Host–Parasite Evolution

General Principles and Avian Models

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Appendix C
Collection and quantification of arthropod parasites of birds

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INTRODUCTION

In recent years, parasite ecologists have adopted a more quantitative approach and data on parasite populations are of interest to a rapidly growing audience. Estimates of parasite load (defined below) are increasingly being used as an independent variable predicting features of host evolution. It is, therefore, important to obtain accurate estimates of parasite load. In principle, ectoparasitic arthropods are relatively easy to quantify accurately, for the simple reason that they are on the outside of the host, where one can see them. They are particularly useful for longitudinal studies, in which both host and parasite need to be studied without undue disturbance. Just as parasite taxonomists are at the mercy of the collector, dependent on properly handled specimens and accurate host data, parasite ecologists are at the mercy of the methods developed for quantifying parasite load. The methodological literature on ectoparasite collecting is extensive, and we do not have space to review all of it here. Instead, we will cover some methods in detail and liberally cite papers devoted to other methods for entry into the literature.

We have tried to address all of the main groups of arthropod parasites of birds with the exception of 'ephemeral' parasites, such as mosquitoes, which complete essentially all of their life cycle away from the host. For these groups, readers must consult reviews such as Service (1993). Table 15.3 provides a list of representative taxa for each group of parasites we cover (Table C.1). In addition to providing practical information, we hope to impress upon readers the diversity of niches occupied by arthropod parasites of birds.

We have mainly concentrated on quantitative methods, but in some cases we present qualitative methods primarily used to obtain specimens of parasites for taxonomic study and for the construction of host–parasite lists. In these cases (e.g. air sac mites), qualitative techniques are, to our knowledge, the only ones available.

We use parasite 'load' as a generic phrase, encompassing three explicit measures of parasites: 'richness', the number of species of parasites present; 'prevalence', the proportion of individuals in a host population that is parasitized; 'intensity', the number of individual parasites harboured by an individual host (Margolis *et al.* 1982). Prevalence and intensity are normally calculated for each species of parasite present.

Measures of parasite load must be appropriate for the kind of parasite studied. Measures of prevalence alone are of limited value for arthropod parasites; intensity provides far more information (see Clayton and Moore, Chapter 1). It is important to use a method with a specified degree of accuracy for predicting parasite intensity (Barnard and Morrison 1985; Clayton 1991), or one that can be shown to be significantly correlated with total intensity (Møller 1990; Clayton and Tompkins 1994; Merino and Potti 1995). Failing this, one should at least try to calculate the repeatability of the method used (Møller 1991). Like most parasites, arthropods typically show an aggregated frequency distribution among individual hosts in a population (i.e. the majority of individuals have few parasites, whereas a few individuals have many parasites; Chapter 1). It is, therefore, important to quantify parasites from as large a sample of hosts as possible. Estimates of parasite richness (Walther *et al.* 1995), prevalence (Gregory and Blackburn 1991) and/or intensity (Poiani 1992) made from small host samples can be very misleading.

Table C.1 Techniques for quantifying arthropod parasites of birds (see text for details)

Principal microhabitat	Parasites	Techniques*
On feathers	Feather mites	VE, AN, DR, (BW, DI)
	Nest mites	VE, TR, AN, DR, (BW, DI)
	Lice	VE, AN, DR, (BW, DI)
	Fleas	AN, DR, (BW, DI)
	Louse flies	VE, TR, AN, DR, (BW, DI)
In feather quills	Quill mites	VE, TR (DI)
	Quill lice	VE, (DI)
On or in skin	Ticks	VE, SC, TR, (DI)
	Nest mites	VE, TR, AN, DR, (BW, DI)
	Skin mites	VE, SC, AN, DR, (BW, DI)
	Chigger mites	VE, SC, TR, (BW, DI)
	Lice	VE, AN, DR, (BW, DI)
	True bugs	VE, TR, AN, DR
	Fleas	AN, DR, (BW, DI)
	Louse flies	VE, TR, AN, DR, (BW, DI)
	Nest flies†	VE, TR
Under skin	Subcutaneous mites	VE, (NE)
	Botflies	VE, TR, (NE)
Under leg scales or bill covering	Scaly face/leg mites	SC, (NE)
In pouch	Lice	VE, (NE)
In nostrils	Nasal mites	(IW, NE)
In air sacs, trachea, and lungs	Respiratory mites	(NE)
In and around the nest‡	Soft ticks	VE, TR, (SS)
	Nest mites	VE, (BT)
	True bugs	VE, TR, (BT)
	Fleas	VE, TR, (BT)
	Nest flies	VE, TR, (BT)

*Methods in parentheses require death of the host or removal of nest material. See Table 15.3 for representative taxa in each group of parasites.
*AN, anaesthesia; BT, Berlese–Tullgren funnel; BW, body wash; DI, dissolution; DR, dust-ruffling; IW, intranasal wash; NE, necropsy; SC, scrapings; SS, scoop samples; TR, traps; VE, visual examination.
†Blow flies, flesh flies, milichiid flies, and neottiophilid flies.
‡Methods not listed include centrifugation, flotation, sedimentation, substrate washing, and ultrasonic cleaning. These methods are normally used in conjunction with one or more of the methods in the table (see text).

