

DUST-RUFFLING: A SIMPLE METHOD FOR QUANTIFYING ECTOPARASITE LOADS OF LIVE BIRDS

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Abstract.—We present a simple, accurate method for quantifying the ectoparasite loads of live birds in the field. Birds were dusted with pyrethrin to irritate their ectoparasites, which were then dislodged from the plumage by ruffling the feathers over a collecting surface for repeated timed bouts until the point of diminishing returns was reached. This method required less equipment and was more accurate and efficient than previously described methods, as we show by comparing our method to other popular approaches. Dust-ruffling is best suited for sampling “permanent” ectoparasites, such as chewing lice (Insecta: Phthiraptera), which pass their entire life cycle on the body of the host.

SACUDIDAS DE POLVO: UN METODO SENCILLO PARA CUANTIFICAR LAS CARGAS DE ECTOPARASITOS EN AVES VIVAS

Sinopsis.—Presentamos un método sencillo y preciso para cuantificar las cargas de ectoparásitos de aves vivas en el campo. Se polvorea las aves con pyrethrina para irritar los ectoparásitos, los cuales son removidos del plumaje al agitar las plumas sobre una superficie de colección en ocasiones consecutivas hasta que se llegue al punto de cargas menores. Este método requiere menos equipo y es más preciso y eficiente que métodos previamente descritos, lo cual evidenciamos al comparar nuestro método con otros métodos comunes. Las sacudidas de polvo es más apropiado para muestrear ectoparásitos permanentes, tales como Phthiraptera, que pasan su ciclo de vida completo en el cuerpo del hospedero.

Recent work shows that some ectoparasites can have a variety of serious detrimental effects on birds (Brown et al. 1995, Clayton and Moore 1997, Clayton and Tompkins 1994, de Lope et al. 1993, Lehmann 1993, Loye and Zuk 1991, Merino and Potti 1995, Møller et al. 1990, Richner et al. 1993). These and other studies have sparked widespread interest in carrying out further tests of the role of ectoparasites in avian behavior, ecology and evolution. In order to conduct such tests, however, it is necessary to have practical methods for accurately measuring the ectoparasite loads of birds.

A variety of methods have been used over the years to quantify the ectoparasite loads of live birds (reviewed by Clayton and Walther 1997). These methods can be divided into two major categories: (1) non-invasive counts made by simple visual examination, and (2) more accurate counts requiring the destructive sampling of ectoparasites. Although the first approach is necessary to collect longitudinal data on ectoparasite population dynamics, the second approach suffices for most studies and is usually more accurate.

Methods for the destructive sampling of ectoparasites from live birds

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range from those aimed at killing parasites *in situ*, to those designed to trap parasites as they escape from a freshly caught host (Clayton and Walther 1997). In this paper we describe a modified method for destructive sampling of ectoparasites, called "dust-ruffling," that is more efficient and accurate than previously described methods. We demonstrate the efficacy of dust-ruffling for quantifying populations of chewing lice (Insecta: Phthiraptera) from Common Swifts (*Apus apus*) and from Rock Doves (*Columba livia*). We also compare dust-ruffling to other methods (visual examination and anesthesia jars).

METHODS

Dust-ruffling.—This method employs pyrethrin dust in a two-step procedure. Pyrethrin is a common insecticide available in a variety of commercial forms (Casida and Quistad 1995). We used Johnson's Rid-Mite Insect Powder (Johnson's Veterinary Products Ltd., Sutton Coldfield, West Midlands, United Kingdom), which contains 0.1% pyrethrin and 0.8% piperonyl butoxide. Pyrethrin is derived from pyrethrum, a natural biodegradable insecticide extracted from the flowers of chrysanthemums. Pyrethrum and its derivatives are "fast knock-down, slow-killing" insecticides that are completely safe for use on birds and mammals (Casida 1973, Jackson 1985). Piperonyl butoxide is a synergist that improves the efficiency of pyrethrin. The piperonyl butoxide-pyrethrin mixture has no effect on the growth or survival of nestling or adult Rock Doves (Clayton and Tompkins 1995).

