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DOES SUNLIGHT ENHANCE THE EFFECTIVENESS OF AVIAN PREENING FOR ECTOPARASITE CONTROL?

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ABSTRACT: Preening is a bird's first line of defense against harmful ectoparasites. Ectoparasites, in turn, have evolved adaptations for avoiding preening such as hardened exoskeletons and escape behavior. Earlier work suggests that some groups of ectoparasites, such as feather lice, leave hiding places in feathers that are exposed to direct sunlight, making them more vulnerable to preening. It is, therefore, conceivable that birds may choose to preen in direct sunlight, assuming it improves the effectiveness of preening. Using mourning doves and their feather lice, we tested 2 related hypotheses; (1) that birds with access to direct sunlight preen more often than birds in shade, and (2) that birds with access to direct sunlight are more effective at controlling their ectoparasites than birds in shade. To test these hypotheses, we conducted an experiment in which we manipulated both sunlight and preening ability. Our results provided no support for either hypothesis, i.e., birds given the opportunity to preen in direct sunlight did not preen significantly more often, or more effectively, than did birds in shade. Thus, the efficiency of preening for ectoparasite control appears to be independent of light intensity, at least in the case of mourning doves and their feather lice.

Birds have a variety of adaptations for combating ectoparasites, ranging from immunological responses (Owen et al., 2010) to morphological and behavioral defenses (Clayton et al., 2010). Preening behavior, which is usually the first line of defense, is effective against different groups of ectoparasites including fleas, lice, flies, mites, and ticks (Marshall, 1981). Preening has an energetic cost (Wooley and Owen, 1978) and it interferes with the ability of birds to engage in other behaviors such as feeding or anti-predator vigilance (Redpath, 1988). Despite these tradeoffs, the ubiquity of preening indicates that it plays a very important role, both for ectoparasite defense and other functions such as straightening and cleaning of the feathers. Across taxa, birds spend an average of 9.2% of their time performing maintenance behavior, the large majority of which consists of preening (Cotgreave and Clayton, 1994). Other maintenance behaviors include scratching, bathing, dusting, sunning, and anting (Simmons, 1964). For a recent review of the role of preening and other maintenance behaviors in ectoparasite control, see Clayton et al. (2010).

Ectoparasites have a variety of morphological and behavioral adaptations for escaping host preening (Marshall, 1981). For example, lateral or dorso-ventral flattening of the body facilitates the rapid movement of parasites across feathers to escape preening. Most ectoparasites also have a thick cuticle that helps protect them from being crushed by the bill. Ectoparasites can also escape host preening by hiding; for example, some feather lice (Insecta: Phthiraptera) hide between the barbs of flight feathers or they burrow into the downy regions of abdominal contour feathers (Bush et al., 2006). Recent work shows that cryptic coloration is yet another way in which feather lice can escape host preening (Bush et al., 2010). Species of host-specific feather lice on light-colored birds are lighter in color than species of lice on dark-colored birds. Interestingly, species of lice confined to the head, which a bird can neither see nor preen, are not cryptically colored.

The work by Bush et al. (2010) indicates that preening for ectoparasite control has an important visual component. The efficiency of preening for ectoparasite control may increase under bright light because most ectoparasites are negatively phototactic (Stenram, 1956; Marshall, 1981). Exposure to bright light causes some groups, such as feather lice, to move out of interbarb spaces and across the feathers. The movement of lice from interbarb spaces increases their vulnerability to preening, but it may also provide a visual stimulus for preening behavior, leading to increased preening when birds are in bright light (Caldwell et al., 2001). These observations yield 2 simple predictions. First, birds in bright light, such as direct sunlight, should preen more frequently than birds in shade. Second, birds given opportunities to preen in direct sunlight should eliminate more ectoparasites than birds kept in the shade.

