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Olfactory Mate Choice and Potential Chemical Signals of the White-Throated Sparrow (Zonotrichia albicollis)

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Olfactory Mate Choice and Potential Chemical Signals of the White-Throated Sparrow (*Zonotrichia albicollis*)

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ABSTRACT

Chemical odor signals are well documented in mammals, and yet almost nothing is known about the use of chemical odor signals in birds due to the traditional view that birds have a no sense or a poor sense of smell. Recent studies have revealed the traditional view to be unfounded, but more work is necessary to 1) expand our knowledge of avian olfaction in passerine species and 2) determine whether birds utilize chemical signals. The aim of this thesis was to 1) test for olfactory-based choice in a passerine species, and examine the chemical composition of preen oil for potential chemical signals. Results suggest that the polymorphic white-throated sparrow does choose between odors from their own bedding and odors from fresh bedding based on their unique disassortative mating, with tan males and white females choosing fresh bedding over their own and white males and tan females choosing their own bedding over fresh bedding. Additionally, a study on captive white-throated sparrows found that multiple preen oil volatile compounds were seasonally elevated during the breeding season, and thus indicate the possibility of these compounds acting as chemical signals. In wild populations, preen oil composition varied by morph-sex classes as well as by year sampled, and some compounds may even change throughout the course of the breeding season. Comparisons between wild populations and captive birds indicate that captive conditions may also alter preen oil composition.

PREFACE

This thesis represents the culmination of over 2 years of study and research at Indiana State University. While at Indiana State University I have had the pleasure of learning about a remarkable passerine species, the white-throated sparrow. In addition, I am grateful for the chance to have learned new skills and techniques both in the lab and field, especially in a field site as beautiful as the Adirondacks of New York. However, one of the most exciting aspects of my time at Indiana State University was exploring the understudied research on avian olfaction and chemical signals. I hope that the reader will find the research presented in this thesis will both be insightful for researchers in the field of avian olfaction as well as inspire more scientists to consider the possible research directions in a field that has vast possibilities.

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CHAPTER 1

INTRODUCTION TO OLFACTORY MATE CHOICE AND POTENTIAL CHEMICAL SIGNALS OF THE WHITE-THROATED SPARROW (*ZONOTRICHIA ALBICOLLIS*)

More animal interactions are mediated by chemical signals than any other type of signal (Wyatt 2003). Animals detect chemical signals using the chemosenses, which are olfaction, taste, and the trigeminal system (Hagelin and Jones 2007), but since olfaction typically involves the perception of chemical cues at a distance (Roper 1999) it is arguably the most important sense for detecting chemical signals. Chemical odor signals are used by mammals (Yamazaki et al. 1976; and see Wyatt 2003), and may even be important to human mate choice (Wedekind et al. 1995). However, our understanding of avian chemical signaling has been hampered by the historical perspective that birds, unlike mammals, have a poor sense of smell, and not until the last few decades have researchers fully realized the potential importance of avian olfaction to chemical signaling (Balthazart and Schoffeniels 1979; Roper 1999; Hagelin 2007; Hagelin and Jones 2007).

Previous evidence for avian olfaction has already strongly refuted the historical perspective that all birds have a poor sense of smell. This evidence includes many studies highlighting the use of olfaction for foraging (Wenzel 1968; Graves 1992; Nevitt et al. 1995; Nevitt 2000; Hagelin 2004; Nevitt et al. 2008) and navigation and orientation (Grubb 1974; Papi

1976; Bonadonna and Bretagnolle 2002; Bonadonna et al. 2003; Wallraff 2004; Nevitt and Bonadonna 2005). Olfaction may even be important to some avian species for nest construction (Petit et al. 2002) and detecting predation risk (Amo et al. 2008). Additionally every bird studied so far has had a functional olfactory system (Bang and Wenzel 1985; Roper 1999; Hagelin 2007) and olfactory receptor genes including a set of receptor genes that is believed to be present in the entire avian class (Steiger et al. 2008). But unlike mammals, it is still not clear as to whether most birds also create and transmit chemical cues that can act as social signals detectable by olfaction (Hagelin 2007; Hagelin and Jones 2007). Specifically, it remains unclear whether birds utilize "pheromones" (Hagelin et al. 2003; Hagelin and Jones 2007), which originally was only applied to chemical cues that were capable of eliciting stereotyped responses in minute quantities (Karlson and Luscher 1959) much like a visual sign stimulus (Johnston 2000).

Some of the best supporting evidence that birds engage in chemical-based communication has come in recent years. Antarctic prions (*Pachiptila desolata*) show a preference for the odor of their partner over that of other conspecifics but prefer conspecific odor to their own (Bonadonna and Nevitt 2004). Meanwhile, the crested auklet (*Aethia cristatella*), which has a tangerine-like plumage odor, prefers conspecific odor to that of a control, even when the odor is simplified to specific feather compounds elevated during the breeding season (Hagelin et al. 2003). An even more recent study has shown a link between olfaction and mate choice in chickens (Hirao et al. 2009). While these studies show intriguing evidence that some birds utilize self-produced chemical cues, similar evidence is scarce in the largest of avian orders: Passeriformes. Previous studies have shown the ability of passerines to detect chemical cues from the environment (Petit et al. 2002; Roth et al. 2008) and recent work by Whittaker et al. (2009, 2010) suggests that preen oil compounds of the dark-eyed juncos (*Junco hyemalis*)

may be used for intraspecific communication. More studies are necessary to determine the identity of conspecific chemical cues across the avian class, and whether these cues act as signals with ecologically and evolutionary relevant roles. Future discoveries on avian social odor usage could drastically alter our understanding of avian communication and behavioral ecology (Hagelin 2007).

The aim of this thesis is to contribute to the current knowledge of avian chemosignals by investigating the polymorphic white-throated sparrow (*Zonotrichia albicollis*). The white-throated sparrow is an especially unique model because it is a polymorphic passerine, with tan and white morphs (Figure 1.1) found in both sexes that mate disassortatively (Lowther 1961; Thorneycroft 1975; Tuttle 1993, 2003) such that white males mate with tan females and tan males mate with white females. The morphs are also correlated to a rearrangement of the second chromosome (Thorneycroft 1966, 1975; Thomas et al. 2008; Romanov et al. 2009) and to alternative reproductive strategies (Tuttle 2003). The genetic differences between the morphs allow for potential genetic variation in both chemical production and preference while the alternative reproductive strategies highlight behavioral explanations for potential chemical signal choice. The unique polymorphism allows for an excellent study model without the need for experimental manipulations. Additionally, studying the morphological differences of the white-throated sparrow can be even more useful than studying differences between closely related species, or even subspecies because they are evolutionarily more related.

In chapter 2, I conducted two separate tests on white-throated sparrows' ability to differentiate and choose one bedding odor over another. In the first test I determined whether white-throated sparrows of both sexes can differentiate between self and non-self odors using

bedding containing the individuals own fecal material as the self odor and fresh unused bedding as the non-self control odor. In the second test, I determined if the same individuals can differentiate and choose between tan and white morph bedding odors of the opposite sex. I conducted these tests utilizing a Y-maze design (Yamazaki 1976; Bonadonna and Nevitt 2004) adapted for indoor trials with Passeriformes. Results gave no indication of morph odor preference, but tan male and white females combined appeared to avoid self odors while white male and tan females combined appeared to prefer the self odor to that of the control.

In chapter 3, I investigated the seasonal shift in preen oil volatile compound composition of captive white-throated sparrows. A seasonal shift in non-volatile compounds of the preen oil in multiple species has been shown (Bohnet et al. 1991, Reneerkens et al. 2002), but Soini et al. (2007) found a seasonal shift in volatile compounds of the dark-eyed juncos (*Junco hyemalis*) between breeding and wintering seasons. An increase, addition, or change to the preen oil composition during the breeding season suggests that preen oil has additional uses during the breeding season. Whittaker et al. (2010) suggests that preen oil during the breeding season may act as a chemical signal that contains information about individual identity, sex, and population. Determining volatile compounds that are seasonally elevated or present can be the first step in nominating potential chemical signals. Additional studies on seasonal shifts in preen oil composition are necessary because each species tested so far has had a unique preen oil composition (Haribal et al. 2005, 2009) and thus each species may have unique specific chemical signals. The results showed that not only do white-throated sparrows also have a unique preen oil chemical composition, but also that specific compounds are seasonally elevated and are candidate compounds for chemical signaling. Additionally, morph-sex classes and pair-types of

the white-throated sparrow differed in the levels of specific wintering condition compounds, which are potentially connected to their genetic behavioral, and environmental differences.

In chapter 4, I continued to investigate the importance of sex and morph on the composition of white-throated sparrow preen oil by analyzing wild-caught preen oil samples from the breeding seasons of 2009 and 2010. The wild-caught data showed that 1) morph-sex class is an important factor in the relative proportion of certain volatile compounds which could be related to the alternative reproductive strategies (Tuttle 2003) of the different morphs, and 2) that individuals within each morph-sex class also vary amongst themselves in volatile compound concentrations, suggesting that each individual may have a unique chemical signature, which can be used for individual recognition and mate assessment (Johansson and Jones 2007, Whittaker et al. 2010). Additionally, year and month of the breeding season were important in determining the preen oil chemical composition. This suggests that individuals might alter their preen oil composition based on environmental factors or an individual's reproductive stage.

Since chapters 3 and 4 both investigate white-throated sparrows in breeding condition, but in captive and wild environments respectively. I examined the differences between wild and captive birds in terms of preen oil chemical composition and found that birds in a high-density captive environment significantly differ in relative proportions of major preen oil compounds compared to wild populations. A new understanding of the effects of captivity on preen oil production should provide a needed caution for represents the first time that preen oil volatile compounds have been examined between wild and captive breeding condition individuals.

Finally, chapter 6 discusses how the results of chapters 2-5 relate to our previous knowledge of the different morphs of the white-throated sparrows' life histories. Specifically, I

will discuss how variation between the morph-sex classes and pair-types discovered in this thesis fit with our current knowledge of white-throated sparrows' behavior, genetics, and environment. Additionally, chapter 6 suggests realistic future studies that would help further our understanding of the white-throated sparrows' olfactory ability and the importance of preen oil compounds.

Together, the chapters of this thesis provide new insights into avian olfactory research. At the conclusion of this thesis I will have provided evidence that the white-throated sparrow (*Zonotrichia albicollis*) has a functioning sense of olfaction and discovered that pair-types (tan males x white females versus white males x tan females) may differ on their olfactory preferences. Additionally, I will have examined the volatile compounds of the white-throated sparrow's preen oil and determined that a variety of factors can affect preen oil composition. Specifically, I will have found evidence suggesting that white-throated sparrow preen oil can vary between winter and breeding seasons (and even the time during the season), years, individuals, morph and sex classes, and pair-types. In the process, I will have discussed the possible roles of preen oil in a passerine species including two non-opposing hypotheses; that preen oil might be important for nest crypsis and might be important for chemical communication. Above all, I hope to have instilled to the reader a greater understanding of avian olfaction and the importance of future research in this field. Chemical signals mediate more animal interactions than any other type of signal (Wyatt 2003), and future studies should consider why birds have traditionally not been included. Ultimately, this thesis does not prove the use of avian olfaction for chemical communication, but does provide a basis for further studies to do just that on a truly unique and useful avian model, the white-throated sparrow.

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Figure 1.1. Images depicting the visual difference between a white and tan-striped morph white-throated sparrow (*Zonotrichia albicollis*).
A) A typical white-striped male with characteristic bright white crown stripes, black lateral crown stripes, clean white throat, and bright yellow supercilliaries.
B) A typical tan-striped male with tan crown stripes, mixed brown and black lateral crown stripes, off-white/tan throat, and dark yellow or mustard supercilliaries.

A)

B)

CHAPTER 2

A Y-MAZE CHOICE TEST FOR ODOR PREFERENCE IN THE WHITE-THROATED SPARROW (*ZONOTRICHIA ALBICOLLIS*)

Abstract

Recent research continues to expand the importance of avian olfaction, despite a long-standing historical view that birds have a poor sense of smell. With the expansion of olfaction-centered avian research, an important question is whether olfaction is used to help mediate mate choice in birds. For this to be possible, individual birds must transmit a chemical signal to help attract potential mates. Bedding from captive birds contain a variety of body odors, which may include proteins known to be involved in mammalian chemical signaling such as major urinary proteins and major histocompatibility complex proteins. The aim of this study is to determine whether a representative passerine, the white-throated sparrow (*Zonotrichia albicollis*), can 1) differentiate between their own bedding odors and a control odor of fresh bedding and 2) differentiate and prefer a particular morph's bedding odor of the opposite sex based on their own morph. The white-throated sparrow was chosen because of its unique polymorphism combined with disassortative mating based on morph, allowing for the possibility of odor choice by morph. I hypothesized that white-throated sparrows can detect their own odor and differentiate between odors of either morph. In order to test the possibility of avian mate choice based on olfaction, I used a Y-maze apparatus to test whether an individual bird can chose between the odor of the

bedding used in its own cage versus that of fresh unused bedding with no intraspecific chemical cues. I then tested if individual birds of both sexes can distinguish between the bedding odor of a white and tan morph of the opposite sex. While there was no indication that either sex preferred one morph over the other, the results suggested that the two pair types differed in their response to the self-odor versus control test with tan males and white females combined choosing the control arm over the self-odor arm (N=5) and white males and tan females combined choosing the self-odor arm over the control (N=7). The sample size was considerably small but a power analysis showed that a sample size of 19 (or 10 individuals of each pair type) would be sufficient for a sample size to accept the outcome of this study. Future trials will increase the sample size to give sufficient power to the results reported here. The results might suggest that tan male and white female pairs have a greater evolutionary need to reduce inbreeding avoidance while white male and tan female pairs are drawn to their own scent due to the higher density of neighbors in their environment.

Key Words- White-Throated Sparrow, Avian Olfaction, Y-maze, Mate Choice, Chemical Cues, Disassortative Mating

Introduction

Social odor signals are well documented in mammals, even in humans (Yamazaki et al. 1976; Wedekind et al. 1995; Wyatt 2003). However, only recently have researchers realized that birds may also utilize olfaction to receive social information (Roper 1999; Hagelin 2007a; Hagelin and Jones 2007). Steiger et al.'s (2008) study of olfactory receptor genes gives plausible evidence that all birds, even poorly studied species, share a class of olfactory receptors, and thus are likely to have a functioning olfactory system. Steiger et al.'s (2008) study is supported by

evidence of birds using olfaction for a variety of purposes pertaining to gaining evidence about their environment: including foraging (e.g. Wenzel 1968; Graves 1992; Nevitt 2000; Hagelin 2004; Nevitt et al. 2008), navigation and orientation (e.g. Grubb 1974; Papi 1976; Bonadonna et al. 2003; Wallraff 2004), nest construction (e.g. Petit et al. 2002), and risk of predation (e.g. Amo et al. 2008, Roth II et al. 2008). However, there is largely a void of information on avian body odor and its putative role as a social chemical signal. More studies are needed that illustrate that birds can transmit odor cues to conspecifics, can differentiate odor cues to and from both other conspecifics and their selves, and that these odor cues play an ecologically and evolutionary relevant role, such as honest signals of fitness.

One of the biggest breakthroughs in avian chemical communication was evidence that Antarctic prions (*Pachiptila desolata*) prefer the body odor of their monogamous partner to that of other conspecifics in Y-maze trials (Bonadonna and Nevitt 2004). Additionally, Antarctic prions also were more likely to choose the odor of unrelated conspecifics than their own odor, suggestive of inbreeding avoidance (See Mateo and Johnston 2000). Work on the crested auklet (*Aethia cristatella*), according to Balthazart and Taziaux's review (2009), is the best evidence that olfaction may play an important role in avian reproduction (See Hagelin 2007b for review). The crested auklet has a noticeable tangerine-like scent and performs a distinctive behavior named the "ruffsniff" display in which individuals will stick their beaks in the neck feathers of other conspecifics (Hagelin et al. 2003), which is a likely mechanism through which they may obtain chemosensory information from conspecifics (Hagelin et al. 2003; Hagelin 2007a, 2007b). Hagelin et al. (2003) ran a series of T-maze experiments and found that crested auklets preferred the odor of conspecifics to the odor of a control, and even showed a preference to a synthetic mix of compounds found in feather scent. The compounds, z-4-decanal and octanal, are either only

present in breeding condition (z-4-decanal) or highly elevated in concentration during the breeding season (octanal) and both have a strong citrus-like scent (Hagelin et al. 2003).