Pyrethrin is dusted onto a freshly trapped bird and worked into its plumage using the fingers of one hand while holding the bird over a smooth collecting surface with the other hand. A large pan lined with a sheet of paper makes a good collecting surface; colored paper provides the best contrast for seeing both light-colored nymphs and dark-colored adults (smaller parasites can be detected with the aid of a hand-held lens or a jeweller's magnifying headset). Dusting works best for sampling ectoparasites like chewing lice, which do not normally leave the body of a trapped host. To collect vagile parasites such as fleas or louse flies, it is necessary to insert the freshly caught bird into a bag for cursory dusting to kill such parasites before they escape (Clayton and Walther 1997). It is important, of course, to search the bag for parasites after the bird has been removed.

The dusting step requires about 3 min for Common Swifts and 5 min for Rock Doves, given the latter's denser plumage. The eyes of the bird should be shielded from dust to prevent irritation, although pyrethrin has no apparent effect on the eyes. When dusting a large number of birds, it is useful to wear a paper mask to prevent sneezing.

After the dust has been distributed throughout the plumage, each feather tract is ruffled to dislodge the dying parasites onto the collecting surface. There is no need to wait between the dusting and ruffling steps because pyrethrin is fast acting. After each bout of ruffling, ectoparasites are counted while being transferred to a vial of 70% ethanol using one

tip of a fine forceps that has been dipped in ethanol to make the delicate parasites adhere. A camel's hair brush dipped in alcohol can also be used, but it is critical to check the brush carefully for parasites lodged between the bristles. This is particularly important if the same brush is to be used for parasites from different host individuals or species. Failure to use caution here inevitably leads to erroneous host-parasite records. The collecting surface must be cleaned thoroughly between birds or, better yet, a new sheet of paper can be used for each bird.

Comparing the number of parasites recovered from each timed ruffling bout enables one to determine the point of diminishing returns at which the procedure can be stopped. For swifts we conducted 60-s bouts of ruffling until two consecutive bouts produced no additional parasites. For Rock Doves we conducted 180-s bouts until achieving two parasite-free bouts. This approach is similar to that devised by Clayton et al. (1992) in which 60-s ruffling bouts were repeated until a bout recovered <5.0% of the total number of parasites obtained during the first three bouts. In short, the decision to stop sampling a host is based on the recovery rate from that host. This criterion should provide a more accurate comparative estimate of parasite load than when hosts are sampled for an arbitrarily chosen period of time (Clayton and Walther 1997).

Comparison of pyrethrin to drione.—Drione is another insecticidal dust that has often been used to collect ectoparasites from live birds. Drione combines pyrethrin with an industrial desiccant known as dri-die that works by abrading the lipid layer of arthropod cuticle, leading to rapid desiccation and 100% ectoparasite mortality within 3 h (Tarshis 1967). Although drione is non-toxic to birds, the silicon it contains can remove plumage oil, causing birds to die from exposure when caught in rainy weather soon after dusting (R. L. Palma, pers. comm.). Clayton and Walther (1997) discuss uses of drione and dri-die in further detail.

To test whether pyrethrin might have a similar detrimental effect on the drying ability of plumage, we subjected 18 domestic quail (*Coturnix coturnix*) to the following treatments (two different birds per treatment per day over three days): (1) 10 min of pyrethrin dust-ruffling, (2) 10 min of drione dust-ruffling, and (3) 10 min of ruffling without dusting. Next we wet the plumage of each quail thoroughly with water by rotating the bird under a gently running tap for 45 s. We then estimated the plumage wetness of each bird at 15 min intervals by pressing six pieces of filter paper on top of, and beneath, the feathers of three plumage tracts (back, breast, and vent). Each piece of paper was then given one of the following wetness scores by the first author, who was blind to each bird's treatment category: 3 = soaked, 2 = moderately wet, 1 = barely wet, 0 = dry. These scores were used to determine the point in time at which each bird had dried out completely. An additional two birds, that were neither dusted nor ruffled, were subjected to the drying procedure to test for an effect of dust-ruffling itself on plumage drying time.