To test these 2 hypotheses, we conducted an experiment with captive mourning doves (*Zenaida macroura*) and their feather lice (*Columbicola baculoides*). Like all such lice, *C. baculoides* are permanent ectoparasites that spend their entire life cycle on the body of the host (Marshall, 1981). *Columbicola* spp. feed primarily on feathers and dead skin and decrease host mating success, thermoregulatory ability, and survival (Clayton, 1990; Booth, 1993; Clayton et al., 1999). They will, therefore, exert selection on the host for efficient preening and other defenses (Clayton et al., 1999).

MATERIALS AND METHODS

Forty-eight mourning doves were captured using mist nets near Tucson, Arizona. They were transported to the University of Utah, Salt Lake City, Utah where they were housed individually in 30 × 30 × 56-cm wire mesh cages in a windowless animal room with a full-spectrum fluorescent lighting. All birds were maintained on a 12 hr light/12 hr dark cycle and provided grain, grit, and water ad libitum. These birds were used to culture lice for other experiments for over a year. They were then used in the experiment described herein before being killed as required by our IACUC committee. All research was conducted under IACUC protocol # 05-08009.

For 15 wk prior to the start of the experiment, we reduced relative humidity (rh) in the animal room to a low level (<30%) to kill 100% of the lice and eggs already present on the birds (Harbison et al., 2008). After the 15-wk period, all birds were visually examined for 30–60 sec in each of the following body regions: head, keel, back, rump, wings, and tail (Clayton and Drown, 2001). No lice were found on any of the 48 birds, confirming the effectiveness of the low-humidity procedure.

We used a 2 × 2 factorial design to investigate the effects of light exposure on preening and louse abundance. Birds were randomly assigned to a sun or shade treatment. Each group was further randomly subdivided into bitted (preening impaired) or not bitted (preening unimpaired) groups for a total of 4 treatments with 12 birds per treatment. Preening was impaired using plastic, C-shaped bits that fit between the mandibles of the

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bird's bill. Bits create a 1–3 mm gap between the mandibles that disrupts the occlusion of the bill tips required for efficient preening but without affecting feeding ability (Clayton et al., 2005). Birds in the unimpaired preening group were handled similarly to bitted birds. One week later, each clean bird was "seeded" with 100 adult *C. baculoides* from a culture stock using methods described in Moyer et al. (2002). Throughout the experiment, the animal room was set at 24 C and 50% rh—conditions at which *C. baculoides* thrive on captive mourning doves (Malenke et al., 2011).

Two days after the 48 birds were seeded with lice, sunlight manipulation treatments were initiated. These treatments involved 2-hr sessions each morning, during which all 48 cages were moved outdoors 2 hr after sunrise. Half of the cages were randomly assigned to the shade group, which had cotton fabric covering the top and side of the cage that faced the early morning sun. The cages of birds assigned to the sun group remained uncovered. The sessions took place in the early morning to prevent any risk of heat stress. Data loggers (HOBO® U12-001) placed directly on a subset of cages in the sun or shade recorded temperature and relative humidity. The experiment lasted 20 days (14 September 2007–3 October 2007). *Columbicola* take a mean (±SE) of 24.4 (±0.3) days to mature to the adult stage from eggs (Martin, 1934). Therefore, the 20-day duration for the experiment was chosen because it allowed us to test for effects of treatment on the survival of a single cohort of lice.

The 48 cages were arranged randomly within a grid each morning. Each cage was in full view of 1 of 2 observers (JAHK or SKH). At the end of the 2-hr period, cages were transferred back to the animal room. On overcast or rainy days (3 days in total), cages were not placed outdoors. During each session, the observers recorded preening and other behaviors using instantaneous scan sampling (Altmann, 1974). They recorded a total of 30 observations per bird per day with each observer making half of the observations for each bird.

At the end of the experiment, all birds were killed, placed individually in plastic bags, and frozen. Later, each bird was thawed and subjected to a body washing procedure that accounts for 99% of the lice on a bird (Clayton and Drown, 2001). A 2-way ANOVA was used to test for an effect of treatment (light exposure and bitting) on preening behavior and louse abundance. All values are presented as the mean (±SE). Analyses were done in Prism v.5.0 (GraphPad Software, Inc).