Hirao et al. (2009) provides recent support for the hypothesis that odor is an important discriminating factor in mate choice. The authors report that male chickens mounted and copulated significantly more with females with intact preen oil glands (sham-operated females) than females with preen glands removed. They also reported that males that lacked olfactory bulbs were equally likely to mount females with or without preen glands. The combined data suggest that the odor from the preen gland, or preen oil, of female chickens is an important stimulus for male mating behavior.

Another important aspect from Hirao et al. (2009) is their implications that preen oil is a source of avian chemosignals, but sources of potential chemosignals include feather compounds and fecal material among others (Hagelin 2007a; Hagelin and Jones 2007). In mammals, urine contains Major Histocompatibility Complex (Mhc) proteins, which not only have an essential function for immune response, but also have been closely linked to both olfaction (Yamazaki et al. 1976, 1979) and mate choice (See Penn 2002 for review). Additionally, mammalian urine contains Major Urinary Proteins (MUPs) that may also transmit individual information via odor (Hurst et al. 2001; Thom et al. 2008). MUPs bind and release volatile compounds (Hurst et al. 2001) and female mice have been shown to prefer MUP heterozygous mates (Thom et al. 2008).

In birds, MUPs are not yet linked to reproduction in birds but the Mhc region has been linked to mate choice (Bonneaud et al. 2006) and extra-pair copulation choice (Richardson et al. 2005). One proposed mechanism for how birds can assess the Mhc compatibility of potential mates is through olfaction, although there are other mechanisms such as improved condition due

to greater immune response (Zelano and Edwards 2002). Bedding samples from captive birds provide an opportunity to gather the overall 'scent' of a bird because they include odorous compounds that rub off from the bird itself, such as preen gland secretions, along with fecal material containing uric acid, and thus may be the avian equivalent to Mhc and MUP-rich mammalian urine.

This study aimed to determine if the polymorphic white-throated sparrow (*Zonotrichia albicollis*) can 1) detect the difference between self and non-self odors and 2) differentiate and choose between potential mates of differing morphs. White-throated sparrows are polymorphic for tan and white morphs and display disassortative mating in which tan males mate with white females and white males mate with tan females (Thorneycroft 1975; Tuttle 1993, 2003). In lab trials, females of both morphs tended to prefer tan males, while males of both morphs tend to prefer white females (Tuttle 1993; Houtman and Falls 1994). Prior lab testing on visual choice preferences gives additional background in preparation for olfactory choice and thus makes the white-throated sparrow a prime study species.

I hypothesized that white-throated sparrows would avoid their own odor in order to minimize the risk of inbreeding. Further, I hypothesized that they would differentiate the morph of potential mates by odor cues in order to determine the suitability of a mate. I tested these hypotheses by utilizing a Y-maze design (Yamazaki 1976; Bonadonna and Nevitt 2004) with different bedding odor stimuli in each arm. Each subject was given a choice between the odor of their own bedding and the odor of fresh, unused, control bedding. Each subject was also given a choice between the bedding odors of white and tan morph individuals of the opposite sex.

Passeriformes is the largest avian order and avian research often incorporates passerine models; yet this is the first published olfactory-mediated choice test with a passerine model.

Methods

Study Species and Housing

The white-throated sparrow (*Zonotrichia albicollis*) is a socially monogamous passerine that breeds mainly in Canada but also the Northeastern United States (Falls and Kopachena 1994). It is an ideal species to test for the role of social chemosignals in mate choice because it is polymorphic, with a white and tan morph found in both sexes (Lowther 1961), and because they mate disassortatively such that white males mate with tan females and tan males mate with white females (Lowther 1961; Thorneycroft 1975). In lab trials based on visual choice, females of both morphs preferred tan males and males of both morphs prefer white females, suggesting that part of the disassortative-mating might be due to white females outcompeting tan females (Tuttle 1993; Houtman and Falls 1994).

Phenotypic differences between the white and tan morphs are absolutely correlated to a chromosomal rearrangement (Thorneycroft 1966; Thomas et al. 2008; Romanov et al. 2009) of the second chromosome (Thorneycroft 1975), which opens the possibility of a genetic basis for any odor differences that might be detectable. The genetic difference between morphs also correlates to behavioral differences (Falls 1988; Lowther and Falls 1968) and alternative reproductive strategies (Tuttle 2003) in which white males sing more than tan males (Lowther and Falls 1968; Falls 1988; Tuttle 1993) and engage in more extra-pair copulations (Tuttle 2003), while tan males guard their female more (Tuttle 2003) and invest more in parental care (Knapton and Falls 1983; Kopachena and Falls 1993a; Tuttle 2003). White males have higher testosterone levels than tan males early in the breeding season (Spinney et al. 2006) and during

nestling and fledgling care stages (Swett and Breuner 2009). Additionally, white males also utilize different habitat types from tan males, preferring habitats with a higher density of neighbors (Formica et al. 2004, Formica and Tuttle 2009). These differences between morphs along with the disassortative mating pattern highlight the motivation for specific morph mate choice.

Four white males, 3 tan males, 2 white females, and 7 tan females for the study were caught using passive mist netting (master banding permit 22296 to E. M. Tuttle at Indiana State University, Terre Haute, IN) between the months of October and December 2008 and stored in the Indiana State University Aviary facility. Uneven numbers of each morph and sex were due to uneven netting success. Birds were fed an *ad libitum* diet of water, sunflower hearts, white millet, thistle, Benebac brand probiotics, as well as biweekly mealworms. Each bird was individually housed in metal cages approximately 0.46 meters cubed. The aviary was kept on a natural winter photoperiod until January 2009 and then slowly brought to a photoperiod of 16 hours light, 8 hours dark in order bring the birds into breeding condition. Bedding, placed at the bottom of the cages, was collected and stored in zip-lock containers after birds had reached breeding condition. Zip-lock containers were tightly sealed and stored in plastic storage bins at room temperature when not being used for trials. Experiments did not begin until mid-march, and after evidence that birds had reached breeding condition i.e. dramatic increase in male singing behavior.

Y-Maze Olfactory Mate Choice

In order to test for chemical signal-based odor choice, I used a modified Y-maze similar to those used in mouse tests (e.g. Yamazaki et al. 1979). The Y-maze apparatus was built of plywood except for the top, which was made of plexiglass for visual monitoring. Each of the

three arms were .46 meters long by .30 meters wide and .30 meters tall (See Figure 2.1). The start arm was connected to a holding chamber for easy bird removal. The other end of the start arm connected to two choice arms, both with different odors blown in via tubing connected to an odor box, both of which connected back to a fan. Wind speed was tested with a wind detector and was found to be steady and evenly entering the apparatus through both arms at a rate of 0.40-0.45 meters per second and could be easily detected by humans at the two points the flow entered the apparatus. Room lights were turned off during trials with rope lights inside the apparatus in order to make the maze appear closed on top to the birds but still allowing for easy videotaping of the birds. The rope lights were installed along the outermost sides from the holding chamber to the tips of the choice arms and then Subject birds were given 15 minutes to acclimate to the apparatus before the fan was turned on and before the odor boxes were connected to the apparatus.

Self versus Control Tests

Males and females were used for two different experimental trials: 1) a choice test of self-odor versus a control, and 2) a choice test of tan odor versus white odor of the opposite sex. For the self versus control, approximately .5 liters of bedding containing easily visible fecal droppings from the subject's own cage were used as the odor cue and placed in one of the odor chambers. Fresh and unused bedding was used as a control for the other choice arm and placed in the opposite odor chamber. To minimize side-bias, odor chambers were alternated between choice arms for each trial. Each trial was videotaped from above with no human presence in the room for one hour once the odor box and fan were connected to the maze (Figure 2.2). In total, five tan females, two white females, three tan males, and three white males were tested for odor choice their own bedding samples and the control bedding.

Morph Choice Tests

The second set of trials conducted were to examine whether males and females would choose the bedding odor of one or the other morph type depending on their own morph. For these tests, both for male and female subjects, bedding samples were collected from the subjects and then paired up into tan and white combinations based on their sex and their cage position. Cage position was considered when choosing pairings in an attempt to ensure that choice could not be based on familiarity to a neighboring cage's odor. Seven tan females and 2 white females were tested for preference to either bedding odor from a tan male or a white male. Three different tan versus white pairs were created and all 9 females were tested for preference to each of the pairs. In total there were 3x9 or 27 female preference trials. Four white males, and 3 tan males were tested for preference to either bedding odor from a tan female or a white female. Two tan female versus white female pairs were used and each of the 7 males was tested for morph preference to both female pairs. In total there were 2x7 or 14 male preference trials. Just like the self versus control tests, the two bedding samples were placed in their respective odor chambers and were alternated between trials to limit side-bias. Each trial was also conducted such that an individual subject would have between two and three days in between one trial and their next.

Video Analysis and Statistics

Videos of both female and male trials were analyzed using Jwatcher Video (Blumstein, Maquari University, UCLA). Each of 54 hour-long videos was analyzed on average at half speed, amounting to a total of 110 man-hours. Key codes were used to code for different behaviors and positions (Table 2.1 lists all behaviors examined in the trials). Amount of total time spent, active time, and time spent perching for each arm was determined based on time

elapsed between specific key codes. Other behaviors such as number of escape attempts and number of vocalizations, as well as songs for males, were recorded but were not found to be common behaviors within most videos. After each video had been scored for behaviors, the videos were analyzed using Jwatcher Video Analysis to create the raw data for each video. Fifteen videos segments of 5 minutes each were scored by a secondary observer, with no knowledge of the odors in each arm, and then analyzed for inter-observer reliability using Jwatcher Video reliability analysis. Since many other behavioral studies sampled populations using 5 minute long focal videos (Blumstein and Daniel 2003, Dettmer et al. 2008), the secondary observer scored 5-minute segments to sample the reliability of the primary observer. The reliability scores between the two observers were very high (N= 15, average kappa = 0.86). The proportion of time spent in each of the odor choice arms was transformed by taking the arcsine square root of the proportion. The difference in response between white and tan males to the self versus control odors was analyzed using a repeated-measures analysis of variance (ANOVA). Additional repeated-measures ANOVA tests were used to test for the responses of white males and tan males to white versus tan female odor, and the responses of females to the same two tests (self versus control and tan male versus white male). A repeated-measures ANOVA was also run to test for differences between pair types, or tan males and white females versus white males and tan females. The data met the assumptions of normal distribution and sphericity necessary for repeated-measures ANOVA. Achieved statistical power was determined using SPSS 19 (SPSS Inc.) and G*Power power analysis program was used to determine ideal sample size (Faul et al. 2007).

Results

Self Versus Control Tests

An analysis comparing the response by pair type (tan males and white females versus white males and tan females) showed that the pair types have a different response to self versus control odors (Ntxw=5, Nwxt=7, F=13.22, p=0.004), with an achieved power of 0.907. Tan males and white females gravitated more towards the control arm and white males and tan females gravitated more towards the self-odor arm.

Initial results of the repeated-measures ANVOA indicated that tan and white males did not differ in their response to either self or control odors (Ntan=3, Nwhite=2, F=19.264, p=0.022). However, One white male video was removed as an outlier because it 1) started the video in the control-odor arm before the odors were added and 2) never left the control-odor arm and thus could not sample both odors. After removing the outlier, the repeated-measures ANOVA indicated that tan and white males did have different responses to self-produced and control bedding odors (Ntan=3, Nwhite=2, F=19.264, p=0.022), with tan males choosing the control over the self-odor and white males choosing self over control. Despite the low sample size, achieved power for the analysis was 0.82. The results of an *a priori* analysis suggested that with a desired power of 0.80 and assuming to find a moderate to high effect size (0.60-0.70), an ideal sample size would be 19-24 total trials, or 10-12 males of each morph. White females and tan females did not differ in their response to the two arms (Ntan=5, Nwhite=2, F=2.298, p=0.190). However, achieved power was only 0.24 and a sample size of at least 19-24 trials (10-12 per morph) with a medium to large effect would be necessary to reach sufficient power (0.80). All groups had even, or almost even (in the case of odd group sizes) numbers of trials with self-odor in the left and right arms.

Tan versus White Tests

Tan and white females did not differ in their response to tan and white males odors (Ntan=21, Nwhite=6, F=0.171, p=0.683). 1 tan female video was excluded based on the same criteria as the white male video in the self versus control trials. After the non-responsive bird was removed, tan and white females still did not differ in their response (Ntan=20, Nwhite=6, F=0.007, p=0.933). Observed power was only 0.051, so there was approximately a 95% chance of a false negative conclusion. Tan and white males also showed difference in their response to tan versus white female odors (Ntan=6, Nwhite=8, F=0.154, p=0.702). Removal of one non-responsive white male did make the results significant (Ntan=6, Nwhite=7, F=0.105, p=0.752). Observed power was also low for males with a power of 0.06, or a 94% chance of a false negative. In order to find an effect size of at least 0.60, which is a medium-high effect size, the sample size should be at least 19 trials, or 10 trials of each morph. While there were plenty of tan female trials to find a medium effect, there were still not enough white females, tan males, or white males to provide enough statistical power.

Discussion

Alternative Response to Self-Odor By Pair Type

Despite small sample sizes, repeated-measures ANOVAs found that the pair type that an individual belongs to affects their choice for their own bedding odor or the control such that tan males and white females were more likely to choose the control odor while white males and tan females were more likely to prefer their own odor. Additionally, when males were considered alone, the two morphs also differed in their choice for self-odor and the fresh bedding control odor. Since male trials, but not female trials, also found a significant difference in response based on morphs, it may be possible that male trials drove the pair type response. However,

males made up less than half of all individuals in the pair type analysis and inclusion of the females strengthened the results. There are two potential explanations that could help explain why tan males and white females would choose the control odor over their own. The first explanation is that since the bedding samples included fecal material, tan males and white females were avoiding aversive odors. Fecal material contains ammonia, which is avoided by domestic fowl in high concentrations (Jones et al. 2005). However, the second explanation is that tan male and white female pairs avoid their own odor as mechanism of inbreeding avoidance (Mateo and Johnston 2000). Tan males are typically less promiscuous then their white male counterparts (Tuttle 1993, 2003) and thus it may be more important for them to secure a mate that is not closely related. Tan by white pairs often nest in territories with fewer neighbors (Formica et al. 2004) and paternity results from a normal density year (2004) indicated that while half of all successful white by tan nests (N=16) contained at least one extra-pair paternity chick, all of the successful tan by white nests (N=14) were completely sired by the social father (Formica and Tuttle 2009). Since tan and white males exhibit alternative reproductive strategies (Tuttle 2003) and tan males are more likely to have fewer extra-pair young, there should be a greater evolutionary stress on tan by white pairs to avoid inbreeding when choosing a mate. The latter of the two explanations may also explain why white male by tan females instead preferred their own bedding to that of the control.

White males utilize a different reproductive strategy in which they attempt more extra-pair copulations than tan males (Tuttle 2003), and thus may be more likely to investigate an odor that may lead to an extra-pair copulation despite chances that the potential mate is a relative. At least one example has been recorded at Cranberry Lake Biological Station, Cranberry Lake, NY of a white male mating with a half-sister (Tuttle, unpublished data). White males had the

strongest aggressive response of any morph-sex class to model white-throated sparrows (Kopachena and Falls 1993b), and thus white males may investigate self-odor over the control as an act of aggression. However, tan females were the least aggressive, and thus an aggressive response does not hold when the response of pair types is considered. However, white by tan pairs nest in territories with higher densities of neighbors, and specifically other white by tan pairs (Formica et al. 2004). In this higher density environment, it may be especially important for white by tan pairs to use their own scent as a marker of their territorial range. Even though statistical power was high for the pair type analysis, future trials will support that the results were not achieved by random chance.

