Responses of *Ornithonyssus sylviarum* (Acari: Macronyssidae) and *Menacanthus stramineus* (Phthiraptera: Menoponidae) to Gradients of Temperature, Light, and Humidity, With Comments on Microhabitat Selection on Chickens

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**ABSTRACT**  Responses of the northern fowl mite (NFM), *Ornithonyssus sylviarum* (Canestrini & Fanzago) (Acari: Macronyssidae), and the chicken body louse (CBL), *Menacanthus stramineus* (Nitzsch) (Phthiraptera: Menoponidae), to variation in temperature, light, and humidity were assessed in bioassays. The location on a continuous thermal gradient at which each ectoparasite arrested was recorded and analyzed. NFM adults arrested at an average temperature of 30.09 ± 0.34°C. Adult CBL and first-instar CBL nymphs arrested at 33.69 ± 0.20°C and 34.99 ± 0.26°C, respectively. Groups of each ectoparasite were placed into clear glass vials (n = 10/vial) with one half shaded, and vials were exposed to three light levels, as follows: high (200 μmol m⁻² s⁻¹), low (4 μmol m⁻² s⁻¹), and nearly no light (0 μmol m⁻² s⁻¹). The vial cap edges provided an opportunity to assess the interactive effect of light with harborage. NFM avoided light and sought harborage. In low light, the harborage preference overrode the tendency to avoid light. CBL avoided the harborage and showed a minimal preference for light. A four-level humidity gradient was established in two separate experimental arenas for NFM and CBL. Trials were run in ambient light (4 μmol m⁻² s⁻¹) for the NFM and in nearly no light for the CBL. The NFM gradient used 38 ± 2%, 54 ± 7%, 73 ± 3%, and 90 ± 4% RH, whereas the CBL gradient used 42 ± 5%, 48 ± 7%, 63 ± 4%, and 73 ± 5% RH. NFM showed no humidity response in the walking bioassay, but the CBL settled at the lowest humidity level. Temperature and humidity on different hen body regions were related to the bioassay results and observed on-host ectoparasite distributions.

**KEY WORDS**  ectoparasite, microhabitat selection, host-parasite interactions, resource partitioning

Ectoparasite infestations, specifically *Menacanthus stramineus*, the chicken body louse (CBL), and *Ornithonyssus sylviarum*, the northern fowl mite (NFM), significantly impact the poultry industry (DeVaney 1976, Mullens et al. 2009). The NFM is regarded as more severe and widespread, and both pests are controlled primarily by biosecurity and pesticide applications (Axtell and Arends 1990, Kells and Surgeoner 1996). Particularly with the onset of resistance (Mullens et al. 2004a) and loss of pesticide options, our ability to manage these important pests long-term depends on an improved knowledge of their biology.

The two ectoparasites show distinctive distributions on the host. NFM reside almost exclusively in the feathers around the vent area of hens, although they can be found in lesser numbers in other body regions (Lemke et al. 1988). CBL use many different regions, but generally are most common on the lower abdomen (Trivedi et al. 1991). Although not well quantified, young CBL nymphs predominate under the wings (Brown 1970). NFM reside on the feathers 1.2–2.5 cm from the skin (DeVaney 1989) and return to the skin to feed periodically (Matthysse et al. 1974), whereas CBL tend to remain on the skin’s surface, but forage into the feathers as well. NFM protonymphs and adults feed on blood (Cameron 1938, Sikes and Chamberlain 1954), and CBL feed on feather barbules, pin-feathers, and the fluids contained within them, or can scrape skin surfaces and thus feed on blood (Crutchfield and Hixson 1943, Stockdale and Raun 1965, DeVaney 1976).

Distinctive ectoparasite distributions on the host are common, and are probably influenced by food or other resources, microhabitat conditions (e.g., temperature or humidity) on the host’s body, and/or the host’s grooming behavior (Clayton et al. 2010, Marshall 1981). For example, 70% of sheep-biting louse *Damalinia ovis* (Schrank) eggs were laid on wool 0.6 cm from the skin of sheep, where the preferred temperature zone of 35–38°C occurred (Murray 1956a).
Ambient conditions change and thus can alter ectoparasite distributions temporally as well. Sheep skin temperatures of exposed, wool-free legs quickly became unfavorable for house development at low ambient temperatures (Murray 1956a). Humidity has been shown to affect development and survival of some lice (Murray 1956b, 1960), but temperature was found to have the greatest influence on *D. ovis* when compared with light and humidity (Murray 1956b). Off-host survival of poultry pests such as NFM and CBL (Abasa 1969, Chen and Mullens 2008) or the red mite *Dermanyssus gallinae* (De Geer) (Nordenfors et al. 1999) is greatly influenced by temperature and humidity, which affects their ability to persist in empty poultry houses between flock transitions. It has also been suggested that morphological adaptations of ectoparasitic lice are related more to areas on birds that are safe from preening than to the abiotic features of the area (Murray 1990).