RESULTS

Temperature and rh varied predictably with treatment. Temperature in the sun, which was 20.79 (±0.18) C, was significantly greater than temperature in the shade, which was 17.01 ± 0.18 C (Mann-Whitney, $U = 89377$, $P < 0.0001$). Over the course of the experiment, maximum temperature in the sun reached 29.7 C and maximum temperature in the shade reached 24.9 C. The rh was significantly lower in the sun, where it was 28.0 ± 0.4%, compared to the shade where it was 34.7 ± 0.4% ($U = 101105$, $P < 0.0001$).

Neither sunlight nor bitting had a significant effect on the frequency of preening observed among groups (2-way ANOVA: light treatment, $F_{1, 44} = 1.22$, $P = 0.28$; bitting treatment, $F_{1, 44} = 0.002$, $P = 0.96$; light × bitting interaction, $F_{1, 44} = 0.08$, $P = 0.78$; Fig. 1). In contrast, there was a strong effect of bitting on adult louse abundance (bitting treatment, $F_{1, 44} = 18.46$, $P < 0.0001$). However, there was no effect of sunlight on adult louse abundance nor any interaction between sunlight and bitting (light treatment, $F_{1, 44} = 0.07$, $P = 0.79$; light × bitting interaction, $F_{1, 44} = 0.04$, $P = 0.84$; Fig. 2).

The frequencies of 2 other bird behaviors were also independent of treatment. There was no significant difference in the frequency of feeding between groups (light treatment, $F_{1, 44} = 1.54$, $P = 0.22$; bitting treatment, $F_{1, 44} = 1.29$, $P = 0.26$; light × bitting interaction, $F_{1, 44} = 0.65$, $P = 0.42$) nor was there a difference in the frequency of resting (light treatment, $F_{1, 44} = 2.78$, $P = 0.10$; bitting treatment, $F_{1, 44} = 0.001$, $P = 0.96$; light × bitting interaction, $F_{1, 44} = 2.63$, $P = 0.11$).

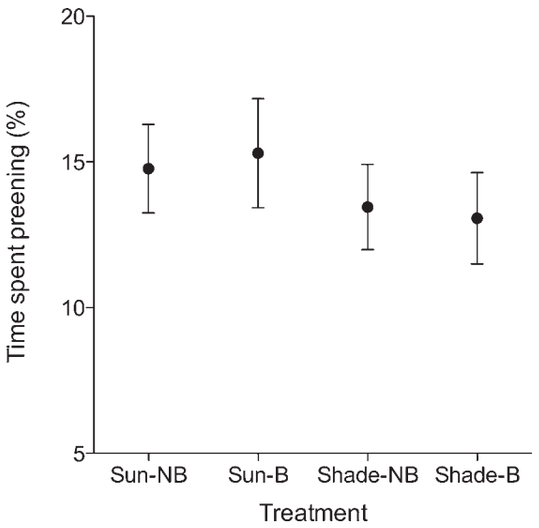

FIGURE 1. Mean (±SE) percent time spent preening for each treatment group: exposed to sun (Sun), exposed to shade (Shade), not bitted (NB), and bitted (B).

In contrast, there was an effect of sunlight on sunning, a behavior in which birds spread their wing and tail feathers while lying prone on the ground (light treatment, $F_{1, 44} = 13.02$, $P < 0.001$). Only birds with access to direct sunlight performed sunning behavior. However, sunning behavior was very uncommon, accounting for less than 1% of all behavior. There was no significant effect of bitting on sunning behavior nor an interaction between light exposure and bitting (bitting treatment, $F_{1, 44} = 2.46$, $P = 0.12$; light × bitting interaction, $F_{1, 44} = 2.457$, $P = 0.12$).