Morph-Choice Tests and Sample Size

Results of this study did not suggest that morphs of either sex had a preference for odors from tan or white potential mates. However, the assumption that odor does not play an important role in white-throated sparrow mate choice should not be made. Both sample size and statistical power were low, which means that there was a good chance of a type II error and false negative results. An *a priori* power analysis determined that to achieve 80% power and find an effect of between 0.60-0.70, future trials should include 19-24 trials total (~10-12 trials of each pair type).

Sample size was limited by a housing limit of 20 cages and an uneven netting ratio that resulted in uneven numbers of morphs and sexes. Two morph-choice trials and one self versus control-choice trial was removed due to unresponsiveness, which further limited the sample sizes. The results of both the *a priori* and achieved power analyses suggest that a larger sample size is necessary to test for odor preference based on the morphs of the transmitter and receiver. Future examination of a similar experimental design based around olfaction-based choice testing should prove very insightful into avian olfactory ability.

Reducing Stress Level of Subjects

The stress level of subjects is another factor essential to gathering meaningful results from behavioral studies. When subjects are not calm they may behave erratically, forcing the observer to remove videos in which a choice could not be reliably made. During the course of this study, only three videos were removed since they started in an odor arm and never left, and thus never sampled both odors. However, with a limited sample size, it is important that each trial reliably shows that a choice was possible. Since this study took considerable measures to maintain birds in a calm state, the best alternative for future studies might be to shorten the trial period from 1 hour to 30 minutes and then double or triple the number of trials performed with each subject. Increased trials may help subjects acclimate to the Y-maze and increase sample size for greater statistical power.

Sources of Odor

Another critical factor in designing olfaction-based choice tests is the odor source. Birds have many plausible odor sources: feces, plumage, stomach oils, blood, preen oil, and skin (Hagelin and Jones 2007). The choice to use bedding was based heavily on the importance of urine and associated proteins as mammalian chemical cues (Hurst et al. 2001; Thom et al. 2008). Avian feces contain uric acid, which may contain the same proteins as mammalian urine (i.e. Mhc proteins and MUPs). A study by Roth II et al. (2008) suggests that house finches have the ability to detect odors from cat and rabbit feces, and thus fecal material could prove to be a useful avian odor source. While there is a possibility that fecal materials contain aversive odors such as ammonia (Jones et al. 2005), it does not explain why white by tan pairs would choose their own odor over that of the control.

Additional studies on fecal material as a source of avian chemical cues are necessary, but future olfaction-based choice tests should also consider the use of preen oil as an odor source. Recent studies on preen oil in passerine species meet many criteria for not only being a chemical cue, but a chemical signal (Johansson and Jones 2007): They have been shown to be repeatable and variable between individuals (Whittaker et al. 2010), they differ between sexes (Soini et al. 2007), and they differ between species (e.g. Haribal et al. 2005). If future work can perfect the methods employed in this study and find a preference for a particular avian chemical cue in a variety of avian species, the way we view avian mate choice will change forever.

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Figure 2.1. A diagram of the Y-maze apparatus used for odor choice trials.
The Y-maze apparatus consists of the main compartment with 2 choice arms and a non-choice arm between with each choice arm connected to a different scent box. The odors from the scent box reach the Y-maze via an air current originating from the same fan. The top of the Y-maze is covered in see-through acrylic for videotaping.

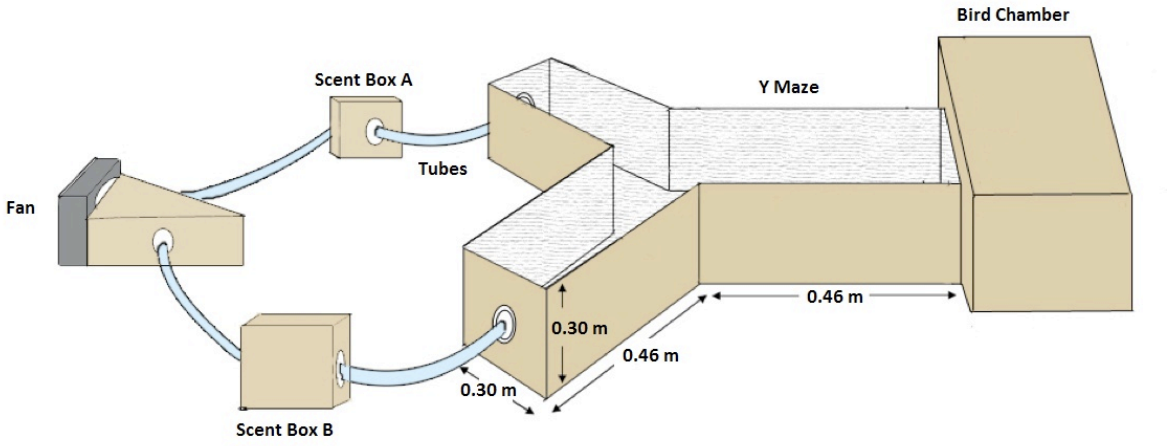

Figure 2.2. A video still that depicts a white-throated sparrow during a Y-maze trial. The subject is perched in the right arm of the maze.

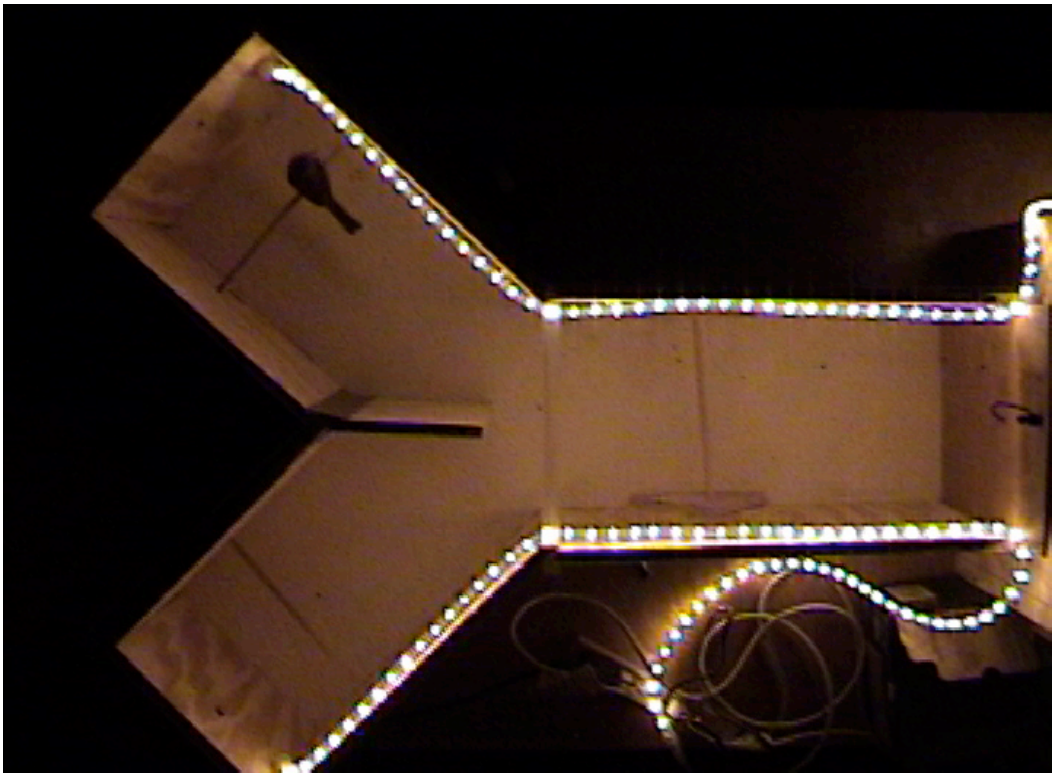

Table 2.1. A Complete List of Behaviors Recorded during Y-maze choice trials.

Key code	Behavior	Behavior Summary
1	Left Arm	Subject enters the left choice arm
2	Middle Arm	Subject enters the middle, no-choice arm
3	Right Arm	Subject enters the right choice arm
8	Active	Subject is actively changing position with no more than 10 seconds of inactivity
9	Inactive	Subject becomes inactive by sitting or standing still with no movement other than fluffing the feathers or slight head turns
p	Perch	Subject begins to perch on the perches in one of the three arms
l	Leave Perch	Subject ends perching bout initiated by (p) behavior
f	Fluff Feathers	Subject expands and fluffs its feathers
b	Bash	Subject hits, or bashes the top of the Y-maze
s	Sing	Subject Sings
a	Another vocalization	Subject makes a vocalization other than singing such as contact call or chip

CHAPTER 3

VARIATION OF PREEN OIL SAMPLES BETWEEN SEASON AND MORPH-SEX CLASSES OF THE WHITE-THROATED SPARROW

Abstract

Within the past two decades, avian olfactory ability has become well supported, and yet little is known about avian intraspecific chemical signals. One potential source of chemical cues is the secretions from the uropygial gland, or preen gland. Preen gland secretions waterproof, maintain, and protect feathers from ectoparasites. Preen oil chemistry also changes seasonally in a variety of birds including waterfowl, sandpipers, and juncos. Therefore, it is possible that preen oil may also contain chemical information applicable to individual recognition or mate choice during the breeding season. The aim of this study was to examine the seasonal differences in volatile compounds of captive white-throated sparrows (*Zonotrichia albicollis*). The polymorphic white-throated sparrow has both tan and white morphs that are found in both sexes and are linked to behavioral and genetic differences. We sampled birds in winter and spring breeding condition to identify candidate chemical signals based on the variation between both season and morph. Our data sampled 22 birds in winter (11 white males, 7 tan males, 2 white females, 2 tan females) and 18 birds in both winter and spring (10 white males, 6 tan males, 2 white females). Linear alcohols with a 10 to 18 carbon chain were the most abundant compounds that make up white-throated sparrow preen oil, and 2-ketones and carboxylic acids

were also common. Twelve compounds were seasonally elevated. Males of the white morph exhibited significantly lower concentrations of 11 compounds during the winter, compared to at least one other morph-sex class. Future work should further investigate the chemical properties of specific preen oil compounds and test whether they act as behaviorally relevant stimuli for birds.

Introduction

Chemical signals mediate more animal interactions than any other signal (Wyatt 2003), but historical assumptions that downplay avian olfactory ability (Roper 1999) have hampered scientific knowledge of avian chemical signals. Since recent studies have shown that birds do employ olfaction for foraging (e.g. Nevitt et al. 1995, 2008; Hagelin 2004), navigation and orientation (e.g. Bonadonna et al. 2003; Wallraff 2004; Nevitt and Bonadonna 2005), nest construction (e.g. Petit et al. 2002), and even assessing predation risk (e.g. Amo et al. 2008; Roth II et al. 2008), there is reason to believe that birds use social chemical signals. Pioneering work by Balthazart and Schoffeniels (1979) indicated that male mallards (*Anas platyrhynchos*) subjected to bilateral olfactory nerve section engaged in significantly less mating behaviors compared to sham-operated males. Similar data have since been obtained in domestic chickens (*Gallus domesticus*, Hirao et al. 2009). 3-hydroxy fatty acids were identified as a seasonally elevated component of female domestic duck preen (Jacob et al. 1979, Kolattukuddy et al. 1987), and thus a plausible chemical signal for males. More recently, multiple avian studies have identified species that detect body odor. For example, Antarctic prions (*Pachiptila desolata*) preferred the body scent of their partner to that of other conspecifics and avoided self-odor (Bonadonna and Nevitt 2004) in a manner consistent with inbreeding avoidance (see Mateo and Johnston 2000). Furthermore, the crested auklet (*Aethia cristatella*) also prefers both the odor of

conspecific plumage and a synthetic cocktail of compounds identified from feather odor (Hagelin et al. 2003). Auklet odor appears to be assessed during "ruffsniff" social displays (Hagelin et al. 2003), which has been implicated as an ornament (Hagelin 2007b) and for its role in parasite-mediated sexual selection (e.g. Douglas 2008).

There are many potential sources of avian chemical signals (Hagelin 2007a; Hagelin and Jones 2007), but preen gland secretions could prove to be one of the most important. Preen oil has been traditionally thought of for its role in waterproofing feathers (Elder 1954) and feather care (Jacob and Ziswiler 1982). It is useful for protection against bacteria, fungi, mites, or other feather parasites (Jacob and Ziswiler 1982; Jacob et al. 1997; Moyer et al. 2003; Shawkey et al. 2003). There is also reason to believe that preen oil is also important as a social chemosignal. Hirao et al. (2009) found that male chickens preferred females with intact preen glands over females with their preen glands removed, but males with removed olfactory bulbs showed no discrimination.

One of the most intriguing discoveries about the properties of preen oil in the past three decades has been the discovery that preen oil of multiple species differ between wintering and breeding seasons (Kolattukudy et al. 1987; Bohnet et al. 1991; Piersma et al. 1999; Reneerkens et al. 2002, 2007; Soini et al. 2007), which suggests unique roles for preen oil during the breeding season. Some researchers have suggested that a seasonal change in compounds could convey information relevant to intraspecific communication, such as an individual's identity (Soini et al. 2007; Mardon et al. 2010; Whittaker et al. 2010) or sexual attractiveness (Bohnet et al. 1991; Mardon et al. 2010; Whittaker et al. 2010). Preen oil often contain odorous volatile compounds that may act as chemical signals detectable by olfaction. Female dark-eyed juncos (*Junco hyemalis*) exhibit different behavioral responses to the scent of conspecific preen oil and

heterospecific preen oil (Whittaker et al. 2009). Preen oil compounds are also species-specific (Haribal et al. 2005, 2009) and meet other criterion to be considered chemosignals (Johansson and Jones 2007), such as exhibiting sexual variation (Jacob et al. 1979; Soini et al. 2007, Mardon et al. 2010) and individual variation and repeatability (Mardon et al. 2010, Whittaker et al. 2010).

The majority of studies suggesting preen oil as a potential source of chemosignals have come in non-passerine species (e.g. Jacob et al. 1979, Bohnet et al. 1991, Hirao et al. 2009, Mardon et al. 2010). Passeriformes is the most specious order of birds and only a handful of studies have investigated volatile compounds in a passerine species (Haribal et al. 2005, 2009; Soini et al. 2007; Whittaker et al. 2010). The dark-eyed junco (*Junco hyemalis*) is the only passerine species to show seasonal variation (Soini et al. 2007) and individual and sexual variation (Whittaker et al. 2010) in preen oil chemistry plus behavioral evidence suggesting olfactory detection of preen oil (Whittaker et al. 2009). The white-throated sparrow has previously been shown to hybridize with the dark-eyed junco (Jung et al. 1994), and the *Junco* and *Zonotrichia* genera are closely related. A study of the preen gland volatile compounds of the white-throated sparrow, therefore, is a logical model to increase our understanding of preen oil chemistry in passerines. The aim of this study is to examine whether seasonal variation of preen oil chemical composition differs between both season and morph-sex class of the white-throated sparrow, in order to identify candidate social chemosignals in a passerine model.

The white-throated sparrow is also an ideal model because of its unique polymorphism, with both sexes occurring as either a tan-striped or white-striped morph depending on the color of their medial crown strip (Lowther 1961; Lowther and Falls 1968). The two morphs mate disassortatively with white males mating with tan females and tan males mating with white females (Lowther 1961; Thorneycroft 1975; Tuttle 1993, 2003). The morphs are absolutely

correlated with a complex chromosomal rearrangement with white birds being heterozygous for the rearrangement (2^m/2) and tan birds homozygous (2/2) for no rearrangement (Thorneycroft 1966, 1975; Thomas et al. 2008; Romanov et al. 2009). The genetic differences between the morph also correlate to alternative reproductive strategies (Tuttle 2003) in which white males trade-off reduced paternity in the home territory for increased extra-pair paternity compared to tan males. The reproductive strategy trade-off and the unique disassortative mating makes the white-throated sparrow an ideal model to test not only the effect of season on preen oil chemistry but also intraspecies variation between morph-sex classes.