The time frame of data collection should also be considered. Ectoparasites are not just another phenotypic feature of the host; they have their own biology and populations that can vary rapidly in both space and time. Errors can be minimized by collecting ectoparasite data over a relatively short time span (Marshall 1981), or in a standardized way that will permit one to document seasonal fluctuations in parasite intensity.

OTHER REVIEWS

Previous methodological reviews include Watson and Amerson (1967), Dubinina (1977), Southwood (1978), Pritchard and Kruse (1982), and McClure (1984*a*). Broader based reviews include Sonenshine (1993) for ticks, Krantz (1978) and Philips (1990, 1993) for mites and Marshall (1981) for insects. Many of these references contain useful drawings

of the main groups of parasitic arthropods of birds. Van Riper and van Riper (1980) provide a listing of anatomical locations of arthropods on and in birds. Good reviews of the arthropods of captive birds are provided by Calnek *et al.* (1991) for poultry and Ritchie *et al.* (1994) for cage birds. Harwood and James (1979) provide an excellent review of the effects of arthropods on human and animal health.

HOST HANDLING AND DATA

Scientific collecting permits are required to handle birds (dead or alive) or their nests. Accurate identification of the host is vital; if the host identification is uncertain, it is important to collect voucher specimens or make detailed photographs. Essential data to record are host identification, collection locality (including elevation), date, and the name of the collector. It is also important to record the number of parasite-free hosts examined, so that prevalence and mean intensity for the host population can be calculated. Additional useful data include host sex, age, body mass, reproductive state, and general condition. The precise anatomical location of the parasite(s) and the collecting method used are also very helpful.

Captured birds should be bagged as soon as possible in the field to pacify them, prevent the loss of vagile parasites, such as fleas and louse flies, and to keep parasites from transferring among hosts. Although workers have traditionally used cloth bags to hold freshly caught birds (McClure 1984*a*), we recommend using paper lunch bags. Cloth bags can be a source of erroneous host–parasite records, even when they are washed between uses. Paper bags eliminate this problem, since they can be thrown away after being used for a single bird. Paper bags breathe well, absorb faeces and are surprisingly resistant to dampness. The flimsy paper bags sold in third world countries also work, but birds need to be double or triple bagged for strength. Staples or clothes pins work well for keeping bags shut and, in a pinch, host data can be recorded on the bag itself and later transferred to a permanent label or notebook. Bags can even be made from newspaper, if necessary, using a stapler.

The equipment needed for collecting ectoparasites is minimal and is generally similar to that outlined by Doster and Goater (Appendix B). Specialized materials are discussed under relevant sections below.

LIVE BIRDS

Visual examination

Visual estimates work best for permanent parasites, like feather mites or lice, which complete their entire life cycle on the body of the host and which are often present in relatively large numbers. It is important to standardize the examination by searching a constant area of the host for a constant amount of time. It is also important to examine body regions of each host in the same sequence, because arthropods often change microhabitat distribution on a captive, struggling bird. It is sometimes helpful to immobilize the bird's legs with a strip of Velcro (Clayton 1991) or surgical tape (Lee and Clayton 1995) before searching it.

Magnification is typically needed when dealing with parasites less than 1 mm in length. It is not practical to use a dissecting microscope or hand lens with live birds, since both hands are required to search the plumage. The best approach is to use a 2–4 × jeweller's headset with a portable light for illumination. It is well worth investing in a good quality one to avoid eyestrain and headaches.

It is critical to check the accuracy of any visual procedure by removing and counting all of the ectoparasites from a subsample of hosts immediately after examination (see methods below). Statistical regression techniques (Barnard and Morrison 1985; Clayton 1991) can then be used to check the accuracy of the visual method against total parasite intensity. The ectoparasites removed should be prepared, identified, and deposited as voucher specimens in a properly curated collection.

Visual examination can be used for the groups of arthropods discussed next.

Hard ticks

Hard ticks are relatively easy to count accurately, since they imbed their mouthparts in the host's skin for several days (Brooke 1985). They tend to attach to naked areas, such as the underwings, bare throat, near the eyes, eyelids, feet, legs, and belly, including the brood patch.

Soft ticks

Soft ticks are much harder to quantify by visual examination, because they are nest-based parasites that normally only spend from 30 minutes to several hours attached to the host, usually at night (larval stages can remain attached for much longer periods, usually days). Nest-based methods are thus a better approach for quantifying soft ticks (see Nests).