The objective of the current study was to gain an understanding of NFM and CBL behavior, specifically their responses to temperature and humidity as well as their reactions to various levels of light. These abiotic factors were manipulated in the laboratory, and measurements of temperature and humidity were made on different parts of a hen’s body. The data gathered offer insights to ectoparasite spatial distributions on the host, and might help predict conditions that would trigger movement to different regions. The data may also relate to their off-host distribution, and thus have relevance to controlling residual ectoparasites in empty poultry houses between flocks.

**Materials and Methods**

**Thermal Gradient.** A thermal gradient was created in the laboratory using an aluminum bar 4 cm wide, 7 mm thick, and spanning a horizontal distance of 40 cm (Fig. 1). The bar was heated at one end by placing it on a controlled temperature hot plate (Corning PC-400D, Corning, Lowell, MA) and cooled at the other end using a chill table (model 1012, Industrial Inventions, Monmouth Junction, NJ). The bar had a total length of 60 cm, and 10 cm of bar was taped onto each of the two temperature control surfaces to ensure there was enough physical contact for thermal conduction. The chill table was set to the coolest setting, 10 (slightly below 0°C). The hot plate was set at 180°C for the trials with the NFM and 170°C for the trials with the CBL. The calibrated portion of the NFM gradient began at 20°C and continued in 1°C increments up to 50°C. A degree difference occurred approximately every 5–10 mm of horizontal distance on the bar. The bar was calibrated using a Physitemp probe (model BAT-12, Physitemp Instruments, Clifton, NJ) held uniformly at 100 μm above the bar surface by using a microscope slide coverslip as a spacer. Two AA batteries attached to the probe provided a steady downward pressure of 51 g. The probe was slowly pushed along the gradient during calibration. The metal’s surface and ambient air temperatures above differed (Bair 1950); we selected the 100-μm distance to approximate the air temperatures encountered by the ectoparasites’ bodies as suspended slightly on their legs.

Both ectoparasites were collected from hens at the University of California Agricultural Experiment Station in Riverside. CBL were collected and used the same day (usually within 2–4 h), because they die within 2–3 d off-host, in contrast to NFM, which survive well off-host (Chen and Mullens 2008). NFM were collected and allowed to acclimate 24–48 h in a chamber held at 75% RH using a saturated NaCl solution (Winston and Bates 1960) and at 30°C, a temperature common in their feather habitat (Combs and Lancaster 1965).

Behavioral criteria for determining arrestment followed in this study are the same as those outlined in Owen and Mullens (2004). NFM adults were first placed individually on the gradient at the 25°C mark with a paintbrush and timed until they arrested. Eventually, groups of five were placed simultaneously for faster data acquisition, and comparisons were made between the two mite groups. The bar was wiped clean with acetone between each trial with the NFM (total N = 124). Adult CBL were always placed one at a time at the 25°C mark (N = 148). First-instar CBL nymphs were placed on the bar one at a time at the 30°C mark (N = 65) because they would not initiate thermotaxis at the 25°C mark like the NFM and adult CBL did. Attempts were made to avoid any extra

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![Fig. 1. Thermal gradient (lateral view), NFM design with clear plastic cylinder to reduce air disturbances; CBL design omitted the cylinder. (Online figure in color.)](https://academic.oup.com/jme/article-abstract/48/2/251/892480/20251)
vibrations, light fluctuations, or disruptions of the air space for all trials.

Because NFM delayed arrestment and were found to be quite sensitive to air currents, vibration (Owen and Mullens 2004), and light in preliminary trials (despite our initial attempts to minimize them), experiments were run in a darkened room (∼0 μmolm⁻²s⁻¹) and the bar was encircled by a clear plastic cylinder to create a still region of air for a radius of 8 cm around the bar (Fig. 1). To see the minute adult mites, NFM were dusted with fluorescent powder (DayGlo Saturn Yellow) before introduction to the walking arena. A mirror was placed behind the bar, allowing the observer to view the NFM on all surfaces of the bar. The observer sat quietly in the room 1.5 m from the test apparatus. A brief inspection of the bar during the trial with a handheld ultraviolet light (about every 5 min) confirmed the mites’ arrested locations on the bar over periods of 5–20 min.

