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Anting Behavior in Birds: Ant Selection and Effect of ant Chemistry on Feather Ectoparasites

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**ANTING BEHAVIOR IN BIRDS: ANT SELECTION AND EFFECT OF ANT
CHEMISTRY ON FEATHER ECTOPARASITES**

by

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B.S. Southern Connecticut State University, May, 1994
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ABSTRACT

ANTING BEHAVIOR IN BIRDS: ANT SELECTION AND EFFECT OF ANT CHEMISTRY ON FEATHER ECTOPARASITES

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Active anting behavior occurs when songbirds apply ants to their feathers. During anting displays, a bird draws its wings forward in front of the head and swings its tail around to the far right or left. Both wing and tail feathers are then rubbed with ants held in the bird's beak. The potential associations among ants, feather ectoparasites and birds make anting behavior an important community-level interaction. Although this behavior is cosmopolitan in distribution and occurs in a wide variety of passerine birds, it remains poorly understood. This dissertation tested hypotheses about anting behavior through controlled experiments with captive songbirds in an aviary. Experimental results revealed that bird responses to ants included either consumption without anting behavior, anting displays without ant consumption, or consumption of manipulated ants following anting displays. Birds were highly selective of ant species for anting displays and chose ants from the subfamily Formicinae significantly more often than from the Myrmicinae. *Camponotus*, *Lasius* and *Aphaenogaster* ants were preferred and *Pheidole* and *Crematogaster* ants were rejected. Preferred ants were significantly more active than ignored ants and lacked aggressive stinging and biting behaviors. Temperature and humidity did not affect anting displays, but significantly less anting episodes occurred on cloudy days. Feather molt

condition was unrelated to anting behavior. Polar and non-polar extracts of preferred and ignored ants were tested for microbial inhibition of potential feather parasites. No extracts inhibited growth of the bacteria *Bacillus subtilis*, *B. licheniformis* strains 138B and 1432B or the fungi *Chaetomium globosum*, *Penicillium chrysogenum*, and *Trichoderma viride* or fungal spore germination. Formic acid inhibited growth of all microbial species and spore germination of *C. globosum*. Dust bathing site location in Wild Turkeys was not related to the presence of preferred ant species, indicating no association between anting behavior and dust bathing activity. These experiments represented the first controlled quantitative study to identify biotic and abiotic factors involved in this widespread and complex behavioral pattern.

This thesis is dedicated in loving memory to my Father:

R. Theadore Revis

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CHAPTER I

INTRODUCTION

Active anting behavior is an enigmatic behavioral pattern in songbirds in which birds apply ants to their feathers. In order to actively ant, a bird draws its wings forward in front of the head and swings its tail around to the far right or left. During active anting behavior, both wing and tail feathers are commonly rubbed with ants held in the bird's bill. Records of anting behavior date as far back as the 1800s identifying members of both families Icteridae, a Tinkling Grackle (*Quiscalus niger*) (Gosse 1847) and Corvidae, a Blue Jay (*Cyanocitta cristata*) (Baskett 1899).

Anting is frequently discussed in the literature; historical references exist from the 19th century (Whitaker 1957). Although this behavior has been documented in over 40 families of the Order Passeriformes, few quantitative studies have been published on anting behavior. The biological implications of anting are still uncertain. Previous studies have been qualitative, observing the behavior of birds. Few published accounts have identified songbird reactions to different ant species or possible defensive mechanisms of ants.

Anting behavior is a community-level interaction. The identification of natural communities and comprehension of system dynamics is a central goal of ecological studies. There are multiple species that make up the community of organisms involved in anting behavioral interactions. In addition to the known involvement of the birds and ants, potential associations may also be formed

The model for this dissertation is The Auk

among ant chemicals, bacteria, fungi, lice, and other organisms living on both the ant and in the bird's feathers (Clayton 1999). These associations may take many different forms, from consumption of ants as food, to parasitic, mutualistic, and commensal relationships. Linkages formed during these potential relationships and the strength of interactions are two aspects of ecology representing minimally required information in community dynamics (Smith 1992).

ANTING BEHAVIOR

Simmons (1966) defines anting behavior as a stereotyped behavior-pattern shown by certain birds when the plumage is treated with foreign organic matter, either by direct application using the bill or by exposure of the bird's body to ants. There appear to be no fundamental differences in active anting behavior among different bird families (Ivor 1943). However, slight variations in the behavior seem to exist among individual species. Individuals within families have been found to differ in their placement of ants during behavioral displays. Most behavioral displays include the treatment of the underside of the wing, particularly concentrating on the primary feathers, and notably away from the cloacal and upper tail regions, the latter being the site of the preening glands (McAtee 1944, Nice 1945, Brackbill 1948, Groskin 1949, Grimshawe 1964, Simmons 1966, Dubois 1969, and Nero 1951). However, many accounts indicate a strong tendency of some birds to apply ants near and around the cloaca, upper tail coverts, and near the uropygial (preening) gland (Edwards 1932, Staebler 1942, Davis 1950, Groskin 1950, Darter 1953, Whitaker 1957, Southern 1963, Clunie 1976, Whyte 1981, and Post and Browne 1982). An

example of these differences in ant placement exists within the Corvid family.

Placement of ants by Magpies (*Pica pica*) included both the top and bottom of tail feathers, whereas Rooks (*Corvus frugilegus*) do not apply ants in the tail region at all (Goodwin 1953). Goodwin (1953) did not find a correlation between these differences in corvid active anting behavior and their phylogenetic relationships.

Accounts of anting behavior in the literature are most common among songbirds. The literature reveals a cosmopolitan distribution of the behavior including references from every biogeographical region: Neotropical (Post and Browne 1982), Nearctic (Potter 1970), Palearctic (Kelso and Nice 1963), Ethiopian (Whyte 1981), Australian (Clunie 1976, Chisholm 1944), and Oriental (Pillai 1941) to cite just a few examples. Over 210 species of songbirds have exhibited anting behavior. Crows (Corvidae), starlings (Sturnidae), troupials (Icteridae), finches (Fringillidae), weavers (Ploceidae), thrushes (Turdidae) and babblers (Timaliidae) are the most common birds seen displaying anting behavior (Simmons 1957).

In addition to songbirds, many birds common to deserts and grasslands engage in anting behavior. Both Wild Turkeys (*Meleagris gallopavo*) and Scaled Quails (*Callipepla squamata*) are known to consume ants as well as exhibit anting behavior (McAtee 1947, Thomas 1957). However, these birds engage in passive anting behavior.

The broad definition of anting behavior actually covers two forms of ant application: active and passive. During active anting behavior the bird controls the placement of ants to its feathers by using its bill. In passive anting behavior,

a bird exposes its entire body to a group of ants that crawl onto the bird. Placement of the ants upon the feathers is not controlled by the bird during passive anting behavior. Records of passive anting behavior usually include ants on or near an underground ant nest. Passive anting behavior has always been recorded on the ground while active anting behavior occurs on both the ground and on perches. Passive anting is usually recorded with numerous ants congregated in a group, a behavior often enhanced by the birds through flopping on or wallowing in the ant nest. In some passive anting behavioral accounts, birds use a tool, such as a small tree branch with leaves, to excite the ants (Goodwin 1955, Southern 1963, Quammen 1985, Potter 1985).

One of the first anting behavioral accounts in the literature is by James Henry Audubon writing about wild Turkeys passively anting in what he thought was a deserted ant nest (Audubon, 1831). Turkeys and Quails are not the only non-passerines to engage in anting behavior. Further, many non-bird species perform anting behavior, including squirrels (Bagg 1952, Hauser 1964), cats (Dennis 1985), primates (Longino 1984), hedgehogs (Burton 1957), snapping turtles (Burke et al. 1993), and wood turtles (McCurdy and Herman 1997).

While true anting behavior is limited to the application of ants to the feathers, birds have been recorded exhibiting similar behavior with non-ant material. Records of these substitutes include the use of caterpillars (family Sphingidae) (Wenny 1998), millipedes (class Diplopoda) (Clunie 1976), flowers (Nero 1984, Dennis 1985), mothballs (Dubois 1969, Clark and Clark 1990), lemons (Johnson 1971), limes (Gosse 1847), other citrus fruits (Clayton and

Vernon 1993), cigarette ends (Miller 1952), hot chocolate and soap suds (Nice 1945), beer, vinegar, cucumber-pickle, cigar-butts, lighted pipe tobacco, matches, onions, and other aromatic leaves (Simmons 1957, Dennis 1985).

These substitutes all elicit stereotypic anting movements. Records of anting behavior with substitutes involve application to wing feathers, and around the tail and cloacal regions.

ANTS AND PRODUCTION OF CHEMICALS

To date, anting behavior has focused on the bird and in many accounts the ant species are not identified. The function of anting behavior might be better understood through studies of the ant species chosen by the bird. Ants are classified as class Hexapoda, order Hymenoptera, family Formicidae, with 297 known genera worldwide. While not universal among ants, the metapleural gland can be used as a distinct diagnostic characteristic separating ants from other Hymenoptera. This gland produces anti-fungal and anti-bacterial chemicals (Holldobler and Wilson 1990). The ant genera lacking metapleural glands include *Camponotus*, *Oecophylla*, *Polyrhachis*, and *Dendromyrmex*, each of which maintains arboreal colonies and is thought to have secondarily lost the metapleural gland (Holldobler and Wilson 1990).

All ants form eusocial colonies with distinct castes of reproductive queen, workers, and larvae. Although the workers are the most abundant caste within a colony, some ant species maintain several actively reproductive queens at a time. Anting behavior has been recorded most often with the worker caste of ants. Almost all ants are female with males developing in the colony only a short

time before their nuptial flight. Ant colonies can range in size from 50 workers, as seen in *Basiceros manni*, a central American ant in subfamily Myrmicinae, to over 100,000 workers, as seen in the army ants of central America, *Eciton burchelli* (Holldobler and Wilson 1990).

Ants vary greatly in both morphology and chemistry (Holldobler and Wilson 1990). Most ant species identified in anting behavioral accounts are from the subfamily Formicinae (Formicidae: Hymenoptera) (McAtee 1938, Staebler 1942, Ivor 1943, Nice 1945, Brackbill 1948, Groskin 1950, Whyte 1954, and Potter 1970, 1981). Ants from the subfamilies Myrmicinae and Dolichoderinae have also been identified in anting behavioral displays (Bourke 1941, Galloway 1948, Poulsen 1956) (Tables 1 and 2). Ants in all three subfamilies produce defensive pheromones, but Formicines and Dolichoderines do not sting (Kugler 1979).

Pheromones, kairmones, and allomones are all types of semiochemicals, substances used in communication. Pheromones produced by ants can function as chemical messengers, released outside the ant's body, that have the ability to generate physiological or behavioral responses in a species. Allomones are substances given off by one individual that communicates across species. These chemicals are usually beneficial to the emitter and function against the receiving organism. Finally, kairmones produce cross species behavioral responses, usually for the benefit of the receiving organism (Holldobler and Wilson 1990).

TABLE 1. Formicinae ants used during anting displays

Subfamily Formicinae			
<i>Oecophylla smaragdina</i>	<i>Lasius</i> (<i>Chthonolasius</i>) <i>Umbratus aphidicola</i>	<i>Formica obscuripes</i>	<i>Lasius</i> (<i>Dendrolasius</i>) <i>fuliginosus</i>
<i>Camponotus pennsylvanicus</i>	<i>Lasius umbratus mixtus aphidicola</i>	<i>Formica exsectoides</i>	<i>Lasius niger neoniger</i>
<i>Camponotus</i> (<i>Myrmophyma</i>) <i>innexus</i>	<i>Acanthomyops claviger</i>	<i>Formica fusca</i>	<i>Lasius</i> (<i>Chthonolasius</i>) <i>mixtus</i>
<i>Camponotus</i> (<i>Myrmobrachys</i>) <i>senex textor</i>	<i>Lasius</i> (<i>Acanthomyops</i>) <i>claviger</i>	<i>Formica fusca subsericea</i>	<i>Acanthomyops mixtus</i>
<i>Camponotus</i> (<i>Myrmepomis</i>) <i>consobrinus</i>	<i>Lasius claviger</i>	<i>Formica fusca</i> var. <i>subaenescens</i>	<i>Formica rufa</i>
<i>Camponotus socius</i>	<i>Acanthomyops interjectus</i>	<i>Formica fusca</i> s. sp. <i>subaenescens</i>	<i>Lasius niger</i>
<i>Camponotus senex textor</i>	<i>Lasius interjectus</i>	<i>Formica</i> (<i>Raptiformica</i>) <i>sanguines</i>	<i>Lasius niger</i> var. <i>americanus</i>
<i>Lasius alienus americanus</i>	<i>Acanthomyops murphyi</i>	<i>Formica</i> (<i>Raptiformica</i>) <i>subintera</i>	<i>Formica sanguines subintegra</i>

Data compiled from: McAtee 1938, Staebler 1942, Groskin 1943, 1950, Nice 1945, Mayr 1948, Hebard 1949, Nero 1951, Darter 1953, Ivor 1956, Poulsen 1956, Whitaker 1957, Kelso and Nice 1963, Southern 1963, Potter 1964 and 1989, Hauser 1973, Potter and Hauser 1974, Willis and Oniki 1978, Ehrlich et al. 1986,

TABLE 2. Non-Formicinae ants used during anting displays

Subfamily Myrmicinae	Subfamily Dolichoderinae
<i>Atta cephalotes</i>	<i>Azteca</i> spp.
<i>Monomorium pharaonis</i>	<i>Iridomyrmex detectus</i>
<i>Pheidole morrisi</i>	<i>Iridomyrmex pruinosus</i>
<i>Crematogaster ashmeadi</i>	<i>Dorymyrmex pyramicus</i>
<i>Pogonomyrmex badius</i>	<i>Tapinoma sessile</i>

Data compiled from: Bourke 1942, Ivor 1943, VanTyne 1943, Galloway 1948, Whitaker 1957, Potter 1964, Potter and Hauser 1974, Post and Browne 1982, Sugihara 1998

There are only a few observations of birds anting with ants from the subfamily Myrmicinae, which include stinging ants (Holldobler and Wilson 1990). In addition, birds have been observed to actively avoid some myrmicine species. For example, Robins (*Turdus* spp.) avoid *Myrmica rubra* (a stinging myrmicinae) and scold them from a distance (Simmons 1966). A captive Orchard Oriole (*Icterus spurius*) rejects four species of myrmicine ants: *Pogonomyrmex barbatus*, *Pheidole bicarinata buccalis*, *Pheidole bicarinata longula*, and *Crematogaster (Acrocoelia) laeviuscula*, when exposed to a variety of ant species within an aviary (Whitaker 1957).

Ants are like small chemical factories. Representatives of the class Hexapoda, especially ants, have evolved a variety of communication systems through the use of pheromones. Known to resemble hormones and circulating in the hemolymph of insects, there are two broad categories of ant pheromones. First are the pheromones that affect development and, in a more general way, behavior. These are slow to produce visible results and are thought by some to have to be eaten by an ant in order to take effect (Sudd and Franks 1987).

The second category of pheromones includes those that have an immediate effect on behavior and affect other ants through their sense organs. These volatile pheromones are released from a gland and diffuse into the atmosphere. The density and effectiveness of these chemicals are reduced in lower concentrations, usually at greater distances from the point of origin (Sudd and Franks 1987). The amount of secretions contained in ant glands varies widely with the ant species and gland. For example, Dufour's glands contain as

little as 50 femtograms of secretion while a metapleural gland contains up to 1.4 μg of secretion (Holldobler and Wilson 1990). The molecular weights of pheromones differ, influencing their outward diffusion rates. Not surprisingly, response to a pheromone will vary with its concentration (Sudd and Franks 1987).

Ant chemicals have been categorized by their effect on ant behavior. Twelve behavioral effects of pheromones are recognized: 1) alarm, 2) attraction, 3) short-range recruitment, 4) long-range recruitment, 5) grooming and assistance, 6) control of competing reproductives, 7) group effect, 8) recognition, 9) territorial and home range, 10) sexual communication, 11) trophallaxis, and 12) the exchange of solid food particles (Holldobler and Wilson 1990). Chemicals commonly produced by ants include esters (Holldobler 1995), alcohols (Nascimento et al. 1998), proteins (Williams and Williams 1965), ketones (McGurk et al. 1966, Scheffrahn et al. 1984), iridoids (Nascimento et al. 1998), nitrogen heterocyclic compounds and acetates (Holldobler and Wilson 1990), acids (Sheridan et al. 1996), terpenoid citronella (Wilson 1971a), and alkaloids (Andersen et al. 1991) (see chapter two for specific examples).

Alarm pheromones are rapidly diffused into the environment to elicit a response from surrounding ants. These often have a molecular weight between 100 and 200 daltons and are most often produced by glands near the main defense structures (Sudd and Franks 1987). Responses to this type of pheromone vary depending on the amount released.

Simple attraction or cluster-initiating pheromones cause ants to congregate and form tight circles (Wilson 1971a). Clustering behavior is usually seen when ants are removed from their nest and placed in a foreign environment. Attraction pheromones can also be medium-to-high molecular weight substances with a relatively slow diffusion rate, so attraction occurs only in ants that are close together (Wilson 1971b).

Recruitment pheromones are focused on identifying the presence of new food or the nest site and are designed to bring ants to food or back to the nest. These pheromones can be divided into those with short-range effects and those with long-range effects. Short-range effects are produced by releasing a low molecular weight pheromone into the air for short durations. Long-range effects are produced by laying chemical trails from the new food to the nest. Most trail pheromones are short-lived and do not lead to mass communication (Holldobler et al. 1978).

Pheromones for grooming and assistance at molting are thought to be spread over the entire surface of the ant's body. These pheromones elicit licking responses that are thought to remove ectoparasites from the epidermis (Sudd and Franks 1987). Queen ants are most commonly identified secreting this type of pheromone. It has been suggested that parasitic queen ants such as *Polyergus breviceps* are able to kill and replace colony queens through grooming and assistance pheromones. This species reduces aggression with a pheromone from the Dufour's glands while attacking the colony queen. After the colony queen has been killed, colony workers respond to this Dufour's secretion

and begin constant grooming of the *P. breviceps* new queen (Regnier et al. 1973, Topoff and Zimmerli 1993).