Feather mites

Feather mites are permanent parasites that look like tiny grains of sand on the feathers and are relatively easy to quantify by visual examination, because they are very sluggish. They are often present in large numbers between the barbs of the flight feathers of the wings and tail. They can be quantified by approximating the number of mites on each flight feather while holding the spread wing or tail against a well lit background (McClure 1989). Feather mite species often show extreme microhabitat specificity on the host, preferring particular feathers or even particular regions of an individual feather (see Janovy, chapter 15). It is, therefore, desirable to make a reference collection of feather mites from each flight feather (and even different regions of the same feather). Feather mites can be collected by snipping off portions of the feather vane with attached mites and placing them in a vial of 70% alcohol. A more painstaking method (but one that is less damaging to the bird) is to pass the tip of a dissecting needle along the barbules to remove the mites.

Nest mites

Nest mites are similar to soft ticks, in that they spend most of their time in the nest and are active mainly at night. Some nest mite taxa cannot be quantified accurately by visual examination (Weatherhead *et al.* 1993), but other taxa spend a lot of time on the body of the host and are relatively easy to count (Møller 1990). Body counts of some taxa are

correlated with nest counts (Møller 1990; Clayton and Tompkins 1995), but a more accurate estimate of nest mite intensity inevitably requires quantification of the number of mites in the nest, as well as on the body (see Nests).

Skin mites

Skin mites include several families with diverse life histories (Janovy, Chapter 15; Philips 1990). Most taxa are difficult to see, because they live in or under the skin or in silken 'nests' constructed on the skin. The best way to search for skin mites is to examine naked regions of skin under magnification. Skin mites often appear as small red or yellow dots in the middle of swellings on the skin and can be collected using a fine brush or probe dipped in alcohol. Skin mites, as well as chiggers and larval ticks, can also be collected by scraping infested areas with a needle, fine forceps, or scalpel. The scrapings should be examined under a dissecting microscope after smearing them onto a slide and adding a little glycerol and a coverslip. Van Riper and van Riper (1980) list regions of the body that should be scraped to collect skin mites.

Scaly face/leg mites

Scaly face/leg mites can sometimes be removed by scraping skin, scabs, lesions, or cysts with a scalpel. Ritchie *et al.* (1994) advise scraping encrusted areas with a dull scalpel and allowing the crusts to fall into a Petri dish of 70% alcohol, which is then examined under a dissecting microscope. However, these mites are often embedded too deeply to be removed by scraping without damaging the bird.

Chigger mites

Chigger mites are parasitic only during the larval stage; post-larval stages are free-living predators. While feeding, they typically attach to the host around the thighs, vent, or under the wings for a period of 3–4 days. They are minute (0.1–0.3 mm) and difficult to observe without magnification. When present in large numbers, they usually cluster on the host's body, greatly facilitating detection. Chiggers can sometimes be removed by scraping.

Subcutaneous mites

Subcutaneous mites encyst too deeply to be retrieved by scraping. They can be detected visually by wetting the skin in the breast area and pushing it back and forth while looking for stationary white nodules of mites under the skin (Philips 1990).

Lice

Lice are permanent parasites that glue their eggs to the host's feathers with a glandular cement (Janovy, Chapter 15). The eggs tend to be congregated in regions that the bird cannot preen, such as the head or the underwing coverts (Nelson and Murray 1971). Louse intensity can sometimes be estimated by counting eggs (Kirkpatrick *et al.* 1991; Lee and Clayton 1995). At times, however, large numbers of hatched eggs may be present in the absence of lice, which have long since emigrated or died (Durden, personal communication). Louse eggs have species-specific microtopography (Balter 1968*a*,*b*; Foster 1969*a*), making it possible to distinguish the eggs of different species of lice from a single host (one must first determine the specific association of egg type and hatched

lice). It is relatively easy to distinguish hatched and unhatched eggs; the former are missing the distal tip and usually appear flattened.

Visual examination has been used by numerous workers to quantify adult and nymphal lice. Clayton (1991) used a stepwise regression approach, originally developed for cattle ticks (Barnard and Morrison 1985), in order to estimate the total intensity of lice on rock doves from counts of lice on particular body regions ($r^2 \geq 0.82$). Hunter and Colwell (1994) used an area–time-constrained search to compare the louse intensities of five shorebird species; they provide a thoughtful discussion of the advantages and disadvantages of this approach. Booth *et al.* (1993) minimized handling time of birds in the field by estimating louse loads using categorical scores.

It is important to be aware of the limitations of visual estimates. Three minute visual estimates of lice on rock doves ($n = 10$) accounted for a mean of only 12% (range 4–26%) of the lice subsequently removed by fumigating the same birds. Three minute estimates of lice on smaller bodied swifts ($n = 36$), which have less dense plumage and larger lice, accounted for a mean of 82% (range 0–100%) of the lice (Walther and Clayton 1996).

In some cases it is possible to estimate the intensity of lice by quantifying the amount of feather damage they cause. Feather mass is correlated with louse intensity (Clayton 1990, 1991), as is the number of holes chewed in the flight feathers (Møller 1991).