Methods

Study Species

The white-throated sparrow (*Zonotrichia albicollis*) is a socially monogamous songbird that breeds throughout Canada and Northeastern United States (Falls and Kopachena 1994). White and tan morphs are found in both sexes, and the phenotypic differences between both morphs are absolutely correlated to a complex rearrangement of the second chromosome (Thorneycroft 1966, 1975; Thomas et al. 2008; Romanov et al. 2009) and the two morphs are maintained in equal proportions through disassortative mating (Lowther 1961; Thorneycroft 1975; Tuttle 1993, 2003).

The morph genotypes additionally correlate with behavioral differences. White males sing more (Lowther and Falls 1968; Falls 1988; Tuttle 1993) and engage in more extra-pair copulations than tan males (Tuttle 2003). Meanwhile, tan males invest more in mate-guarding (Tuttle 2003) and in parental care (Knapton and Falls 1983; Kopachena and Falls 1993; Tuttle 2003). These behavioral differences highlight the alternative reproductive strategies each morph invests in, where white males may gain extra-pair paternity, but often loose paternity in their

home territory (Tuttle 2003). Since the different morphs of the white-throated sparrow vary in their genetics, behavior, and reproductive strategies, the morphs should also vary in their preen oil compound concentrations if preen oil secretions are used as intraspecific chemosignals to convey identity or influence mate choice. The genetic variation between morphs provides a pathway for preen oil variation while behavioral differences between morphs allow for differential selection.

Sample Collection

White-throated sparrows were captured at Indiana State University, Terre Haute, IN using passive mist netting (master banding permit 22296 to E. M. Tuttle at Indiana State University, Terre Haute, IN) between the months of October 2009 and February 2010. 11 white males, 7 tan males, 2 white females, and 2 tan females were captured for a total of 22 birds. Winter samples were successfully collected from all 22 birds, and 18 of the 22 birds were sampled again in breeding condition (10 white males, 6 tan males, and 2 white females). All captured birds were housed in indoor aviaries at Indiana State University and fed an *ad libitum* diet of water, sunflower hearts, white millet, thistle, probiotics (Benebac), and meal and wax worms. Daily bird care averaged 30 minutes per day over ~ 150 days for a total of 75 man hours. Preen oil were sampled at least 10 days after being housed in lighting conditions set to a natural Indiana winter day length. The aviary was kept on a natural winter photoperiod until late February 2010, and slowly brought to a photoperiod of 16 hours light, 8 hours dark to bring the birds into breeding condition. Preen oil was then again sampled at least 10 days after reaching this breeding photoperiod. Preen oil was collected by gently squeezing the preen gland with the fingers (Kolattukudy et al. 1987) while holding a 80ul glass capillary tube (Scientific Products) up to the end of the preen gland. Volume was calculated by measuring height of the sample with

digital calipers and using the average internal diameter of the glass capillary tube (1.1 mm). Samples were then kept frozen at -20^{o} C until they could be shipped on dry ice to Indiana University, Department of Chemistry, Institute of Pheromone Research, Bloomington, IN.

Sample Preparation

Sample preparation followed the methods of Soini et al. 2007 and Whittaker et al. 2010 utilizing Soini's stir bar sorptive extraction technique (Soini et al. 2005). Thawed preen oil samples were pushed out of the microcapillary glass pipettes into 20-ml glass vials. Then, 2.0 ml of water (high-purity OmniSolv, EMD Chemicals, Inc., Gibbstown, NJ), 100 mg of ammonium sulfate (99.991%from Sigma-Aldrich, St Louis, MO), and an internal standard of 8 ng of 7-tridecanone (Sigma-Aldrich) dissolved in 5 ul methanol (Baker Analyzed, Mallinckrodt Baker, Inc., Phillipsburg, NJ) were added to each vial. A Twister™ stir bar (10 x 0.5 mm polydimethylsiloxane) was then added and stirred at 800+rpm for 60 minutes (Gerstel GmbH, Mu¨lheim an der Ruhr, Germany). After 60 minutes, the stir bar was then removed and rinsed with distilled water (high-purity OmniSolv), dried gently, and placed in the thermal desorption autosampler tube for Gas Chromatography-Mass Spectrometry analysis.

Analytical Instruments

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted using the Agilent 6890N gas chromatograph connected to the 5973iMSD mass spectrometer (Agilent Technologies, Inc., Wilmington, DE) with the thermal desorption autosampler and cooled injection system (TDSA-CIS 4 from Gerstel) as used in Soini et al. 2007 and Whittaker et al. 2010. All analytical instruments were set to the same specifications as Whittaker et al. 2010. Specifically, positive electron ionization (EI) mode at 70eV was used with a scanning rate of 2.47 scans/sec, and mass range of 40-350 amu. The mass spectrometric detector (MSD) transfer

line temperature was 280°C, the ion source was 230°C and quadrupole temperature was150°C. Samples were thermally desorbed in a TDSA automated system and then injected into the column with the CIS-4 cooled injection assembly. Splitless mode was used for desorption with a temperature program of 20°C for 0.5 min, then 60°C/minute up to 250°C for 3 min. Transfer line temperature was set at 280°C and CIS was cooled using liquid nitrogen to -80°C. After desorption and cryotrapping were complete, CIS was heated at 12°C/sec until 270°C and held for 12 min. Solvent vent mode was used for CIS inlet, with a vent pressure of 9.1 psi, a vent flow of 50 ml/min, and a purge flow of 50 ml/min. During GC operation the carrier gas head pressure was 9.1 psi at a constant 1.1 ml/min flow, and temperature started at 50°C for 2 min, then increased to 200°C at the rate of 3°C min, and held for 12 min.

Quantitative Comparisons

Major volatile compounds in the preen oil samples were positively identified by comparison with standard substances of known mass spectra and retention times. The peak areas for each identified compound were used for quantitative comparisons and were integrated either from the total ion current (TIC) profiles or from post-run, single ion current (SIC) profiles at either m/z 55 for linear alcohols, m/z 58 for methyl ketones, or m/z 60 for fatty acids. The internal standard, 7-tridecanon, peaked at m/z 113 and peak areas were normalized by dividing each peak area by that of this internal standard. Total chemical analysis for all captive samples took 1.5 months.

Statistics

Peak areas, or abundances, of compounds were non-normally distributed and thus nonparametric wilcoxon-signed rank tests were used to compare the concentration of each compound in winter samples to samples collected under conditions that simulated spring

breeding. Since captive bird populations were heavily male-biased, wilcoxon-signed rank tests were run separately all males (N=16) and for white (N=10) and tan morph males (N=6). Next, Mann-Whitney U Tests were run to determine if there were any within season variation in compound abundance between sexes and male morphs, but did not include a Kruskal-Wallis ANOVA comparing morph-sex classes since only two winter samples were available for white females and tan females, and no breeding condition samples were available for tan females. The wilcoxon-signed rank tests and Mann-Whitney U tests run dependent variables in a univariate fashion in SPSS 19.0 with only one independent variable comparison, and thus did not cause need for Bonferroni or sequential Bonferroni adjustments. Statistically significant values less than 0.05 were accepted. Statistical analysis was completed using SPSS 19.0 (SPPS Inc.).

Results

A selected ion current profile of mz/55 revealed the major components of white-throated sparrow preen oil to be primarily 9 different linear 1-alkanols with a carbon chain ranging from 10-18. The secondary compounds that are also commonly found included 9 different 2-ketones from 2-nonanone to 2-heptadecanone and 9 carboxylic acids including pentanoic acid and nonanoic acid through stearic acid (octadecanoic acid). Of the 27 total compounds identified, some compounds were found in the majority of samples while others were found in only a handful of individuals (Table 3.1). Pentanoic acid (1 sample), 2-nonanone (4 samples), and 2-Decanone (5 samples) were especially rare compounds that were only observed in some breeding condition samples, but not in any wintering condition samples.

Nonparametric Wilcoxon-signed rank tests discovered that breeding season samples contained higher abundances of 12 different compounds that were seasonally elevated in spring breeding condition compared to winter condition. Seasonally elevated compounds included

linear alcohols from 1-decanol to 1-pentadecanol, dodecanoic acid, stearic acid, 2-decanone, 2-tridecanone, 2-tetradecanone, and 2-pentadecanone (Table 3.1). Additional Wilcoxon-signed rank tests were run to determine if males (N=16), white males (N=10), and tan males (N=6) also exhibited higher compound abundance in breeding condition, since males made up almost the entire sample size. All 12 of the seasonally elevated compounds for the entire sample were also seasonally elevated in males, plus 1-hexadecanol was also seasonally elevated (Table 3.2). Ten compounds seasonally elevated in males were also seasonally elevated in white male samples (N=10, Table 3.3), but no compounds were seasonally elevated in tan male samples (N=6).

Results of Mann-Whitney U tests determined that 11 different compounds found in winter samples were more abundant in females (N=4) than males (N=18, Table 3.4). Additionally, 3 linear alcohols were more abundant in tan males (N=7) than white males (n=11, Table 3.5). During the winter season, tan males also appeared to be more abundant in 2-tridecanone and 2-tetradecanone than white males, but the results were marginally non-significant (2-tridecanone: $U=17.5$, $Z=-1.908$, $p=0.056$; 2-tetradecanone: $U=17$, $Z=-1.954$, $p=0.056$).

Discussion

Twenty-seven volatile compounds were present in the uropygial oil of the captive white-throated sparrows in breeding condition while only 23 volatile compounds were present in winter condition preen oil. 1-Decanol, 2-Decanone, 2-Nonanone, and Pentanoic Acid were only present when sampled during the breeding condition. Compounds of white-throated sparrow preen oil contained primarily linear alcohols, 2-ketones, and carboxylic acids that typically ranged from 9-18 carbons (Table 3.1). Twelve of these compounds were found to be seasonally elevated for the whole sample size, including 7 linear alcohols, 4 ketones, and dodecanoic acid (Table 3.2), and

13 compounds were seasonally elevated for males (Table 3.3). The results strongly support a basis for seasonal variation in the preen oil of white-throated sparrows, which also suggests that has additional roles, or increased importance for preen oil use during the breeding season. Explanations for a seasonal shift in preen oil include nest crypsis (Reneerkens et al. 2002, 2007), increased protection from ectoparasites (Moyer et al. 2003; Shawkey et al. 2003; Soini et al. 2007) and use as social chemosignals (Mardon et al. 2010; Whittaker et al. 2010). Recent papers on avian olfaction or preen oil are optimistic that avian social chemosignals exist (Hagelin 2007; Hagelin and Jones 2007; Bathazart and Taxiaux 2009; Mardon et al. 2010; Whittaker et al. 2010).

Almost all of the compounds found in white-throated sparrow preen oil were also found in dark-eyed junco preen oil, and many of the same compounds were found to be seasonally elevated in both species (Soini et al. 2007). Both species had higher concentrations during breeding condition of linear alcohols with 10-15 carbons in at least one sex. White-throated sparrows and male juncos had higher levels of 2-tridecanone, 2-tetradecanone, and 2-pentadecanone, while white-throated sparrows and female juncos had higher concentrations of dodecanoic acid during the breeding condition. However, junco uropygial secretions also contained compounds not present in those of white-throated sparrows such as aldehydes. White-throated sparrow uropygial secretions meanwhile contained seasonally elevated 2-decanone and stearic acid that was not present in junco secretions (Soini et al. 2007). Therefore, the previous claim that all previously studied volatile preen compounds vary between species (Haribal et al. 2005, 2009; Soini et al. 2007; Whittaker et al. 2010) is still supported since juncos neither share the same number of volatile compounds or the all of the same compounds. Additionally, volatile compounds may be combined in many unique ways to create larger non-volatile compounds

(Haribal et al. 2005), and it is beyond the scope of this study to examine the non-volatile compound variation between species.

Within season analysis strongly suggested that both sexes and morphs vary in multiple preen oil compound abundances. Females had higher abundances of 8 different linear alcohols from 1-Undecanol to 1-Octadecanol as well as higher abundances of 2-tetradecanone, 2-pentadecanone, 2-hexadecanone, and 2-heptadecanone than males (Table 3.4). The reasons why females have higher levels of multiple linear alcohols and 2-ketones during the winter remain unclear. One potential explanation is that males have lower concentrations of sexually selective compounds during the winter to create a greater winter to breeding season shift, and the strong shift in preen oil compounds is the actual cue that females are detecting. White-throated sparrow winter plumage is also much more difficult to distinguish (Atkinson and Ralph 1980; Piper and Wiley 1989) and it has been proposed that the reduced plumage brightness of white males helps maintain stable social relations within wintering flocks (Atkinson and Ralph 1980). Similarly, the lower levels of preen oil compounds in white-throated sparrow males may also help maintain social flocks. An analysis with more female samples could provide further support since female sample sizes (N=4 in winter, 2 in breeding condition) were much lower than that for males (N=18 in winter, 16 in breeding condition).

White males in winter condition (N=11) also had significantly lower abundances of three different linear alcohols from tan males in winter condition (N=7, Table 3.5). 1-tetradecanol, 1-pentadecanol, and 1-hexadecanol were lower in white males than tan males during the winter sampling, which suggests that different morphs may utilize preen oil compounds differently even during wintering months when reproductive benefits and parasite protection benefits unnecessary. However, the abundance of chemicals may not be as important during the breeding

season as the change in abundance between winter and breeding condition. This is not unlikely after considering that white males also have significantly duller wintering plumage than spring plumage (Atkinson and Ralph 1980; Piper and Wiley 1989). Alternative production of preen oil compounds, even in winter condition, is genetically warranted since white morph individuals are heterozygous for a complex rearrangement of their second chromosome (Thorneycroft 1966, 1975; Thomas et al. 2008; Romanov et al. 2009), which includes upwards of 86% of the chromosome (Romanov et al. 2009). Future gene mapping studies are required to reveal whether preen oil production is coded on a gene on the second white-throated sparrow chromosome or a gene that interacts with the second chromosome.

While winter samples showed variation between sexes and male morphs, breeding condition samples showed no indication of between groups variation. All individuals were fed on the same diet. Some evidence in white-throated sparrows suggests that different dietary supplements can affect preen oil nonvolatile compounds (Thomas et al. 2010), and thus may affect volatile compounds as well. There is also a correlation between the pair types of the white-throated sparrows, the type of habitat used, and the number of neighbors surrounding a pair's territory (Formica et al. 2004). If territory type and the density of neighbors affects preen oil in wild populations, it is possible that high-density captive conditions altered any alternative response in preen oil production between morphs.

In conclusion, preen oil of the white-throated sparrow contains volatile compounds that are elevated during simulated breeding condition. Some seasonally elevated compounds differed between sexes and male morphs, but future work should include a greater sample of females of both morphs in order to make complete morph-sex class comparisons. Variation due to morph reflects the white-throated sparrow's unique behavioral and genetic differences and its

importance to future studies on the role of preen oil. Future studies can utilize the discovery of seasonally elevated uropygial compounds to investigate olfactory mate-choice based on the odor choice for synthetic compounds found in preen oil.

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Table 3.1 Preen oil compounds used for comparison of breeding and nonbreeding condition in the white-throated sparrow.
Significant z-scores and p-values are listed for wilcoxon-signed rank test between seasons.

Compound	Molecular Weight (amu)	% Winter Samples with Compound (N=22)	% Spring Samples with Compound (N=18)	Z-Score (N=18)	P*
1-Decanol	158.28	0%	39%	-2.366	0.018
1-Undecanol	172.31	82%	89%	-3.375	0.001
1-Dodecanol	186.33	86%	94%	-3.245	0.001
1-Tridecanol	200.36	82%	94%	-3.288	0.001
1-Tetradecanol	214.39	86%	94%	-3.180	0.001
1-Pentadecanol	228.41	82%	94%	-2.765	0.006
1-Hexadecanol	242.44	86%	94%		
1-Heptadecanol	256.47	68%	61%		
1-Octadecanol	270.49	50%	78%		
2-Nonanone	142.24	0%	22%		
2-Decanone	156.27	0%	28%	-2.023	0.043
2-Undecanone	170.29	86%	89%		
2-Dodecanone	184.32	82%	78%		
2-Tridecanone	198.34	91%	94%	-2.440	0.015
2-Tetradecanone	212.37	86%	94%	2.221	0.026
2-Pentadecanone	226.40	91%	94%	-2.287	0.022
2-Hexadecanone	240.42	73%	72%		
2-Heptadecanone	254.45	73%	67%		
Pentanoic Acid	102.13	0%	6%		
Nonanoic Acid	158.24	5%	17%		
Decanoic Acid	172.26	5%	11%		
Dodecanoic Acid	200.32	41%	94%	2.592	0.010
Tridecanoic Acid	214.34	14%	11%		
Tetradecanoic	228.37	64%	94%		
Pentadecanoic	242.40	27%	33%		
Palmitic Acid	256.42	77%	94%		
Stearic Acid	284.48	5%	39%	0.017	0.016

Table 3.2 Significant results of the wilcoxon-signed rank test for male white-throated sparrows (N=16), to determine which compounds are seasonally elevated during the breeding season.