Comparison of dust-ruffling to other methods.—We compared pyrethrin

dust-ruffling to two other common methods for estimating the ectoparasite loads of live birds, anesthesia jars and visual examination.

Anesthesia jars expose the body of a bird (but not its head) to chloroform or other fumes in a sealed jar, thus causing ectoparasites to drop out of the plumage. We compared dust-ruffling to the anesthesia jar designed by Fowler and Cohen (1983), which has a cap replaced by a rubber diaphragm with a hole in it, through which the bird's head protrudes. We filled the jar with chloroform fumes by placing a few drops of liquid chloroform on a piece of filter paper in the bottom of the jar. Birds were exposed to the fumes for 5 min, and the ectoparasites were allowed to fall onto the filter paper. We also compared dust-ruffling to a more recent design in which the jar has an attached bellows that increases circulation of the fumes, presumably improving their penetration of the bird's plumage (Bear 1995).

Visual examination (i.e., direct counting of ectoparasites) is often used to estimate the parasite loads of live birds without harming the parasites themselves (Clayton and Walther 1997). We conducted 3 min visual examinations by checking all regions of the body, including the under-surface of each wing. The visual examinations, which were done for all Common Swifts and Rock Doves in the study, preceded the other methods of quantifying lice. Feathers were displaced during the examination with forceps.

We carried out three combinations of sampling methods on 12 swifts each: (1) pyrethrin dusting followed by a Fowler and Cohen (1983) anesthesia jar, (2) Fowler and Cohen jar followed by pyrethrin dusting, and (3) Bear (1995) anesthesia jar followed by pyrethrin dusting.

RESULTS

Dust-ruffling.—This method recovered large numbers of chewing lice (*Dennyus hirundinis*) and small numbers of louse-flies (*Crataerina pallida*) from swifts, as well as large numbers of chewing lice (*Columbicola columbae* and *Campanulotes bidentatus*) and small numbers of feather mites (unidentified) from Rock Doves. Only lice were collected in numbers adequate for statistical comparisons of dust-ruffling to other methods (see below).

Pyrethrin dusting alone, without ruffling, recovered 57 (66%) of a total of 86 lice collected from swifts, once they were also exposed to ruffling. Birds were ruffled for repeated bouts until the point of diminishing returns was reached (see Methods), which required a mean of 3.5 min of ruffling (range = 2–8 min; $n = 14$ birds). In the case of Rock Doves, dusting recovered only 171 (32%) of a total of 542 lice collected from the same birds when they were ruffled to the point of diminishing returns, which required a mean of 18 min of ruffling (range = 12–36 min; $n = 10$ birds). Ruffling times do not include the time required to first dust the bird (3 min for swifts, 5 min for Rock Doves).

Comparison of pyrethrin to drione.—Birds dust-ruffled with drione ($n = 6$) invariably required at least 15 min longer to dry than birds dust-ruffled

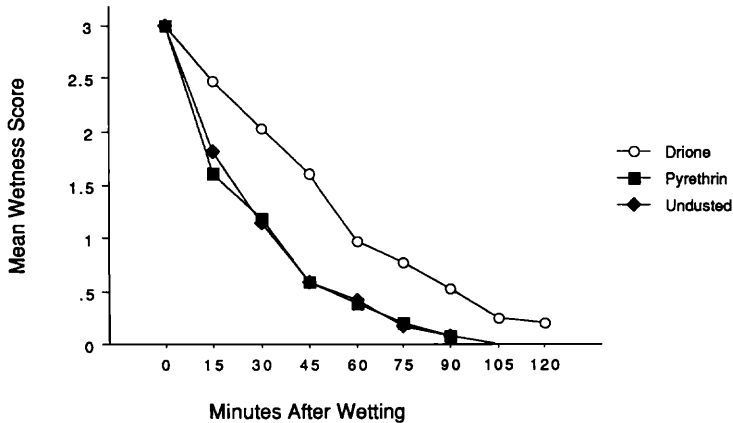


FIGURE 1. Drying times of quail dusted with drione or pyrethrin compared to undusted controls (six birds per treatment). Each score is the mean wetness of six pieces of filter paper applied to three different regions of the plumage (see Methods).