Quill lice, quill mites and pouch lice

Three orders of birds (Procellariiformes, Charadriiformes and Galliformes) are known to have quill lice that live inside the quills of flight feathers, and several orders of birds have quill mites that occupy the same microhabitat (see Janovy, Chapter 15). To count these by visual examination requires dissection of the quills with a microscalpel under alcohol, although both quill lice and quill mites can also be detected by holding the translucent quills up to a strong light.

Pelicans and cormorants have large lice that live inside their gular pouches.

Fleas

Fleas cannot be quantified accurately using visual examination, because they quickly abandon the body of a captured host (Stark and Kinney 1962). They also move too fast through the feathers to be sure of counting each individual only once. It is feasible, however, to count fleas on the bodies of nestling birds before they acquire their feathers (Brown and Brown 1986). It is also straightforward to count 'sticktight' fleas, which spend up to several weeks firmly attached to the face, wattles, vent, and other unfeathered regions (Marshall 1981).

Louse flies

Louse flies are temporary ectoparasites that spend the adult stage on the host, later pupating in the host's nest or general surroundings. Visual examination is an effective means of quantifying wingless species (Lee and Clayton 1995), as well as certain winged species (Young *et al.* 1993), but most louse flies move too quickly to be censused visually. Like fleas, they tend to abandon a struggling host, so it is important to use walk-in traps, rather than mist nets (McClure 1984*b*), and to move freshly caught birds into a more

secure enclosure as soon as possible. Agitating the feathers of a freshly caught bird in a fine meshed net bag is a good way to encourage flies to leave the host, after which they can be killed by squirting alcohol on them with a dropper. If a large number of birds is to be examined, a screened enclosure with sleeved armholes is a good method for agitating feathers and capturing flies (Klei and DeGiusti 1975). Tarshis (1952) provides detailed designs for portable insectaries that facilitate the collection of louse flies from freshly trapped birds in the field. Anaesthesia jars and dust-ruffling also work well (see below).

True bugs

True bugs are nest-based parasites that can be estimated by counting the number of feeding individuals attached to unfeathered nestling birds. Such counts are correlated with the total number of bugs in the nest (Brown and Brown 1996). Because they are active mainly at night, infestations need to be relatively high for many bugs to remain attached to nestlings during the daytime (Brown, personal communication). Methods for estimating the number of bugs in the nest are discussed later.

Nest flies

Nest flies (Table C.1) have larvae that spend most of their time in the host's nest, periodically emerging to feed on the nestlings. They are easy to quantify by counting the number of larvae and pupae in the nest material after the nestlings have fledged (see Nests).

Botflies

Botflies, in contrast, have body-based larvae that develop between the dermis and musculature of the nestling host. They breathe by means of spiracles protruding through a hole in the skin and are therefore easy to locate and count (Arendt 1985). Botflies drop from the host as third instar larvae and pupate in the nest or close to it.

Trapping

Ectoparasites like ticks, chiggers, and some nest mites can be trapped from live birds by placing the host in a cage over a large pan of water into which the parasites fall after feeding (Krantz 1978; Sonenshine 1993). Castro (1973) developed a clever method for trapping quill mites in a funnel as they emerged from the feathers of live house sparrows.

Anaesthesia

When removal of ectoparasites does not conflict with the goals of the study (e.g. collection of longitudinal data), then a more automatic method for determining parasite load is to anaesthetize or fumigate the parasites. This approach yields a higher fraction of the parasite population than visual examination (Walther and Clayton 1996). It is also less prone to error than visual examination, especially when dealing with large numbers of hosts. However, one cannot tell what fraction of the ectoparasites were alive at the time of collection. This can be a problem in the case of parasites such as Ischnoceran lice, which tend to clamp their mandibles shut around a feather barb when they die.

Birds have occasionally been anaesthetized along with their ectoparasites (see for example Chandra *et al.* 1990). However, this procedure is dangerous to the bird, as one must be certain to use the correct dosage. Wolfensohn and Lloyd (1994) review anaesthesia by injection or inhalation. It is, of course, essential to make sure the bird has recovered fully prior to release.

A much safer approach is to anaesthetize parasites of conscious birds using a glass or plastic jar with a modified cap, the middle of which has been replaced by a rubber diaphragm. A piece of filter paper is cut to fit the bottom of the jar and a few drops of anaesthetic are added to the paper. The bird is then placed in the jar with its head protruding through a slit of appropriate size cut in the diaphragm. The usual procedure is to wait 20 minutes for the fumes to penetrate the plumage, then release the bird, and carefully remove the filter paper for examination under magnification. This design, described by Fowler and Cohen (1983), replaced an earlier less efficient design known as the 'Fair Isle Apparatus' (Williamson 1954; Southwood 1978; Marshall 1981). Bear (1995) recently suggested further improvements, but Walther and Clayton (1996) were unable to detect any significant difference in the performance of the Fowler–Cohen and Bear designs, at least for collecting lice.