Compound Family	Compound	Z-Score (N=10)	p
1-alkanols	1-Decanol	-2.201	0.028
1-alkanols	1-Undecanol	-3.103	0.002
1-alkanols	1-Dodecanol	-2.947	0.003
1-alkanols	1-Tridecanol	-3.051	0.002
1-alkanols	Tetradecanol	-2.896	0.004
1-alkanols	1-Pentadecanol	-2.689	0.007
1-alkanols	1-Hexadecanol	-2.043	0.041
2-Ketones	2-Decanone	-2.023	0.043
2-ketones	2-Tridecanone	-2.328	0.020
2-ketones	2-Tetradecanone	-2.198	0.028
2-ketones	2-Pentadecanone	-2.224	0.026
Carboxylic Acid	Dodecanoic Acid	-2.300	0.021
Carboxylic Acid	Stearic Acid	-2.384	0.017

Table 3.3. Significant results of the wilcoxon-signed rank tests for white males, N=10, to determine which compounds are seasonally elevated in white-striped males.

Compound Family	Compound	Z-Score (N=10)	p
1-alknaols	1-Undecanol	-2.803	0.05
1-alkanols	1-Dodecanol	-2.803	0.05
1-alkanols	1-Tridecanol	-2.803	0.05
1-alkanols	1-Tetradecanol	-2.803	0.05
1-alkanols	1-Pentadecanol	-2.803	0.05
1-alkanols	1-Hexadecanol	-2.599	0.009
2-ketones	2-Tridecanone	-1.988	0.028
2-ketones	2-Tetradecanone	-1.988	0.047
2-ketones	2-Pentadecanone	-2.090	0.037
Carboxylic Acid	Dodecanoic Acid	-2.393	0.017

Table 3.4. Results of the Mann-Whitney U tests highlighting the differences between males and females in the abundance of preen oil compounds found during the winter season. Females displayed higher abundances than males of all 12 compounds listed below.

Compound in Winter Sample	More Abundant in Females	Mann-Whitney U	Z-Score (N=18)	Exact Significance p*
1-Dodecanol	F>M	12	-2.046	0.042
1-Tridecanol	F>M	7.5	-2.434	0.010
1-Tetradecanol	F>M	10	-2.216	0.026
1-Pentadecanol	F>M	7	-2.476	0.010
1-Hexadecanol	F>M	8	-2.386	0.014
1-Heptadecanol	F>M	4	-2.791	0.003
1-Octadecanol	F>M	4	-2.912	0.003
2-Tetradecanone	F>M	9.5	-2.261	0.019
2-Pentadecanone	F>M	9	-2.300	0.019
2-Hexadecanone	F>M	12	-2.065	0.042
2-Heptadecanone	F>M	12	-2.064	0.042

Table 3.5. Results of Mann-Whitney U tests comparing abundance of winter compounds between white and tan male white-throated sparrows.
All 3 compounds below were more abundant in the winter samples of tan males than of white males.

Compound in Winter Sample	More Abundant in Tan Males	Mann-Whitney U	Z-Score (N=18)	Exact Significance p*
1-Tetradecanol	T>W	16	-2.043	0.041
1-Pentadecanol	T>W	13.5	-2.276	0.023
1-Hexadecanol	T>W	16	-2.042	0.041

CHAPTER 4

PREEN OIL VOLATILE COMPOUNDS OF BREEDING WHITE-THROATED SPARROWS (*ZONOTRICHIA ALBICOLLIS*): INDIVIDUAL AND MORPH-SEX CLASS VARIATION

Abstract

Uropygial gland secretions, or preen oil, have long been known to help waterproof and protect feathers from degradation and ectoparasites. Preen oil also has been shown to vary between seasons in multiple species, suggesting that it has additional roles in breeding condition. Two non-opposing hypotheses have gained recent evidence: 1) that preen oil contains social chemosignals and 2) that preen oil enhances nest crypsis from olfactory-keen predators. We examined the variation of volatile compounds in the preen oil between individuals, and morph-sex classes of a polymorphic passerine, the white-throated sparrow (*Zonotrichia albicollis*). Our aim was to examine whether the variation of preen oil found in white-throated sparrows was suggestive of chemical signatures and social chemosignals, nest crypsis, or both. Adult white-throated sparrows were sampled during breeding condition over a two-year period. Capillary gas chromatography-mass spectrometry was used to compare the relative abundance of linear alcohols, 2-ketones, and carboxylic acids found in the white-throated sparrow preen oil, between each combination of morph and sex. Results showed that individuals and morph-sex classes both significantly varied in their relative concentrations of preen oil compounds. Specifically, tan females had a smaller relative proportion of 2-pentadecanone compared to all other morph-

sex classes. Preen oil analysis of breeding white-throated sparrows showed results that could be supportive of both the social chemosignal and nest crypsis hypotheses.

Introduction

Secretions from the uropygial or preen gland form a complex wax or oil which contains both volatile and non-volatile compounds, and the exact compounds can be considerably diverse between species (Haribal et al. 2005, 2009). Preen oil has historically been credited with a major role in waterproofing (Elder 1954; Jacob and Ziswiler 1982) as well as feather protection from normal wear and tear (Stettenheim 1972, Jacob and Ziswiler 1982) and from ectoparasites such as bacteria and mites (e.g. Jacob et al. 1997; Moyer et al. 2003). However, it has been discovered in multiple species that preen oil contains different compounds, or elevated levels of compounds during the breeding season (Jacob et al. 1979; Kolattukudy et al. 1987; Bohnet et al. 1991; Reneerkens et al. 2002, 2007; Soini et al. 2007). The seasonal shift in preen oil compounds suggests that preen oil has additional roles for breeding adults. Two non-opposing hypotheses have been recently proposed to explain this seasonal shift in preen oil: 1) that preen oil contains social chemosignals capable of transmitting individual identity or some measure useful for sexual selection, and 2) that preen oil contains compounds involved in nest crypsis from olfactory-keen predators.

Avian olfactory ability has been traditionally underappreciated, but recent discoveries support the premise that birds emit odors capable of transmitting social chemical signals. A chemical signal is a chemical compound or mixture produced by the sender, which causes a social or physiological effect on the intended receiver (Johnston 2000; Hagelin 2007a). Therefore, chemical signals should contain necessary variation to distinguish either the identity or quality of the sender (Johansson and Jones 2007). Antarctic prions (*Pachiptila desolata*,

Bonadonna and Nevitt 2004) and blue petrels (*Halobaena caerulea*, Mardon and Bonadonna 2009) both preferred the odor of their partner to that of conspecifics and the odor of conspecifics to their own (Bonadonna and Nevitt 2004), which supports the concept of inbreeding avoidance (See Mateo and Johnston 2000). The crested auklet (*Aethia cristatella*), preferred both conspecific odor and a synthetic cocktail of compounds identified from feather odor (Hagelin et al. 2003). Auklet odor has also been implicated as an ornament (Hagelin 2007b).

Recent studies have shown supporting evidence of preen oil as a potential source of social chemosignals in birds. Hirao et al. (2009) found that males preferred females with intact preen glands, but males without olfactory bulbs showed no preference for females with or without preen glands. Dark-eyed Junco preen oil not only vary by season and sex (Soini et al. 2007) but also show individual repeatability (Whittaker et al. 2010). Results of Mardon et al. (2010) demonstrate that preen oil from both Antarctic prions and blue petrels contain variation capable of determining an individual's identity, gender, and species.

Preen oil may also contain compounds that help in nest crypsis from predators. The nest protection hypothesis was originally applied to parasite load reduction (Wimberger 1984; Clark and Mason 1985; Clark 1991). However, Reneerkens et al. (2005) applied the concept reduction of nest predation from olfactory-keen predators by masking the scent of the nest. Diesters found in the preen oil of breeding red knots (*Calidris canutus*) were harder for sniffer dogs to detect than the monoesters found in winter condition (Reneerkens et al. 2005). Additionally, in multiple species of sandpipers, the sex or sexes responsible for incubation were more likely to switch the preen oil composition from monoesters to diesters during the incubating period (Reneerkens et al. 2007). Under the nest crypsis hypothesis, the better the ability of an individual to mask its scent and that of its nest will lead to a higher likelihood of successful

broods and higher fitness. Preen oil compounds that mask the scent of a nest should be naturally selected in the sex responsible for nest incubation, especially in birds with nests accessible to olfactory-keen predators.

The aim of this study is to identify volatile compounds found in the preen oil of the polymorphic white-throated sparrow (*Zonotrichia albicollis*) that differ between individuals and morph-sex classes in order to determine the likelihood of the both the social chemosignal and nest crypsis hypotheses. The white-throated sparrow is a unique species to study volatile compounds because it is polymorphic, with white and tan morphs that mate disassortatively (Lowther 1961; Thorneycroft 1975; Tuttle 1993, 2003). The morphs are correlated to a rearrangement of the second chromosome (Thorneycroft 1966, 1975; Thomas et al. 2008; Romanov et al. 2009) and they also exhibit alternative reproductive strategies (Tuttle 2003), which gives basis for variation between the combinations of morph and sex. Individuals should show significant variation in the relative proportion of preen oil compounds if preen oil of the white-throated sparrow contains unique chemical signatures. White-throated sparrows are also a ground nesting species like sandpipers (Reneerkens et al. 2007) and dark-eyed juncos (Whittaker et al. 2010) and thus provide an ideal species to test the nest crypsis hypothesis. Assuming both hypotheses are true, white-throated sparrows should exhibit a trade-off between more volatile and less volatile preen oil compounds depending on of incubation and nestling care and sexual selection for each morph and sex class.

Methods

Study Species

The white-throated sparrow (*Zonotrichia albicollis*) is a socially monogamous passerine that breeds in Northeastern United States and Canada (Falls and Kopachena 1994). The white-

throated sparrow is dimorphic with a white and tan morph that can most notably be separated by the color of the median crown stripe (Lowther 1961; Lowther and Falls 1968). The phenotypic differences between the white and tan morphs are absolutely correlated to a chromosomal rearrangement (Thorneycroft 1966, 1975; Thomas et al. 2008; Romanov et al. 2009) of the second chromosome such that whites are heterozygous for the rearrangement and tans are homozygous for no rearrangement. The morphs are also kept in equal proportions through disassortative mating, in which white males mate with tan females and tan males mate with white females (Lowther 1961; Thorneycroft 1975; Tuttle 1993, 2003). Females nest on the ground or near the ground in small shrubs that are accessible to predation from olfactory-keen predators such as garter snakes, and thus face a similar need for nest crypsis as sandpipers (Reneerkens et al. 2007) and juncos (Soini et al. 2007). Females build, incubate, and care for young in the nest, while males feed nestlings and fledglings. However, tan males invest more heavily in parental care than white males (Knapton and Falls 1983; Kopachena and Falls 1993; Tuttle 2003), placing higher pressure for parental care on tan females than white females.

The morphs utilize alternative reproductive strategies (Tuttle 2003) in which white males sing more than tan males (Lowther and Falls 1968; Falls 1988; Tuttle 1993) and engage in more extra-pair copulations (Tuttle 2003), but tan males guard their mates more (Tuttle 2003) along with their increased parental care. Additionally, the two disassortative pair-types differ in their habitat with white males x tan females preferring bog vegetation with a high-density of neighbors while tan males x white females prefer lower-density ponds (Formica et al. 2004).

Sample Collection

White-throated sparrows were sampled from late May to early August 2009 and 2010 at Cranberry Lake Biological Station (44^{0} 15’ N, 74^{0} 78’ W), Cranberry Lake, NY. Birds were

caught and banded using passive mist netting and playback (master banding permit 22296 to E. M. Tuttle at Indiana State University, Terre Haute, IN). Preen Oil was collected by gently squeezing the area around the preen gland in order to discharge a small amount (Kolattukudy et al. 1987) into either a 1ul glass microcapillary (Drummond) for the 2009 samples, or an 80 ul glass microcapillary glass pipette (Scientific Products) for the 2010 samples and volume was calculated using digital calipers for height and the internal diameter of the pipette (0.2 mm Drummond, 1.1 mm SP). Samples were kept frozen at -20^{0} C until shipped on dry ice to Indiana University, Department of Chemistry, Institute of Pheromone Research, Bloomington, IN. Total sample size was 55 individuals with 22 white males, 14 tan males, 10 white females, 9 tan females, and 3 juveniles.

Sample Preparation

Sample preparation followed the methods of Soini et al. 2007 and Whittaker et al. 2010 utilizing Soini's stir bar sorptive extraction technique (Soini et al. 2005), and can be found in chapter 3 methods. The thawed preen oil samples were pushed out of the microcapillary glass pipettes they were collected in and into a 20-ml glass vial using a Teflon plunger. 2.0 ml of high purity water, 100 mg of ammonium sulfate (99.991%from Sigma-Aldrich, St Louis, MO), and the internal standard of 8 ng of 7-tridecanone (Sigma-Aldrich) dissolved in 5 ul methanol (Baker Analyzed, Mallinckrodt Baker, Inc., Phillipsburg, NJ) was added to every vial. A Twister™ stir bar (10 x 0.5 mm polydimethylsiloxane) was then added and stirred at 800+rpm for 60 minutes (Gerstel GmbH, Mu¨lheim an der Ruhr, Germany). After 60 minutes, the stir bar was then removed and rinsed with water (high-purity OmniSolv), dried gently on paper tissue and placed in the thermal desorption autosampler tube for Gas Chromatography-Mass Spectrometry analysis.

Analytical Instruments

Analytical instruments used were the same as used in Soini et al. 2007, Whittaker et al. 2010 as well as chapter 3, which were the Agilent 6890N gas chromatograph and the 5973iMSD mass spectrometer (Agilent Technologies, Inc., Wilmington, DE). Additionally, instrument specifications were followed according to Whittaker et al. 2010 and are outlined in chapter 3.

Statistics

All of the major volatile compounds in the preen oil samples were positively identified by comparison with standard substances of known mass spectra and retention times. Peak areas were integrated either from the total ion current (TIC) profiles or from post-run, single ion current (SIC) profiles at either m/z 55 for linear alcohols, m/z 58 for methyl ketones, or m/z 60 for carboxylic acids. The peak areas for each identified compound were normalized by dividing each peak area by the peak area of the internal standard. Normalized peak areas for each individual were converted into a proportion of the total observed peaks and then were logit transformed by taking the natural logarithm of (p/(1-p), where p is the proportion (Armitage and Berry 1994; Whittaker et al. 2010).

A principal component analysis (PCA) (SPSS 18.0) was conducted to help create potential chemical to simplify the overall chemical composition. Seventeen compounds were below detectable levels in a considerable amount of individuals and thus eleven compounds found in almost every individual were used for the PCA. One-sample t-tests were run to test for within group variation of the relative concentrations of each of the 11 compounds used in PCA analysis. Discriminant function analyses classified individuals based on sex, morph, morph-sex class (i.e. white male, tan male, white female, tan female), year and month using the principle components as independent variables. Chi-squared test for independence determined the

accuracy of the discriminant function classification. Three Multivariate analyses of variance (MANOVA) were run to determine the variation between 1) sex and morph, 2) year and month, and 3) morph-sex classes with the three principle components as dependent variables according to the methods from Whittaker et al. (2010). The MANOVAs met the assumptions of random sampling, and the data was normally distributed with variance between groups approximately equal. Statistically significant values less than 0.05 were accepted but p-values were corrected using a sequential Bonferroni correction (Holm 1979). After a MANOVA was conducted with the synthesized principle components as dependent variables, a follow-up MANOVA was conducted on all compounds that associated to significant components with a post-hoc Bonferroni correction applied for morph-sex class since morph-sex class was the only independent variable with more than two groups.