with pyrethrin ($n = 6$), or birds ruffled without dusting ($n = 6$; Fig. 1). The drione-treated birds took significantly longer to dry than pyrethrin-treated or undusted birds (exact probability — trials done on separate days combined according to Sokal and Rohlf [1981]; $df = 6$, $X^2 = 17.5$, $P < 0.01$). The pyrethrin and undusted birds took nearly identical times to dry (Fig. 1). Birds that were neither dusted nor ruffled ($n = 2$) required as much time to dry as the pyrethrin and undusted birds, showing that ruffling itself does not delay plumage drying.

Comparison of pyrethrin dust-ruffling to anesthesia jars.—Dust-ruffling removed a mean of 17.9 lice from swifts (range = 1–38; $n = 12$ birds), while the Fowler-Cohen anesthesia jar removed a mean of only 11.3 lice (range = 3–26; $n = 12$ birds). The methods do not differ statistically if all birds are included in the analysis (Mann-Whitney $U = 88$, $P = 0.35$); however, when the five lowest-load birds (1–7 lice) are excluded from each method, the difference is significant despite the small samples ($U = 43$, $P = 0.02$; $n = 7$ birds per method). Differential performance of dust-ruffling and Fowler-Cohen anesthesia is thus apparent only for birds with more than a few lice.

Dust-ruffling removed a mean of 15.2% more lice (range = 0–25%) when used following the Fowler-Cohen anesthesia jar (Fig. 2a). The anesthesia jar removed a mean of only 2.8% more lice (range = 0–12%) when used after dust-ruffling (Fig. 2b). The difference in the two methods is statistically significant even when all of the birds are included in the analysis ($U = 119$, $P = 0.0004$).

The Bear (1995) anesthesia jar did not remove more lice than the Fowler-Cohen jar. The Bear jar recovered a mean of 8.1 lice (range = 1–19; $n = 12$ birds), compared to the data already shown for the Fowler-Cohen jar. The two methods did not differ significantly when all 24 birds