Group-effect pheromones function to alter physiology or behavior and are produced continuously (Wilson 1971b). Insecticidal, antibiotic and repellent activities are the results of the group-effect alkaloid pheromone produced by the ergatoids of *Megalomyrmex goeldii*. These pheromones are highly adaptive in the nest environment; they function in part to keep the nest clean (Jones et al. 1991).

Recognition pheromones function to identify ants of specific colonies and are similar to caste determination pheromones. Both pheromones are unique identification tags for ants and are important to the social, colonial structure in which ants live (Holldobler and Wilson 1990).

Territorial and home-range signals and nest markers are pheromones applied to the ground and areas around the nest. These chemicals are usually applied in spot patterns or streaks and are used as homing devices and warnings to other species. Worker ants of colonies are responsible for this signal (Holldobler and Wilson 1990).

Both sexual communication between a queen and sexually mature male ants and the glands from which these pheromones originate are not as well understood as other types of pheromones. Although sexual activities are initiated by factors other than pheromones, including time of year, temperature, and time of day (Holldobler and Wilson 1990) pheromones, like the poison gland secretions of *Solenopsis invicta* (Vargo 1999), function as a form of sexual

communication. Male ants have many exocrine glands whose function is not well understood (Holldobler and Wilson 1990). The components of specific behavior-releasing pheromones have not been established through experimentation (Holldobler and Wilson 1990).

Control of competing reproductives is an effect derived from the queen indicating both her presence and level of fecundity. This pheromonal control is thought to increase with colony size, thus decreasing both physical aggression of the queen and cannibalism of eggs (Bourke and Franks 1995).

Finally, two actions, most often initiated with a touch, are thought by Wilson and Holldobler (1990) to be re-enforced through pheromones: trophallaxis, a form of food transfer in a liquid form (either from the anus or the mouth), and the exchange of solid food particles.

Holldobler and Wilson's (1990) definitive work identifies the many glandular and pheromone-producing organs in ants. The types of glands and their placements include the cloacal gland, which is found in the base of the VIIth abdominal sternite (ventral side). The Dufour's gland, in the gaster, is typically under the poison gland reservoir. The poison compounds are found in vesicles in the poison gland reservoir that is connected to the sting at the tip of the gaster. The pygidial gland is at the tergite (dorsal side) of the gaster in the VIth segment, whereas the sternal gland (Pavan's gland) is found in the sternite of the gaster in the Vth and sometimes the VIth segments. Mandibular glands are directly behind the mandibles in the head. The rectal gland, most often found when a sting is absent, is located at the tip of the gaster. The metapleural glands, bulla

and orifice, are behind the propodeal spiracle between the second and third legs. The postpygidial gland (spiracle plate gland) is found in the VIIth segment on the tergite side of the gaster. The propharyngeal gland is behind the frontal lobes of the head. The maxillary gland is on the ventral side of the head behind the mouth. The labial gland is located in the pronotum within the alitrunk. The postpharyngeal gland is in the head behind the occiput. The quadrate plate gland is behind the quadrate plate on the gaster. The triangular plate gland is found behind the triangular plate in the gaster.

Ants can mix pheromones by releasing chemicals from more than one gland. An example of ants mixing glandular secretions to achieve a single response can be found in the alarm pheromone of the widely distributed ant *Forelius pruinosus*. This ant produces 2-heptanone and *cis,trans*-iridodial in the mandibular glands which are mixed with iridodial produced by the gaster (Scheffrahn et al. 1984). The purpose of mixed pheromones is the same as that of single source secretions. Not all ants communicate through pheromones at the same level of complexity. Ants are covered with glands from head to gaster, and some genera have evolved many chemically complicated pheromones. The number of different types of chemicals and their glandular sources vary among genera (Holldobler and Wilson 1990).

HYPOTHESES CONCERNING ANT BEHAVIOR IN SONGBIRDS

Anting behavior can be described as either a primitive form of behavior, lost by some birds but retained by others (Ivor 1956), or as a highly adaptive derived behavior. Definitions that classify anting behavior as highly adaptive

include its possible use as a method of zoopharmacognosy (self-medication by animals) (Clayton and Wolfe 1993, Burke et al. 1993).

There are seven hypotheses concerning why birds perform anting behavior. These include to: 1) reduce feather mites, ticks, and lice (Goodwin 1955, 1956, Simmons 1966, and Clayton and Vernon 1993,); 2) remove stale lipids from the skin and feathers (Kelso 1946, Kelso and Nice 1963, Simmons 1966); 3) provide autoerotic stimulation (Whitaker 1957); 4) store ants in feathers as a reservoir food supply (Groskin 1943); 5) facilitate molting and sooth feathers (Potter 1970); 6) prepare food by removal of chemical secretions of ants, including formic acid (Judson and Bennett 1992); and 7) act as a fungicide and bactericide (Ehrlich et al. 1986, Clayton 1999).

According to the first hypothesis, songbirds may apply ants to their feathers to remove ant chemicals used to reduce feather mites, ticks, and lice (Goodwin 1955, Simmons 1966, Clayton and Vernon 1993). The reduction of arthropod ectoparasites tends to attract widespread attention, remaining the most qualitatively accepted hypothesis among birders (personal communications with Cape Hennerly and Outer Banks Audubon Societies 1997 and Zuni Hunt club 2001) This is one of the few hypotheses quantitatively examined; however, experimental results do not support it (Judson and Bennett 1993, Clayton 1999). Though minimal quantitative data for the ants used during anting behavior exist, records indicate a higher number of anting behavioral displays with worker caste formicine ants, which produce formic acid (Whitaker 1957). The use of ants as self-medication is supported by the type of ants most commonly found to be used

during the behavior. *In vitro*, formic acid kills feather mites, lice and ticks (Judson and Bennett 1992).

A review of the literature of the effect of anting behavior on feather mites, ticks, and lice shows minimal support for the reduction of arthropod ectoparasites. Early studies conducted by Dubinin (1951 *in* Kelso and Nice 1963) identify heavy mortality of feather mites on Meadow pipits (*Anthus pratensis*) when exposed to workers ants of *Formica rufa*. However, Dubinin found no significant mortality of feather mites in the plumage of two Wood Hoopoes (*Upupa epops*) after active anting behavior (1951 *in* Kelso and Nice 1963). Whitaker (1957) also finds no support for ectoparasite mortality after active anting episodes. Recent controlled aviary experiments show no effect of ant chemicals on feather mites or lice (Judson and Bennett 1992, Clayton 1999). Experiments testing an anting substitute, lime fruit juice containing citric acid, also produced no detrimental effect on feather lice (Clayton and Vernon 1993). These results give additional support to the null effect of mild acids (similar in nature to ant-derived formic acid) on feather ectoparasites. Potter and Hauser (1974) observe no correlation between anting behavior and the removal or reduction of ectoparasites. However, they did find a strong seasonal correlation between peak anting behavior and low ectoparasitic loads in wild songbirds.

The hypothesis that anting removes stale lipids from the skin and feathers (Kelso 1946, Kelso and Nice 1963, Simmons 1966) classifies the behavior with other feather-grooming methods. Dust bathing is a feather maintenance behavior like preening and water bathing in birds. Birds engage in dusting

behavior by covering themselves with dust, a practice thought to replace bathing behavior in arid or upland habitats. Dusting behavior usually leaves a distinctive round, bowl-like depression in the ground. Frequent dusting helps maintain optimal feather conditions, because the dust absorbs excess lipids and preening oils from the plumage (Ehrlich et al. 1986). No correlations have been found between anting behavior and the removal of stale lipids from feathers (Potter and Hauser 1974, Potter 1989).

Although anting behavior is well known, recordings of the behavior by amateur ornithologists seem to be rare. For example, only one person from the Cape Henry Audubon Society in Norfolk, Virginia, an active society with over 60 members, remembers seeing even a single anting display (personal communication David Clark 1997). Preening behavior is very similar to anting behavior, and these similarities have led to speculation that anting is comparatively common and just overlooked by ornithologists in the field (Nice 1945, Brackbill 1948). Most previous reports of anting behavior have related events viewed from a backyard over breakfast or have been observations made of pet birds displaying anting behavior.

There have been disputes in the literature as to the existence of a functional sequence combining the anting behavior with bathing, oiling and preening. Ivor (1943) and Poulsen (1956) both agree that no normal preening behavior occurs after anting, yet Goodwin (1956) writes that preening and bathing in water often follow anting behavior. Clearly there is much variation

surrounding anting behavior, which could be due to the species of birds or ants, or both.

It has been proposed that anting behavior provides autoerotic stimulation to songbirds (Whitaker 1957). These accounts appear anthropomorphic. Whitaker's Orchard Oriole (*Icterus spurius*) was thought to have derived sensual pleasure from anting. Mendez (1984) supports the theory of self-stimulation with his description of trogons (Trogonidae) "closing their eyes and trembling as if about to swoon" after anting behavior. Finally, Quammen (1985) addressed the theory of self-stimulation by birds through anting behavior with crows. He compares anting behavior in birds to smoking by humans, a "stimulation and soothing of the body."

The storage of ants in feathers as a reservoir food supply is an hypothesis put forth by Groskin (1943). When eaten, the formic acid from the ants could increase the bird's muscular energy and fitness. Anting behavior would then facilitate this storage through placement of the ants into the plumage. This theory is supported by observations from Ramsden's (1914) observations of birds carrying snails under their wings during migration. However, the energy requirements of birds are so great that a few ants carried in their feathers would represent a tiny fraction of the total daily energy requirements. Additionally, formic acid is corrosive and toxic (Bennett et. al 1996).

Potter (1970) concludes that anting behavior could facilitate molting and soothe feathers in songbirds. Her observations relates high seasonal anting behavioral displays to periods of feather molt. Potter and Hauser (1974) suggest

that anting behavior (as well as sunning behavior) relieves skin irritation and stress during feather molt. These authors define anting behavior as a comfort-motivated behavior, increasing a bird's sense of well being during seasonal molt.

Anting behavior might act as a food preparation method used to remove ant chemical secretions including formic acid (Judson and Bennett 1992). Formicine ants derive their name from the main venom constituent they produce, formic acid. Formic acid is a corrosive and cytotoxic fatty acid capable in large quantities of causing dermal necrosis (Budavair 1989). Formicine ants have a vestigial sting, and they use the secretion of formic acid as a primary defensive mechanism (Kugler 1979). This volatile organic compound is produced as a spray, usually a 60% aqueous solution containing formic acid, free amino acids, and small peptides (Blum 1992).

Ant-derived formic acid is toxic when ingested by birds (Bennett et al. 1996). Willis and Oniki (1978) find that only subordinate antbirds display anting behavior. Antbirds are South American birds that follow ant swarms of army ants to catch insects flushed from cover (Stouffer and Bierregaard, 1995). This suggest that ants represent a distasteful prey item that subordinate, hungry birds are forced to consume. According to this scenario, these hungry birds prepare ants for consumption through anting behaviors, which remove toxic secretions. Willis and Oniki (1978) propose that dominant antbirds do not display anting behaviors because dominant birds tend to consume better prey items, not ants. Perhaps anting behavior is not frequently observed because ants, though eaten, are not a preferred food item for most birds (Willis and Oniki 1978).

A final hypothesis is that ant-derived chemicals can act as fungicides and bactericides reducing the microbial loads of feathers (Ehrlich et al. 1986, Clayton 1999, Furlow 2000). Ants used during anting displays tend to secrete some form of acidic chemicals, often formic acid. However, there are recorded sightings of ants that do not produce formic acid being used by songbirds in the stereotypical movements of active anting displays (Groskin 1950). For every hypothesis about anting behavior there seems to be a countering argument.

Although anting behavior has been shown to have no effect on feather mites or lice, the behavior could reduce the number of microbial colonies on feathers (Ehrlich et al. 1986, Clayton 1999). Ant metapleural glands produce antibiotic secretions that inhibit the growth of both bacteria and fungi (Ehrlich et al. 1986). Skatole (3-methylindole) of army ants inhibits both the gram-negative enteric bacteria, *Escherichia coli*, and the fungus *Aspergillus parasiticus* (Tittler et al. 1935, Brown et al. 1979).

Even though there are numerous accounts of anting behavior in the literature, there remains no clear answer as to why songbirds apply ants to their feathers. Ivor (1956) describes anting behavior best when he calls it an "enigma." The experiments conducted here represented one of the few quantitative efforts seeking answers to the question: Why do birds display anting? This dissertation focused on the effect of active anting behavior on possible food preparation (removing formic acid), soothing and facilitation of feather molt, bactericidal, and fungicidal properties of ant chemicals, and storage of ants. The experiments described here seek to identify: 1) factors affecting

selection of ant types during active anting behavior, 2) effects of abiotic conditions (temperature, cloud cover, and humidity) on behavioral displays, 3) possible bactericidal and fungicidal properties of ant secretions, and 4) correlations between anting behavior and the location of Wild Turkey dust-bathing bowls.

CHAPTER II

THE ROLE OF ANT SPECIES IN ACTIVE ANTING BEHAVIOR IN CAPTIVE SONGBIRDS

INTRODUCTION

The factors that elicit active anting behavior in songbirds are relatively unknown. Anting behavior is the self-application of ants to feathers. Reasons for this obscurity are two-fold. First, anting behavioral displays are rarely recognized even by avid birdwatchers. The second reason results from unbalanced accounts of anting behavior in the literature. By not standardizing information from witnessed accounts, the literature produces a muddled overall picture of the behavior. Important factors sometimes included in anting behavioral accounts are the temperature, humidity, season, condition of feather molt in birds, ant activity, and use of available ant species. Among these factors, the ant species used in anting displays are most often missing from literature accounts.

At least 24 species of ants have been identified in the literature as being used during anting displays (Tables 1 and 2) (Whitaker 1957). The majority of these are classified within the Formicinae subfamily of the Formicidae (Potter 1970). The pheromones produced by ants may play a role in the selection of ant species during active anting displays. Unlike some other ant subfamilies (i.e., Myrmicinae), formicines have vestigial stingers and produce chemicals for their defensive mechanisms (Blum 1992). Most formicines produce volatile semiochemicals including formic acid, a corrosive compound (Blum 1992).

Chemicals produced by ants, like formic acid, are well documented in the literature (Holldobler and Wilson 1990, Blum 1992, Holldobler 1995). These chemical secretions are produced by glands for communication between species (allomones and kairmones) and within species (pheromones) (Holldobler and Wilson 1990). Many exocrine glands are identified for specific behavioral functions (Holldobler and Wilson 1990). Chemicals produced within each ant type can be specific to their genera (Blum 1992). Below is a list known chemicals for some of the ant genera included in these experimental.

Within the subfamily Myrmicinae, the cephalic gland of *Aphaenogaster* species produces methyl anthranilate (an ester $C_8H_5NO_2$). Its poison gland produces (S)- and (R)-4-methyl-3-heptanone, (+)-4-methyl-3-heptanone, (R)-1-phenylethanol, (S)-4-methyl-3-heptanone, 2,5-dimethylpyrazine, 4-methyl-3-hexanone, 4-methyl-3-heptanol and 3-ethyl-2,5-dimethylpyrazine (Holldobler 1995, Holldobler et al. 1995). *Aphaenogaster* recruitment pheromones include 1-phenylethanol, 4-Methyl-3-heptanone, and 4-Methyl-3-heptanol (Holldobler et al. 1995). Alpha-farnesene is produced from the Dufour's gland (Wilson 1971b).

Crematogaster species secrete alarm pheromones such as 2-hexenal, 3-octanone, 3-octanol, and 3-nonanone from the mandibular glands. Mandibular glands of *Myrmica* species produce 3-decanol (an alcohol, $C_{10}H_{22}O$), 3-octanol (an alcohol $C_8H_{18}O$), 3-nonanone (a ketone $C_9H_{18}O$), and 3-octanone (a ketone $C_8H_{16}O$). The poison gland produces 3-ethyl-2,5-dimethylpyrazine, a nitrogen heterocyclic compound $C_8H_{12}N_2$ (Holldobler and Wilson 1990).

Within the subfamily Formicinae, *Formica* species have Dufour's glands which produce the esters decyl acetate ($C_{12}H_{14}O_2$), dodecyl acetate ($C_{14}H_{18}O_2$), tetradecyl acetate ($C_{16}H_{22}O_2$), undecyl acetate ($C_{13}H_{16}O_2$), n-decane ($C_{10}H_{22}$), and n-undecane ($C_{11}H_{24}$). These function in trail production (Holldobler and Wilson 1990). Recognition pheromones produced by *Formica* include the hydrocarbons: *n*-heneicosane, *n*-Docosane, *n*-Tricosene, *n*-Tetracosene, *n*-Tetracosane, *n*-Pentacosene, *n*-Pentacosane, *n*-Hexacosene, *n*-Hexacosane, *n*-Heptacosene, *n*-Heptacosane, *n*-Octacosene, *n*-Octacosane, *n*-Nonacosene, *n*-Nonacosane (Henderson et al. 1990).

Camponotus species secrete methyl 6-methylsalicylate and 2,5-dimethyl-3-isopentylpyrazine as sex pheromones (Wilson 1971b). The alkane 11, 17-dimethylhentriacontane is produced as a trail pheromone (Pempo et al. 2000). Their Dufour's glands secrete n-undecane (Holldobler and Wilson 1990), geranylinalol and geranylfarnesol (Brand and Morgan 1999), while poison glands produce formic acid (a carboxylic acid CH_2O_2) (Holldobler and Wilson 1990). Their mandibular glands release pentane-2,4-dione, *n*-hexanal, 4-methyl-3-heptanone, and mellein (Brand and Morgan 1999).

The hindgut glands of *Lasius* species produce fatty acids that function in trail production. These include decanoic acid ($C_{10}H_{20}O_2$), dodecanoic acid ($C_{12}H_{24}O_2$), heptanoic acid ($C_7H_{14}O_2$), hexanoic acid ($C_6H_{12}O_2$), nonanoic acid ($C_9H_{18}O_2$), and octanoic acid ($C_8H_{16}O_2$) (Wilson 1971b). The Dufour's glands secrete n-undecane while the mandibular glands produce the terpenoid

citronellal ($C_{10}H_{18}O$), and 2,6-dimethyl-5-hepten-1-al as excitement pheromones (Holldobler and Wilson 1990).