Results

Individual Variation

One-sample t-tests were run to ensure that there was significant within-group variation for each of the 11 compounds used in PCA analysis in order to test whether preen oil composition can significantly differ between members of the same morph-sex class. Variation within the tan male group (N=14) was significant for all 11 compounds used in the PCA analysis (t-value range=-13.040, -35.334; p-values= 0.000 for all). The same was true for white males (N=21, 20 for 1-decanol, 2-tridecanone, 2-pentadecanone; t = -17.018, -47.297; p= 0.000 for all), white females (N=10; t= -7.642, -46.479; p= 0.000 for all), and tan females (N=9; t= -11.114, -53.959; p =0.000 for all).

Principle Component Analysis and Discriminant Function Analysis

Three principle components from the PCA were included because they had eigen values over 1 and explained 34.99, 23.82, and 20.01% of the variance respectively (Table 4.1). Component 1 positively associated with 1-decanol, 1-undecanol, 1-dodecanol and negatively associated the larger, less volatile 1-pentadecanol, and 1-hexadecanol. Component 2 positively associated with 1-tridecanol and negatively associated with tetradecanoic acid and palmitic Acid. Component 3 positively associated with 2-tridecanone and 2-pentadecanone (Table 4.1).

A series of discriminant function analyses (DFA) classified individuals on their PCA scores based on sex, morph-sex class, year, and month. The sex DFA contained 1 significant discriminant function with an eigen value of 0.234 that explained 100% of the variance. Discriminant function 1 did successfully classify a significant number of individuals based on sex (Wilk's Lambda= 0.810, X^2= 10.417, p=0.015) with females scoring higher for principle component factor 1 (Wilk's Lambda =0.909, F= 5.133, df=1, 51, p= 0.028; Figure 4.1). Chi-squared tests for independence between actual and predicted found that the predicted groups were successful as a predictive tool (X^2= 12.755, p= 0.000). A DFA analysis for morph resulted in 1 discriminant function that did not successfully classify individuals by morph (Wilk's Lambda = 0.935, X^2= 3.340, p= 0.342). However, the DFA for morph-sex classes created 3 discriminant functions with eigen values of 0.301, 0.099, and 0.023 that explained 71.2%, 23.4%, and 5.4% of the variance respectively. Functions 1-3 were significant for classification (Wilk's Lambda= 0.684, X^2 18.425, p= 0.031; Figure 4.2) but functions 2-3 alone were not (Wilk's Lambda= 0.890, X^2= 5.663, p= 0.226). Chi-squared test for independence was significant (X^2= 17.682, p=0.039). One discrimant function with eigen value of 0.485 explained 100% of the variance for classifying by year. Function 1 for year classification was

significant (Wilk's Lambda= 0.673, X^2= 19.584, p= 0.000; Figure 4.3). Chi-squared test for independence was significant (X^2= 15.987, p= 0.000). One discriminant function for month alone could not significantly classify individuals (Wilk's Lamda= 0.857, X^2=7.335, p=0.062) but was marginally non-significant (Figure 4.4).

MANOVA Results

A MANOVA analyzed morph and sex in the same model for the three synthesized PCA variables found that variation between sexes was significant (F= 3.837, df= 3, 47, p= 0.015) but not for morph (F= 1.110, df = 3, 47, p= 0.355) or the interaction between morph and sex (F= 1.736, df=3, 47, p=0.172). Principle component 1 scores were higher in females (F= 5.133, p= 0.028) while principle component 3 scores were higher in males (F= 4.392, p= 0.041), but neither p-value was lower than the sequential Bonferroni adjusted p-value of 0.016. Post-hoc analysis of the compounds that associate with principle components 1 and 3 revealed that 2-pentadecanone varied between groups before Bonferroni sequential correction (F=7.305, p=0.009) but did not meet the corrected alpha value of p= 0.007. Variation between sexes appears to relate more to the overall relationship between compounds rather than a single compound.

A MANOVA analysis of morph-sex classes based on PCA components 1-3 showed that the four morph-sex classes vary by class (F=2.112, df= 9, 147, p= 0.032). Since there are 6 possible post-hoc class comparisons, a sequential Bonferroni adjustment of the alpha level requires that the most significant class comparison for any principle component be under an alpha level of 0.008 and the second lowest p-vale be under an alpha of 0.01. A LSD post-hoc showed that tan females scored lower on component 3 than tan males (Mean Difference =-1.02, p= 0.007, 95% C.I. [-1.75, -.029]) and tan females also scored lower on component 3 than white

males (MD= -0.93, p=0.009, 95% C.I. [-1.63, -0.24]). A post-hoc ANOVA analyzed class variation of 2-tridecanone and 2-pentadecanone that associate with component 3 and found that 2-pentadecanone varied between groups (F= 4.592, df= 3,50, p=0.006). The LSD post-hoc showed that tan females (N=9) had lower relative concentrations of 2-pentadecanone than white males N=20, p=0.001) and tan males (M=.034, p=.003, 95% C.I. [.027, .043]) and both post-hoc scores were lower than the sequential Bonferroni adjusted alpha levels of 0.008 and 0.01 respectively.

One more MANOVA analyzed temporal variation between the principle components by using year and month as independent factors. Variation between the year (F=7.882, df=3, 45, p=0.000) and month (F=3.517, df=3,45, p=0.022) collected was significant. Years varied in their scores on principle component 1 (F=20.903, p=0.000; Figure 4.3) while the months of June and July varied on their principle component 2 scores (F= 7.736, p=0.008; Figure 4.4). Both p-values were below the adjusted alpha value of .016. A post-hoc ANOVA revealed that Year 2010 (N=28) samples contained higher relative concentrations of 1-decanol (F= 9.759, p=0.003), 1-undecanol (F=23.999, p=0.000), and 1-dodecanol (F= 13.252, p=0.001), but lower concentrations of 1-pentadecanol (F= 16.530, p=0.000) and 1-hexadecanol (F=10.591, p=0.002) than 2009 samples (N=26). A post-hoc ANOVA showed that June samples (N=33) contained a significantly higher relative proportion of tetradecanoic (F= 5.701, p=.021) and palmitic acids (F=4.676, p=.035) than July (N=20).

Discussion

Volatile compounds found in preen oil have previously been suggested to be chemical signals useful for reproductive behaviors in a closely related genus to *Zonotrichia* (Soini et al. 2007; Whittaker et al. 2010). Whittaker et al. (2010) showed that individual juncos (*Junco*

hyemalis) have repeatable preen oil compound profiles, and individuals vary between each other in their profiles. One-sample t-tests for each morph and sex group of white-throated sparrows also showed individual variation for each of the 11 compounds used in the PCA, which were found in all, or almost all, individuals. The 11 compounds found in the majority of wild, breeding condition white-throated sparrows regardless of morph and sex were 1-decanol, 1-undecanol, 1-dodecanol, 1-tridecanol, 1-tetradecanol, 1-pentadecanol, 1-hexadecanol, tetradecanoic acid, palmitic acid, 2-tridecanone, and 2-pentadecanone. To date, all species analyzed have had unique preen oil volatile compound compositions (Haribal et al. 2005, 2009; Soini et al. 2007; Whittaker et al. 2010), and despite white-throated sparrows and juncos both being abundant in linear alcohols (Soini et al. 2007; Whittaker et al. 2010), the white-throated sparrow and dark-eyed junco are not identical in total number of compounds, or abundance of the compounds they share.

Morph and Sex Variation

White and tan morphs did not show any significant variation in preen oil composition, but males and females did. The difference between the preen oil composition of the sexes is not so much variation between individual compounds, but a difference in the overall chemical signature. The overall difference between then signatures was that males tended to invest more in heavier, less volatile linear alcohols and 2-ketones like 1-pentadecanol, 1-hexadecanol, 2-tridecanone, and 2-pentadecanone, while females invested more in lighter, more volatile linear alcohols like 1-decanol, 1-undecanol, and 1-dodecanol. Sexual variation could be supportive of either hypothesis: preen oil chemosignals or nest crypsis. Sexual selection of song and plumage act greatest on males, so if preen oil contains reproductive signals, then it would make sense for those signals to be sexually selected in males as well. Visual cues like feather coloration and hue

are correlated to fitness in white-throated sparrow males (Rathbun 2010), so if sexual variation is to explain the difference between male and female preen oil investment, either odor-preference for specific linear alcohols or 2-ketones should be tested or there should be a correlation between male fitness and preen oil composition.

The sexual variation between males and females could also be explained by the nest crypsis hypothesis (Reneerkens et al. 2005, 2007). Females may be investing more in shorter chain alcohols to help protect the nest from olfactory-keen predators. Since the three shorter chain alcohols are more volatile than the two longer chain alcohols, one might assume that females should have oil profiles more similar to males. However, while shorter chain alcohols are more volatile, they break down faster, causing them to loose their odors quicker than less volatile compounds. From personal observation, it is appears that females adjust to higher predation risk by nesting higher off the ground in shrubs as predation increases. The linear alcohols 1-decanol, 1-undecanol, and 1-dodecanol may also better match the scent of surrounding plant material (Soini et al. 2007) that also contain linear alcohols (Vioque and Kolattukudy 1997). Studying the olfactory preferences of predators, such as the common garter snake (*Thamnophis sirtalis*), would reveal which compounds females should invest in under the nest crypsis hypothesis.

Additionally, analysis of morph-sex classes found that tan females scored lower on component 3, and specifically 2-pentadecanone than both morphs of males (Figure 4.5). 2-pentadecanone has previously been shown to be an aggregation pheromone in *Drosophila busckii* flies (Schaner et al. 1989). The revelation that elephants and some insects share the same sex pheromones (Rasmussen et al. 1997) emphasizes that the same compounds can act as chemosignals in very different animals. Whether low concentrations of 2-pentadecanone are

useful for nest crypsis, or high levels of 2-pentadecanone in male white-throated sparrows leads to greater sexual attractiveness cannot currently be determined. However, since white males invest less in parental care than tan males (Knapton and Falls 1983, Kopachena and Falls 1993, Tuttle 2003), tan females are forced to carry the heaviest parental care load of any morph-sex class. Lower levels of 2-pentadecanone may create more cryptic odors that help tan females compensate for the reduced parental care of the white males. The parental care load of tan females should not be overlooked, because in years of high predation, tan male x white female nests are the most successful (Tuttle, unpublished data).

Temporal Variation

Evidence consistent with seasonal variation has been found in preen oil of the white-throated sparrow (see chapter 3) just as other avian species have previously shown (Jacob et al. 1979; Kolattukudy et al. 1987; Bohnet et al. 1991; Reneerkens et al. 2002, 2007; Soini et al. 2007). However, preen oil also exhibited monthly variation during the breeding season. Birds sampled in June contained higher proportions of tetradecanoic and palmitic acid than July. Fatty acids, like tetradecanoic and palmitic acid, have previously been proposed to make up part of an individual's olfactory signature (Nicolaides 1974). Tetradecanoic and palmitic acid were recently used as analogs to porcine appeasing chemical cues (Guiraudie-Capraz et al. 2005), and could potentially be chemical signals in birds as well. Females start the breeding season with synchronous nesting but females become more asynchronous as the season progresses (Tuttle 2003). Tuttle (2003) previously found that the synchronicity index, as used by Bjorklaud and Westman (1986), was around 44% for the first clutch but dropped to approximately 24% for the second clutch. If tetradecanoic acid and palmitic acid are chemical signals, then adults that have had successful nests by July may no longer need as much investment in tetradecanoic acid or

palmitic acid. Based on the month differences, both the social chemosignal and nest crypsis hypothesis could be possible. The differences in preen oil composition between months could also represent within season variation based on an individual's reproductive stage (i.e. nest building, egg laying, incubation, nestling care, or fledgling care). While the principle component scores did not vary between stages, 2-tridecanol peaks at multiple points throughout the breeding season, which may align with a specific stage.

Preen oil composition also varied between the years 2009 and 2010 with 2010 samples containing higher proportions of lower chain alcohols and lower proportions of higher chain alcohols compared to 2009. The Cranberry Lake Biological Station white-throated sparrow population has been studied for over 20 years and it has been proposed that individuals might use more monogamous or more promiscuous mating strategies depending on the availability of resources or predation in a given year (Tuttle 1993). If preen oil does contain compounds that act as social chemosignals, they may make up less of the overall composition in years when a less promiscuous strategy will lead to higher fitness. Additionally, the variation in the proportion of different compounds may accurately reflect different predation threats since 2010 had more nest predation than 2009 (pers. obs.). According to the nest crypsis hypothesis, one may expect that in years with increased predation, preen oil should be more similar to that of the incubating sex, females, which is what was observed.

Reproductive Chemical Signals in Preen Oil?

Chemical compounds must meet many requirements before they can be labeled as chemical signals, especially ones used to convey reproductive information. That is because chemical signals should vary between both species and sexes to ensure that the intended receiver can respond correctly to the proper receiver (Johansson and Jones 2007; Whittaker et al. 2010).

The chemical signal may also convey information about the individual's fitness, compatibility, or sexual attractiveness (Wyatt 2003; Johansson and Jones 2007). The preen gland has been shown to be important for proper reproductive behavior (Hirao et al. 2009) and more studies, including this study, are continuing to expand our knowledge of preen oil secretions at a chemical level (Reneerkens et al. 2002, 2005, 2007; Haribal 2005, 2009; Soini et al. 2007; Whittaker et al. 2010). Recent reviews of avian olfaction suggest that avian chemical cues may be more common than ever thought (Hagelin and Jones 2007; Balthazart and Taziaux 2009). Future studies should further test the hypotheses of preen oil as a chemosignal and or the involvement of preen oil in nest crypsis. Y-maze odor-choice experiments could test the attractiveness of synthetic compounds (Hagelin et al. 2003) found in white-throated sparrow preen oil or whether birds can recognize the preen oil of their social mate over that of another conspecific (Bonadonna and Nevitt 2004; Mardon and Bonadonna 2009). Odor experiments that test the behavioral response of a potential predator, such as tongue-flicking in common garter snakes, could provide evidence that preen oil compounds are ecologically relevant as nest masking compounds.

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Table 4.1 Principle component analysis synthesized factors and their eigen values, % variance explained, and rotated component matrix (varimax).
Any components scoring higher than .600 (in bold) were deemed strongly associated with that principle component.

	Component		
	1	2	3
Eigen Values	3.848	2.621	2.209
% Variance Explained	34.99	23.82	20.01
1-Decanol	**.827**	.037	-.138
1-Undecanol	**.939**	-.055	-.066
1-Dodecanol	**.869**	.406	.039
1-Tridecanol	.044	**.844**	.147
1-Tetradecanol	-.484	.471	-.157
1-Pentadecanol	**-.852**	.224	-.252
1-Hexadecanol	**-.726**	-.003	-.458
Tetradecanoic Acid	.016	**-.802**	.164
Palmitic Acid	-.036	**-.897**	.168
2-Tridecanone	.179	-.076	**.950**
2-Pentadecanone	-.067	-.114	**.952**

Figure 4.1. Visual representation of the discriminant function analysis classification for female and male samples.
95% confidence interval circles surround the mean for each group. Direction and distance of the principle component axes represent the direction and importance of each principle component score towards the discriminant function scores respectively. Males, on average scored higher for discriminant function 1, which positively associates with principle component 3.