The CBL experiments were run in the same room, but both adult and first-instar CBL were far less sensitive to light (in this experiment) and disturbance, and trials were run in ambient light without the cylinder. On the flat metal surface, running adult CBL frequently fell off the bar. To contain them to the bar’s surface, a square-channeled aluminum bar of equal length was placed onto the original bar, and two Teflon-coated walls of plastic were placed along the vertical sides of the channel. The new bar setup was recalibrated. First-instar CBL nymphs were able to walk on all surfaces of the aluminum bar, allowing for trials to be run on the same bar used for NFM trials. The CBL were observed until they arrested (usually <5 min).

Light. Ectoparasites were placed in groups of 10 into small, clear glass shell vials that were covered in black electrical tape (shaded) on one half. A 2- to 3-mm hole was punched in the tight-fitting, translucent plastic cap of each vial for ventilation, and a 2-cm square piece of Kimwipe tissue (Kimberly Clark, Roswell, GA) was pushed into the vial with the cap, confining the NFM or CBL while minimizing any shading effects. This also provided a small groove that served as a potential physical harborage cue. Paired vials were used in each bioassay, with one vial covered by tape at the end where the cap was and one covered at the closed glass end, leaving the cap end exposed. The possible harborage (cap) × light interaction thus was tested simultaneously. Trials were run on a heavy granite table that was specifically designed to damp out vibrations.

At 5-min intervals through to 30 min and then 10-min intervals through to 60 min, the numbers of NFM or CBL seen in the untaped, visible ends of the vials were recorded. Each pair of vials was exposed to one of three levels of light intensity. The first light level was called high light, in which all lights in the laboratory were on and a 13 W compact fluorescent light fixture was held 3.5 cm above the vial arena (the two vials rested side by side on clear plastic stands on top of a mirror). Light intensity was measured with a Quantum Meter (model QMSW, Apogee Instruments, Logan, UT) in units of Photosynthetic Photon Flux, with high light being 200 μmolm⁻²s⁻¹. The next light level was low light (4 μmolm⁻²s⁻¹), in which all the lights in the laboratory were on, but the compact fluorescent light fixture was turned off. The lowest light level was no light (0 μmolm⁻²s⁻¹), in which the vial arena was moved to a darkened room and placed on a thick metal plate resting on cotton. There was some very dim light present in the room, but it was not detectable by the sensor. Mostly adult NFM (N = 140 mites high light, N = 120 low light, and N = 100 no light) and adult and later-instar CBL nymphs (N = 120 lice high light, N = 120 low light, and N = 120 no light) were observed by carefully and quietly entering the room every 5 min for the first half hour and then every 10 min for the rest of the hour. A flashlight outfitted with a red filter was used for viewing the ectoparasites in the no light treatment.

Humidity Gradient. Two different humidity gradient bioassay designs were used. For NFM, a transparent acrylic box 40.5 cm long × 15.5 cm wide × 15.5 cm tall was partitioned into four separate, watertight chambers each 10 cm long (Fig. 2A). Holes 7 mm in diameter were drilled to allow a 3-mm-diameter glass rod to pass through the entire length of the box (through all chambers) centered 5 cm down from the top. Interior to the rod’s wire suspenders on each end, a thin film of Tanglefoot (The Tanglefoot Company, Grand Rapids, MI) was applied onto the glass rod to confine the NFM to the box’s chambers. Only the opposite ends of the rod made any contact with the box, and the NFM could pass freely among chambers, but had no means of walking off the rod. A lid with 10-mm-diameter holes above each chamber was placed on the top to create still air columns. The holes were covered by transparent adhesive tape, except when a RH probe (Vaisala HUMICAP model HM34, Helsinki, Finland) was inserted through them to check the relative humidity. To create the gradient, saturated salt solutions were added to the bottom of each chamber (Winston and Bates 1960) to fill them up to a depth of 2 cm. Trials were run at 22–23°C on days with ambient humidity of 45–60%. The humidity levels for the trials were 38% (NaCl), 54% (amphib, no solution), 73 ± 3% (NaCl), and 90 ± 4% RH (H₂O). For each trial, five NFM adults were placed on the rod by passing a paintbrush through the 10-mm-diameter hole into a chamber (N = 100 mites). The lid was taped on to ensure the best seal. The number of NFM on the rod in each chamber was noted at 5-min intervals over the course of 1 h. The glass rod was then removed and thoroughly cleaned with acetone for the experiments to be repeated. The paintbrush used was also cleaned with acetone. Because we did not analyze arrestment, trials were run at ambient light levels (4 μmolm⁻²s⁻¹). NFM were introduced into a different chamber for each trial, and the entire box was also rotated 180° halfway through the course of the trials to control for any possible light gradients.

