THE ROLE OF CHEMICAL INFORMATION IN THE BREEDING BIOLOGY OF THE LEACH'S STORM-PETREL (*Oceanodroma leucorhoa*)

By

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TABLE OF CONTENTS

ABSTRACT

Chemical communication has been understudied in vertebrates, but no group has been more overlooked than birds, where scent guided behaviors are cryptic, and until recently, olfaction was considered unimportant. It is now well established that birds have a functional sense of smell, yet due to the relative infancy of this field, there is still little known about how chemicals inform the social lives of birds. The overarching objectives of this dissertation were two-fold. First, to increase our understanding of how chemical information aids avian breeding behavior; and second, to develop improved analytical methods for identifying and measuring bird-produced odors, with a focus on the volatile chemicals that may function in communication.

With these two objectives in mind, I completed three projects to investigate how chemicals facilitate the reproductive lives of the Leach's storm-petrel (*Oceanodroma leucorhoa*). This long-lived, monogamous seabird species is an ideal system for studying avian chemical communication. Leach's storm-petrels perform their reproductive behaviors under the cover of darkness, but lack good vision in low light conditions, indicating a reliance on non-visual communication. Moreover, they have a large olfactory anatomy, an excellent sense of smell, and a pungent plumage odor.

In the first chapter of this dissertation, I used gas-chromatography mass spectrometry to examine the social information present in the strong odor of Leach's storm-petrels. I discovered that despite interannual variation, the feather chemicals reflected individual identity, which may help storm-petrels maintain their long-term mate bonds.

In vertebrates, the immune genes of the Major Histocompatibility Complex (MHC) influence mate choice decisions and enable individual recognition. Moreover, MHC shapes

body odor and organisms may use olfaction to evaluate the MHC genotype of conspecifics. In the second chapter of this dissertation, I tested the hypothesis that the plumage chemicals of Leach's storm-petrels contain information about MHC. This study was motivated by a recent finding that male Leach's storm-petrels make MHC-based mate choice decisions to select high quality females. I found that feather odors reflected two aspects of MHC genotype: similarity and diversity. These findings were particularly strong for females, which implicates olfaction as a likely mechanism enabling MHC-informed mate choice by males.

Making trips to and from their nest is an important behavior that all birds must accomplish to ensure reproductive success. In the final chapter of this dissertation, I investigated how chemicals inform olfactory homing in Leach's storm-petrels. I analyzed the odors associated with the colony floor, storm-petrel nests, and their avian occupants. I identified gradients of environmental chemicals across the colony, which may aid homing in storm-petrels. Furthermore, I discovered that storm-petrel chemicals are deposited inside the nest, creating unique nest odors that reflect the specific storm-petrel occupants. This is the first evidence of bird nests being scented by the owner's body odor, which likely facilitates olfactory nest recognition in this species.

In summary, the integrative research presented in this dissertation connects the fields of animal behavior, analytical chemistry, and molecular biology to highlight chemicals as an important source of information for breeding birds.

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Instructions for living a life:
Pay attention.
Be astonished.
Tell about it.

Mary Oliver

I have learned many important lessons over the course of my graduate school experience, but two in particular that I will carry forward. The first is that my ability to persist in the face of adversity is much stronger than I ever imagined. Almost everything about this experience has been unexpected, both the enjoyable and the trying. I have learned that challenging does not mean impossible, and that hard work and commitment do eventually pay off. I have also realized the importance of having a solid team. The research process inevitably presents challenges, but when surrounded by supportive colleagues, peers, and loved ones, these experiences can offer amazing opportunities for growth and discovery. I would like to thank all the individuals who have helped me on this journey.

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Artwork by Amy E. Miles. Used with the artist's permission

INTRODUCTION

Chemicals are the oldest and most widespread mechanism that organisms use to evaluate their environment and to communicate with others (Bradbury and Vehrencamp 2011; Stevens 2013). Two main chemical senses provide organisms with information: the gustatory system for taste, which perceives a handful of sensations at close range, and the olfactory system for smell, which has been implicated in many aspects of animal behavior because it detects a multitude of odorants across a range of spatial scales (Müller-Schwarze 2006). Chemicals allow organisms to move through a landscape, locate food, avoid predators, find mates, and recognize kin (Wyatt 2014). However, our understanding of how chemicals mediate behavior comes mainly from invertebrates and has been studied to a much lesser extent in vertebrates. In particular, the role chemicals play in the lives of birds has been notably neglected, despite birds serving as important models for animal communication and sensory ecology research.

Scent guided behaviors are difficult to identify in birds; they do not crinkle their nose in response to an odor, their beak is a relatively fixed structure, and their nares are often inconspicuous. Consequently, it is not surprising that the idea that birds lack a functioning sense of smell persisted for centuries. Famous ornithologist, John James Audubon, was in part to blame for the pervasive "birds-can't-smell" fallacy. In the 1820s, he was intent on proving that vultures use sight, not scent, to locate food. In a series of experiments, Audubon found that vultures were attracted to a visible stuffed deer skin, but not to the smell of a concealed decaying pig carcass. He presented the results to the Natural History Society of Edinburg in 1826 in a paper titled "An Account of the habits of the turkey buzzard (*Vultur aura*), particularly

with the view of exploding the opinion generally entertained of its extraordinary power of smelling" (Audubon 1826).