Carbon dioxide, chloroform, ether, and ethyl acetate have all been tried in anaesthesia jars. Carbon dioxide has been used successfully with Bear-type anaesthesia jars (Visnak and Dumbacher, personal communication). Chloroform is also effective, but dangerous to work with, and can cause the birds themselves to become drowsy or comatose, even in jars with tightly fitted diaphragms (Fowler 1984). Ethyl acetate is safer to use, but is somewhat less effective than chloroform or ether (Poiani, personal communication; Walther, unpublished data). Brown *et al.* (1995) used ether to fumigate individually more than 5000 cliff swallows and found it to work well, with no side effects on the birds, many of which were recaptured in subsequent years. Unfortunately, ether is a serious fire risk and is so volatile that it tends to evaporate even from tightly sealed vessels.

Anaesthesia jars have been used for many bird species to collect a variety of ectoparasite groups, including lice, mites, and even vagile parasites such as louse flies and fleas (Fowler and Cohen 1983; Wheeler and Threlfall 1986; Poiani 1992). The principal advantage of using jars is that they enable one person to sample 10 or even 20 birds at the same time, given a sufficient number of jars (Fowler and Cohen 1983). The main disadvantage is that the method removes only up to 80% of the ectoparasites (Fowler and Cohen 1983; Poiani 1992) and misses the head entirely, which is the main site of attachment for certain groups such as hard ticks. It is thus necessary to do a visual search of each bird's head, reducing the standardization of the technique. Returns are greatly improved by ruffling the bird's feathers as it is removed from the jar (Walther and Clayton 1996). Ideally, the feathers should be ruffled for repeated bouts until the point of diminishing returns is reached (Clayton *et al.* 1992; Walther and Clayton 1996).

Anaesthesia jars are further limited in that they cannot be used for very large birds (although Fowler and Shaw 1989 used modified buckets for Manx shearwaters), jars and liquid anaesthetic are cumbersome to transport, particularly to remote field sites, and the birds look uncomfortable in the jars, which can be disturbing to some field assistants and members of the public.

Dust-ruffling

A simpler, more thorough method of removing ectoparasites from live birds is to dust them with an insecticidal powder, then ruffle their feathers over a collecting surface, such as a large pan, piece of paper, cotton sheet, or into a plastic bag. A careful search of the paper or bag is then made under magnification from a jeweller's headset or dissecting microscope, while transferring the parasites to a vial with a brush dipped in alcohol (Walther and Clayton 1996). A coloured collecting surface provides the best contrast for seeing both light coloured immatures and darker adults.

Dust-ruffling was introduced by Floyd and Tower (1956) for poultry and Malcomson (1960) for wild birds, although the latter encouraged birds to flutter beneath an inverted paper carton, rather than ruffling their feathers by hand. Both studies used pyrethrum powder, a 'fast knock-down, slow killing' insecticide with no side effects on birds or mammals (Casida 1973; Jackson 1985). Pyrethrum is a biodegradable derivative of chrysanthemums, which breaks down within hours or days in the environment, making it ideal for studies of re-infestation rates (Casida and Quistad 1995). The kill rate of pyrethrum is not 100%, so most commercial insect powders use a combination of pyrethrin, a derivative of pyrethrum, and the synergist piperonyl butoxide. This combination is extremely effective and pyrethrin is considered 'the safest of all mite killers' (Bates and Busenbark 1963). Aerosal sprays containing these ingredients are produced by the pet industry and may actually work better than dust, so long as they do not wet the plumage.

Another fumigant used extensively for collecting bird ectoparasites is the silica aerogel powder known as Dri-Die 67 (Tarshis 1961; Dalgleish 1966; Watson and Amerson 1967; Kettle 1975; McClure 1984*a*). This substance is an extremely fine grained, chemically inert, industrial desiccant that works by abrading and absorbing the lipid layer of insect cuticle, leading to rapid desiccation and 100% ectoparasite mortality within 3 hours (Tarshis 1967). Unlike pyrethroids, Dri-Die is an extremely long lasting fumigant which should not be used in studies of re-infestation rates. An aerosol spray of silicon aerogel is available, but it should not be used on birds, as it coats them with crystals (McClure 1984*a*). A product called Drione dust is a silica aerogel mixed with pyrethrin and synergized with piperonyl butoxide; it works more quickly than Dri-Die alone (Dalgleish, personal communication). Although Dri-Die and Drione are non-toxic to birds (Tarshis 1961; Jackson 1985), the silicon they contain can remove oil from the plumage, causing birds to die from exposure when caught in rainy weather soon after dusting (Palma, personal communication; Walther and Clayton 1996).