Like the thermal gradient, the humidity gradient had to be redesigned to compensate for differences in

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CBL walking behavior. CBL were incapable of hanging onto the glass rod, or even consistently to a wooden dowel of equal diameter. It was observed that CBL also tended to climb to the highest point when in an enclosure. This behavior was exploited for the trials in a circular plastic arena (Fig. 2B). The top of the arena had a fine mesh screen to allow them to adhere to the ceiling. The rest of the chamber beneath was composed of a 9 cm diameter \times 5.1 cm tall cylinder divided into quarters by plastic vertical partitions. The bottoms of the quarters were water tight and contained the same saturated salt solutions as the NFM gradient, but in a 1-cm-deep layer. Each quarter had a sealable hole in the side to insert the RH probe. The humidity levels for the trials were 42 \pm 5\% (\text{MgCl}_2), 48 \pm 7\% (\text{ambient}), 63 \pm 4\% (\text{NaCl}), and 73 \pm 5\% (\text{H}_2\text{O}). A coarse wire screen was placed above the solutions in each quarter to catch any falling CBL, where they could then climb back up to the ceiling. The top of the plastic partitions came to within 2 mm of the top, allowing the CBL to walk freely along the circumference of the top while still being able to pass through each differently humidified air space. Given the degree of contact between the air spaces and the size of the spaces relative to the NFM chamber, the range of the gradient was comparably smaller versus that of the NFM bioassay. Both late-stage CBL nymphs and adults were introduced 10 at a time for each trial.
through a specified quarter’s RH probe entry hole on a side of the chamber (N = 150 lice). They were introduced into a different quarter (RH level) for each consecutive trial. Because the CBL were fairly responsive to light in this experiment, the gradient was placed in a darkened room (≈0 μmolm⁻²s⁻¹). To control for any minor imbalances of light, the gradient was rotated one-quarter turn every 15 min during the course of the hour-long trial.

High Temperature Limits on NFM Egg Hatch. Adult NFM were collected early in the day from the aforementioned donor hens and allowed to oviposit in glass shell vials throughout the day. The oviposition vials were kept at ambient laboratory conditions (25°C and 50% RH). After ≈5 h, eggs were collected with a fine paintbrush and placed up to seven at a time into clean vials. The duration of the NFM egg stage was reported to be 30.4 h at a temperature range of 37.7–40°C and at 90–100% RH (Cameron 1938). Each day, new vials of eggs went into each one of four plastic containers, and vials were held above saturated NaCl solutions. There were four incubators set to yield different temperatures immediately adjacent to the egg vials in the containers: 37°C, 39°C, 41°C, and 31°C (because the latter is close to the allegedly preferred temperature, it served as the control). Each incubator held one container. Temperatures were measured using a YSI Tele-Thermometer probe (model 43TD, Yellow Springs Instruments, Yellow Springs, OH). After being placed in the incubators, the relative humidity in each of the 37°C, 39°C, 41°C, and 31°C containers was checked with the RH probe and ranged from 56.7 to 95.5% RH. The NaCl solution did yield a RH closer to the expected 75% with a range of 72.5–78.5% RH in the control (31°C) incubator. To obtain some control tests with the higher humidity level, water was used in the slightly ventilated container, which yielded 78–89.1% RH. Over the course of 3 d postoviposition, the vials of eggs were checked daily for hatching.

On-Host Humidity and Temperature Readings. Using the Vaisala RH probe and the YSI temperature probe above, readings were taken from each of five 6-to12-mo-old, fully feathered Isa Brown hens at the University of California Agricultural Experiment Station in Riverside. Skin surface temperature readings were taken just anterior to the vent, under the wing, on the anterior keel, and on the back. The temperature probe was allowed to rest on the skin surface, and the feathers were allowed to cover the probe in a normal orientation for ≈2–3 min to obtain each reading. A temperature reading was also taken within the vent feathers 2 cm from the skin’s surface by temporarily clipping the probe tip to a vent region feather of each hen. Humidity readings were taken from the same four locations. The RH probe was pushed into the feathers as close to the skin as possible, and feathers were returned to the normal position to allow the RH probe reading to stabilize. Ambient air temperature and humidity were 25–29°C and 40% RH, respectively. Temperature readings were conducted on two separate days, whereas the RH readings were conducted only on the second day.