DISCUSSION

The goal of this study was to explore the relationship between sunlight and preening for ectoparasite control. First, we tested the

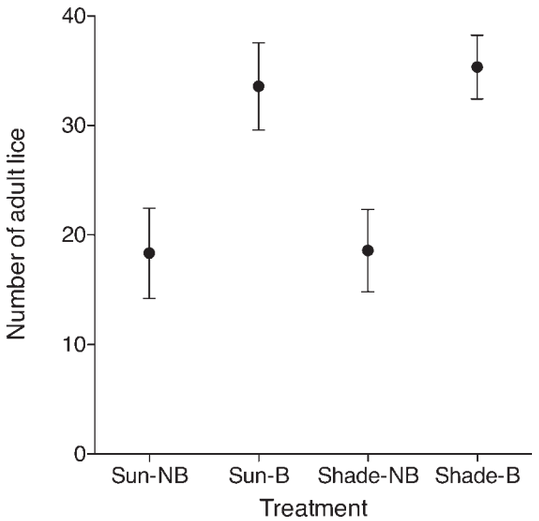

FIGURE 2. Mean (±SE) number of adult lice at the end of the experiment: exposed to sun (Sun), exposed to shade (Shade), not bitted (NB), and bitted (B).

hypothesis that birds with an opportunity to preen in sunlight would preen more frequently than birds in shade. We also tested whether preening in sunlight is more effective at controlling ectoparasites than preening in the shade. To eliminate variation in parasite load at the start of the experiment, parasite-free birds were "seeded" with identical numbers of lice. We increased the probability of detecting effects on parasite load by infesting birds with 100 lice each—4-fold the number found on wild mourning doves in Utah (mean = 23.8, Malenke et al., 2011). At the end of the experiment, birds were killed and louse populations measured using a washing method that quantifies parasite load very accurately (Clayton and Drown, 2001). In summary, our experimental approach should have allowed us to detect even small treatment effects. By manipulating both access to sunlight and preening ability, the design of this experiment allowed us to test for direct and indirect effects of each factor on preening efficiency.

Sunlight may cause lice to move on feathers, increasing their vulnerability to host preening and, in doing so, providing an additional visual stimulus for preening behavior. Therefore, we predicted that birds in sunlight would increase their preening frequency. However, our results show that birds in sunlight do not, in fact, preen more than birds in shade (Fig. 1). This result suggests that sunlight is not a stimulus for preening behavior, at least in captive mourning doves.

While the amount of time birds spent preening between treatments did not differ significantly, birds that preened in sunlight might still have been more effective at controlling their ectoparasites. However, our study further showed that birds with access to sunlight did not have significantly fewer lice at the end of the experiment than did birds in shade (Fig. 2). Thus, sunlight does not appear to increase preening efficiency and, thus, the ability of birds to control their ectoparasites.

In our study, birds were exposed to direct sunlight or shade for 2 hr each day. Although this was only 17% of the diurnal phase of the experiment, it is more than the 11–12% of time adult pigeons (*Columba livia*) spend preening in nature (Clayton, 1990) and more than the average 9.2% of time birds spend performing general maintenance behaviors (Cotgreave and Clayton, 1994). Of course, birds also spent time preening in the animal room. However, light intensity in the windowless animal room was clearly lower than in the light outdoors. Therefore, we expected birds to capitalize on conditions of direct sunlight outdoors, such that the 2-hr period each day would have been sufficient to detect any difference in preening frequency, the effectiveness of preening between treatments, or both.

Sunlight is also central to the ability of birds to combat ectoparasites with sunning behavior, during which birds often lie prone on the ground with their wing and tail feathers spread and their head feathers erect, facing directly into the sun (Simmons, 1986). Moyer and Wagenbach (1995) showed that the surface temperatures of feathers during bouts of simulated sunning are lethal to feather lice. However, these authors also found that birds perform sunning behavior only when the air temperature exceeds 29 C, which seldom occurred in our experiment. Sunning behavior was rare in our study, comprising less than 1% of all recorded behaviors. Not surprisingly, only birds with access to direct sunlight engaged in sunning. Our experiment was purposefully carried out during relatively cool autumn weather, which avoided the potentially confounding effects of high temperature on host behavior and parasite survival. Future studies could use a similar experimental design, under conditions known to elicit more frequent sunning behavior, in order to test the effectiveness of sunning, per se, in controlling ectoparasites.

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