Dust-ruffling with pyrethrin or Dri-Die can be irritating to the investigator, unless one uses a paper face mask to prevent sneezing. It is important to shield the bird's eyes from as much dust as possible, although it has no long-term effects. The dust can be distributed throughout the plumage either with the fingers or by using a plastic squeeze bottle to 'puff' the dust under the feathers. Distributing dust through the dense plumage of a rock dove can take up to 5 minutes (Walther and Clayton 1996). Harshbarger and Raffensperger (1959) encased chickens in open, inverted plastic bags to ensure that ectoparasites fell straight down onto the collecting surface during ruffling. Pyrethrin and Dri-Die are very irritating to parasites, causing even ischnoceran lice to drop out of the plumage, instead of dying with their mandibles clamped shut around barbules. And, because neither substance kills the parasites outright, they continue to twitch on the collecting surface, making them easier to see.

It is necessary to combine dusting with ruffling for best results. Kettle (1983) found that Drione dusting alone removed only 75% of the lice on starlings. Walther and Clayton (1996) tripled the number of lice initially removed from pigeons dusted with pyrethrin by subsequently ruffling them for repeated bouts until reaching the point of diminishing returns (Clayton *et al.* 1992). Dust-ruffling removes up to 25% more lice than anaesthesia jars and returns are a more accurate reflection of total load (Walther and Clayton 1996).

When vagile parasites, like fleas or louse flies, are to be collected, birds should be inserted quickly into plastic bags after a cursory dusting. If necessary, the dust can be distributed more thoroughly to ensure killing of other parasites after vagile taxa have died. The materials required for this method are portable and it can conceivably be used on birds of any size, although the idea of dust-ruffling an ostrich is rather daunting! Palma (personal communication) has ruffled large albatrosses with excellent results. Unlike anaesthesia jars, dust-ruffling allows thorough sampling of the head, which is sometimes the most heavily infested part of the bird (Marshall 1981).

DEAD BIRDS

More accurate parasite counts can be made from dead birds than from live ones. Indeed, groups such as air sac mites are difficult to collect from live birds at all. Killing large numbers of birds to quantify their parasite loads is undesirable on ethical grounds. However, opportunities often exist for collecting the ectoparasites of dead birds by teaming up with museum expeditions, hunters, pest control authorities, or other sources of freshly killed material.

Several methods are aimed at collecting and quantifying arthropods from hosts in the field. Sealander and Hoffman (1956) devised a funnel for collecting fleas and other vagile ectoparasites from freshly killed mammals and birds. Clayton *et al.* (1992) anaesthetized the parasites of dead birds with ethyl acetate fumes, then ruffled their feathers for repeated bouts until reaching the point of diminishing returns.

Although the best results are usually obtained from freshly killed hosts, permanent parasites can also be collected from refrigerated hosts for up to several days following death. If refrigeration is unavailable, it is possible to delay collecting for a day or two by preserving the host temporarily with an injection of formalin into the body cavity (Mohr 1959) or embalming fluid (Nelson and Murray 1971). Birds can also be frozen for long-term storage. If aetiological agents are to be isolated, then the parasites must be kept alive or frozen in an ultra-cold or on dry ice (Marshall 1981). Museums often preserve some of their bird specimens in alcohol after injecting them with formalin to fix the internal tissues. The ectoparasite faunas of such birds can easily be quantified, so long as the birds have been bound in *several layers* of cheesecloth or otherwise isolated prior to preservation. Isolation is a critical step, since birds destined for alcoholic collections are normally transported from the field in containers containing more than one species of bird.

It is essential to keep dead birds bagged and isolated from one another in the field, because many ectoparasite groups will quickly abandon a dead host in search of a new one. The literature is replete with erroneous host records owing to the secondary

transfer, or 'straggling', of arthropods among hosts on the skinning table. It is also essential to be sure that the working surface and one's hands are thoroughly cleared of ectoparasites before switching individual hosts. The best approach is to examine birds on a sheet of paper in a large, shallow pan or tray. The paper is then shaken clean or, better yet, changed entirely each time a new host individual is examined. One cannot overemphasize the importance of avoiding contamination in the field.

Visual examination

Ectoparasite loads of dead birds can be quantified by searching the entire skin and plumage while deflecting the feathers with forceps. This is normally done under magnification, although it is difficult to examine entire carcasses, even small ones, under a dissecting microscope. A magnifying glass on a light stand (Eveleigh and Threlfall 1976) or a jeweller's headset and lamp are the best approaches. An easier way to keep track of one's progress during a visual examination is to remove the feathers a few at a time and examine each under a dissecting microscope (Doster *et al.* 1980). This approach is also an excellent way to map the microhabitat distributions of ectoparasites (Nelson and Murray 1971; Choe and Kim 1989), so long as the parasites are 'frozen' in place immediately upon death of the host. This is a necessary step to prevent post-mortem migration of the parasites, which takes place very quickly after the host dies. Several approaches have been used, including chloroform-soaked towels wrapped around the body of the host (Nelson and Murray 1971), quick freezing (Choe and Kim 1989), and fumigation of freshly killed birds in the field.