Results

Thermal Gradient. Minitab version 14 was used for data analysis (www.minitab.com). The thermal gradient data sets had different variances and unequal sample sizes; therefore, a nonparametric test was used to determine whether the three medians were different (pairwise Mann-Whitney tests). Arrestment temperatures for NFM (median = 31.00°C; mean = 30.09 ± 0.34°C) differed from adult CBL (median = 34.00°C; mean = 33.69 ± 0.20°C) and first-instar CBL nymphs (median = 35.50°C; mean = 34.99 ± 0.26°C); the two CBL stages also differed from each other (W = 8,531; P < 0.001) (Fig. 3). The NFM tested in groups of five (n = 57) were lumped together and compared with those tested singly (n = 67). The arrestment temperature for the single NFM (median = 31.00°C; mean = 30.56 ± 0.46°C) did not differ significantly from the grouped NFM (median = 30.00°C; mean = 29.54 ± 0.49°C) (W = 4,344.0; P = 0.4341); therefore, the two were combined and analyzed as one NFM group.

Light. A general linear model analysis of variance was used to analyze the data from the light experiments. Data were cap-side adjusted (proportions in the cap end when it was concealed were calculated as: 1 minus proportion visible in other end), and square root arcsine-transformed proportions were used for the general analysis. Factors considered in the analysis were the light effect, the cap effect, time (multiple time observation points over the 1-h period), and interactions between them.

The NFM demonstrated thigmotaxis by showing a tendency to use the small recess created by the outer circumference of the cap and Kimwipe tissue meeting the vial wall (positive cap effect). Main effects of both light (F = 32.45; df = 2, 311; P < 0.001) and cap (F = 387.55; df = 1, 311; P < 0.001) were highly significant for NFM; however, the time effect was not (F = 0.69; df = 9, 311; P = 0.719). The light × cap interaction (F = 153.74; df = 2, 311; P < 0.001) and the time × cap interaction (F = 6.15; df = 9, 311; P < 0.001) were both significant. The light × time interaction was not sig-
significant \( F = 0.98, \text{df} = 18, 311; P = 0.484 \). The light \times \text{cap} interaction for the NFM is evident in Fig. 4A. The bar values (means) and standard error on the graph were generated from raw data. In all light treatments, there was a significant difference between the cap-exposed and the cap-concealed ends. Note that the treatments for each light level show mean NFM proportions in the somewhat preferred cap end (positive cap effect) only; the proportion in the other end is 1 minus the cap end proportion. At high light intensity, NFM markedly avoided the light. At high light (cap exposed vial), nearly 80\% of NFM avoided light even when the harborage was there. In low light (cap exposed vial), 60\% of the NFM tolerated the light to gain the harborage. This tendency to settle in relative darkness and select harborage nearly disappeared at the no light intensity, but some vestiges of the thigmotactic behavior remained even at this minimal light level.

Analysis of all factors of the transformed CBL data showed a somewhat positive (i.e., attractive) main effect of light, but it was not quite statistically significant \( F = 2.82; \text{df} = 2, 317; P = 0.061 \). The main cap effect was negative and significant \( F = 40.62; \text{df} = 1, 317; P < 0.001 \), and the time effect was not significant \( F = 0.31; \text{df} = 9, 317; P = 0.970 \). However, the light \times \text{cap} interaction was significant \( F = 3.92; \text{df} = 2, 317; P = 0.021 \).

In the high and low light treatments, there was a significant difference in CBL distribution between the cap-exposed and the cap-concealed ends. There was no difference between the two conditions for the no light treatment (Fig. 4B).

**Humidity Gradient.** Both NFM and CBL distribution data were analyzed using a one-way analysis of variance. The average numbers of NFM at each count up to and including the 30-min count were compared with each other to see whether there was a difference in the counts of NFM in the different humidity levels. In the majority of trials, the NFM continued movement between the chambers for up to 30 min. After that point, they began arresting and we no longer assumed independence between each count (even though counts continued for another 30 min). Although there were four counts for the CBL trials (one every 15 min), the CBL arrested fairly quickly and only the average numbers in each chamber from the first count were compared for differences. There was no significant difference in NFM counts among the different humidity treatments \( F = 2.14; \text{df} = 3, 76; P = 0.103 \) (Fig. 5A). The CBL, however, showed a tendency to gather in somewhat lower humidity areas \( F = 5.93; \text{df} = 3, 56; P = 0.002 \) (Fig. 5B).

**High Temperature Limits on NFM Egg Hatch.** The lethal temperature for egg hatching occurred between 37°C and 39°C. In the 37°C incubator, 37 of 41 eggs...
they are solitary or gregarious, and whether they are hosts, such as host size, grooming behavior, whether mites are linked to the ecological adaptations of their (Marshall 1981). The ectoparasites’ lifestyles are ultimately linked to the ecological adaptations of their hosts, such as host size.