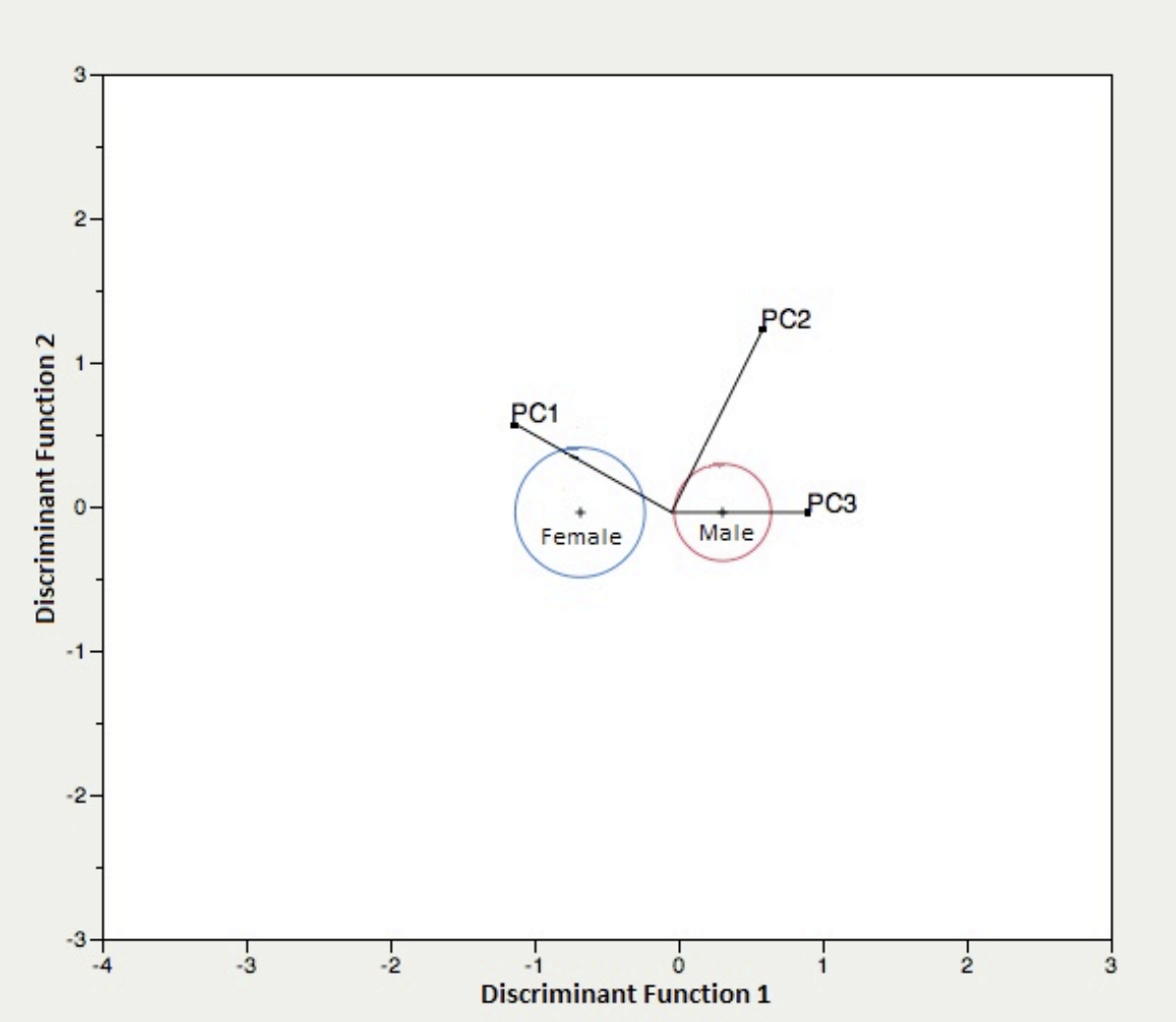


Figure 4.2. Visual representation of the discriminant function analysis classification for morph-sex classes.
95% confidence interval circles surround the mean centroid for each group. Direction and distance of the principle component axes represent the direction and importance of each principle component score towards the discriminant function scores respectively. Average centroids are close enough for 95% confidence intervals to overlap between all groups. However, tan females associate less with principle component 3.

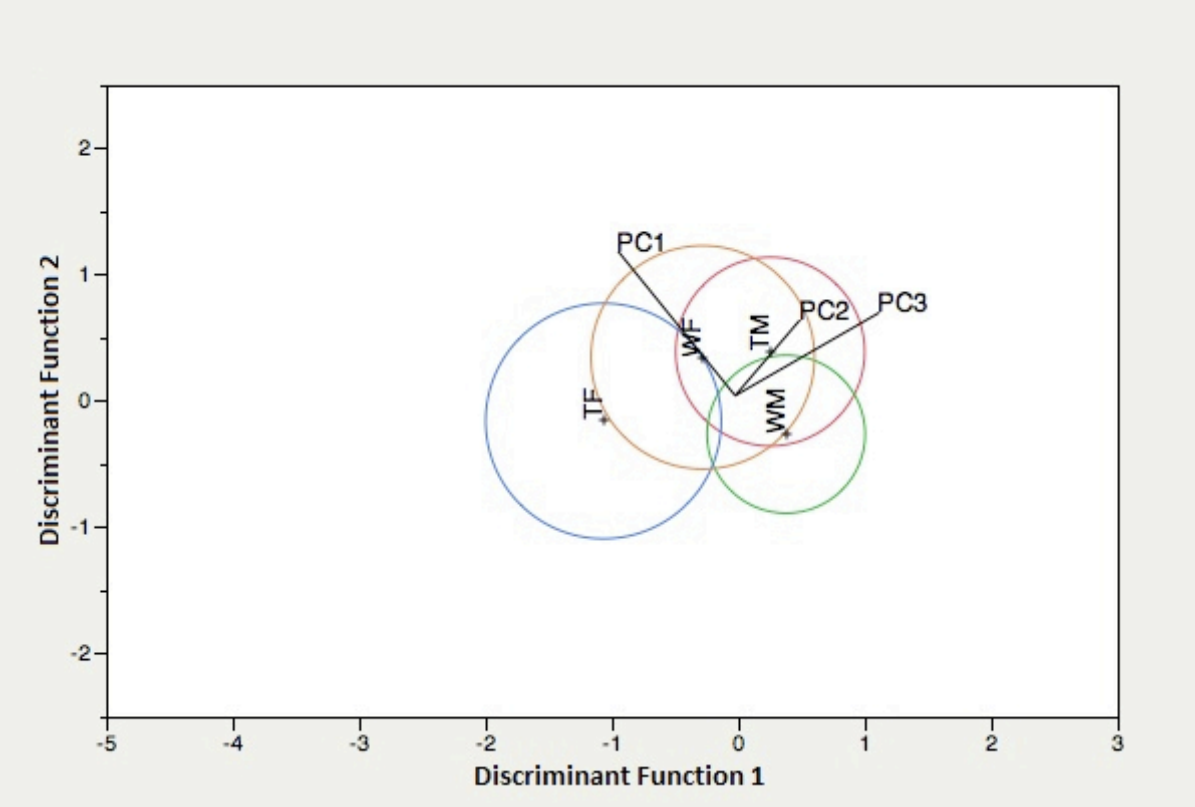


Figure 4.3. Visual representation of the discriminant function analysis classification for 2009 and 2010 samples.
95% confidence interval circles surround the mean for each group. Direction and distance of the principle component axes represent the direction and importance of each principle component score towards the discriminant function scores respectively. Average 2010 scores are higher on discriminant function 1 than 2009, and function 1 associates with principle components 1 and 3.

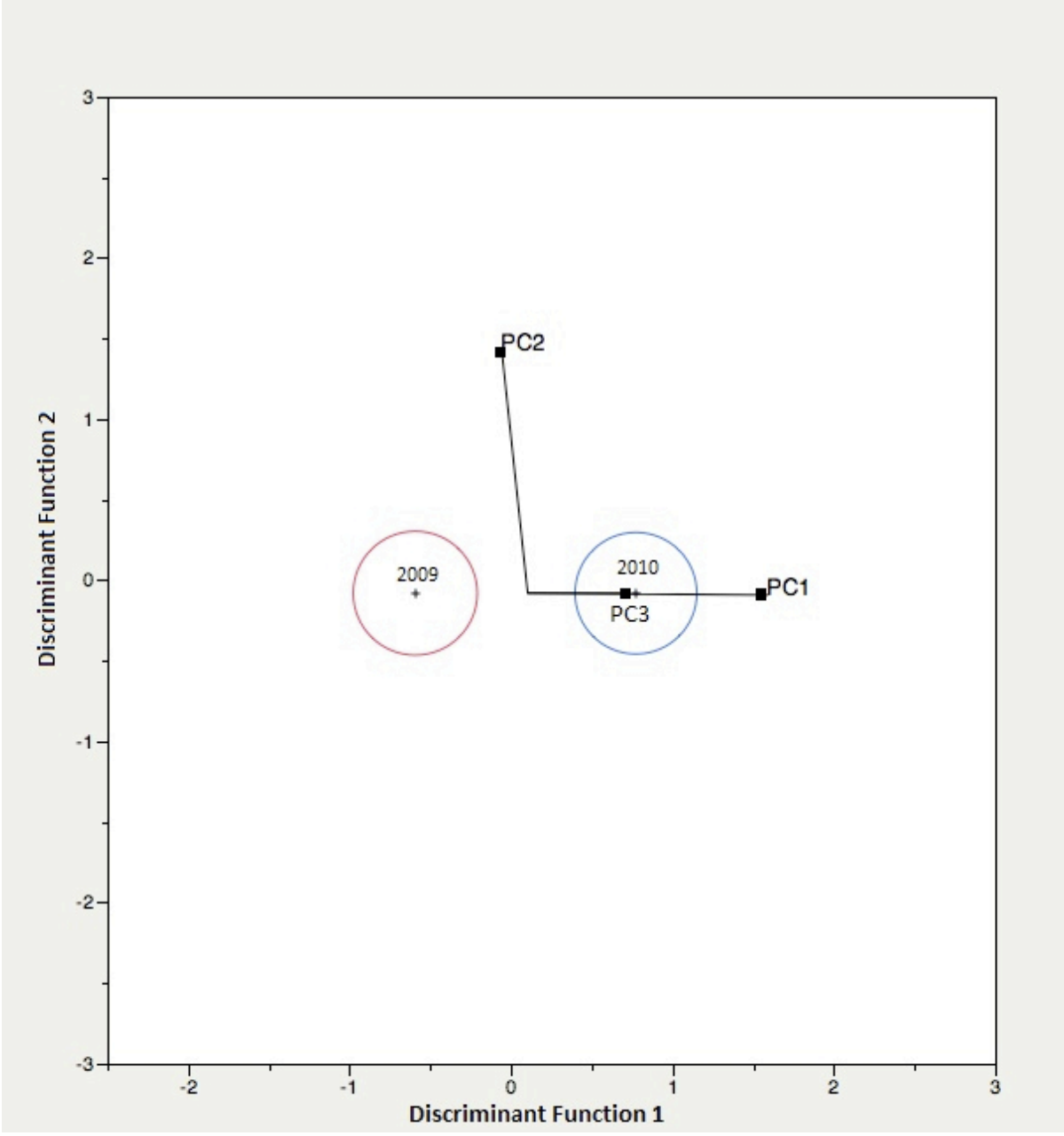


Figure 4.4. Visual representation of the discriminant function analysis classification for June and July samples.
95% confidence interval circles surround the mean for each group. Direction and distance of the principle component axes represent the direction and importance of each principle component score towards the discriminant function scores respectively. Average July scores are higher on discriminant function 1 than June, and function 1 associates with principle component 2.

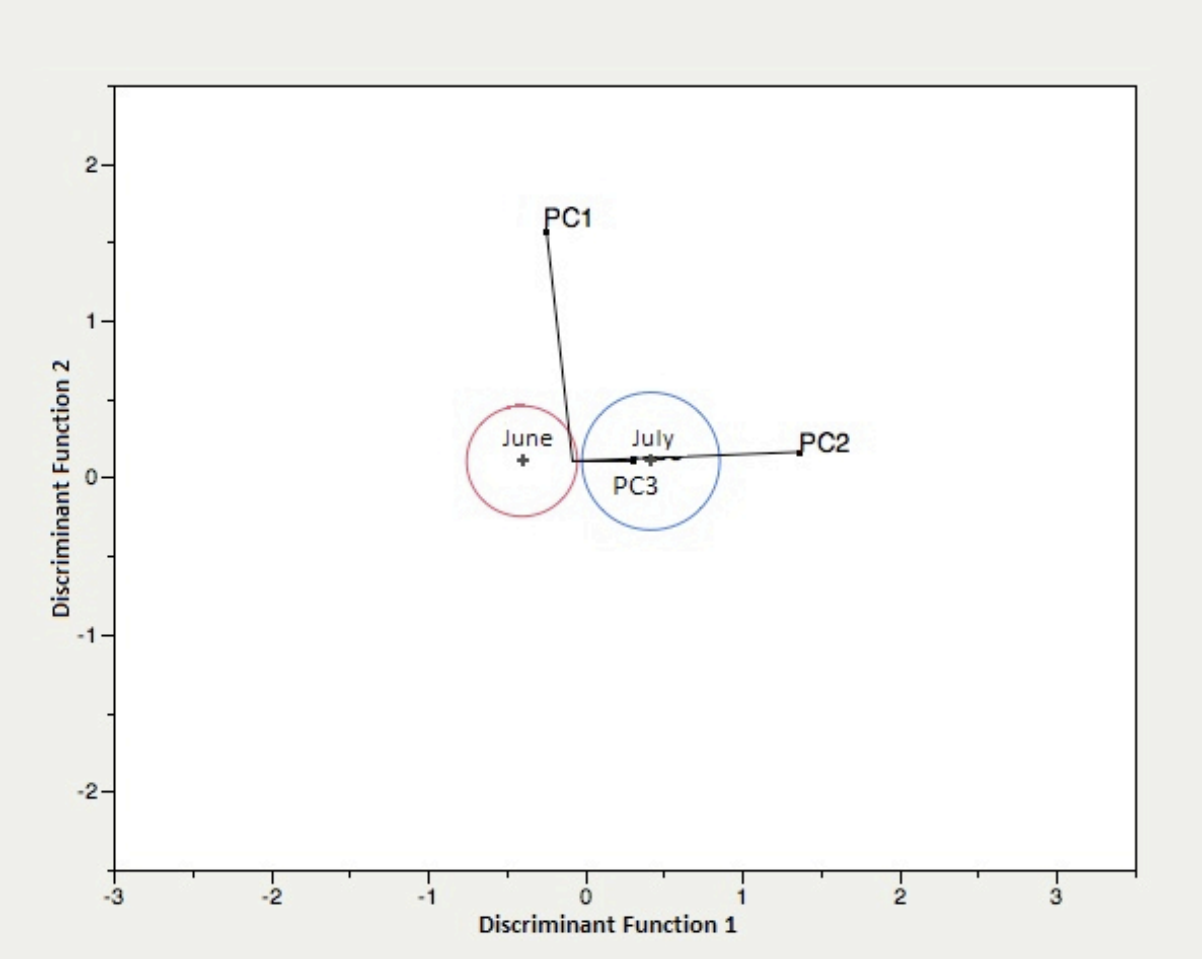
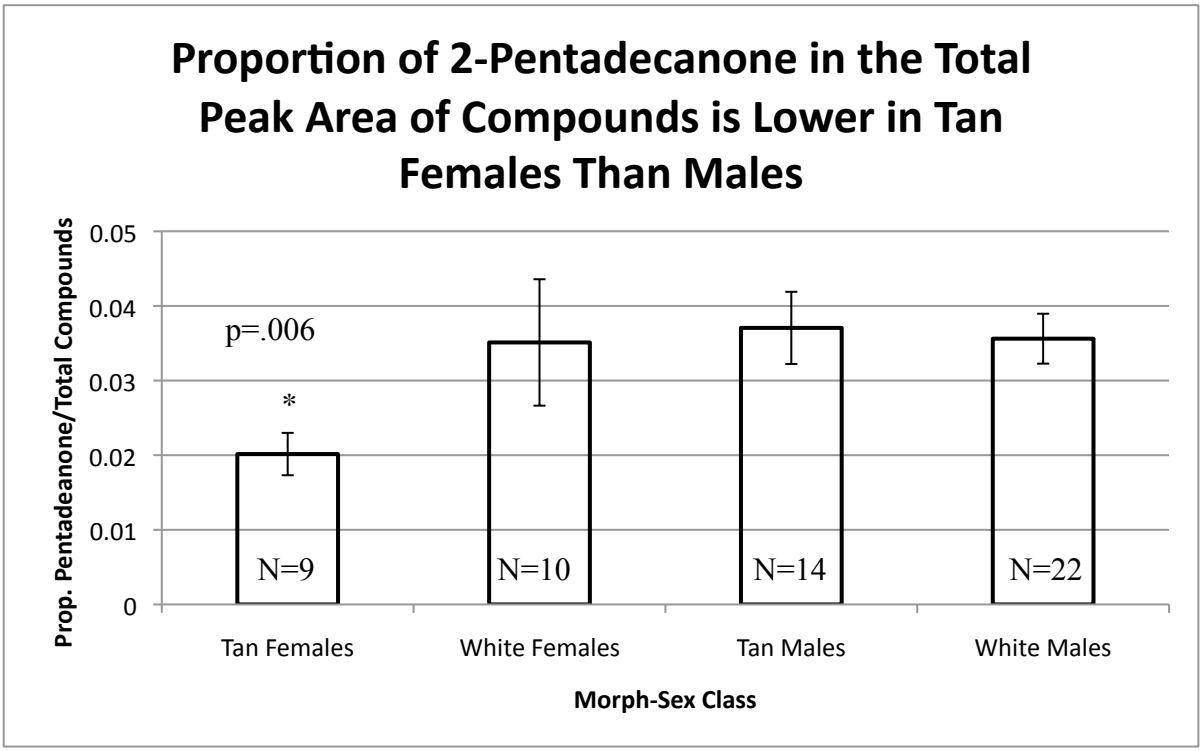


Figure 4.5. Graph showing the proportion of 2-pentadecanone to that of the sum of all peak areas from all major compounds for each morph-sex class.
Tan females have a significantly lower proportion of 2-pentadecanone in their preen oil composition than tan males and white males. Error bars represent standard error.