There are many hypotheses put forth in the literature relating to possible effects of ant chemistry on active anting behavior. Groskin (1943) predicts that the formic acid produced by many ants could increase muscular energy and fitness in a bird. This hypothesis suggests that birds display anting behavior in order to store ants in feathers as a reservoir food supply (Groskin 1943). Potter (1970) suggests that the chemicals produced by ants could facilitate molting and soothe the skin around a bird's feather tracts. Ehrlich et al. (1986) and Clayton (1999) suggest that ant chemicals could act as a fungicide or a bactericide killing feather ectoparasites. The hypothesis that ant chemicals could remove stale lipids from the skin and feathers as well as the idea that ant chemicals could reduce feather mites, ticks, and or lice have been suggested by multiple authors (Goodwin 1955, Simmons 1966, Clayton and Vernon 1993). Finally, Judson and Bennett (1992) suggest that anting behavior functions as a food preparation by removing by harmful ant chemicals before ingestion. The experiments reported here examined the role of ant species in eliciting active anting behavior in captive songbirds.

METHODS

Worker caste ants, collected from the Hampton Roads area, were presented to songbirds in an indoor aviary at the Virginia Living Museum in Newport News, Virginia. Single tray trials were conducted at both the indoor aviary and at an outdoor feeding station at the Weyanoke sanctuary, Norfolk,

Virginia. Ants were contained in clear plastic trays (30 cm X 15 cm X 10 cm) edged with fluon, an F.D.A.-approved chemical that is not harmful to organisms. Video records of anting behavior and the molt conditions of the birds exhibiting the behavior were produced for each ant presentation. Abiotic factors such as the time of day, temperature, relative humidity and cloud cover were recorded. Biotic factors, including species of ant, action of ants, actions of the bird after anting behavior, and the length of time the behavior was exhibited were also recorded.

The indoor aviary has ten species of songbirds (Table 3), each either hand reared or rehabilitated. Their ectoparasitic loads were removed with Sevin dust® before release in the aviary. The birds are fed a combination of wild bird feed and fresh produce from three feeding stations within the room. The aviary is a semicircular design (11.4 m maximum length, 5.7 m maximum width, and 7.7 m at the maximum height). Three of the four walls and the ceiling are glass. The foundation of the room is concrete pilings in a dirt floor. The room is divided into multiple tiers. The basal level has a concrete walkway and water collection ponds. Five additional levels produce a stepwise descent from the entrance and upper walkway to the base floor level. Each tier is filled with dirt and is planted with a mixture of herbaceous and deciduous plant species. Nothing blocks access to the interior aviary from underground through the topsoil. A pump-run stream descends from the top (fifth) tier down one side of the room into a collection pond on the basal level. Stumps of fallen trees and drift wood were brought into the aviary to provide bird perches. Although the room is

temperature controlled, temperature fluctuations ranged between 15 to 40 C. Continuous water is supplied in the form of an artificial stream running down one wall. The aviary is washed down with a hose-directed spray of water from the ceiling every Tuesday. This action simulates rainfall in nature and functions to wash the plant life and provide weekly baths for the captive birds. The aviary contains active colonies of *Crematogaster lineolata*, thought to have been introduced with downed rotting logs.

The Weyanoke Sanctuary in Norfolk, Virginia is a songbird preserve owned by the Nature Conservancy and maintained by the Cape Henry Audubon Society. The sanctuary is 1.01 hectares of natural and planted oak/maple woodland. The songbird feeder is maintained year-round with sunflower seed. A bird-watching blind is situated near the feeder for an unobstructed view of bird behavior.

Single tray ant trials. - Single tray ant presentations were conducted in the aviary and at the sanctuary to determine if anting behavior can be solicited from captive and wild songbirds. Different species of live worker ants representing the Formicinae, Myrmicinae and Dolichoderinae subfamilies were randomly selected from species common to southeastern Virginia (Table 4). A tray containing a single species of ant was placed first at the Virginia Living Museum indoor aviary and later that same day it was placed at Weyanoke Sanctuary for a total of 38 days. A total of 68 ant presentations were conducted at the Virginia Living Museum. Numbers of ants contained within plastic trays ranged from 15 - 200

workers. Ant trays were observed for one hour or until all ants had been removed.

Ant choice trials. - Ant genera in choice trials: Ant choice trials were conducted to determine if songbirds display a preference for any ant genera. Two trays, each containing a minimum of 20 live worker ants, were presented to the captive songbirds. Only ants from the Formicinae and Myrmicinae subfamilies were presented in these trials. Dolichoderinae workers were excluded because these ants were not found to elicit anting behavioral responses in the single ant tray presentations. Species presented during these trials included *Lasius flavus*, *Camponotus pennsylvanicus*, *Camponotus subbarbatus*, *Pheidole dentata*, *Aphaenogaster rudis*, *A. fulva*, and *C. lineolata*. Both fluon-lined trays were placed side-by-side on the lowest two tiers of the aviary. Ants were presented only between 0630 and 1000 hours. These morning hours are traditionally when birds are active and represent the quietest time in the songbird aviary before guests are admitted to the museum. Fifty choice trials were conducted. Each of the five ant genera was paired with itself and every other genus to determine if songbirds displayed a preference for any genus of ant. Paired tray trials were conducted twice for each ant species. Trays containing ants were placed side by side in the aviary in a left/right and then right/left orientation to control for any placement effect.

Cloud cover was recorded during each choice trial as cloudy or not-cloudy. Cloudy trials were identified when my hand held between 12.5 to 17.5

TABLE 3. Birds contained in the Virginia Living Museum indoor aviary.

Common Name	Scientific name	Sex	Date placed in the aviary
Hooded Merganser	<i>Mergus cucullatus</i>	Female	3/1/1997
Hooded Merganser	<i>Mergus cucullatus</i>	Male	3/1/1997
Northern Cardinal	<i>Cardinalis cardinalis</i>	Male	3/1/1997
Killdeer	<i>Chadrius vociferous</i>	Unknown	7/1/1997
Northern Bobwhite	<i>Colinus virginianus</i>	Male	7/1/1997
Eastern Meadowlark	<i>Sturnella magna</i>	Unknown	7/1/1997
Northern Mockingbird	<i>Mimus polyglottos</i>	Unknown	7/1/1997
Mourning Dove	<i>Zenaidura macroura</i>	Unknown	3/1/1997
Eastern Towhee	<i>Pipilo erythrophthalmus</i>	Female	7/1/1997
American Robin	<i>Turdus migratorius</i>	Unknown	7/1/1997
American Robin	<i>Turdus migratorius</i>	Female	7/1/1997
Clapper Rail	<i>Rallus longirostris</i>	Unknown	7/1/1997
American Green-winged Teal	<i>Anas crecca carolinensis</i>	Female	3/1/1997
Blue Jay	<i>Cyanocitta cristata</i>	Female	7/1/1997
Northern Bobwhite	<i>Colinus virginianus</i>	Female	7/1/1997
Clapper Rail	<i>Rallus longirostris</i>	Unknown	7/1/1997
Northern Bobwhite	<i>Colinus virginianus</i>	Female	7/1/1997
Gray Catbird	<i>Dumetella carolinensis</i>	Male	3/1/1998
Evening Grosbeak	<i>Coccothraustes vespertinus</i>	Male	1/1/1999
Rose-breasted Grosbeak	<i>Pheucticus ludovicianus</i>	Male	1/1/1999

cm over white paper did not produce a distinct shadow. Clear days were identified from the presence of a clear hand shadow with all four fingers and the thumb outlined on the paper.

Ant movement in choice trials: Ant choice trials were conducted to determine if songbirds displayed a preference for live (moving) ants or dead ants. Factors such as lack of ant movement due to mortality might regulate the frequency of anting behavioral displays in songbirds. This trial tested songbird responses to ant movement. Two trays, each containing either live, active ants or dead ants were placed on the lower tiers of the aviary. Workers of *C. pennsylvanicus*, an ant species previously demonstrated to elicit active anting behavior, were used. Dead ants were frozen to preserve structural integrity. Twenty ants were presented in each tray. Paired tray trials consisting of live versus frozen ants were conducted four times, each choice placed in the aviary in a left/right and then right/left orientation to control for any placement effect.

Ant chemistry trials: Ant choice trials were conducted to determine if songbirds displayed a preference for either live ants, freshly-frozen ants, or critically dried (CPD) ants. Factors such as ant chemistry production might regulate the frequency of anting behavioral displays in songbirds. Live ants can actively release semiochemicals. Freshly frozen ants maintained their wet weights and non-volatile chemicals, while CPD ants were washed with organic solvents and dried, reducing both volatile and non-volatile chemicals as well as their water weight. Critically-point drying procedures involved soaking

TABLE 4. Ants presented in single trays at both the Virginia Living Museum and the Weyanoke Sanctuary.

Myrmicinae	Formicinae	Dolichoderinae
<i>Aphaenogaster</i>	<i>Acanthomyops</i>	<i>Tapinoma</i>
<i>Crematogaster</i>	<i>Camponotus</i>	
<i>Monomorium</i>	<i>Formica</i>	
<i>Pheidole</i>	<i>Lasius</i>	
<i>Solenopsis</i>		
<i>Tetramorium</i>		

frozen ants in 100 ml acetone for 48 hours. Ants were then placed in a CPD chamber and dried through a series of CO₂ baths at high temperatures and extreme pressures, reaching a maximum of 35 C and 1250 p.s.i. Two trays, each containing either 20 live, or frozen, or CPD worker caste of *C.*

pennsylvanicus, were offered to the birds. Trays were paired by live/frozen, frozen/CPD, live/live, frozen/frozen, live/CPD and CPD/CPD. Each arrangement was presented to captive songbirds in a left/right and then right/left orientation to control for potential placement effects, for a total of twelve trials.

Spatial pattern of ants in trays trials: Ant choice trials were conducted to determine if songbirds displayed a preference for spatial patterns of ants in clear plastic trays. Observations during the previous experiments indicated that some ant species clumped together whereas others dispersed in the trays. Spatial patterns of ants may affect the attraction of the captive songbirds to the ant trays and subsequently affect the frequency of anting displays. Two trays, each containing frozen worker caste *C. pennsylvanicus*, were presented to captive songbirds. Twenty-seven ants were placed in clumped, uniform, or random arrangements in each tray. Random patterns were achieved through the use of a temporary grid on the bottom of the tray and a random numbers table; uniform patterns were achieved using the same grid pattern. Clumped patterns were produced by dividing ants into three groups (8, 8, and 9 ants) in the tray. This configuration of multiple small groups simulated grouping patterns observed during single ant trials. Trays were presented in the following pairs: clumped/random, clumped/uniform, and uniform/random, each

arranged in a right/left and left/right orientation. Paired tray trials were conducted twice, for a total of twelve trials, each controlling for potential placement effects.

In all ant choice trials, ants were observed for one hour or until all ants had been removed. Data were analyzed using SPSS, Statview and Microsoft Excel software.

RESULTS

Single tray ant trials. - Anting behavior was observed 14 times during 12 of the 68 days of total tray presentations in the aviary. Two of the eight presentations resulting in anting behavior included two separate birds displaying the behavior in a single tray. Both American Robins (*Turdus migratorius*) displayed anting behavior in the same tray of Lasius ants. The Blue Jay (*Cyanocitta cristata*) and one American Robin displayed anting behavior in the same tray of Formica ants. In all, 12 anting behavioral displays were recorded. Anting behavior was exhibited by two American Robins, a Blue Jay, a Gray Catbird (*Dumetella carolinesis*), and an Eastern Towhee (*Pipilo erythrophthalmus*) in the aviary. No anting displays were observed at the outdoor Weyanoke Sanctuary. Formicine and myrmicine ants were used for active anting behavior (Table 5 and Fig. 1). Dolichoderine worker ants were not selected for anting displays. During these trials, anting behavior always was followed by consumption of the ants.

A significant difference was seen in anting behavior between formicine and myrmicine ants ($\chi^2 = 1.333$, $df=4$, $P=0.05$) (Fig. 2). All anting behavior was observed only in ants with a chemical defense mechanism. No anting behavior

was seen in ants with aggressive stinging or biting defensive responses.

Songbirds were able to discriminate between ants during these trials, consistently performing anting behavior with preferred ants. These trials provide the first significant results on selection of formicine ants by songbirds for anting displays in choice test.

Ant behavior differed within the single tray presentation. Ants were either clumped within the tray or active. Active ants usually remained consistently active throughout each trial. A tray of ants was considered active if more than 50 % of the ants were moving. Similar numbers of trials with clumped and active ants, 34 and 38, respectively were observed. A two-factor ANOVA identified differences between the songbird responses to the ants' motion (Table 6). If ants were active within the tray, songbird interactions increased (Fig. 3).

Temperature inside the songbird aviary ranged from 15 to 40 C during the single tray trials. An analysis of variance identified no significant effect of temperature on songbird activity during these trials (Table 7 and Fig. 4).

Humidity, which ranged from 37 % to 72 % in the aviary, had no significant effect on songbird/ant interactions as identified by an analysis of variance (Table 8 and Fig. 5).

The bird interaction with ant species presented was classified as none, eating and anting behavior with the consumption of ants. Songbirds ate all ant types presented except *Monomorium* (Fig. 6). *Solenopsis* ants were consumed by the songbirds 100% of the trials when they were presented. During anting

behavioral displays, the songbirds consumed ants after application to their feathers.

Ant choice trials. - Ant genera choice trials: Thirty-three active anting displays were recorded during the 50 trials. Anting displays were conducted by American Robins, a Blue Jay, a Gray Catbird, an Eastern Towhee and a Northern Mockingbird. Anting behavior was conducted using ants from species of *Aphaenogaster* and *Crematogaster* (Myrmicinae), *Lasius* and *Camponotus* (Formicinae). Ant/songbird interactions recorded during these choice trials included consumption of ants (eating), active anting displays (anting) and immediate consumption of manipulated (anted with) ants (both eating and anting). Species of ants utilized during active anting displays included: *L. flavus*, *C. subbarbatus* and *C. pennsylvanicus*, *C. lineolata*, *A. fulva* and *A. rudus*. A Friedman's analysis revealed a significant difference among responses to ants in paired combinations of five ant genera ($\chi^2 (0.05, 5, 5) = 37.8$, $df=4$, $P = 0.05$) (Fig. 11). The ant species exposed to the songbirds were consumed in the course of these trials. Position effects (right trays versus left trays) did not significantly affect anting displays as calculated by a two-tailed t-test ($t = 0.9181$, $df = 117$, $P = 0.05$).

Temperatures in the songbird aviary ranged from 13 to 27 C. The abiotic factors, temperature and humidity, had no effect on anting behavior. These were no significant differences among the songbird interactions in the choice trials related to temperature (Table 9 and Fig. 7) or related to percent

TABLE 5. Ant genera used during anting behavioral displays in single species ant experiments.

Formicinae	Myrmicinae
<i>Lasius</i>	<i>Aphaenogaster</i>
<i>Formica</i>	<i>Pheidole</i>
<i>Camponotus</i>	

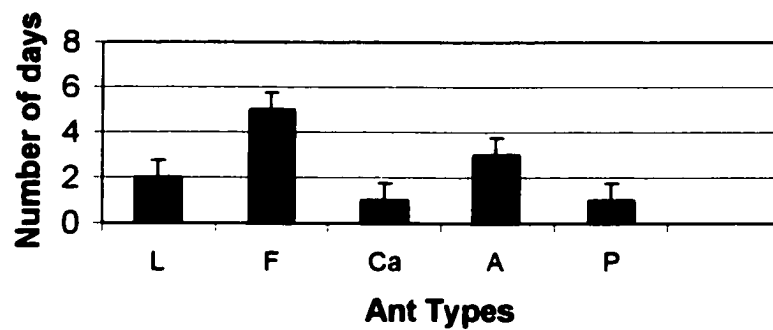


Fig. 1. Ant types used during anting behavior in single tray displays by the number of days anting behavior was recorded, \pm S.E. Ant types are L = *Lasius*, F = *Formica*, Ca = *Camponotus* (formicine), and A = *Aphaenogaster*, P = *Pheidole* (myrmicine).

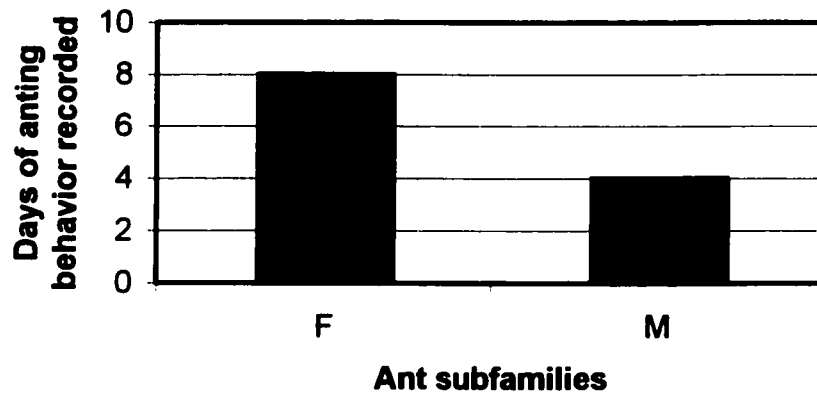


Fig. 2. Occurrences of anting behavior between Formicinae (F) and Myrmicinae (M) in single ant subfamilies species experiments. Ant subfamilies F = Formicinae and M = Myrmicinae.

TABLE 6. Songbird interactions with active versus non-active ants.