Fig. 6. (A) Average temperatures (±SE) on hen skin surface or 2 cm out on the vent feathers. (B) Average relative humidity within the feather coat near the skin’s surface. Histogram bars with the same letter are not significantly different using Tukey’s honestly significant difference (P < 0.05).

hatched. In the 39°C incubator, 0 of 42 eggs hatched (χ² = 67.25; df = 1; P < 0.001). Likewise, there was no hatching in the 41°C incubator (0 of 36 eggs hatched). There was no significant difference between the control (31°C) incubator (31 of 33 eggs hatched) and the 37°C incubator (χ² = 0.335; df = 1, P = 0.563).

On-Host Humidity and Temperature Readings. Although skin temperatures differed between the 2 d (day 1, N = 5 hens; day 2, N = 5 hens) during which readings were taken, the results of the relative comparisons between the various body locations were consistent. The average vent skin surface temperature was significantly lower than the average under-wing skin surface temperature on both days (day 1, F = 6.95; df = 3, 12; P = 0.006; day 2, F = 43.32; df = 4, 16; P < 0.001). There was no significant difference between the average anterior keel and back skin surface temperatures. The average vent feather temperature 2 cm from the skin was significantly cooler than the other skin surface temperatures (day 2, F = 43.32; df = 4, 16; P < 0.001) (Fig. 6A). The vent region had a significantly higher average RH than the other three body regions (day 2, F = 8.76; df = 3, 12; P = 0.002) (Fig. 6B).

Discussion

Although the host is the primary habitat for many ectoparasites, there are often specific regions on the body where they are most commonly encountered, and several species of ectoparasites can be found simultaneously on one host in different body regions, which implies some level of resource partitioning (Marshall 1981). The ectoparasites’ lifestyles are ultimately linked to the ecological adaptations of their hosts, such as host size, grooming behavior, whether they are solitary or gregarious, and whether they are covered with an insulating layer (e.g., hair, feathers) (Murray 1990). The skin and associated integumentary processes form a microclimate in which ectoparasites spend most of their lives; however, this can vary at small scales on the surface of the host (Marshall 1981).

Ectoparasites that have physically close associations with their hosts show a marked tendency to occupy specific temperature zones in particular body regions (Marshall 1981). Temperature can also be critically important for development or survival of particular life stages. The sheep-biting louse D. ovis showed a tendency to lay eggs within a certain range of temperature (Murray 1956b). Egg development and hatching for the louse were also dependent on temperature, but the temperature range at which the eggs hatched was found to be greater than the range at which they successfully developed (Murray 1960).

Our findings suggest NFM eggs will hatch within a range of 31–37°C, but somewhere between 37°C and 39°C lies a lethal temperature that prevents the eggs from hatching and/or developing. Cameron (1938) stated that NFM eggs hatched in 30.4 h in an incubator that reportedly varied between 37.8 and 40.0°C, but it is not clear how accurate or controlled the temperatures actually were. Similarly, he reported that all stages of NFM died between 40.0 and 42.5°C. Maurer and Baumgartner (1992) found that relative egg production by the red mite, D. gallinae, was greatest at 30°C, and the egg and immature stages of that mite died at 40°C.

Temperature gradients on the host and the possibility of being dislodged from the host present a challenge to the mobile stages of ectoparasites in locating the appropriate temperature region or in finding their way back to the host. Thus, thermal sensitivity may be markedly acute. D. gallinae is activated by a temperature gradient as low as 0.005°C/s (Kilpinen 2001), but the degree of sensitivity may vary with physiological condition. The duration of D. gallinae movement activated by the heat cue increased up to ~8 d off-host, and then declined with increasing levels of starvation (Kilpinen and Mullens 2004). Several experimental studies have investigated responses in walking ectoparasites to thermal gradients (e.g., Bair 1950, Stenram 1956, Wigglesworth 1941). NFM orientation toward a radiant heat source is thought to be idiothetic, that is, the mites use internally stored (programmed) information for course control, executing larger angular displacements when a heat cue diminishes and smaller angular displacements as it nears a heat cue. They do not actually move consistently toward the source, but end up doing so (Owen et al. 2005).

The average experimental NFM arrestment temperature of 30.09°C on our gradient matches previous on-host observations. Combs and Lancaster (1965) observed that the mite occupation zone in the feathers 1.3–2.5 cm from the skin was ~30°C, and this feather region is approximately the zone reported as favored by NFM in DeVaney (1989). Our findings show temperatures on the vent feathers 2 cm from the skin surface are cooler than skin surface temperatures on
the vent, the anterior keel, the back, or under the wings. The cooler temperature profile of the vent feathers may also provide the proper environment for the hatching and development of NFM eggs. Feathers on other body regions would likely also yield cooler temperatures when compared with the skin surface temperatures in those body areas. However, a thicker feather coat and the thick downy feathers found in the vent probably contribute to enhanced temperature stability relative to regions with thinner feathers or no feathers at all.