Necropsy

Respiratory mites

Respiratory mites in the lungs, trachea, and air sacs are most easily collected by necropsy, although this is not an exercise for the impatient. Burley *et al.* (1991) failed to detect any air sac mites in 31 necropsied birds. On the other hand, Tidemann *et al.* (1992) removed respiratory mites from 62% of wild caught gouldian finches ($n = 26$); sites of infection included the nasal and buccal cavities, trachea, syrinx, bronchi, lungs, air sacs, and body cavity.

Tracheal mites, which can be detected in coughed up mucous, appear as small black spots in the trachea and bronchi; large numbers are also often seen in the air sacs around the base of the heart. The mites can sometimes be located using transillumination of the trachea or a transtracheal wash (Ritchie *et al.* 1994). Van Riper and van Riper (1980) list sites to be examined for respiratory mites during necropsy.

Nasal mites

Nasal mites live in the nasal passages of the host and feed on mucous, blood, and tissue. Some taxa are fairly large and reddish or white in colour and are, therefore, easily seen. The traditional way to collect nasal mites is by necropsy, in which the bill is split between the nostrils and examined under a dissecting microscope with a probe. If the host is to be preserved as a museum specimen, the culmen can be left intact by removing the palate to expose the nasal mucosa for examination (Watson and Amerson 1967). Nasal mites can also be collected using the following method of non-destructive washing.

Intranasal washing

In this procedure, the nasal cavity of a dead bird is flushed with a fine stream of water from a hypodermic syringe or bulbed pipette. The method, originally devised for mammals (Yunker 1961), is rapid and provides good returns from birds (Wilson 1964). Wilson (1964) reported that visual examination, in conjunction with necropsy of the nasal passages, yielded higher prevalences of nasal mites than intranasal washing. However, the prevalences he reported from the two approaches are not significantly different [mites detected in 32 of 89 (36%) dissected birds and 62 of 200 (31%) washed birds; $\chi^2 = 0.69$, $P = 0.41$]. Spicer (1984) used intranasal washing to collect nasal mites from a large number of tropical bird species. The method also can be used on fluid-preserved museum specimens.

Body washing

This is an efficient technique, but one that can only be used on birds that are to be preserved in alcohol, skeletonized, or discarded (Watson and Amerson 1967). The ectoparasites are removed by shaking the bird in a plastic jar or tin containing a 1–2% solution of detergent or soap. The soap serves merely as a wetting agent and must be used in small quantity to prevent excessive foaming. The brand of soap is not critical; good results have been obtained with Cold Water All® (Henry and McKeever 1971), Alconox® (McGroarty and Dobson 1974) and Palmolive Dishwashing Liquid® (Wicht and Crossley 1983). Optimal results are obtained by shaking the immersed host on a paint shaker or other mechanical shaker for 5–10 minutes. After reducing the surface tension and foam with a stream of 95% alcohol, the solution is filtered through an 80 mesh (0.180 mm) screen or filter paper. The latter normally requires several changes of paper, particularly in the case of birds with soiled plumage. Vacuum filtration with a Buchner funnel (Krantz 1978) can speed the process, or one can try methods such as sedimentation, flotation, and/or centrifugation (see sections on Dissolution and Nests).

Henry and McKeever (1971) removed > 90% of mites, fleas and lice from rats using the washing technique and a paint shaker. However, only 66% of ticks were removed, the others presumably remaining attached to the host. Lipovsky (1951) refrigerated hosts for 24 hours, then warmed them to room temperature to encourage chiggers to detach before washing. McGroarty and Dobson (1974) removed > 95% of lice and > 85% of feather mites from house sparrows using the washing method and a paint shaker.

Clayton (unpublished data) used a simple form of body washing to collect lice and mites from freshly killed birds subsequently prepared as museum skeletal specimens. Each freshly killed bird was immersed in alcohol in a medical Whirlpack® bag, shaken vigorously for 60 seconds, then rinsed with a stream of alcohol as it was removed from the bag. The bags were rolled shut and transported back to the laboratory for examination.

Dissolution

This approach is like burning down a haystack to find its needles. The feathers and skin of the host are completely dissolved in potassium hydroxide (KOH), leaving behind the

exoskeletons of arthropods, which are made of chitinous carbohydrates that do not dissolve. The following protocol is a combination of steps from Choe and Kim (1987), Lemke *et al.* (1988) and Clayton (1991).

The dead bird is carefully skinned and the skin with attached plumage is incubated at 37–38°C for 24 hours in a beaker containing 0.5% trypsin (4 × USP Pancreatin) buffered to pH 7.5–8.3 with 0.2 M disodium phosphate (Na_2HPO_4). Following incubation, KOH is added to a concentration of 5.0%. The solution is then boiled on a hot plate until both skin and feathers are dissolved. The hot solution is filtered through an 80 mesh (0.180 mm) bronze or stainless steel screen and the exoskeletons are rinsed first with 95% alcohol, then with xylene to dissolve any fat remaining from the host's skin. Next the specimens are washed into a gridded Petri dish with 95% alcohol and stained with acid fuchsin for counting under a dissecting microscope. Adult arthropods collected by this method are often in good enough shape to be identified by taxonomists after mounting on microslides (but immature stages are often badly damaged).