Source	df	Sum of Squares	Mean Square	F	P
Between groups	1	9	9	21	0.0001
Within groups	71	31	0.4		
Total	72	40			

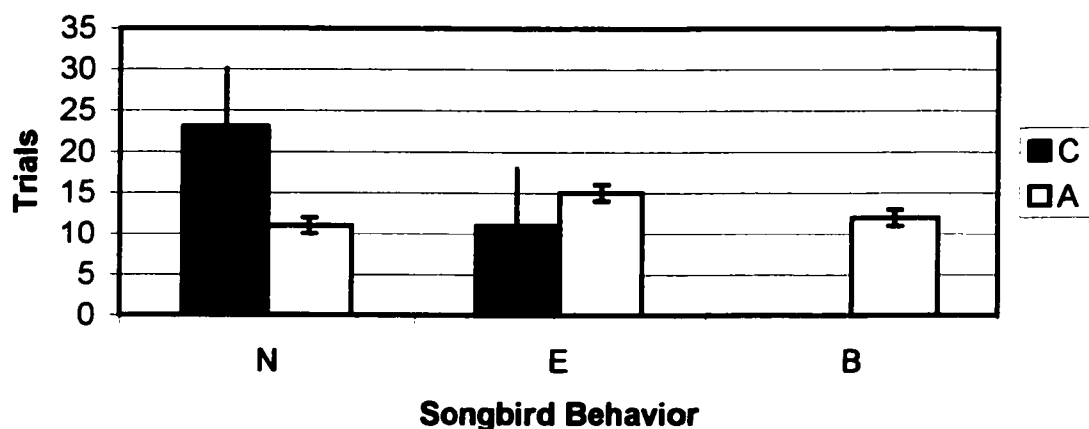


Fig. 3. Songbird interactions with active versus non-active ants. Ant activities are C = clumped and A = active, +/-S.E. Songbird behaviors are N = none, E = eating, B = both anting behavior and eating.

TABLE 7. Effect of temperature on songbird behavior in single tray trials.

Source	Sum of Square	df	Mean Square	F	P
Activity	0.723	2	0.361	0.013	0.987
Error	1751.899	63	27.808		
Total	37453.0	66			

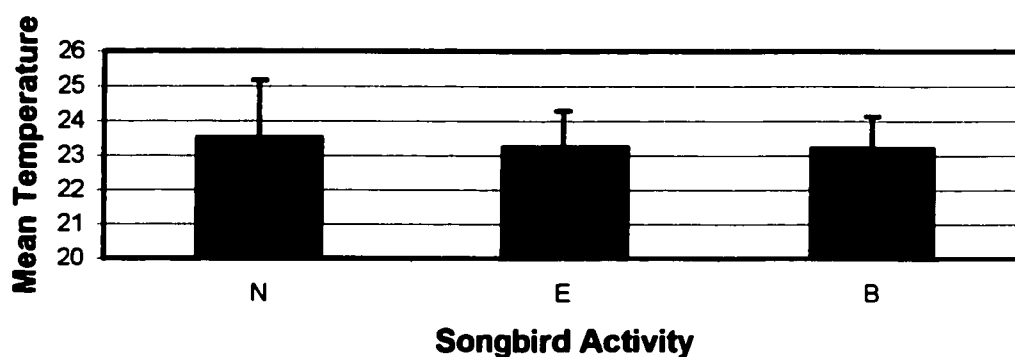


Fig. 4. Mean temperature (°C) +/- S.E. related songbird activity in single tray trials. Songbird activities are N = none, E = eating, B = both anting and eating.

TABLE 8. Percent humidity's effect on songbird/ ant interactions in single tray trials.

Source	df	Sum of Square	Mean Square	F	P
Between	2	243	122	1	0.3049
Within	21	2032	97		
Total	23	2275			

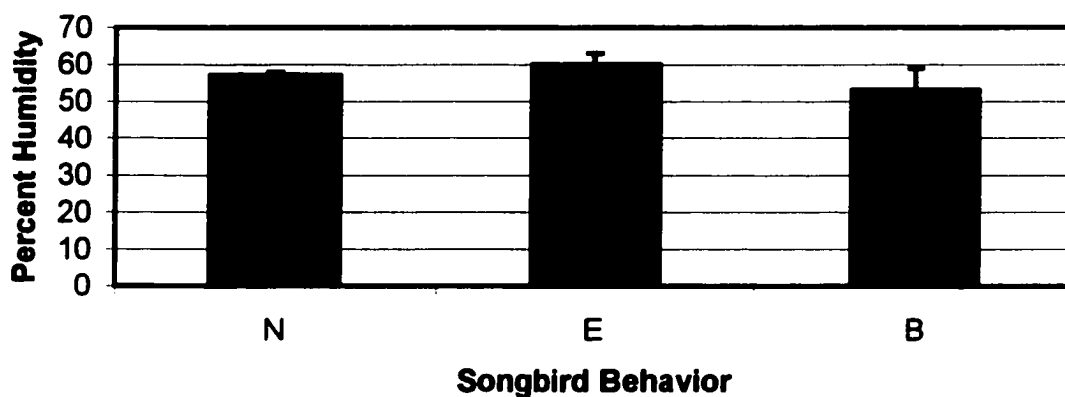


Fig. 5. Songbird/ant interactions by mean percent humidity \pm S.E. in single tray trials. Songbird behaviors are N = none, E = eating, B = both eating and anting behavior.

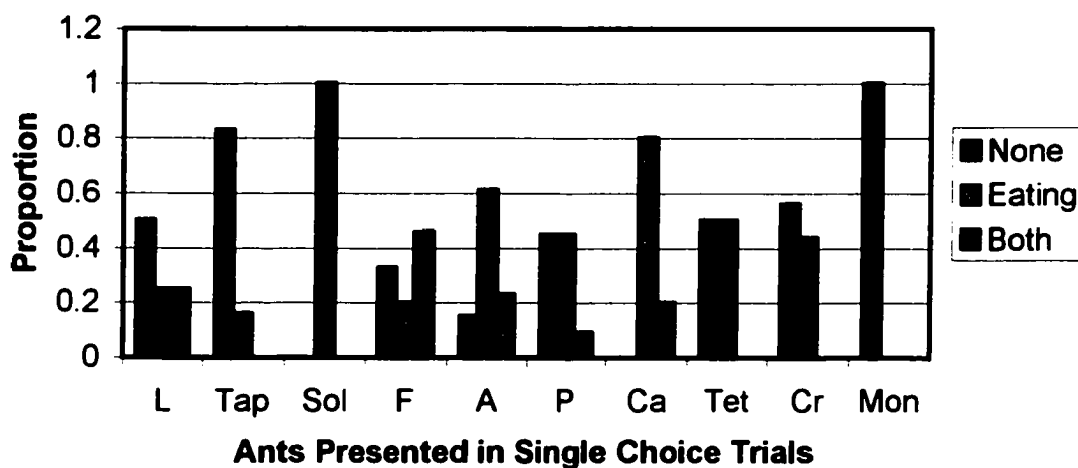


Fig. 6. Proportion of songbirds' interaction with ant genera in choice trials. Ant genera are L = *Lasius*, Tap = *Taponoma*, Sol = *Solenopsis*, F = *Formica*, A = *Aphaenogaster*, P = *Pheidole*, Ca = *Camponotus*, Tet = *Tetromorium*, Cr = *Crematogaster*.

humidity (Table 10 and Fig. 8) as calculated with a one-factor analysis of variance.

Sky conditions during these choice trials significantly affected the songbird interaction with the ants, calculated with a Fisher's Exact test ($P = 8.1409 \times 10^{-10}$, $df=1$). There were significantly more interactions between the songbirds and ants during clear days. A clear day was defined as few clouds and no rain. Additionally, anting displays occurred at a proportionally higher rate during clear days as compared to cloudy or rainy days. Songbird consumption of ants occurred more often on cloudy or rainy days (Fig. 9).

Songbird responses varied among different ant species. A one-factor ANOVA revealed a significant difference in the remaining ant numbers of different species after exposure to songbirds (Table 11). *Aphaenogaster*, *Lasius*, and *Camponotus* ants were consumed significantly more often than *Pheidole* and *Crematogaster* (Fig. 10). A Scheffe F-test revealed significant differences between numbers of *Pheidole* and *Aphaenogaster*, *Pheidole* and *Lasius*, and *Camponotus*, and *Crematogaster* and *Aphaenogaster* ants remaining after exposure to the songbirds during choice trials and between (Table 12).

The ant species chosen for these trials represent a broad spectrum of sizes and colors in both subfamilies. The *C. pennsylvanica* and *C. subbarbatus* are black ants 7 - 11 mm long. Both *L. flavus* and *P. dentata* are yellow 1.5 – 3 mm ants. *A. rudis* and *A. fulva* are both red ants 5 – 8 mm. *Crematogaster lineolata* is a black ant 3 - 5 mm (Smith 1965).

There was a significant difference among the reactions of songbird species to different ant types as identified by a three-factor ANOVA (Table 13). The Blue Jay, Gray Catbird and American Robin had the most interactions with ant types presented in the aviary. *Aphaenogaster* and *Camponotus* elicited the greatest number of interactions with the songbirds. The largest number of ant/songbird interactions, where ants were consumed, occurred between the American Robin and *Camponotus*. Anting behavior occurred most often between the Gray catbird and *Lasius*. The greatest number of both anting and consumption of ants occurred between the Blue Jay and *Camponotus* (Fig. 11).

The numbers of anting displays differed significantly among ant types presented to the songbirds (Table 14). Within songbird displays, *Camponotus* ants were used the most and *Crematogaster* ants the least after *Pheidole* ants, which were never selected at all (Fig. 12). A Scheffe F-test produced a significant difference between selection of *Pheidole* versus *Aphaenogaster*, *Lasius*, and *Camponotus* and between *Crematogaster* versus *Aphaenogaster*, *Lasius*, and *Camponotus* (Table 15).

The behavior of different ant species varied during choice trials. Ants in fluon-lined trays were classified as either clumped or active. Clumped ants were grouped together in small clusters, not actively moving about in the trays, whereas active ants moved freely. A one-factor ANOVA revealed significant differences among ant species in their actions in the trays (Table 16 and Fig. 13). A Scheffe F – test revealed significant differences between the actions of

TABLE 9. Effect of temperature on songbird activities in the songbird aviary in choice trials.

Source	df	Mean Square	F	P
Bird action	2	21408.965	1.023	0.367
Error	53	8.283		
Total	56	8.099		

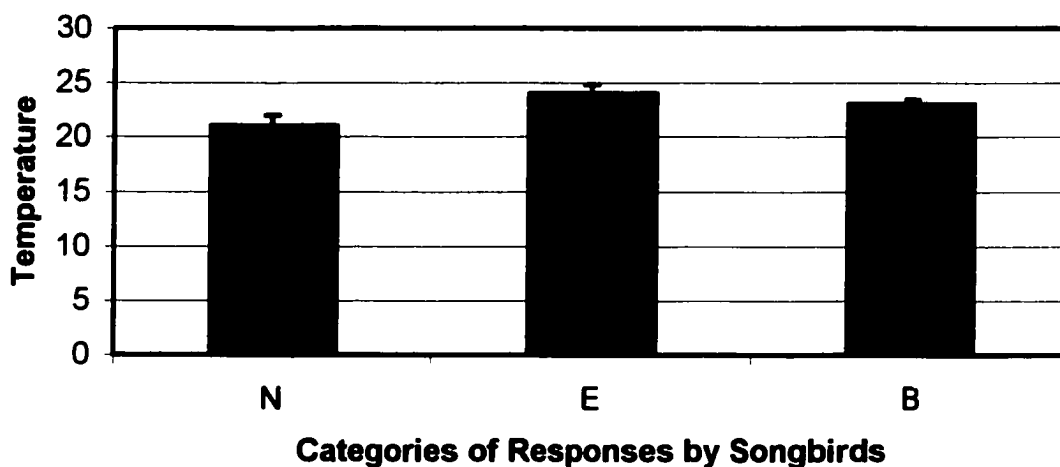


Fig. 7. Songbird/ant interactions related to mean temperature \pm S.E. in choice trials. Songbird behaviors are N = none, E = eating, B = both eating and anting behavior.

TABLE 10. Effect of humidity on songbird activity in the songbird aviary in choice trials.

Source	df	Sum of Squares	Mean Square	F	P
Between	2	239	119	1	0.2784
Within	97	8944	92		
Total	99	9183			

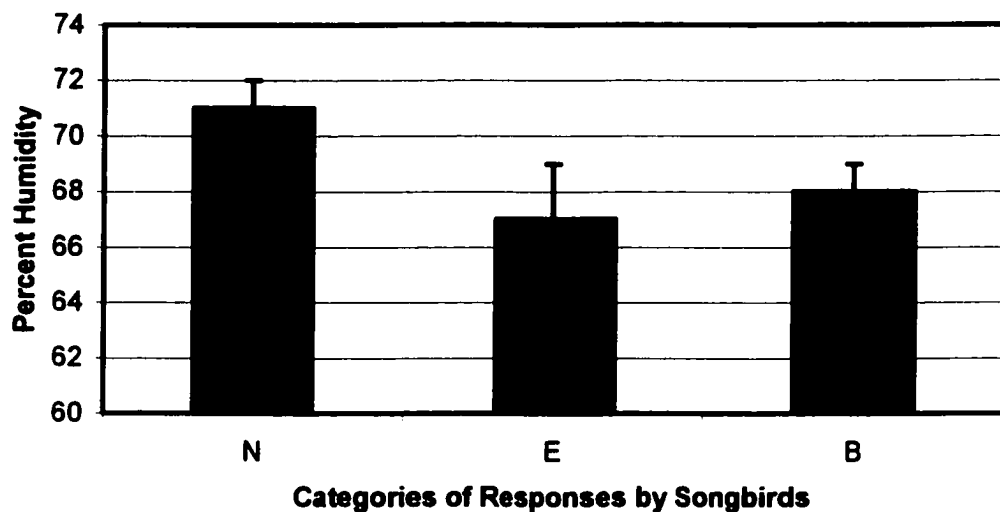


Fig. 8. Songbird/ant interactions related to percent humidity \pm S.E. in choice trials. Songbird behaviors are N = none, E = eating, B = both eating and anting.

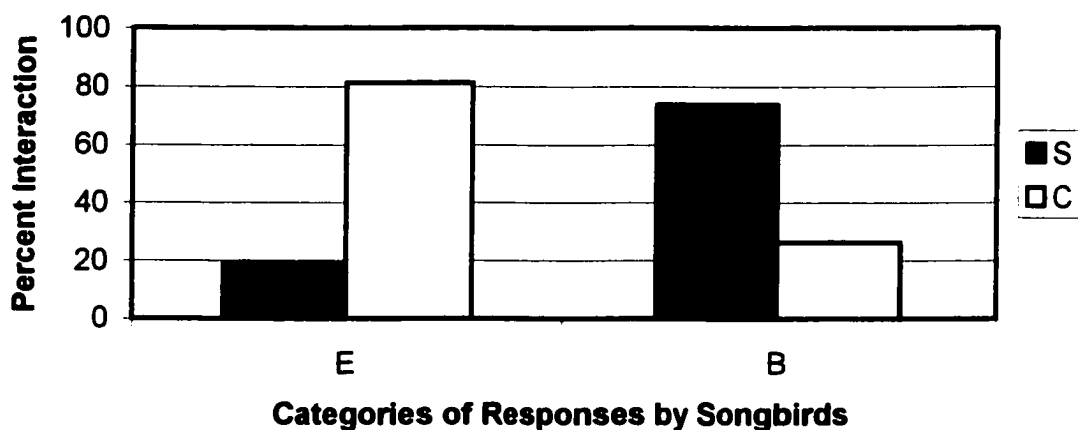


Fig. 9. Songbird/ant interactions related to sky conditions during choice trials. Songbird behaviors are E = eating, B = both anting behavior and eating, S = clear sky conditions, C = cloudy or rainy sky conditions.

TABLE 11. Removal of ant type by songbirds in Virginia Living Museum songbird aviary.

Source	df	Sum of Squares	F	P
Between groups	4	2978.956	14.974	0.0001
Within groups	94	4675.226		
Total	98	7654.182		

TABLE 12. Scheffe F-test identifying significant differences among ant species removed by songbirds during choice trials, $P < 0.05$.

Comparison	Scheffe F – test
<i>Pheidole</i> versus <i>Aphaenogaster</i>	5.305
<i>Pheidole</i> versus <i>Lasius</i>	4.289
<i>Pheidole</i> versus <i>Camponotus</i>	6.542
<i>Crematogaster</i> versus <i>Aphaenogaster</i>	6.881

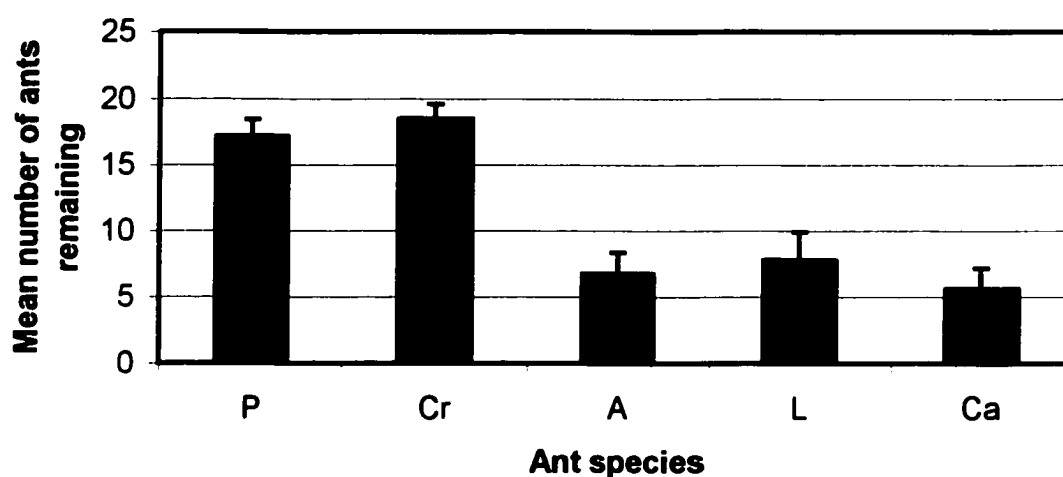


Fig. 10. Mean \pm S.E. number of ant species remaining uneaten after choice trials. Ants are P = *Pheidole*, Cr = *Crematogaster*, A = *Aphaenogaster*, L = *Lasius*, Ca = *Camponotus*.

Crematogaster versus *Aphaenogaster*, and *Crematogaster* versus *Camponotus* during paired ant choice trials (Table 17).