Both the average adult and first-instar CBL nymph arrestment temperatures (33.69°C and 34.99°C, respectively) were higher compared with that of the NFM. This reflects the fact that the skin surface is the primary habitat for CBL. (B.A.M., unpublished data), although they will forage up into the feathers. Brown (1970) reported perianal and under-wing skin temperatures, respectively, as 35.6°C and 39.5°C. Our findings indicate the average under-wing skin temperature is significantly higher than the average vent skin temperature. First-instar CBL nymphs arrested at a higher temperature than adults did, and this appears to agree with their frequent occurrence under the wings (Brown 1970).

Aside from temperature varying along the surface of the host, light and suitable locations for harborage are also likely to vary. Some parasitic mites have been known to respond to light (Camin 1953, Kilpinen 2005, Pegler and Wall 2004), and even though the NFM does not have obvious eyes, it will move away from light. NFM fed readily on a chick in the partially lit or darkened half of a feeding chamber (Sikes and Chamberlain 1954). The astigmatid mite, Psoroptes ovis (Hering), likewise showed a marked tendency to avoid light even though no light-detecting organs have been identified (Pegler and Wall 2004). When exposed to light, D. gallinae responded to CO2 by ceasing movement, but movement continued when CO2 was presented in the absence of light, indicating both the ability to detect light and a possible adaptation to avoid being eaten by the host (Kilpinen 2005). In a diel trapping study, tube traps deployed at night collected far more D. gallinae than did traps deployed during the day (Sokol et al. 2008).

NFM tended to be more restless on the thermal gradient in ambient light, and they arrested faster in the dark. Owen et al. (2005) stated that NFM did not appear to have distinct reactions to normal room level light. Our results showed that NFM reactions to ambient light (4 μmol m⁻² s⁻¹) are subtle relative to high light (200 μmol m⁻² s⁻¹).

There was some tendency for CBL, which have distinct eyes, to be attracted to light, although it was not statistically significant. If not provided a temperature gradient, the louse D. ovis chose to oviposit in the darkened side of an experimental arena (Murray 1956b). The CBL relative lack of sensitivity to light compared with the NFM could reflect their broader distribution on the hen (Trivedi et al. 1991), including the neck, which would be more exposed to light. At high densities, CBL even have been observed to drink from the eyes of hens (i.e., are completely exposed) during midday (Mullens 2010).

NFM had a strong tendency to arrest with harborage, which was present in the vials as the groove created by the outer circumference of the cap and Kimwipe where they met the glass wall (cap effect). This thigmotactic response is strong in a variety of temporary, nest-dwelling ectoparasites. It has been seen in D. gallinae, for example, and aggregation pheromones also influence the response (Entrekin and Oliver 1982). It is interesting to note the CBL showed a tendency to avoid the harborage of the cap end.

Several mesostigmatid mites, including selected hematophagous, predatory, saprophagous, and polyphagous species, absorb water from air at RH levels ranging from 76 to 97% (Gaede and Knüll 2000). Within the Acari, this ability can be attributed to glands in the mouth that secrete a hygroscopic fluid, which absorbs water from the air and is subsequently swallowed (Arlian and Veselica 1979). A relative humidity of 90% was found to be the equilibrium humidity at which the mite Echinolaelaps echidninus (Berele) neither lost nor gained weight (Wharton and Kanungo 1962). This ability may have an effect on mite survival off-host or in the absence of its food source.

NFM did not show any relative humidity preference in the present adult activity bioassay, despite the fact that humidity affects off-host survival. NFM survive longer off-host at higher humidities (Abasa 1969, Chen and Mullens 2008), but survival was adversely affected by very high humidity, i.e., 98% RH (Abasa 1969). The related poultry mite, D. gallinae, also survives better off-host at higher humidities (Nordenfors et al. 1999). It is possible the fitness of certain stages not tested, such as the protonymph and egg, might be affected by this factor, or the NFM walking bioassay may not have been able to show such a tendency. Clearly, the humidity in the vent feathers of a domestic hen is significantly higher than in other body regions (by ~15% RH), and this could significantly influence survival and fitness of the NFM living in the vent region. The vent feathers of a hen are distinctly thicker and much denser (feather down layer) than other body regions. Clipping vent region feathers reduced NFM numbers on hens (DeVaney 1986) by possibly modifying the humidity or temperature regimes and/or denying the mites appropriate structure for oviposition, etc. Heavy mite infestations result in visibly damp, encrusted feathers that are often almost glued together with mites and mite debris (cast skins, eggs, feces) that may be several mm thick. It was not possible to measure using our probe, but it is likely that the humidities in such dense mite aggregations approach saturation.