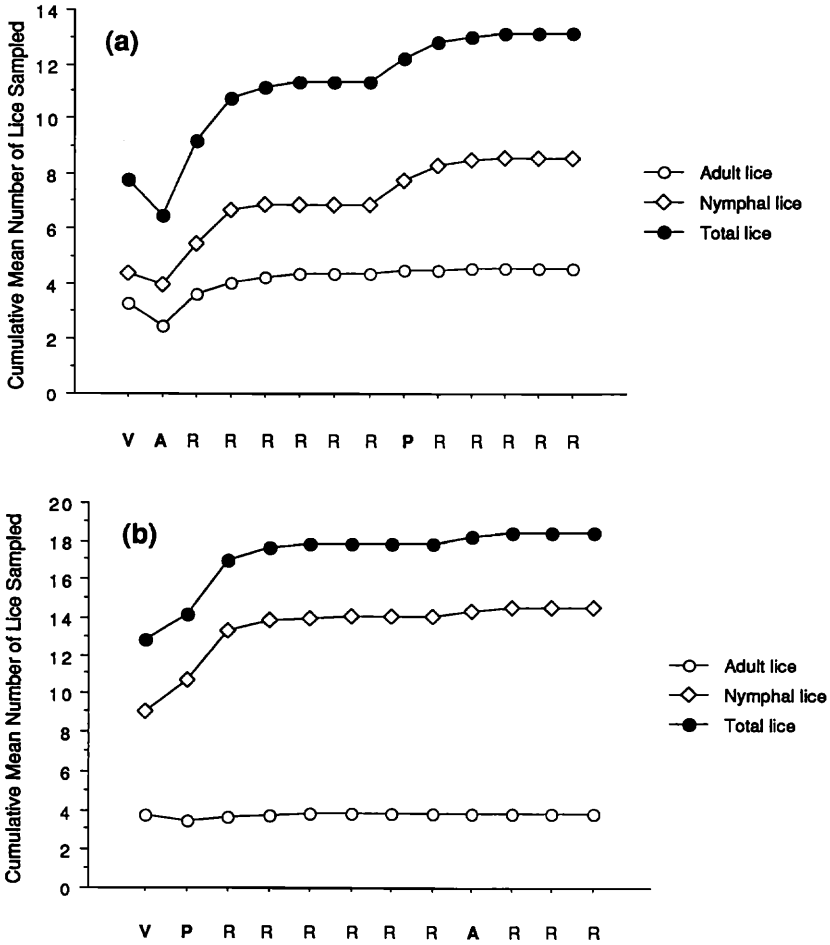


FIGURE 2. Cumulative number of chewing lice (*Dennysus hirundinis*) removed from swifts subjected to consecutive methods: (a) anesthesia jar followed by dust-ruffling ($n = 12$ birds); (b) dust-ruffling followed by anesthesia jar ($n = 12$ birds). V = visual examination; A = anesthesia jar without ruffling (Fowler and Cohen design); R = 60-s bout of ruffling; P = Pyrethrin dusting without ruffling.

were included in the analysis ($U = 92, P = 0.25$), nor when the five lowest-load birds in each category were omitted ($U = 21, P = 0.57$).

Comparison of pyrethrin dust-ruffling to visual examination.—Rapid visual examination consistently underestimated the final number of lice removed from swifts using a combination of more thorough methods (Fig. 3). A mean of 82% (range 0%–100%) of swift lice was detected during 3 min counts; these counts were significantly correlated with the final louse loads (Spearman rank correlation corrected for ties, $n = 36, r_s = 0.90, P < 0.0001$). However, a mean of only 12% (range 4–26%) of

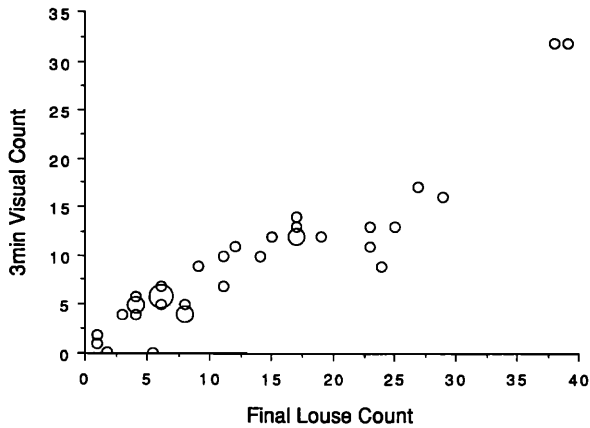


FIGURE 3. Number of lice counted during visual examinations in relation to the final number removed from the same birds ($n = 36$). Final loads were removed through the combinations of dust-ruffling and anesthesia shown in Figs 2a and 2b, in addition to another 12 birds that were dust-ruffled after being placed in a Bear-type anesthesia jar. Circle size is proportional to the number of equal-value data points, with three being the maximum.

Rock Dove lice was detected during 3 min counts; these counts were not significantly correlated with final loads (dust-ruffling only; $n = 10$, $r_s = 0.24$, $P = 0.46$).

DISCUSSION

We have shown that dust-ruffling is a simple, efficient method for quantifying the ectoparasite loads of live birds in the field. It is best suited for sampling "permanent" ectoparasites, which pass their entire life cycle on the body of the host (e.g., chewing lice). The method is not suitable for ectoparasite taxa that are found inside the throat pouch, feather quills, under the skin, or in other areas inaccessible to dust-ruffling (discussed below). Our results demonstrate the utility of repeated ruffling of the feathers for identifying the point of diminishing returns at which parasite accumulation curves reach an asymptote. Malcolmson (1960) suggested that the movement of a bird inside a paper cone is sufficient to dislodge ectoparasites following dusting, but we maintain that ruffling of the feathers is essential. Fig. 2 shows that large-bodied ectoparasites tend to drop out of the plumage sooner than smaller ones with continued ruffling. For example, nearly all adult chewing lice were removed from swifts at the first sampling bout, whereas most of the nymphs were removed only after one or two bouts of ruffling (Fig. 2). Similarly, the larger-bodied Rock Dove louse *Columbicola columbae* tended to drop out of the plumage before the smaller-bodied louse *Campanulotes bidentatus*.