Ant movement in choice trials: Songbirds interacted with both the frozen and live *C. pennsylvanicus* ants. These preliminary trials showed that songbirds will display anting behavior with dead ants (Fig. 14). The Blue Jay displayed anting behavior with frozen ants 25 % of the total anting displays.

Ant chemistry trial: Ant chemical production might regulate the frequency of anting in songbirds. The effect of different levels of ant chemicals on songbird interactions was tested using live, frozen and CPD ants. Live ants can actively release pheromones, but freshly frozen ants maintain their wet weights and non-volatile chemicals. By contrast, CPD ants were washed with organic solvents, reducing levels of both volatile and non-volatile chemicals. Average wet and dry weights of 20 *C. pennsylvanicus* are 0.0172 g and 0.0122 g, respectively.

There was no significant difference between songbird/ant interactions during in these trials (Table 18 and Fig. 15). Although songbirds consumed all three ant presentation types, songbirds did not display anting behavior using CPD ants. Frozen ants were used in anting displays during 60% of these choice trials, despite being presented in only 36% of the trials. Songbirds rejected the CPD ants for anting displays when given a choice between live, frozen and CPD ants.

TABLE 13. Songbird reactions to ant types. Songbird species (A) were the Blue Jay, Gray Catbird, American Robin, Eastern Towhee, and Evening Grosbeak. Ant species (B) were *Lasius flavus*, *Crematogaster lineolata*, *Aphaenogaster rudis*, *A. fulva*, *Camponotus pennsylvanicus*, and *C. subbarbatus*. Songbird/ant interactions (AB) evaluated each songbird species interaction versus each ant species (B). Interactions were identified as Activities (C) eating of the ants, anting behavior, both eating and anting behavior.

Source	df	Sum of Square	Mean Square	F	P
Bird spp A	3	3	1	4×10^{19}	0.001
Ant spp B	4	2	1	2×10^{19}	0.001
AB	12	4	0.3	1×10^{19}	0.001
Activity C	2	4	2	6×10^{19}	0.001
AC	6	3	0.4	2×10^{19}	0.001
BC	8	2	0.2	7×10^{18}	0.001
ABC	24	4	0.2	6×10^{18}	0.001
Error	61	2×10^{18}	3×10^{20}		

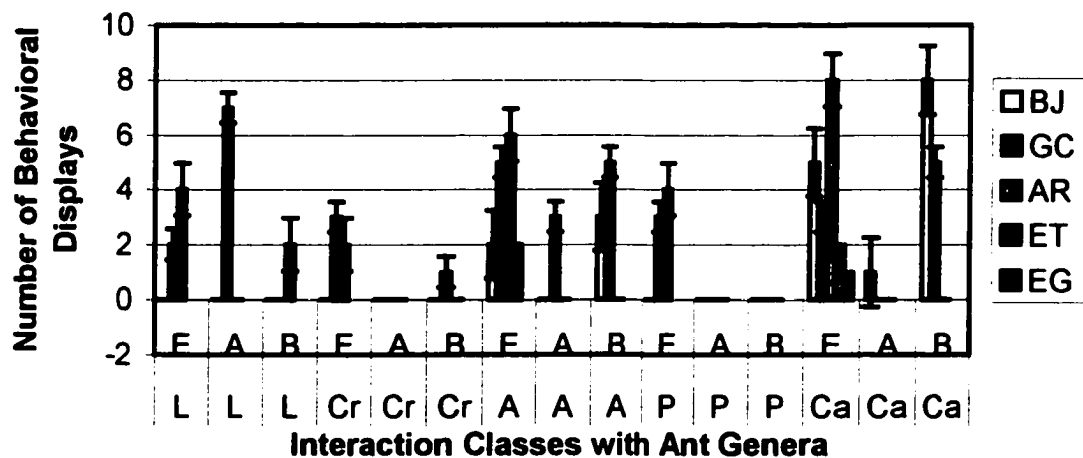


Fig. 11. Songbird interactions with ant genera in choice trials. Ant genera are L = *Lasius*, Cr = *Crematogaster*, A = *Aphaenogaster*, P = *Pheidole*, and Ca = *Camponotus*. Songbirds are BJ = Blue Jay, GC = Gray catbird, AR = American Robin, ET = Eastern Towhee, and EG = Evening Grosbeak. Interactions are E = Eating, A = anting behavior, B = both anting and eating behavior.

TABLE 14. Selection of ants for anting behavioral displays among different ant genera. Ant genera are L = *Lasius*, Cr = *Crematogaster*, A = *Aphaenogaster*, P = *Pheidole*, and Ca = *Camponotus*.

Source	df	Sum of Square	Mean Square	F	P
Between gps	4	7.04	1.76	10.857	0.0001
Within gps	95	15.4	0.162		
Total	95	22.44			

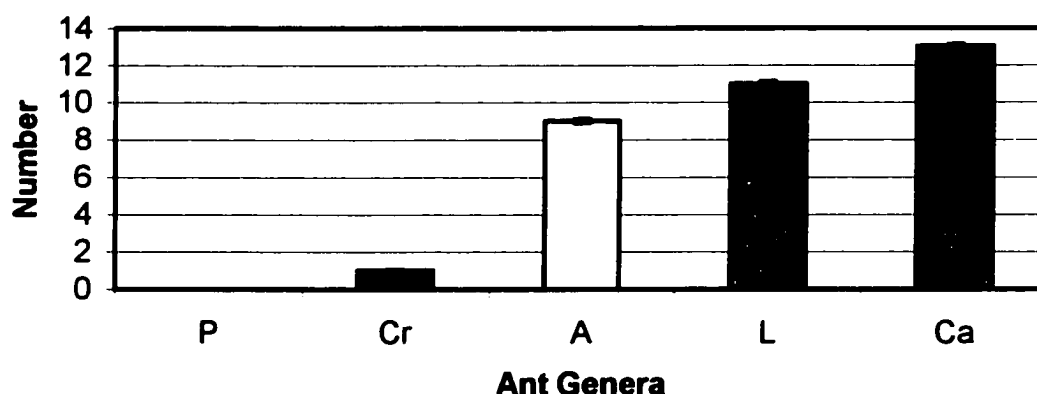


Fig. 12. Average anting behavioral displays with each ant genera +/- S.E. Ant genera are P = *Pheidole*, Cr = *Crematogaster*, A = *Aphaenogaster*, L = *Lasius*, Ca = *Camponotus*.

TABLE 15. Scheffe F-test showing significant differences between ant species selected for anting behavioral displays by songbirds, $P < 0.05$.

Comparison	Scheffe F-test
<i>Pheidole</i> versus <i>Aphaenogaster</i>	3.123
<i>Pheidole</i> versus <i>Lasius</i>	4.665
<i>Pheidole</i> versus <i>Camponotus</i>	6.516
<i>Crematogaster</i> versus <i>Aphaenogaster</i>	2.468
<i>Crematogaster</i> versus <i>Lasius</i>	3.856
<i>Crematogaster</i> versus <i>Camponotus</i>	5.552

TABLE 16. Clumping versus active movement among ant genera during choice trials. Ant genera are L = *Lasius*, Cr = *Crematogaster*, A = *Aphaenogaster*, P = *Pheidole*, and Ca = *Camponotus*.

Source	df	Sum of Squares	F	P
Between groups	4	4.2	5.377	0.0006
Within groups	95	18.55		
Total	99	22.75		

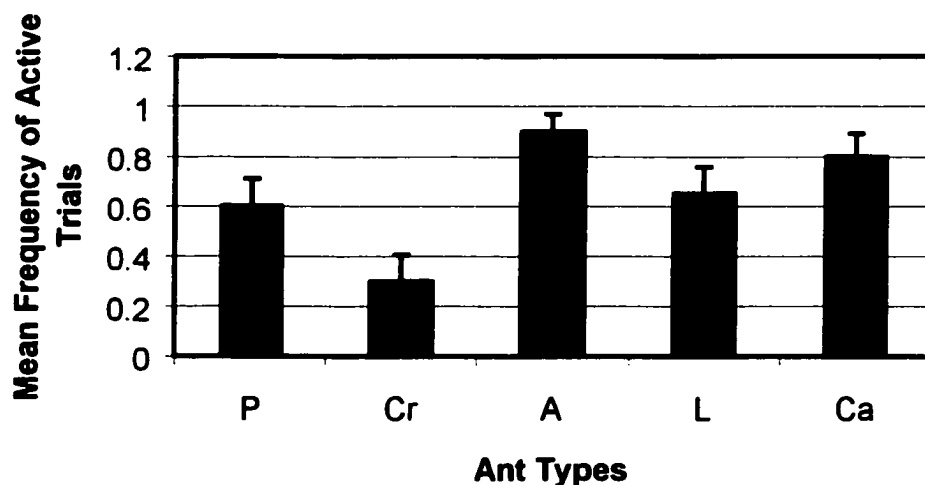


Fig. 13. Frequency of choice trials in which ants were active +/- S.E. Ant species are P = *Pheidole*, Cr = *Crematogaster*, A = *Aphaenogaster*, L = *Lasius*, Ca = *Camponotus*.

TABLE 17. A Scheffe F – test showing significant differences in activity between ant genera, $P < 0.05$.

Comparison	Scheffe F – test
<i>Crematogaster</i> versus <i>Aphaenogaster</i>	4.609
<i>Crematogaster</i> versus <i>Camponotus</i>	3.201

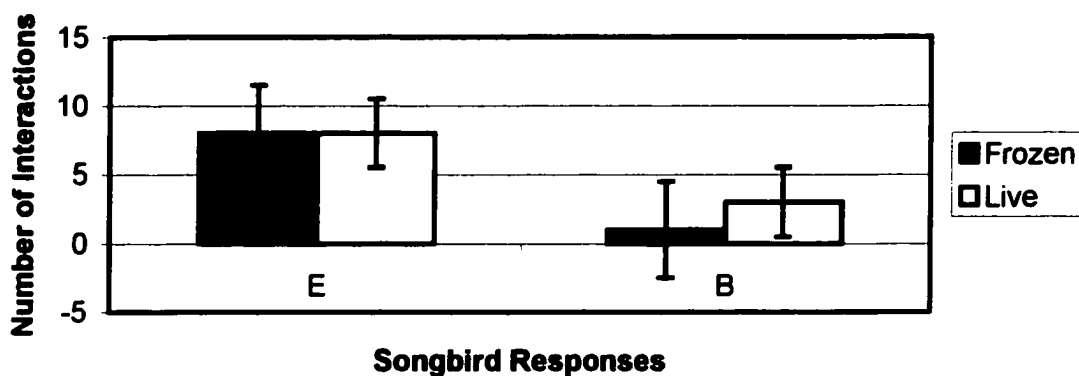


Fig. 14. Songbird responses to live and frozen ants during ant movement choice trials +/- S.E. Songbird behaviors are E = eating, B = both anting and eating.

TABLE 18. Songbird reactions to ant chemistry trials. Reactions were none, eating or both anting behavior and eating

Df	2
Number in Sample	3
Number of Cases	3
Chi-Square	2
P	0.3679

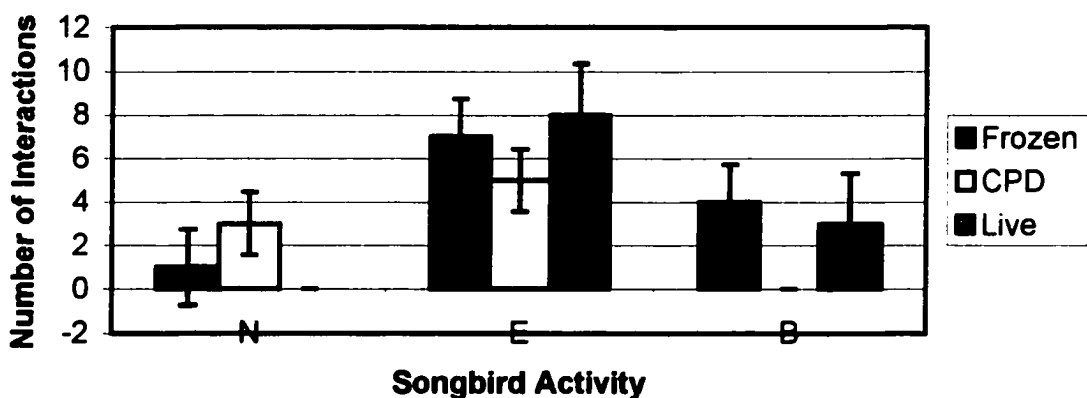


Fig. 15. Songbird activity in ant chemistry trials +/- S.E. Songbird activities are N = none, E= eating, B= both anting behavior and eating. Ants were either frozen, critically point dried (CPD), or live.

Spatial pattern of ants in trays trials: Songbirds displayed anting behavior twice during these spatial pattern trials. Both displays were conducted with ants in a random pattern. The interactions among the songbirds and ants did not differ significantly among the three spatial patterns, random, uniform and clumped, during the presentations as identified by a Friedman's test (Table 19, Fig. 16).

Finally, over all trials, molting conditions of the songbirds did not seem to influence ant/songbird interactions. Seven records of songbirds in early or late molting stages with few visible pin feathers were observed eating ants placed out in the trays and nine observations were made of molting songbirds displaying anting behavior and consuming ants. During two trials with birds in heavy stages of molt, where the birds have multiple, visible pin feathers, songbirds were not observed interacting with the ants.

DISCUSSION

Captive songbirds in the Virginia Living Museum songbird aviary displayed active anting behavior with multiple ant types. Of the ants presented to the songbirds, anting displays were recorded more often with ants in the subfamily Formicinae. These ants have vestigial stings and spray toxic chemicals such as formic acid as defense mechanisms. Every ant species presented in the aviary was consumed by the songbirds during the course of these experimental trials. The combination of anting behavior and the consumption of ants differed by the ant and bird species during the choice trials. The highest frequency of ant consumption occurred with the American Robin

eating *C. pennsylvanicus*. The Gray Catbird and *Lasius* were identified in the highest frequencies of anting behavior without consumption of the ants. The highest frequency of both anting behavior and consumption of ants was seen with the Blue Jay and *C. pennsylvanicus*.

Anting behavior has been reported irrespective of common adult diet preferences. The behavior is most common in Crows (Corvidae), starlings (Sturnidae), troupials (Icteridae), finches (Fringillidae), weavers (Ploceidae), thrushes (Turdidae) and babblers (Timaliidae) but is observed in over 40 families of birds (Simmons 1957). The songbirds represented within the aviary also differed in respect to adult diets. The songbirds identified interacting with ants during these trials include the Blue Jay, Gray Catbird, American Robins, Eastern Towhee, Northern Mockingbird, and Evening Grosbeak (*Coccothraustes vespertinus*). However, the Evening Grosbeak was recorded interacting with the ants only once and thus its interaction was considered an anomaly and omitted from this discussion. Blue Jays are known to consume a wide range of foods, including acorns, fruit, nuts and seeds as well as insects, other invertebrates, carrion, bird eggs, nestlings, and small vertebrates. Gray Catbirds frequently consume spiders, as well as berries and other fruit. American Robins consume fruit along with earthworms and snails. Eastern Towhees consume acorns, grasses and forb seeds the most often as well as berries and terrestrial invertebrates (Ehrlich et al. 1988).

The foraging techniques for the captive songbirds can be divided into two uneven groups. The first of the two groups contains the Gray Catbird, American

TABLE 19. Songbird Interaction with ants in differing spatial patterns.

df	2
Number of Sample	3
Number of Cases	3
Chi-Square	4.167
P	0.1245

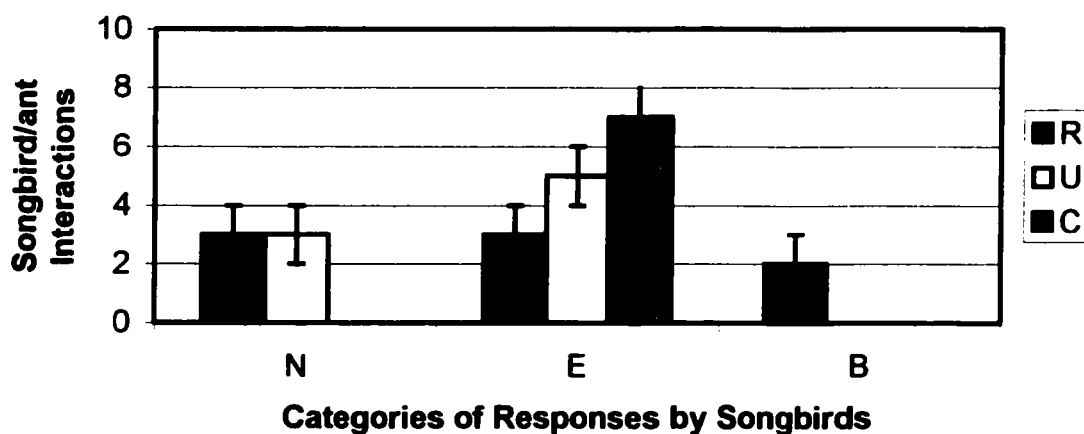


Fig. 16. Songbird responses to ants by ant spatial patterns +/-S.E. Songbird responses are N = none, E = eating, B = both eating and anting behavior, Ant spatial patterns are R = random, U = uniform, C = clumped.

Robin, Eastern Towhee, and Northern Mockingbird. The second group includes the Blue Jay. The Blue Jay is considered a ground gleaner, picking up items from the surface of the soil, turf, and sand. Blue Jays are known to scavenge as well as take short sallying flights to capture flying insects. The first group all glean from foliage and occasionally from branches. The Gray Catbird, American Robin, Eastern Towhee, and Northern Mockingbird all consume fruit and nuts from vegetation, rarely from the surface of the ground. Anting behavior is known to occur across feeding guilds (Simmons 1957) and this was evident in these experiments.

The captive nature of the songbirds recorded during these experiments must be considered. Although the songbirds have been conditioned to eat from bowls placed in the room, the bowls are placed at multiple levels and in tree branches. Having once lived in the wild, all of the songbirds have had some exposure to ant species. Additionally, a colony of *Crematogaster* ants was identified as living in the aviary.

