consider is that the *in vivo* experiment may not have been of sufficient duration. For example, it is conceivable that small amounts of residual oil on the feathers of glandectomized birds may have suppressed louse populations, meaning that removing glands is not equivalent to removing oil. Although we think that possibility is unlikely, the only way to be sure would be to remove glands and wait for birds to molt while continuing to monitor the lice.

A third factor is that subtle effects of preen oil may not be detectable in captive birds. Such effects may show up only in combination with the stresses experienced by birds in the field, such as having less time for preening. A gland removal experiment involving free-ranging Rock Doves would be informative in that regard.

A fourth and final factor to consider is that preen oil may simply have no effect on lice under natural conditions. The higher mortality of lice on oiled feathers in our *in vitro* experiment may have been caused by an unnatural placement or quantities of oil on the experimental feathers, compared to the way in which birds oil their own feathers. It is worth noting that lice are also killed *in vitro* by the application of tiny amounts of mineral oil (B. R. Moyer unpubl. data), probably because the oil clogs the spiracles (breathing pores) of the lice. In short, the detrimental effect of preen oil on lice *in vitro* may have been a fortuitous effect with no relevance to wild populations.

Even if preen oil has no effect on feather lice, it may still help protect birds from other plumage-degrading organisms such as bacteria and fungi. Feather-degrading bacteria and fungi reside in the plumage of many bird species (Pugh and Evans 1970a, b; Hubalek 1978; Burtt and Ichida 1999). Several studies have shown that preen oil inhibits *in vitro* growth of plumage microorganisms (Baxter and Trotter 1969, Pugh and Evans 1970b, Pugh 1972, Bandyopadhyay and Bhattacharyya 1996, Jacob et al. 1997, Law-Brown 2001).

Law-Brown (2001) recently synthesized 17 chemical compounds found in the preen gland secretions of the Red-billed Woodhoopoe (*Phoeniculus purpureus*). She tested the *in vitro* activity of each compound against a dozen avian pathogens (e.g. *Salmonella gallinarum*, *Staphylococcus aureus*, *Streptococcus faecalis*), as well as the feather-degrading bacterium *Bacillus licheniformis* (Burtt and Ichida 1999). Seven of the 17 compounds significantly inhibited bacterial growth. What has not yet been tested is whether uropygial secretions deter bacterial or fungal pathogens *in vivo*.

If preen oil is important for keeping plumage in good condition, why do members of so many families lack a preen gland, for example, ostriches (Struthionidae), rheas (Rheidae), cassowaries (Casuariidae), mesites (Mesitornithidae), bustards (Otididae), pigeons and doves (Columbidae), parrots (Psittacidae), frogmouths (Podargidae), and woodpeckers (Picidae) (Elder 1954, Johnston 1988)? The diversity of glandless taxa has puzzled researchers, who have been unable to correlate the presence or absence of a gland with factors such as distribution, climate, ecology, or flightlessness (reviewed in Johnston 1988). One possible explanation for how these birds manage without the gland is that they use alternative strategies for plumage maintenance. Glandless birds might compensate with dusting behavior, powder down, or other adaptations for keeping their plumage in good condition (Moyer et al. 2003).

Even if alternative strategies are used by some birds, however, the preen gland does not appear to be critical for all birds, even within Rock Doves. At the start of our *in vivo* experiment we captured a Rock Dove that had no

preen gland. That bird was a banded individual that was originally captured on a nest containing eggs a year earlier. Despite having no gland, its plumage was in good shape and the bird had one of the lowest louse loads of all the birds we captured (275 lice). Assuming the gland was never present, the condition of that bird suggests that some Rock Doves are able to survive, breed, and control their louse loads without preen oil. More work is needed on the function and distribution of preen glands in Rock Doves and across other species of birds.

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