CHAPTER 5

DIFFERENCES BETWEEN CAPTIVE AND WILD PREEN OIL IN WHITE-THROATED SPARROWS, THESIS CONCLUSIONS, AND FUTURE DIRECTIONS

Introduction

Recent studies on uropygial, or preen gland, secretions have investigated its use as a social chemical signal (Hirao et al. 2009; Mardon et al. 2010; Whittaker et al. 2009, 2010) and as source of nest masking compounds (Reneerkens et al. 2002, 2007). Mardon et al. (2010) recently looked at volatile preen oil compounds in two species of Procellariiformes, and other studies have examined non-volatile precursors in other bird populations (e.g. Piersma et al. 1999; Reneerkens et al. 2002, 2007; Haribal et al. 2005, 2009, Thomas et al. 2010). Meanwhile, volatile compounds from dark-eyed juncos (*Junco hyemalis*) have been studied in captive conditions that simulated natural winter and breeding photoperiods (Soini et al. 2007; Whittaker et al. 2010). However, there remains a large gap in our understanding of the differences between captive and wild preen oil composition. Whittaker et al. (2010) states that wild and captive individuals showed similar chemical signatures, but the authors state unpublished data. Meanwhile, Thomas et al. (2010) provides evidence of captive and wild individual variation in white-throated sparrows, but only for non-volatile compounds. Here we analyze the difference between samples collected from captive white-throated sparrows in artificial breeding condition and wild caught white-throated sparrows during the breeding season from 2 different years. Bird

models such as the Antarctic prion *Pachyptila desolata* (Bonadonna and Nevitt 2004; Mardon et al. 2010), blue petrel *Halobaena caerulea* (Mardon and Bonadonna 2009; Mardon et al. 2010), crested auklet *Aethia cristatella* (Hagelin et al. 2003), and dark-eyed junco *Junco hyemalis* (Soini et al. 2007, Whittaker et al. 2009, 2010) are rare in that they offer both behavioral and chemical evidence for avian chemical signals. The aim of this chapter is to review the results of the behavioral and chemical studies presented in this thesis to determine what can be learned from viewing the previous chapters together as well as what are the most logical future directions.

Methods

Captive and Wild samples in breeding condition were analyzed for volatile compounds using GC-MS as in chapters 3 and 4. Peak areas or abundances for each compound were standardized by the peak area of the internal standard 7-tridecanone (Soini et al. 2007, Whittaker et al. 2010). Peak areas were then divided by the total sum of peak areas for each compound identified to create proportions and the proportions were logit transformed according to Whittaker et al. (2010). Ten of 28 compounds were present in the majority of individuals, and thus were chosen to be variables in a principle component analysis (PCA). The 10 compounds were also 10 of the 11 compounds used in the PCA for wild birds (Chapter 4), but 1-decanol was not used since it was present in only 39% of captive breeding condition samples. 1-nonanol was unique in that it was only found in wild samples, but not with very high consistency. Discriminant function analysis was used to classify individuals based on whether they were sampled during breeding condition in captivity in 2010 (N=15), the wild population during 2009 (N= 26), or the wild 2010 population (N= 27). Multivariate analysis of variance (MANOVA) was used to determine whether the three groups varied based on the synthesized principle

components. Sequential Bonferroni adjustments were made to the alpha values of the overall significance of each principle component (Holm 1979). A Bonferroni post-hoc was run to determine which groups varied from each other on each significant principle component.

Results

Principle component analysis created 3 synthetic components with eigen values over 1 that explained 43.11%, 21.49%, and 17.56% of the variance respectively. Component 1 positively associated with the lower-chain alcohols (1-undecanol, 1-dodecanol, 1-tridecanol) but negatively associated with higher-chain alcohols (1-tetradecanol, 1-pentadecanol, 1-hexadecanol). Component 2 positively associated with 2-pentadecanone and 2-tridecanone while component 3 positively associated with tetradecanoic acid and palmitic acid (Table 5.1).

The discriminant function analysis synthesized two separate functions with eigen values of 2.886 and .100 and % variance explained of 96.6% and 3.4% respectively. Functions 1 through 2 were significantly able to categorize the three groups (Wilk's Lambda =0.234, X^2=92.986, p=0.000), and function 2 alone also could categorize the groups (Wilk's Lambda= 0.909, X^2=6.109, p=0.047). A graph with function 1 by function 2 illustrates the separation between group mean centroids and that principle component 1 scores positively associate with discriminant function 1 scores (Figure 5.1). A Chi-square Test of independence between the actual group and the predicted group classification indicated that classification was significantly better than chance (X^2= 76.578, p= 0.000).

A MANOVA with year as the independent and the three principle components as the independents found that there was significant variation between the year groups for both component 1 (F=55.755, p=0.000) and component 2 (F=4.753, p=0.012) even after the sequential Bonferroni adjustment. A Bonferroni post-hoc revealed that captive samples scored

significantly lower on principle component 1 (N=15, Mean= -1.39, SD= 0.90) than wild years 2009 (N= 26, M=0.08, SD= 0.54, p=0.000) and 2010 (N=27, M=0.70, SD=0.48, p=0.000). Additionally, wild samples from 2009 scored significantly lower than wild samples from 2010 (p=0.002), which fits with the year differences found between only wild samples (Chapter 4). For principle component 2, captive samples (N=15, M=0.51, SD=0.90) scored significantly higher than wild 2009 samples (N=26, M=-0.41, SD= 1.14, p=0.012), but not significantly higher than wild 2010 samples (N=27, M=0.11, SD= 0.75, p=0.58). 2009 and 2010 wild samples did not differ in their principle component 2 scores (p=0.155).

Discussion

Captive Versus Wild Samples

DFA and MANOVA of the synthesized principle components revealed that there is a difference in the investment of principle component 1, or linear alcohols between wild and captive breeding populations. A trade-off is apparent in which wild samples invested more in the 3 lower-chain linear alcohols than the 3 higher-chain linear alcohols, but captive samples invested in the opposite. When the shorter-chain alcohols make up more of the overall preen oil composition, it comes at a cost to the longer-chain alcohols and vice versa. The exact reasons behind this difference are unknown, but diet could be an important factor (Haribal et al. 2005, Thomas et al. 2010). Thomas et al. (2010) showed evidence that white-throated sparrow non-volatile compounds from preen oil can be enhanced with enhanced diet. Photoperiod has previously been used as a cue of breeding-condition for preen oil studies (e.g. Soini et al. 2007; Whittaker et al. 2010; Thomas et al. 2010) and for behavioral studies on white-throated sparrows (e.g. Tuttle 1993). However, there may be other cues that affect preen oil composition during the breeding season in wild populations such as environmental factors. Captive males in breeding

condition sang, but could not engage in typical wild breeding condition behaviors, such as territorial aggression, mate guarding, and copulation. If preen oil compounds do increase nest crypsis (Reneerkens et al. 2002, 2007) or contain social chemosignals (Whittaker et al. 2010; Mardon et al. 2010), captive populations may differ from wild populations simply because they are not able to nest or fully investigate potential mates and competitors.

Pair types of the white-throated sparrow (tan male by white female and white male by tan female) typically nest in different habitat types that differ in the density of neighbors (Formica et al. 2004; Formica and Tuttle 2009). The captive population in this study featured an artificially high level of neighbors (N=18) while typical wild individuals have only 1-2 neighbors to their territory (Formica et al. 2004). With such a large increase in neighbor density in the captive study, captive birds may have over adjusted their preen oil composition in favor of the longer-chain alcohols. 2010 featured higher levels of predation than 2009 (Tuttle, unpublished data), which may have had a direct effect on the importance of particular compounds such as longer or shorter linear alcohols. White-throated sparrows typically nest on the ground, but may also nest slightly off the ground (Tuttle 2003). Increased predation as the season progresses may cause a behavioral change in the height at which females nest, and thus have an indirect effect on the preen oil composition. Captive populations face no threat of nest predation, and thus may not alter their preen oil as much. Future preen oil studies on the preen oil of birds should consider all the added factors that can explain variance in captive populations.

Thesis Conclusions

Few avian models have shown behavioral and chemical evidence for chemical cues (Hagelin et al. 2003; Bonadonna and Nevitt 2004; Soini et al. 2007; Mardon and Bonadonna 2009; Whittaker et al. 2009, 2010; Mardon et al. 2010). This thesis has investigated behavioral

odor preference for fecal material as well as preen oil composition in captive and wild individuals, and because of this is able to make further inferences than one study alone. Sexual variation in preen oil was evident in both captive winter samples and wild breeding condition samples. Preen oil may have very different uses between the sexes since females are responsible for nest incubation (Reneerkens et al. 2002, 2007). Social interactions and social dominance (Wiley et al. 1999) may cause males and females to maintain variation in volatile compounds of preen oil, even in the winter. An increased sample size of females would also determine whether females differ from males in captive breeding conditions.

Morph-sex classes varied in preen oil chemistry and in their odor choice between self and control odors. Tan males and white females together exhibited a choice for the control odor while white males and tan females exhibited a choice for self odor, which suggested that even odor preference is maintained by alternative strategies between pair types (Tuttle 2003). In captive winter conditions, tan males had higher abundances of 1-tetradecanol, 1-pentadecanol, and 1-hexadecanol than white males. In the wild population, tan females had on average the lowest proportion of 2-pentadecanone to the overall chemical signature. White-throated sparrows morph-sex classes have unique naturally and sexually selective pressures (Tuttle 1993, 2003; Formica et al. 2004, Rathbun 2010). Even subtle differences in preen oil chemistry or odor preference between the morph-sex classes can add to the growing understanding of the white-throated sparrows polymorphism (Thorneycroft 1966, 1975).

Finally, both the captive study and the wild study illustrated that temporal variables can have an important role in preen oil chemistry, whether it be season, year, or month. The results of this thesis suggest that preen oil chemistry is constantly changing, and may vary based on

environmental factors. Future studies on preen oil chemistry should be careful to account for as much temporal variation as possible.

Future Directions

The results of the multiple experimental chapters in this thesis are suggestive of various recent hypotheses regarding avian chemical cues. The first hypothesis is that birds transmit chemical signals that may alter or influence the response of the receiver (Johnston 2000; Hagelin 2007). This thesis has shown some behavioral evidence that avian feces contain chemical cues, and chemical evidence of potential chemosignals in preen oil. However, to further test this idea, further odor-choice tests are necessary. Bonadonna and Nevitt (2004) and Mardon and Bonadonna (2009) tested odor preference for 1) mate versus conspecific, 2) own versus mate, and 3) own versus conspecific. Future odor-preference trials on white-throated sparrows could also be done on wild individuals and examine these additional odor choices.

What was unclear from the white-throated sparrow trials was whether tan by white pairs were avoiding their own scent as an inbreeding avoidance tactic (Mateo and Johnston 2000; Bonadonna and Nevitt 2004; Mardon and Bonadonna 2009) or due to the aversiveness of fecal odors such as ammonia (Jones et al. 2005). Additionally, a choice between white and tan odors may not result in a preference, but a choice between the odor of a mate and the odor of a conspecific may result in a preference for the odor of the mate for tan males, but a preference for the odor of a conspecific for white males due to the white-throated sparrows alternative reproductive strategies (Tuttle 2003). Y-maze choice trials could be done with preen oil as the odor source instead of fecal material. Recent evidence suggests that volatile compounds in preen oil may act as social chemosignals (Mardon et al. 2010; Whittaker et al. 2010). Additionally,

future studies should investigate the correlation between preen oil composition and fitness since there is a correlation between plumage and fitness in white-throated sparrows (Rathbun 2010).

The second hypothesis suggested in this thesis is that birds utilize intraspecifically produced chemicals for nest crypsis. Sexual and class variation from chapter 4 suggests that volatile preen oil compounds in the white-throated sparrow may play a role in nest crypsis from olfactory-keen predators. Reneerkens et al. (2005) used a trained sniffer dog to suggest that seasonally produced diesters were harder for predators to locate than the monoesters found in winter preen oil. However, trained dogs are not an ecologically relevant predator for white-throated sparrow populations. Garter snakes (*Thanophis sirtalis*) are an ecologically relevant nest predator at Cranberry Lake Biological Station. Previous studies on garter snakes have shown their ability to detect odors from prey through the use of tongue flicking (Halpern et al. 1997). Halpern et al. (1997) used a series of simple tests to test for garter snake olfactory ability that could be used to determine whether they also prefer the volatile odors emitted from white-throated sparrows. The tests used were tongue flick counts when presented with prey odor versus tongue flick counts when presented with a control odor, and a Y-maze odor choice for either the control or the prey odor (Halpern et al. 1997). Tongue flicking response of garter snakes could be compared between compounds elevated in females such as 1-undecanol or 1-dodecanol, and compounds that make up more of a male preen oil profile such as 1-hexadecanol. Further Y-maze trials with snakes could test for a preference between a single volatile compound and a control, or one volatile compound versus another.

The results presented in this thesis show exciting evidence that white-throated sparrows may utilize intraspecific chemical cues, and that white-throated preen oil varies between seasons in captive populations, sex, morph-sex class and time in wild populations. Future directions will

be able expand upon data presented here in order to better understand 1) whether songbirds such as the white-throated sparrows use chemical signals, and 2) the complete benefits of preen oil during the breeding season.

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Table 5.1 Principle component analysis synthesized factors and their eigen values, % variance explained, and rotated component matrix (varimax) for wild and captive breeding condition samples combined.
Any components scoring higher than .600 (in bold) were deemed strongly associated with that principle component.

	Component		
	1	2	3
Eigen Values	4.311	2.149	1.756
% Variance Explained	43.11	21.49	17.56
1-Undecanol	**.926**	-.216	.162
1-Dodecanol	**.956**	-.193	-.003
1-Tridecanol	**.830**	-.088	-.322
1-Tetradecanol	-.361	-.264	-.494
1-Pentadecanol	**-.851**	-.080	-.268
1-Hexadecanol	**-.797**	-.216	-.299
Tetradecanoic Acid	.273	-.126	**.830**
Palmitic Acid	-.194	.006	**.903**
2-Tridecanone	.084	**.968**	.024
2-Pentadecanone	-.238	**.937**	-.021

Figure 5.1. Visual representation of the discriminant function analysis classification for captive 2010, wild 2009, and wild 2010 samples.
95% confidence interval circles surround the mean for each group. Direction and distance of the principle component axes represent the direction and importance of each principle component score towards the discriminant function scores respectively. Wild 2010 samples scored the highest on principle component 1 and discriminant function 1, but 2010 captive samples were further separated than 2009 and 2010 wild samples.