The songbirds came to recognize the ant trays presented during these experiments and would inspect each tray upon its placement in the room. Ant trays differed from food trays in shape, size, color, and opacity. Food trays were removed from the aviary each evening and all trials were conducted before food was placed in the room. Ant trays were placed on the ground in the aviary at least 1 m away from the vicinity of food trays.

Chisholm (1944) and Judson and Bennett (1992) believe anting behavior functions as a food preparation mechanism in songbirds. Although songbirds do

select formicine more often for anting behavior and these ants produce chemicals known to be corrosive, causing chronic toxicity (Judson and Bennett 1992), songbirds also consumed formicine ants without displaying anting behavior. During these trials, all of the ants presented during the ant genera choice trials were consumed more often and separately from anting behavior.

Songbirds interacted with *Camponotus* and *Lasius* of the subfamily Formicinae, and *Aphaenogaster*, subfamily Myrmicinae. These ants differ in length and color. Both *Camponotus* and *Aphaenogaster* are larger ants, usually greater than 5 mm. *Lasius* and *Aphaenogaster* are light colored, yellow or red ants whereas *Camponotus* are dark black ants. All songbirds' preferred ants that were active in the tray; however, the spatial arrangement of still ants within the trays did not matter. The visual inspection of ant trays by the birds, combined with the types of ants preferred for anting behavior, identifies the importance of visual cues for the behavior.

Although ant movement was a significant factor in the selection of ant species during active anting displays, pheromones produced by ants may play a role. However, the ant chemistry experimental trials did not support this conclusion with significant results. Frozen ants were selected for anting behavioral displays while CPD ants were only consumed during these trials; the non-significant difference in songbird selection of *C. pennsylvanicus* between fresh frozen and CPD ants does not suggest, that the presence of chemical compounds affected ant selection. The songbirds might have selected the frozen ants based upon their wet weight, rejecting the lighter, dry, CPD ants. Frozen

ants maintain their water weight and higher amounts of chemical residue. The process of critically drying ants removed water mass and reduced the presence chemicals through acetone and carbon dioxide washes. The preference for ant chemicals and formicine ants that produce multiple acids, terpenoids and alcohols may affect whether birds eat or perform anting behavior with the ants.

The selection of *Aphaenogaster* but not *Pheidole* (both Myrmicinae) could have resulted from differences in ant activity rather than chemistry. This is supported by the avoidance of *P. dentata*. *Pheidole* were not as active as *Aphaenogaster* in the experimental presentation trays (Fig. 13). It is possible that songbirds make poor myrmecologists.

The abiotic factors examined in these experiments did not play a significant role in the display of anting behavior. Although both temperature and humidity had wide ranges during the trials, neither affected the anting displays of songbirds. In temperate climates where ants usually are not active during the winter season, these abiotic factors will naturally limit ant availability and anting behavior.

Sky conditions did seem to play an important role. Songbirds displayed anting behavior more frequently than ant consumption during clear sunny days (Fig. 9). The opposite was true for non-sunny days when either cloud cover or rain limited sunlight in the aviary. The selection of formicine ants, producing toxic chemicals such as formic acid known to have bactericidal effects, suggest that one purpose of anting behavior could be reduction of ectoparasites.

Sunning behavior in songbirds has been shown to reduce ectoparasites (Moyer and Wagenbach 1995). Although songbirds did not enter into a trance-like state while performing anting behavior similar to that induced during sunning behavior, the prevalence of anting behavioral displays during these experimental trials on clear sunny days suggested that songbirds might combine anting and sunning behaviors for a similar purpose: reducing feather ectoparasites. Additionally, anting behavior has been reported to induce trance-like states in birds, similar to sunning behavior (Ivor 1943, Groskin 1950).

Although anting and sunning behaviors were not observed together in my studies, the captive conditions might have influenced these observations. Ultra-violet light, with a wavelength between 4 nm and 400 nm, does not penetrate the treated glass of the songbird aviary. Thus, the glass walls and roof of the aviary might have reduced the prevalence of sunning behavior in the captive songbirds. During these experimental trials from 1996 to 2001, I observed sunning behavior once.

It has been suggested that anting behavior might facilitate molt in songbirds (Potter and Hauser 1974); however, there was little difference in anting behavioral displays by songbirds in molting and non-molting conditions during these experimental trials.

The experimental trials conducted herein represented the first controlled, quantitative study of ant selection during anting behavior. These results provided clear evidence for songbird preference and avoidance of specific ant species. Songbirds displayed a significant preference for *Camponotus*, *Lasius*, and

Aphaenogaster and they rejected *Pheidole* and *Crematogaster*. Preferred ants differed from rejected ant types in their activity levels. The songbirds also avoided aggressive ants, with stinging and biting defensive responses.

CHAPTER III

POSSIBLE BACTERICIDAL AND FUNGICIDAL ACTIONS OF ANT CHEMICALS ON FEATHER PARASITES.

INTRODUCTION

Songbirds may apply ants to their feathers to extract ant chemical secretions in order to control feather parasites. Although anting behavior has been shown to have no effect upon feather mites or lice (Kelso and Nice 1963, Potter 1974, Judson and Bennett 1992, Clayton 1999), the behavior might reduce microbial growth on feathers (Ehrlich et al. 1986, Clayton 1999). Beer (1963) reviewed the frequency and kinds of microorganisms on feathers. Both fungi and bacteria can be harmful to feathers, breaking down the structural integrity through keratinolytic activities (Pugh 1965, Burt and Ichida 1999, Muza et al. 2000). Further, the types of ants most commonly used during anting behavior support the self-medication hypothesis. I have collected preliminary data indicating that a greater number of anting displays occurred when worker ants of the Formicinae subfamily were used by songbirds.

Formicine ants secrete a variety of chemicals, including formic acid, a corrosive and cytotoxic acid capable of causing dermal necrosis in large doses (Blum 1992). Formicine ants have a vestigial sting, and they use formic acid as a primary defensive mechanism. This volatile organic compound is produced as a spray, usually a 60% aqueous solution containing formic acid, free amino acids,

and small peptides (Blum 1992). Formic acid may have both bactericidal and fungicidal qualities, which the birds can use to remove microorganisms from their feathers (Ehrlich et al. 1986).

Ants produce numerous chemicals in addition to formic acid. For example, growth of both *Escherichia coli* bacteria and *Aspergillus parasiticus* fungi is repressed by the 3-methylindole produced by *Neivamyrmex nigrescens* army ants (Brown et al. 1979). Although pheromone secretions are usually species specific, other species, including parasitoids, have evolved the ability to identify ant locations through the pheromonal secretions of ants (Brown and Feener 1991). Watkins et al. (1969) found that 3-methylindole produced by *N. nigrescens* repels insectivorous snakes. *Apocephalus paraponerae*, a phorid parasitoid, is attracted to whole body extracts of *Paraponera clavata*, a large tropical ant (Feener et al. 1996). Additionally, some colony parasites use ant chemicals to identify appropriate prey. The myrmecophilic beetle *Atemeles pubicollis* identifies suitable ant colonies through the host odor (Holldobler 1969).

The objectives of this research were to identify possible bactericidal or fungicidal properties of both polar and non-polar ant secretions and formic acid on microbial ectoparasites of feathers. Hypotheses tested herein included: 1) Ant chemical secretions have an inhibitory effect on feather bacteria, fungi, and fungal spore germination. 2) Formic acid has an inhibitory effect on feather bacteria, fungi, and fungal spore germination. 3) Ant preferences displayed by songbirds for selection in anting behavior are related bactericidal and fungicidal properties of preferred ant chemical secretions.

METHODS

The effect of ant chemicals on feather microbes was examined using agar-plate inhibition assays with bacterial and fungal species likely to occur on feathers. These included the bacteria *Bacillus subtilis*, *B. licheniformis* strains 138B and 1432B, the fungi *Chaetomium globosum*, *Penicillium chrysogenum*, and *Trichoderma viride*. Ant chemicals were derived from *Camponotus pennsylvanicus*, *Pheidole dentata*, *Aphaenogaster rudis*, *Crematogaster lineolata*, and *Lasius flavus* worker caste ants collected from the Hampton Roads Virginia area. The feather fungi and *B. subtilis*, identified as feather inhabitants through the literature, were ordered from Fisher's Scientific. *Bacillus licheniformis* strains were provided by Edward H. Burt, Department of Zoology at Ohio Wesleyan University. Each bacterial and fungal species was first subcultured to produce individual colonies and to confirm identity through visual examination.

Bacillus colonies and mature fungal colonies and spores were suspended in sterile 0.85 % NaCl solution. The *Bacillus* suspension was produced using a loop-full of a mature colony (over 24 hours incubation time) with 1 ml of saline mixed in a test tube by using a vortex. The mature fungal hyphae (over 72 hours incubation time) suspension was produced with 1 ml of agar cut from a mature fungal spread plate combined with 1 ml saline in a tissue homogenizer.

The fungal spore suspension was produced by tapping the bottom of three spread plates containing mature fungal colonies onto a 6.5-cm diameter round filter paper. Collected spores were then mixed with 1 ml de-ionized water in a

glass test tube in a vortex. The presence of spores was confirmed through visual inspection of resulting spread plates under 20X Nikon dissection microscope.

Spread plates used in inhibition tests were produced with 10 μ l suspension of each *Bacillus* strain, 100 μ l mature fungal suspension, and 40 μ l fungal spore suspension. All microbial work was conducted with autoclaved equipment in sterile conditions under a Type II A Laminar flow hood.

Inhibition assays were conducted using 6 mm diameter filter paper disks testing for possible bactericidal and fungicidal activity of ant secretions and pure formic acid. Each agar plate contained four filter paper disks; two disks contained 10 μ L each of the experimental treatment solution and two disks contained 10 μ L each of the control solution (Fig. 17).

Ant chemical treatments were performed by two different methods. The first treatment, hexane extract, was performed using 50 frozen ants per species. Each ant species was frozen and then placed in a beaker of 5 ml of hexane for 30 minutes. After the 30-minute soak, 5 ml of hexane was added to the beaker and allowed to sit for an additional 30 minutes. Ant carcasses were removed from the hexane extract solution after soaking for 1 h. Thus, 10 ml hexane-ant extract was produced for each ant species. Hexane-ant extract was pipetted onto 6 mm diameter filter paper disks. Preliminary trials identified no flooding or leakage from 6 mm filter paper disks when absorbing 10 μ l of de-ionized water. Controls for the hexane-ant extract consisted of 10 μ l pure hexane. Paper disks with hexane-ant extract treatment and hexane-control were allowed to dry before placement onto agar spread plates containing microbial cultures.

The second ant chemical treatment was produced as an ant suspension in deionized water. Three frozen ants of each species were ground in separate tissue homogenizers with 700 μ l deionized water. In cases where ant castes are divided into soldier and worker castes, one soldier and two worker ants were selected. Ten μ l of ant suspensions were pipetted onto paper disks and placed on agar spread plates. Control solutions for the ant suspensions consisted of deionized water.

The combination of three ants to 700 μ l deionized water versus 50 ants to 10 ml hexane resulted in the same proportion of solute to solvent: 4.3×10^{-3} and 5×10^{-3} , respectively. These concentrations represented the application of a single ant to one feather covering an approximate 1.5 cm by 4 cm area.

Formic acid trials used sequencing grade pure formic acid, ordered from Fishers Scientific. Controls for the formic acid trials were untreated filter paper disks.

For each experiment and each ant species/microbe species combination, five replicate plates were prepared. For the fungal spore experiment, three replicate plates were prepared for each ant species/fungal species combination. Bacterial growth was monitored at the 1st, 4th, 12th and 24th hour after inoculation. Fungal growth cultured from hyphal strands was monitored at the 4th, 5th and 6th day after incubation. Zones of inhibition were visually measured in millimeters as

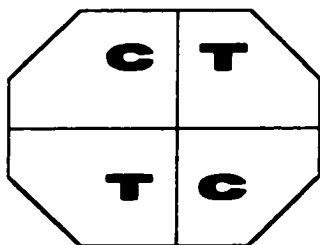


Fig. 17. Experimental agar plate design. (C) Control filter paper disk. (T) Treatment filter paper disk.

the diameter of no growth around each filter paper disk. Spore inhibition trials were monitored at the 1st, 24th, and 36th hour after incubation. Spore germination was documented using a Nikon inverted microscope with a phase contrast lens at 200X magnification. Counts were made one hour and 36 hours post-incubation for germinating and non-germinated spores in three fields of each agar plate.

Culture conditions for the bacteria tested included. -

- *Bacillus subtilis*

These motile, gram positive rods were cultured aerobically at 30 °C on tryptic soy agar (TSA) growth medium (Burt and Ichida 1999).

- *Bacillus licheniformis* strains 138B and 1432B

These keratinolytic, gram-positive rods (known to degrade feathers) were cultured from wild birds on TSA. They were cultured at 30 °C on tryptic soy agar growth medium (Burt and Ichida 1999).

Fungi tested included. -

- *Chaetomium globosum* (Ascomycetes)

This cellulose decomposer was cultured at 25 °C on potato dextrose agar (PDA) growth medium (Hubalek 1978).

- *Penicillium chrysogenum* (Deuteromycetes)

This species was cultured at 25 °C on PDA growth medium (Hubalek 1978).

- *Trichoderma viride* (Deuteromycetes)

This species was cultured at 25 °C on PDA growth medium (Hubalek 1978).

Estimates of the number of microbial endospores carried on the exoskeleton and in the gut of each ant species were produced by pipetting 10 μ L of homogenized ant suspension onto PDA plates. Two plates were produced for each of the five ant genera. Agar plates were visually inspected for bacterial colonies after 24 hours incubation at 30 °C. Bacterial colonies were heat-fixed onto glass microscope slides and gram stains were performed for each slide.

Data collected from all experimental trials were assessed through visual inspection. Paired t-tests and ANOVA were calculated using the SPSS statistical package. Percentages, standard error, and histograms were produced using Microsoft Excel software.

RESULTS

Formic acid was a strong inhibitory agent for all the bacteria and fungal hyphae tested. Average inhibition (n = 10) zones produced by formic acid were: 30.7 mm after 12 hours for *B. subtilis*, 44.5 mm after 12 hours for *B. licheniformis* strain 1432B, 47.1 mm after 12 hours for *B. licheniformis* strain 138B, 32.0 mm after 4 days for *C. globosum*, 22.0 mm after 4 days for *P. chrysogenum*, and 49 mm after 4 days for *T. viride* (Fig. 18). None of the hexane-ant extracts nor the whole ant suspension in deionized water produced inhibition in any of the microbial species. Neither the preferred ants (*Camponotus*, *Lasius* and *Aphaenogaster*), nor the non-preferred ants (*Pheidole* and *Crematogaster*) selected for anting behavior produced chemicals that inhibited common feather microbes.

Concentrations of mature spore suspensions varied among fungal types. Forty μ l suspensions cultured on agar spread plates and analyzed through three 200X fields of view produced an average of five spores of *T. viride*, an average of 88 spores of *P. chrysogenum*, and an average of 54 spores of *C. globosum*. Thus, fungal spore germination in formic acid was moderately inhibited.

All of the *C. globosum* spores were inhibited, an average of three of the total five spores were inhibited for *T. viride*, and 87 of the 88 spores were inhibited for *P. chrysogenum* (Fig. 19). Germination of *C. globosum* fungal spores was significantly inhibited by formic acid (paired t-test=-2.936, df=8, P=0.019). The formic acid treatment did not have a significant effect on spore germination in *P. chrysogenum* (paired t-test=-2.137, df=5, P=0.086) or *T. viride* (paired t-test=-1.437, df=8, P=0.189) (Table 20).

DISCUSSION

Feather ectoparasites have the ability to reduce host fitness (Burt 1999). These potential effects are present in every aspect of avian life including mate selection (Clayton and Tompkins 1995), fecundity and successful rearing of hatchlings (Burt and Ichida 1999), and host survival (Booth et al. 1993). Bird feathers serve important functions for both flight and thermal protection (Proctor and Lynch 1993). Microorganisms can potentially degrade feathers and could act as a fundamental selective force in the evolution of molt (Burt and Ichida 1999). The ability to suppress ectoparasites through self-medication has the potential to increase fitness levels among individuals and within populations (Clayton and Wolfe 1993).

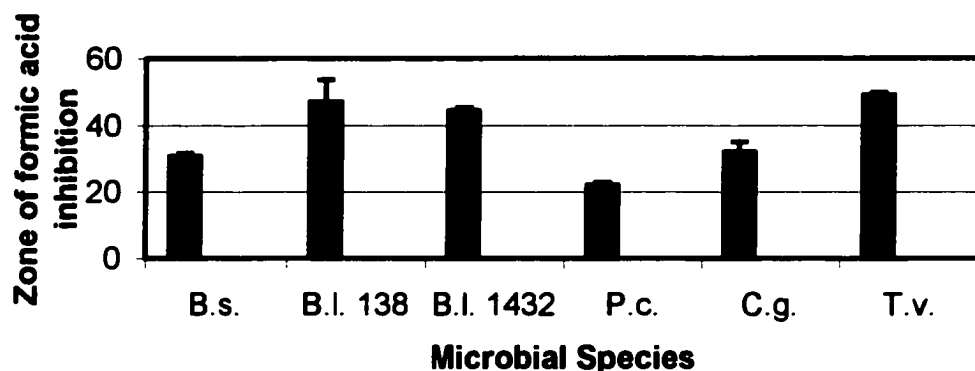


Fig. 18. Mean diameter (mm) +/- S.E. of inhibition in microbial inhibition trials. B.s. = *Bacillus subtilis*, B.I.1432B = *Bacillus licheniformis* strain 1432B, B.I. 138B = *Bacillus licheniformis* strain 138B, C.g. = *Chaetomium globosum*, T.v. = *Trichoderma viride*, and P.c. = *Penicillium chrysogenum*.

TABLE 20. Paired t-test results showing effects of formic acid on *Chaetomium*, *Penicillium* and *Trichoderma* spore germination. Mean germination represents the mean number of spores contained in three 200X microscopic fields of view.