Tests of this technique (Clayton 1991), using known numbers of adult lice added to clean feathers, showed a mean recovery of 95% (range 91–100%). However, the mean recovery of nymphal lice was only 82% (76–93%), which accords with the findings of other workers who found few nymphs (Ash 1960; Lemke *et al.* 1988). Hence, the method is not as reliable for immature stages as for adults, because nymphal instars apparently lack sufficient chitin or pass through the screen during filtration.

As in the case of washing, it is possible to use methods other than filtration for isolating ectoparasites following dissolution. Hilton (1970) suggested allowing hot KOH solution to stand for 12 hours until the ectoparasites and other fine particles have settled to the bottom of the beaker. The supernatant can then be decanted and the sediment transferred to a tube where it is centrifuged at 1200 r.p.m. After decanting again, the tube is filled with a zinc sulfate solution (386 g $ZnSO_4$ in 1.0 litre of water, specific gravity 1.18) and the mixture is again centrifuged for 5 minutes. This procedure causes the ectoparasites to float on the surface of the solution, from which they can be decanted or aspirated into a Petri dish and counted under a dissecting microscope. The disadvantage of this approach is that, with so many additional steps, the probability of losing ectoparasites increases, unless one is very careful. Diligent checking of the supernatants and final sediment under a dissecting microscope are essential to prevent losses.

Dissolution can be used to collect parasite microhabitat data by dividing the skin of the host into regions which are then incubated and dissolved in separate beakers. Choe and Kim (1988) used this approach to plot the microhabitat distributions of ticks, mites, and lice on the bodies of seabirds.

The principle drawback of dissolution is that, like body washing, it can only be used with dead birds that are not needed for museum specimens, although skeletons can be saved. Dissolution is also a relatively slow procedure and has an offensive smell (to put it mildy). The boiling step must be performed under a fume hood, as KOH fumes are toxic.

PRESERVED BIRDS

Foster (1969*b*) studied the demographics of warbler lice by counting unhatched louse eggs on museum skins. This approach could be useful for other groups of permanent

parasites, like feather mites. Museum skins are also a source of dried lice for taxonomic study (Ward 1957). One must be cautious, however, to avoid erroneous host records, because skins are routinely moved from drawer to drawer as collections expand. It is conceivable that louse intensity might be estimated from dried lice on bird skins, assuming the skins have been collected and prepared in exactly the same way.

Fluid-preserved birds are also a useful source of ectoparasites, so long as they have been isolated from other birds when collected, as described earlier. Indeed, fluid-preserved specimens are one of the best sources of ectoparasites like skin mites, which are difficult to collect, much less quantify, under field conditions.

NESTS (*IN SITU*)

Nest-based ectoparasites can be quantified more accurately from the nest than from the body of the host. Many methods have been used (Marshall 1981) and a comprehensive treatment is beyond the scope of this review. Generalizations are difficult, because birds' nests come in a wide variety of shapes and sizes (Collias and Collias 1984).

Visual examination

It is sometimes possible to obtain accurate estimates of large bodied parasites by counting the number of individuals visible in the nest (Lee and Clayton 1995). Rannala (1995) found that the number of bugs visible on the outside of cliff swallow nests was highly correlated with the total collected by subsequent destruction and sifting of the nest. Møller (1990) estimated the number of mites in barn swallow nests from the number swarming on his hand when it was placed on the rim of the nest for 10 seconds; these estimates were significantly correlated with the number of mites collected in Berlese–Tullgren funnels (see below). Samples of parasites can be removed from nests with a bulb-operated aspirator (Singer 1964). 'Pooters' or other aspirators requiring mouth suction should not be used, because of the possibility of inhaling arthropods capable of vectoring human pathogens (or the pathogens directly).

Traps

Bates (1962) devised a series of ingenious traps for capturing fleas, including pitfalls placed at the entrances of burrow nests, funnel traps attached to nest boxes, and artificial birds constructed from bottles of hot water for attracting fleas. Brown and Brown (1986) sampled flea populations over-wintering in cliff swallow nests by holding a black, honey-coated card up to the nest entrance. The fleas instinctively jumped onto such cards, as they would onto hovering cliff swallows returning from their wintering grounds. Chapman and George (1991) bolted ectoparasite harbourages to cliff faces in order to compare the densities of bugs and ticks at different colonies of cliff swallows. Drummond (1957) attached funnels to the bottoms of nest boxes to trap mites. Loye (1996) collected protocalliphorid fly pupae from nest boxes using inserts made of corrugated cardboard.