It is interesting to note that CBL (later-stage nymphs and adults) seemed to prefer low to moderate relative humidities, and unlike NFM, CBL arrested rather quickly in the bioassay chamber. In general, bird lice do better at higher humidities (Moyer et al. 2002). Those authors measured the humidity in feathers on the rump of captive pigeons, and the humidities were similar to ambient levels over a wide range of RH.
This tendency for feather coat humidities to resemble ambient humidity also showed on hens in the current study, for all regions except the vent. *D. ovis*, a biting louse of sheep, avoided ovipositing in high humidity zones. Extremely low humidities had little effect on egg development, but very high humidities prevented eggs from hatching (Murray 1960). Humidity ranging from 31 to 85% RH did not affect the relatively short off-host survival of CBL (Chen and Mullens 2008), but it still may be an important on-host factor. All mobile stages of *M. stramineus*, and the adults and protonymphs of *O. sylviarum*, feed at least periodically on host fluids, which may to some degree exempt them from stricter dependence on high humidity. The adaptive significance of the apparent tendency of CBL to occupy lower humidity zones is unclear. One fungal pathogen, *Tremomyces histophorus* (Chatton and Picard) (order Laboulbeniales), was reported infecting and possibly regulating populations of *M. stramineus* and other poultry-biting lice by Meola and DeVaney (1976), and such fungi typically do better under higher humidity conditions. However, we commonly observed dense aggregations of *M. stramineus* in the higher-RH vent region of hens in the current study, which does not support the idea that CBL avoid higher humidity zones on the host.

**Conclusion**

Even though both NFM and CBL are permanent ectoparasites, their response to abiotic factors and host cues is important for orientation and is probably related to habitat suitability, both on- and off-host. The feather coat is key to this. Nonfeathered areas on a hen can vary as much as 20°C over an ambient range of 20–40°C, whereas feathered areas vary only 2–5°C over the same ambient range (Richards 1971). Feather structure buffer temperature extremes at the skin’s surface, even though they are influenced by ambient temperature (Wilson and Plaister 1951). Although not yet documented, we have observed that NFM move farther out on the feathers when ambient temperatures are high. This is probably a symptom of their seeking optimal temperatures, resulting in their lodgment and making them more obvious to poultry workers (abundant on eggs) when the weather first becomes hot (Mullens et al. 2004b). The mite, *Protophyllodes stylicher* (Buckholz), also changed its feather position on blue tits with changes in ambient temperatures (Wiles et al. 2000).

To our knowledge, only Matthyssse et al. (1974) attempted to measure humidities within the feather coat of hens. They did so by removing small air samples from the feather coat using a syringe and then injecting them into a gas chromatograph. In those studies, the humidities in the vent feather coat near the skin actually were lower than ambient, which disagrees with our more direct observations using the Vaisala RH probe. We do not have experience with their technique, however. We have also not tested humidity effects on NFM egg hatch. Preliminary observations (B. L. Chen, personal communication) suggested that 31% RH was not conducive to NFM egg viability. Similarly, we did not specifically test younger CBL nymphs on the humidity gradient; they have a relatively higher surface area:volume ratio and have been noted gathering under the wings (Brown 1970). Our data do not suggest the humidities are higher beneath the wings, however.

When organisms are used in bioassays, trade-offs among multiple, potentially influential cues may influence experimental outcomes. For example, temperature had the most potent influence on *D. ovis* distribution when combined with either light or humidity in experimental trials (Murray 1956b). In our experiments, avoiding intense light appeared to be more important than seeking harborage for NFM, although at lower light levels, harborage was somewhat preferred despite the exposure to light. While logistically challenging, gathering light readings from the various surfaces of the hen’s body for comparison with the results gathered in this study would be of great interest.

Interestingly, roosters are reported to support higher numbers of NFM, over more of their body, than hens do (Hall et al. 1978, Matthyssse et al. 1974). This is probably hormonally influenced and related to immune function, but further studies of mite distribution on roosters could also yield more insights about physical habitat requirements for both NFM and CBL. The restriction of NFM to the vent region feathers of hens is likely due in part to the role of those unique feathers in increasing humidity. The relatively deep feather layer and considerable structural heterogeneity probably provide the mites with a generally favorable and stable environment, and the opportunity to find proper feather structure and zones in the range of 30°C. The extent to which that range may optimize mating, oviposition, and development remains to be determined. Similar information also is lacking for CBL. For either species of ectoparasite, much remains to be learned about what constitutes optimal conditions for their populations to thrive. Such knowledge could be helpful in poultry breeding programs, for example, which may allow us to develop host strains that lack characteristics needed by the ectoparasites.

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