	<i>Chaetomium</i>	<i>Penicillium</i>	<i>Trichoderma</i>
Mean Germination	0.667	1.116	1.22
Mean Non-Germination	54.11	86.333	2.556
T	-2.936	-2.137	-1.437
df	8	5	8
P	0.019	0.086	0.189

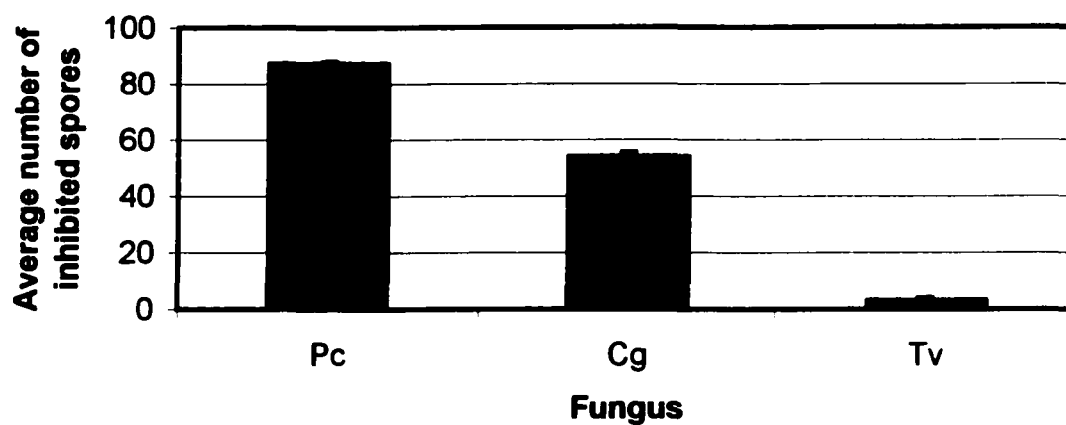


Fig. 19. Mean \pm S.E. numbers of fungal spores inhibited by formic acid for *Chaetomium globosum*, *Penicillium chrysogenum*, and *Trichoderma viride*

Although the microfibrils of twisted keratin in feathers can be resistant to biological degradation, keratinolytic bacteria and fungi have been identified as potential destructive agents (Burt and Ichida 1999). High frequencies of feather microorganisms are identified by Brittingham et al. (1988). Most of these feather-degrading bacteria also commonly occur in soils (Hubalek 1978, Brittingham et al. 1988, and Burt and Ichida 1999). Thus, irrespective of their feeding guild, most songbirds come in contact with soil particles and will potentially acquire multiple microorganisms harmful to their feathers.

Preening behavior in birds, including bathing in water or dusting and coating feathers with oils from preen glands, is commonly thought to improve feather condition. These behaviors may not substantially reduce feather ectoparasites. Clayton and Wolfe (1993) document that the surgical removal of the preen gland did not lead to increased populations of feather lice. It is possible that anting behavior combined with preening activities can decrease harmful feather parasites (Goodwin 1955, 1956, Simmons 1966, Ehrlich et al. 1986, Clayton and Vernon 1993, and Clayton 1999,). The songbirds in genus *Pitohui* from New Guinea have evolved a chemical defense against feather ectoparasites in the steroidal alkaloid homobatrachotoxin (Dumbacher et al. 1992). This toxin, sequestered in their feathers and muscle tissue, is the same chemical found in *Phyllobates*, the Dendrobatid poison-dart frogs. Thus, *Pitohui* is able to deter ectoparasitic feather lice as homobatrachotoxin has been found to be lethal to chewing lice (Dumbacher 1999).

My experiments tested the hypothesis that ant chemical secretions inhibit microbial growth. Anting behavior might be considered adaptive if the amount of bacterial growth, hyphal strands of fungi and fungal spores could be reduced by the application of ant chemicals.

Although metapleural glands of ants have been found to produce anti-fungal and anti-bacterial chemicals (Holldobler and Wilson 1990), neither the hexane-ant extracts nor the homogenized ant in deionized water inhibited bacterial colony growth, fungal spore germination or hyphal growth. In contrast, formic acid inhibited all microbial growth including *B. subtilis*, *B. licheniformis* strains 138B and 1432B and hyphal growth of *C. globosum*, *P. chrysogenum*, and *T. viride*. Formic acid significantly inhibited spore germination in *C. globosum* but not *P. chrysogenum* or *T. viride* spores.

The results of this study suggest that ants are not selected for anting behavior based upon their ability to inhibit microbial growth. The compounds secreted by highly preferred ants [*Aphaenogaster* (Myrmicinae), *Lasius*, and *Camponotus* (Formicinae)], and the ants avoided for displays of anting behavior [*Pheidole* and *Crematogaster* (Myrmicinae)] had no effect on microbial growth.

CHAPTER IV

DIFFERENCES IN HABITAT AND ANT SPECIES AMONG WILD TURKEY DUST BOWL AND NON-DUST BOWL LOCATIONS

INTRODUCTION

Wild Turkeys (*Meleagris gallopavo*) are one of the first species to be linked with anting behavior (Whitaker 1957). During an account detailed by James Audubon, Wild Turkeys rolled in apparently deserted ant nests (Audubon 1831). A wide range of birds, including many native grassland and prairie species, have been recorded as displaying anting behavior (McAtee 1947, Thomas 1957, Healy and Thomas 1973). Many of these same species engage in dusting as well as anting behavior.

Dusting behavior has been identified as a form of preening, possibly used to facilitate molt and clean feathers (Ehrlich et al. 1986). Birds engage in dusting behavior by covering themselves with dust, a practice thought to replace bathing behavior in dry habitats (Brennan et al. 1987). Dusting behavior leaves a distinctive round, bowl-like depression in the ground. Frequent dusting helps maintain optimal feather conditions, because the dust absorbs excess plumage lipids and preening oils (Slessers 1970, Ehrlich et al. 1986). It is possible that dusting behavior is often combined with passive anting behavior. Additionally, ant colonies in habitats chosen by wild birds to dust might influence site selection for dusting behavior.

Active dusting locations were identified in Zuni Pine Barrens in Isle of Wight County, Virginia. Three areas of high dust bathing activity by Wild Turkeys were located within the preserve through multiple sightings, call identifications, tracks, and dust bowls. Each of the three sites contained multiple, large dust bowls with molted feathers from Wild Turkeys. Wild Turkeys were observed during dusting behavioral displays at a single study site (personal communication, Tolson 2001). I sought to determine whether dust bowl locations were influenced by ant species, vegetation types, overhead canopy cover or soil type. If anting behavior is linked with dusting activity, then ant species known to elicit anting behavior should be common in dust bathing locations.

METHODS

Ant collections. - At each site, one 20-m transect was placed across a Wild Turkey dust bowl, centering over the bowl location. A second 20-m transect was placed 50 m from the end of each dust bowl transect in locations with no visible dust bathing activity (non-dust bowl location). Ant species were collected from each 20-m transect via ant baits and pitfall traps. Twenty ant baits consisting of 5-cm² white paper smeared with a bait (mixture of equal parts: Skippy peanut butter, peanut oil and granulated white sugar) were placed 1 m apart along the transect. Ant baits were allowed to sit for one hour. Ants recruited to the bait were aspirated into a containment jar and the bait was removed. Each site was baited for ants on three consecutive days, October 14th, 15th and 16th, 2000. Ten pitfall traps were left overnight at each transect. Pitfall traps consisted of 16 mm X 50 mm glass test tubes, partially filled with soapy

water and sunk level with the ground. Traps, placed 1 m apart along each transect, were checked for ants every 24 hours, for a total of two collections on October 15th and 16th, 2000. Ants collected were transported to the laboratory and frozen. Ants were identified to species using taxonomic keys and comparison with specimens at the Smithsonian Natural Museum of Natural History. Voucher specimens were donated to the Department of Biological Sciences Entomological Laboratory at Old Dominion University. Ant species diversity and community similarity were calculated using the Shannon diversity index and the Sorensen's coefficient of community similarity. The significance of statistical differences between Shannon indices was identified using a two-sample t-test.

Soil samples. - Five soil samples were selected randomly along both transects at each sample site. Soil cores were collected using a bulb corer to a 15-cm depth. Samples were transported to the laboratory and dried for three days in a drying oven at a minimum of 30 °C. Soil was separated and weighed for particle size using a soil sieve kit with 5 graduated mesh screen sizes: >0.5 cm, 0.25 cm < 0.5 cm, 0.04 cm < 0.25 cm, 0.01 cm < 0.04 cm, and < 0.01 cm.

Vegetation samples. - Community vegetation type and numbers of plant species were collected for three 1-m² plots selected randomly along both transects at each sample site. Vegetation identified in each m² was classed in one of three different categories: trees, shrubs, and herbaceous. Vegetation was collected from each site along each transect using a cross-line transect method. If the plant touched the transect line it was collected and pressed for

identification. Vegetation samples were identified to species using taxonomic keys and were prepared as voucher specimens donated to the Department of Biological Sciences Herbarium at Old Dominion University.

Canopy cover. - Forest overstory density canopy cover was measured three times at random points along each transect using a spherical densiometer. The percent of overhead area not occupied by canopy was determined for each site and an ANOVA was calculated to identify any significant differences between dust bowl and non-bowl locations for canopy cover.

Study site. - The Blackwater Ecological Preserve is a 128.6-ha preserve owned by Old Dominion University (ODU), and jointly managed with the Nature Conservancy, the Virginia State Department of Conservation and Recreation, International Paper, and the Virginia Department of Forestry. The area was dedicated a State Natural Area Preserve in 1995 (Blonder and Erdle 1999). It is located north of and adjacent to Route 614, west of Horse Swamp and east of the Blackwater River. This preserve is the northernmost reproducing longleaf pine (*Pinus palustris*) stand. The history of land use in the preserve includes timber harvesting and planting from unknown seed sources in the late 1950s (Blonder and Erdle 1999). The surrounding land uses are agriculture and silviculture with some residential areas.

Located on a remnant estuarine terrace, the preserve has elevations ranging from near sea level to approximately 11.4 m above M.S.L. I examined three sites on the preserve, designated A, B, and C. Site A was on an open dirt road, site B was on a woodland trail, and site C, the most complex habitat, was in

a vegetated area. Soils at study sites B and C included both a very deep, poorly drained Leon soil and deep moderately well-drained Chipley soil. These soils can be characterized as sandy surface approximately 15 cm deep, variable sandy subsurface from 15 to 77 cm, and a firm sand subsoil from 54 to 77 cm. The soil at study site A is a very deep, poorly drained Kingston loam. This soil can be characterized as loamy surface approximately 10 cm deep, and mottled sandy clay from 23 to 54 cm (Kitchel et al. 1986).

The vegetation sites have been characterized as longleaf pine / turkey oak (*Quercus laevis*) which has been replaced by loblolly pine (*Pinus taeda*) and turkey oak in places in site A. Pond pine (*Pinus serotina*) and vegetation types in the family Ericaceae characterize site B. Site C is composed of Black Gum swamp vegetation types. The vegetation community is maintained through prescribed, low intensity forest fires (Blonder and Erdle 1999).

RESULTS

Ant species. - Seventeen species of ants were collected at the Zuni site (Table 21). Three species of ants were present in dust bowl locations only: *Aphaenogaster lamellidens*, *Lasius umbratus*, and *Leptothorax pergandi*. Four species of ants were found only at a single site: *Monomorium minimum* was identified in site B non-bowl location; *Formica* spp. was found in two non-bowl sites, B and C; and *Neivamyrmex opacithorax* was in only site C. Shannon diversity index revealed differences between bowl and non-bowl locations in the ant species collected (Agosti et al. 2000) (Table 22). The ant species diversity was greatest in Wild Turkey dust bowl areas located in the Black Gum swamp,

site C. The open dirt road (site A) contained the fewest different ant species and the lowest ant diversity. A two-sample t-test for differences between two Shannon diversities indices revealed significant differences in the ant species diversity between dust bowl and non-bowl locations for site A ($t(2) 0.05 = 0.482$, $v = 15.3$, $p = 0.05$) and site C ($t(2) 0.05 = 2.437$, $v = 105$, $p = 0.05$). There was no significant difference between the Shannon diversity indices for ant species identified in Wild Turkey dust bowl and non-dust bowl locations at site B ($t(2) 0.05 = -0.15$, $v = 91.5$, $p > 0.05$). Overall, other than a single species in site C dust bowl, there was no real difference among preferred and non-preferred ants identified between dust bowl and non-dust bowl locations (Table 22). The Wild Turkeys did not select dust-bathing sites based upon the presence of preferred ants.

The total number of ant collections was not significantly different between sites, calculated with a two-factor ANOVA (Table 23). The number of ant species collected did not significantly differ between bowl and non-bowl locations, calculated with a two-factor ANOVA (Table 24). However, where species were located by site A, B, and C, the ants differed significantly by the number of times they were found, as calculated with a two-factor ANOVA (Table 25).

Soil particle size. - Soil was sorted into five classes: 1.5% greater than 0.5 cm, 2.0% between 0.5 and 0.25 cm, 90.3% between 0.25 and 0.04 cm, 5.7% between 0.04 and 0.01 cm, and 0.4% less than 0.01 cm. A three by two factor ANOVA identified no significant differences across sites A, B, and C for particle size greater than 0.5 cm, 0.2 cm < 0.5 cm, and 0.01 cm < 0.04 cm. Soil particle

sizes were significantly different across sites for $0.04 \text{ cm} < 0.25 \text{ cm}$ and $< 0.01 \text{ cm}$. Soil particle sizes were significantly different between Wild Turkey dust bowl and non-dust bowl locations for class $0.04 \text{ cm} < 0.25 \text{ cm}$. The interactions of sites and locations were not significant (Table 26). A Scheffe F-test revealed a significant difference for soil particle size $0.04 \text{ cm} < 0.25 \text{ cm}$ between sites A and C, sites B and C, and site C with A and B, and for soil particle size $< 0.01 \text{ cm}$ in site A with sites B and C, site B with site A, and site C with site A (Table 27, Fig. 20).

Community vegetation type. - Community vegetation types identified along the cross-line transects varied between the Wild Turkey dust bowl and non-dust bowl locations in both sites A and B, but they were similar for site C (Table 28). Shannon diversity indices were calculated for the numbers of trees, shrubs and herbaceous plants identified in m^2 -sample plots for each Wild Turkey dust bowl and non-dust bowl location at sites A, B, and C (Table 29). A two-sample t-test for differences between two Shannon diversities indices revealed no significant differences in the vegetation sampled from m^2 -plots in bowl versus non-bowl locations for sites: A ($t(2)_{0.05} = -0.218$, $v = 18$, $p > 0.05$), B ($t(2)_{0.05} = -0.024$, $v = 15$, $p > 0.05$), and C ($t(2)_{0.05} = -0.084$, $v = 1223$, $p > 0.05$).

Percent canopy cover. - A 3 by 2 factor ANOVA identified a significant difference between the overstory canopy cover between sites as well as between Wild Turkey dust bowls and non-dust bowl locations (Table 30). A significant difference was observed in the overstory canopy cover among sites ($F=7.633$, df 2, 12, $P = 0.007$), and a significant difference existed in the overstory canopy

cover between Wild Turkey Bowl and non-bowl locations ($F=14.501$, df 1, 12, $P = 0.002$) (Fig. 21). There was no significant interaction between site and bowl versus non-bowl locations (Table 30).

DISCUSSION

Wild Turkeys in Zuni Pine barrens displayed active dust bathing during the year 2000, as evidenced by dust bowl formations. The number of ant species collected from Wild Turkey dust bowls was not significantly different among the three sites and between areas with and without Wild Turkey dust bowls. However, there was a significant difference among sites in ant abundance. The diversity of ants significantly differed between bowl and non-bowl locations for both sites A and C. Site C, the most structurally complex and with the greatest plant diversity, contained the highest numbers of ants. *Lasius umbratus* is the only species previously recorded in anting behavioral displays and unique to Wild Turkey dust bowl locations (Whitaker 1957). Of the three ants identified in locations with Wild Turkey dust bowl locations, two genera were previously recorded in anting behavioral displays, *Aphaenogaster* (from personal observations at Virginia Living Museum) and *Lasius* (McAtee 1944, Whitaker 1957, Simmons 1957). Ants in the *Aphaenogaster* genus were identified at both dust bowl and non-dust bowl locations. Three of the four ant species found only in non-dust bowl locations have been identified in anting behavioral displays. The genera *Monomorium* and *Formica* are common in anting behavioral observations (Stabler 1942, Groskin 1943, Ivor 1943, Brackbill 1948, Hebard

TABLE 21. Ant species identified at Blackwater Ecological Preserve study sites. Site A = Open dirt road, Site B = Woodland trail, Site C = Black Gum swamp vegetation, Bowl = locations with Wild Turkey dust bowls, Non-bowl = locations without Wild Turkey dust bowl locations.

Site A		Site B		Site C	
Bowl	Non-bowl	Bowl	Non-bowl	Bowl	Non-bowl
<i>Forelius pruinus</i>	<i>Monomorium viride</i>	<i>Pheidole bicarinata</i>	<i>Pheidole davis</i>	<i>Paratrechina parvula</i>	<i>Pheidole bicarinata</i>
<i>Pheidole dentata</i>	<i>Pheidole bicarinata</i>	<i>Pheidole dentata</i>	<i>Forelius pruinus</i>	<i>Pheidole dentata</i>	<i>Pheidole dentata</i>
<i>Monomorium viride</i>		<i>Pheidole davis</i>	<i>Aphaenogaster treatae</i>	<i>Pheidole davis</i>	<i>Aphaenogaster treatae</i>
<i>Pheidole bicarinata</i>		<i>Aphaenogaster treatae</i>	<i>Monomorium Minimum</i>	<i>Forelius pruinus</i>	<i>Solenopsis</i> spp.
<i>Pheidole davis</i>		<i>Solenopsis</i> spp.	<i>Solenopsis</i> spp.	<i>Aphaenogaster treatae</i>	<i>Leptothorax texanus davis</i>
		<i>Aphaenogaster lamellidens</i>	<i>Pheidole dentata</i>	<i>Leptothorax texanus davis</i>	<i>Paratrechina parvula</i>
		<i>Leptothorax pergandi</i>	<i>Formica pallidefulva</i>	<i>Solenopsis</i> spp.	<i>Formica nitidiventus</i>
			<i>Pheidole bicarinata</i>	<i>Lasius umbratus</i>	<i>Pheidole davis</i>
					<i>Neivamyrmex opacithorax</i>

TABLE 22. Shannon diversity indices comparing bowl versus non-bowl locations for ant species. Site A = Open dirt road, Site B = Woodland trail, Site C = Black Gum swamp vegetation.