Our study also shows that dust-ruffling is more accurate than visual examination, especially in the case of Rock Doves, and provides better returns than anesthesia jars. One reason is that, unlike anesthesia jars,

dust-ruffling samples ectoparasites from the bird's head as well as its body. Chloroform or other anesthetics are presumably also less efficient at penetrating the plumage than dust that has been distributed thoroughly by hand.

Pyrethrin dust provides excellent results and is preferable to drione, given that drione increases the drying time of plumage (Fig. 1). Drione is also abrasive, which may harm the bird's plumage, especially as it remains on the plumage for weeks or even months following dusting (Clayton and Walther 1997). Like drione, pyrethrin irritates parasites causing them to drop from the plumage while still alive. The twitching parasites are easier to locate on the collecting surface than are dead parasites on filter paper removed from the bottom of an anesthesia jar.

Dust-ruffling requires good bird-handling abilities. Flapping wings must be restrained to prevent parasites from being blown off the collecting surface. A large piece of cloth or fine insect mesh with a hole for the bird's head helps to ensure that parasites fall straight down onto the collecting surface. Birds held in the hand during dust-ruffling appear to be less distressed than birds suspended by the neck inside an anesthesia jar. Some minor damage to the plumage is inevitable during the ruffling procedure. However, fluttering birds inside an anesthesia jar may also damage their feathers.

Dust-ruffling requires less equipment than anesthesia jars. It eliminates the need to carry jars of different sizes for different sized birds, not to mention the need to carry a jar containing liquid anesthetic. It is particularly well suited for work at remote locations (Clayton, unpublished data).

Dust-ruffling is a fairly rapid procedure with small-bodied birds, but it is more labor intensive than using anesthesia jars. The latter offers the possibility of sampling several birds simultaneously. If the objective of a study is merely to determine the number of (permanent) ectoparasite species present in a bird population, rather than quantifying parasite population size, then the simultaneous use of several anesthesia jars may prove more efficient. The number of individual hosts to be sampled for studies of parasite species richness depends on a variety of factors (reviewed by Walther et al. 1995). Anesthesia jars are better than dust-ruffling in windy environments where no tent or other wind-protected space is available.

Note that in this paper we have not determined the fraction of the *total* louse population on a bird that is removed by dust-ruffling. It is possible in the case of swifts, and probable in the case of Rock Doves, that dust-ruffling fails to remove some lice—particularly immature stages—even with prolonged bouts of ruffling. A more thorough test would have required killing birds in order to search them with more comprehensive methods (see Clayton and Walther 1997), which was not an option in this case. A. Poiani (pers. comm.) recently dust-ruffled a sample of 28 House Sparrows (*Passer domesticus*), all of which were then kept in bags for 30 min following the dust-ruffling procedure. In nearly all cases additional

ectoparasites were found in the bags. Dust-ruffling alone removed 85% of the total number of parasites obtained. Therefore, holding birds in bags following the completion of dust-ruffling is advisable to maximize returns.

In conclusion, avian ectoparasites are an extremely diverse assemblage of taxa (Janovy 1997), and no single method can effectively quantify them all. Dust-ruffling is accurate and efficient for sampling relatively permanent ectoparasites, but it is not reliable for sampling ectoparasites such as fleas that quickly abandon a freshly captured host. Dust-ruffling will also seriously undersample taxa such as ticks or skin mites, which anchor their mouthparts or entire bodies under the skin of the host. Ectoparasites such as nasal mites, air sac mites, quill mites, quill lice and pouch lice will also be missed entirely by dust-ruffling. To collect such groups, other methods must be used (Clayton and Walther 1997).

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