Site Location	Wild Turkey Dust Bowl	Non-Dust Bowl
A	0.537	0.177
B	0.724	0.727
C	0.767	0.6

TABLE 23. Number of ant species collected by site for Site A = Open dirt road, Site B = Woodland trail, Site C = Black Gum swamp vegetation.

Source	Df	Sum of Squares	Mean Square	F	P
Site (A)	2	3.4	1.733	0.019	0.9808
Bowl (B)	1	98.174	98.178	1.099	0.2975
AB	2	16.089	8.044	0.09	0.914
Error	84	7504.267	89.337		

TABLE 24. Number of ant species collected by location, Wild Turkey dust bowl versus non-dust bowls.

Source	Df	Sum of Squares	Mean Square	F	P
Bowl (A)	1	98.178	98.178	1.271	0.2641
Ants (B)	14	2049.667	146.405	1.895	0.451
AB	14	839.489	59.963	0.776	0.6897
Error	60	4634.667	77.244		

TABLE 25. Abundance of ant species collections by site for Site A = Open dirt road, Site B = Woodland trail, Site C = Black Gum swamp vegetation

Source	Df	Sum of Squares	Mean Square	F	P
Site (A)	2	3.467	1.733	0.028	0.9724
Ants (B)	14	2049.667	146.405	2.364	0.0148
AB	28	2781.887	99.352	1.604	0.0771
Error	28	2787	61.933		

TABLE 26. Relationships between soil particle size classes, site, and locations. Sites are A = dirt road, B = woodland trail, and C = Black Gum swamp, Locations are Wild Turkey dust bowl areas and non-dust bowl areas, Soil particle size classes are Soil A = > 0.5 cm, Soil B = 0.2 cm < 0.5 cm, Soil C = 0.04 cm < 0.25 cm, Soil D = 0.01 cm < 0.04 cm, and Soil E = < 0.01 cm.

Source	Dep. Variable	Sum of Square	df	F	P
Site	Soil A	28.721	2	0.411	0.667
	Soil B	589.822	2	0.945	0.403
	Soil C	544056.963	2	13.532	0.000
	Soil D	5312.401	2	3.008	0.068
	Soil E	55.934	2	13.489	0.000
Location	Soil A	43.923	1	1.258	0.273
	Soil B	92.225	1	1.940	0.176
	Soil C	16902.880	1	10.383	0.004
	Soil D	37.632	1	0.725	0.403
	Soil E	5.125	1	2.472	0.129
Site by Location	Soil A	209.646	2	3.002	0.069
	Soil B	326.701	2	3.436	0.049
	Soil C	10045.579	2	3.085	0.064
	Soil D	15.998	2	0.154	0.858
	Soil E	8.953	2	2.159	0.137
Error	Soil A	838.004	24		
	Soil B	1140.944	24		
	Soil C	39070.092	24		
	Soil D	1246.188	24		
	Soil E	49.760	24		
Total	Soil A	1863.310	30		
	Soil B	3061.480	30		
	Soil C	3025842.09	30		
	Soil D	12110.4	30		
	Soil E	183.720	30		

TABLE 27. Scheffe F-test of significant relationships between soil particle size classes and sites. Sites are A = dirt road, B = woodland trail, and C = Black Gum swamp, Soil particle size classes are Soil A = > 0.5 cm, Soil B = 0.2 cm < 0.5 cm, Soil C = 0.04 cm < 0.25 cm, Soil D = 0.01 cm < 0.04 cm, and Soil E = < 0.01 cm.

Soil class	Site locations		P
Soil C	A	C	0.003
	B	C	0.000
	C	B	0.003
Soil E	A	B	0.003
		C	0.003

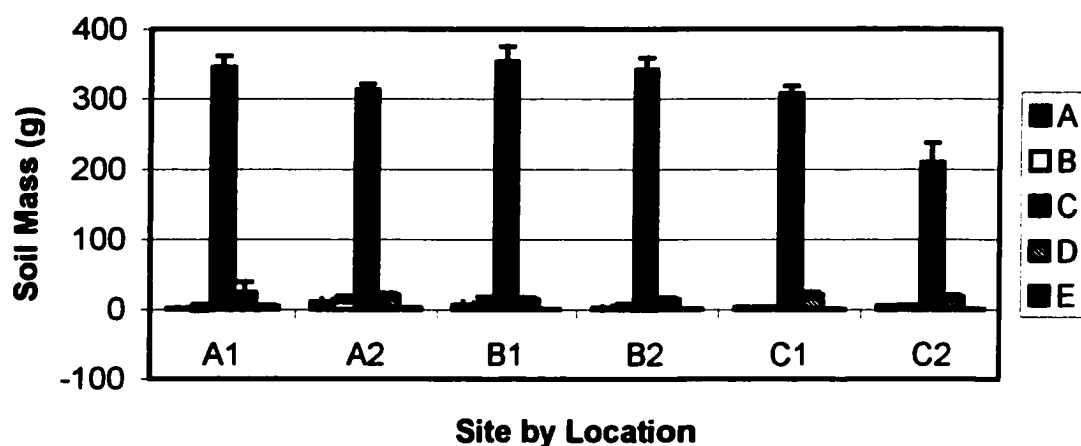


Fig. 20. Mean +/- S.E. for soil class weight in grams by site and locations. Sites are A = dirt road, B = woodland trail, and C = Black Gum swamp, Locations are Wild Turkey (1) dust bowl areas and (2) non-dust bowl areas, Soil particle size classes are Soil A = > 0.5 cm, Soil B = 0.2 cm < 0.5 cm, Soil C = 0.04 cm < 0.25 cm, Soil D = 0.01 cm < 0.04 cm, and Soil E = < 0.01 cm.

TABLE 28. Plant species present along entire sample transect. Site A = open dirt road, Site B = woodland trail, Site C = Black Gum swamp vegetation, bowl = samples from locations with Wild Turkey dust bowls, non-bowl = sample from locations without Wild Turkey dust bowls.

Site	Species	
A Bowl	Herbaceous	Shrub
	<i>Chrysopsis graminifolia</i> , <i>Juncus tenium</i> , <i>Paspalum setaceum</i> , <i>Eragrostis spectabilis</i> , <i>Andropogon ernarium</i>	<i>Hypericum gentianoides</i>
A Non-Bowl	Herbaceous	
	<i>Andropogon ternaries</i> , <i>Eragrostis spectabilis</i>	
B Bowl	Herbaceous	
	<i>Pteridium aquilinum</i>	
B Non-Bowl	Herbaceous	Shrub
	<i>Cnidocolus stimulosus</i>	<i>Gaylussacia frondosa</i>
C Bowl	Herbaceous	Shrub
	<i>Pteridium aquilinum</i> , Juncacea spp.	<i>Myrica cerifera</i> , <i>Vaccinium corymbosum</i>
C Non-Bowl	Herbaceous	Shrub
	<i>Pteridium aquilinum</i> , <i>Pyxidanthera barbulata</i>	<i>Antennaria plantaginifolia</i> , <i>Gaylussacia dumosa</i> , <i>Vaccinium ellioti</i> , <i>Lyonia mariana</i>

TABLE 29. Shannon diversity indices of plant species in Wild Turkey dust bowls versus non-dust bowl locations. A = Site A open dirt road, B = Site B woodland trail, C = Site C black gum swamp vegetation.

Site Location	Wild Turkey Dust Bowl	Non-Dust Bowl
A	0	0.319
B	0.132	0.195
C	-0.193	1.435

TABLE 30. Analysis of variance between sites, Wild Turkey dust bowl versus non-dust bowl locations, and possible interactions for canopy cover. Bowl = locations with Wild Turkey dust bowls and Non-bowl = locations without wild Turkey dust bowls.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Site	0.364	2	0.182	7.633	0.007
Bowl v. Non-bowl	0.364	1	0.346	14.501	0.002
Interaction	0.064	2	0.032	0.334	0.300
Error	0.286	12	0.024		

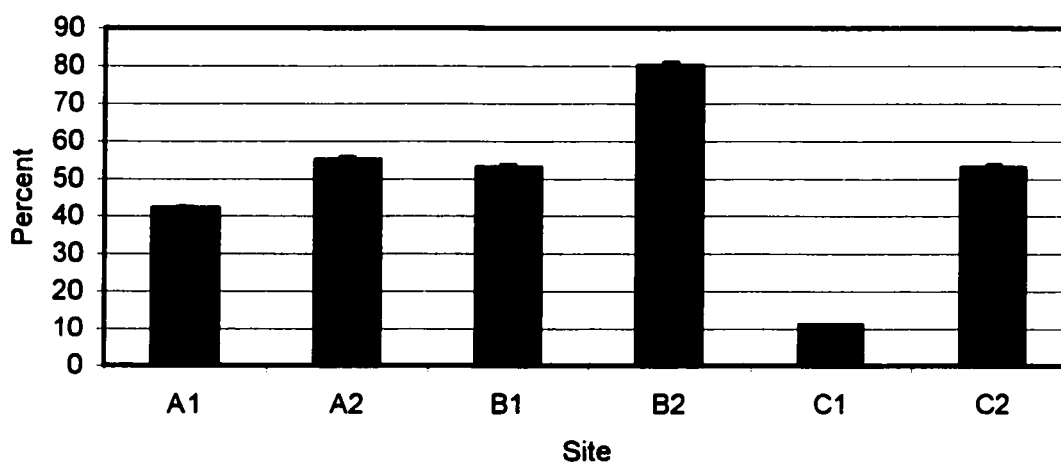


Fig. 21. Mean +/- S.E. percent canopy cover. Site A = Open dirt road, Site B = Woodland trail, Site C = Black Gum swamp vegetation, 1 = Samples from locations with Wild Turkey dust bowls, 2 = Sample from locations without Wild Turkey dust bowls.

1949, Nero 1951, Goodwin 1953, McAtee 1954, Poulsen 1956, Kelso and Nice 1963, Potter 1970). These data suggest that Wild Turkeys are not selecting areas for dusting behavior based on the presence of ant species known to elicit anting behavior.

Locations with Wild Turkey dust bowls were not significantly different in ant numbers compared to non-dust bowl sites. The National Research Council (1977) states that Turkey poults require 28 % dietary protein, achieved by eating arthropods. My results did not suggest Wild Turkeys select dust bathing locations by the general presence of ants for their consumption.

Two soil particle size classes significantly differed across sites A, B, and C: class $0.04 \text{ cm} < 0.25 \text{ cm}$ and class $< 0.01 \text{ cm}$, and soil particle sizes were significantly different between Wild Turkey dust bowl and non-dust bowl locations for the class $0.04 \text{ cm} < 0.25 \text{ cm}$. The difference between site and location of class $0.04 \text{ cm} < 0.25 \text{ cm}$ suggests that this class does not affect Wild Turkey dust bowl site selections. There was no significant difference between site and location for soil particle sizes. Soil particle size did not seem to affect the selection of dust bathing locations.

Nor did plant species or plant diversity differ between Wild Turkey dust bowls and non-dust bowl locations. However, sites selected for dusting behavior did have significantly less overstory canopy cover (Table 31, Fig. 21). The selection of open areas for dusting behavior is common in many gallinaceous birds including Ruffed Grouse (*Bonasa umbellus*), Northern Bobwhite (*Colinus virginianus*), Scaled Quail (*Callipepla squamata*), Ring-necked Pheasant

(*Phasianus colchicus*), Chukar Partridge (*Alectoris chukar*), and Japanese Quail (*Coturnix japonica*) (Healy and Thomas 1973). The difference in overstory canopy cover was the greatest at site C where the forest shrub/scrub understory was the thickest. Sites A, B, and C contained 14 %, 24 %, and 42 % greater open canopy space over the dust bowl location than over the non-dust bowl locations, respectively. Both sites A and B had low numbers of trees and shrubs, probably as a result of vehicular use and foot traffic on the road and path. Wild Turkeys select open areas with little ground cover for dust bathing activities (Mosby and Handley 1943).

CHAPTER V

CONCLUSIONS

The potential association among ants, feather ectoparasites and birds make anting behavior an important community-level interaction. Although this behavior is cosmopolitan in distribution and occurs in a wide variety of passerine birds, it remains poorly understood (McAtee 1938, Whitaker 1957, Simmons 1957 and 1966, Ivor 1956, Potter 1970, Judson and Bennett 1992, Clayton and Wolf 1993). Anting behavior requires investigation at multiple scales spanning from the molecular chemistry of ants to parasitism and predation. To identify the associations intrinsic to anting behavior, several factors must be considered. These include 1) possible selection of ant types during active anting behavior, 2) effects of abiotic conditions on anting behavioral displays, 3) possible bactericidal and fungicidal properties of ant secretions, and 4) correlations between anting behavior and alternative feather care such as dust bathing.

The selection of ant types identified in these experimental trials for active anting behavioral displays included both Myrmicinae and Formicinae subfamilies. Anting behavior was not restricted to ants that produce formic acid, nor was the behavior always associated with the consumption of ants. However, there was a significantly higher rate of anting behavioral displays using formicine ants. This selection preference may relate to the degree of movement and physical activity

of formicine ants during the trials. Two formicine species were active whereas only one myrmicine ant species was active during the ant genera choice trials.

Ant species eliciting significantly more of songbird interactions were active in containment trays. Anting behavior appeared to be initiated in part based upon visual cues like movement of the ants. Because active production of ant chemicals was not necessary to elicit anting behavior, as demonstrated by the anting episodes with frozen ants, ant chemistry seems to play a minor role in ant species selection for anting behavior.

Previous studies have indicated that anting behavior may relate to the removal of ant chemicals before consumption (Judson and Bennett 1992). The frequency of ant consumption and lack of food preparation through anting displays during my study did not support the hypothesis that anting behavior might act as a food preparation method. Songbirds displayed anting behavior irrespective of adult diets and their common foraging modes. The occurrence of anting behavior and ant consumption by songbirds varied among ant types, just as the defensive mechanisms of different ant types varied. Songbirds avoided aggressive ants with stinging and biting defensive mechanisms, and the preferred formicine ants which have a vestigial sting.

Sky conditions also affected the relative frequencies of ant consumption versus anting behavior. More ants were consumed without first having been rubbed on the bird's feathers during cloudy or rainy days. The importance of sunny sky conditions for the display of anting behavior suggests a possible relationship between sunning and anting behavior. The type of ants most often

selected for anting behavior supports this hypothesis. Formicine ants produce caustic chemicals, such as formic acid, proven *in vitro* to inhibit some ectoparasites (Judson and Bennett 1992). Sunning behavior has been shown to reduce ectoparasitic loads (Moyer and Wagenbach 1995). It is likely that sunning and anting behavior are combined to reduce feather ectoparasites. Further research is needed to relate sunning and anting behaviors to their possible effects on ectoparasites.

Temperature and humidity were not related to anting behavior. Neither affected the display of anting behavior by captive songbirds in the Virginia Living Museum. Anting behavior in wild songbirds is undoubtedly limited by ant availability. The lack of ants during cold and wet conditions accounts for seasonal variations previously recorded in the literature.

Ant chemical extracts had no effect upon microbial feather ectoparasites tested in my experiments. Neither hyphal strands, bacterial colonies, nor spore germination were inhibited by chemical extracts from ants known to elicit anting behavior in songbirds. In contrast, 85% sequencing-grade formic acid inhibited all microbial growth. My study did not support the possible self-medication of songbirds through anting behavior for *Bacillus subtilis*, *B. licheniformis* strains 138B and 1432B, *Penicillium chrysogenum*, *Chaetomium globosum*, and *Trichoderma viride*.

The selection of dust bathing locations was not related to ant species commonly preferred in anting behavioral displays. Only one species and two genera recorded in anting behavioral display observations were present in

locations selected by wild turkeys for dust-bathing behaviors. However, the presence of these ant types was not consistent among the dust bowl sites. Additionally, three of the four ant species found only in non-dust bowl locations were used in anting behavioral displays in the laboratory. The diversity of ants significantly differed between two study bowl versus non-bowl locations. The availability of open space and low canopy cover influenced selection of dust bathing areas.

Four broad hypotheses established from the literature were addressed in this work. The selection of ant types was experimentally evaluated and the results lead to the conclusion that songbirds are highly selective among ants for anting displays. Environmental such as temperature and humidity did not significantly affect the prevalence of anting behavior, although these factors likely affect the availability of ants in temperate climates. A significant relationship did exist between cloud cover and anting behavioral displays.

Both non-polar and polar ant chemicals and formic acid were examined for their bactericidal and fungicidal properties as well as inhibition of fungal spore germination. Common feather microbes and fungal spore germination were not inhibited by ant chemicals. Nor was there a relationship between preferred and non-preferred ants selected for anting displays the effect of ant chemicals on feather microbes. Formic acid was a strong inhibitory agent for all feather microbes tested and significantly inhibited spore germination for one fungal spore type.

Finally, the potential relationships between dust bathing site selection and the presence of preferred ant species were examined. No relationship existed between these two factors. Site selection for Wild Turkey dust bathing was significantly related to the availability of open canopy cover.

These experiments were designed to help answer the question: "Why do birds apply ants to their feathers?" As one of the few quantitative studies published on anting behavior, this work represented the only controlled experimental examination of ant selection during anting behavior. Clear preferences were identified in captive songbirds for select ant species. Ant activity was identified as an important factor in ant selection. Additionally, abiotic factors such as cloud cover significantly affected the frequency of anting displays. There is likely no single answer to the question of: "Why do birds ant?" My results have identified complex interactions between multiple factors involved in anting behavior.

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Revis, H. and D. Waller, 2001. Abstract: Bactericidal effects of formic acid and ant extracts on *Bacilius licheniformis*. Proceedings of the Tidewater Virginia Chapter Sigma Xi 46th Annual Student Research Poster Session 4:18. Awarded Honorable Mention for graduate student presentation.

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