While not everyone was convinced by Audubon's conclusions, it took until the 1960s before a number of studies provided compelling evidence for functional olfaction in birds. Kenneth Stager of the Los Angeles County Museum played a major role in debunking Audubon's ideas about vultures. He showed that scavenging turkey vultures use ethyl mercaptan, a compound emitted by rotting flesh, as an olfactory cue to locate freshly dead prey (Stager 1964). Stager also found that the turkey vultures lose interest in the carcass when it is more than four days old, which likely explains why the far-from-fresh pig used by Audubon failed to entice any birds.

At the same time as Stager, two female scientists, Betsy Bang and Bernice Wenzel, made important discoveries about the avian sense of smell. In a series of papers, Bang described the olfactory anatomy of over 150 bird species (Bang 1960, 1971; Bang and Cobb 1968). She noted that while all bird species possessed olfactory anatomy, the size of the olfactory bulb relative to the cerebral hemisphere varied widely across species from as little as 3% in the black-capped chickadee (*Parus atricapillus*) and up to 37% in the snow petrel (*Pagadroma nivea*; Bang 1971). Bang's contemporary, Bernice Wenzel, was an electrophysiologist who studied olfactory perception. In her first experiment on rock doves (*Columba livia*), she found that the heart rate of doves increased after exposure to an odor indicating that the stimulus had been centrally processed by the brain (Sieck and Wenzel 1969). Wenzel went on to perform experiments on 6 additional bird species, showing that all of them responded to odors (Wenzel and Sieck 1972).

Inspired by the findings of Stager, Bang, and Wenzel, other researchers began to explore how chemicals inform avian behavior, with efforts primarily focusing on non-social contexts.

The findings of this research uncovered a wide array of functions for olfaction in birds. In addition to turkey vultures, a number of species use their sense of smell to locate food (Grubb 1972; Hutchison and Wenzel 1980; Nevitt et al. 1995). Olfaction also facilitates navigation (for reviews see Gagliardo 2013; Papi 1989) and homing to the nest (Bonadonna and Bretagnolle 2002; Grubb 1974). At the nest, some birds maintain an aromatic environment by adding plant materials that they select using olfactory cues (Clark and Mason 1985, 1987; Gwinner and Berger 2008; Petit et al. 2002). Moreover, several bird species use odors to detect and avoid predators (Amo et al. 2008; Roth et al. 2008).

Ornithologists also began to consider the strong scents produced by birds, which were initially explored as possible chemical defenses (Weldon and Rappole 1997). The acrid scent of members of the genera *Pitohui* and *Ifrita*, which sequester toxic chemicals in their feathers and skin, was investigated as a predator deterrent (Dumbacher et al. 1992, 2000; Dumbacher and Pruett-Jones 1996). Other birds adopt an alternative strategy and shift to producing fewer scented chemicals during the breeding season to conceal themselves from predators (Reneerkens et al. 2002, 2005). Bird-produced chemicals can also act as repellents for lice, ticks, and feather degrading bacteria (reviewed in Hagelin and Jones 2007). Growing interest in the function of bird odors spurred research into their chemical make-up (Campagna et al. 2012; Haribal et al. 2009; Nevitt and Prada 2015; Soini et al. 2013). These studies uncovered the large diversity of compounds produced by birds, which is a crucial step for disentangling how chemical information influences avian ecology.

Much of the research into olfactory mediated behavior in birds has focused on chemical information present in the form of cues. Cues provide relevant information because they are

correlated with a condition of interest, but they are produced inadvertently as a byproduct of non-communication behaviors or processes (Bradbury and Vehrencamp 2011). In contrast, signals have evolved specifically for communicating information. Signals are produced by a sender and alter the behavior of the receiver in a way that benefits both parties (Bradbury and Vehrencamp 2011). Birds are largely assumed to use visual and acoustic signals for communication. However, the idea that birds may also use chemicals to communicate has recently emerged. Two landmark studies propelled this idea to the forefront. Hagelin et al. (2003) showed that crested auklets (*Aethia cristatella*), a citrus scented seabird, are attracted to conspecific odors and have a preference for chemicals that become elevated during the breeding season. The following year, partner specific odor recognition was described in another species of seabird, the Antarctic prion (*Pachiptila desolata*; Bonadonna and Nevitt 2004).

There are a multitude ways birds could use chemicals for communication, with the vast majority relating to some aspect of their breeding biology. Chemicals may aid in species recognition; allow individuals to identify members of the opposite sex; facilitate the evaluation of potential mates; and enable recognition between mates or parents and offspring (Johansson and Jones 2007a). To date, studies have found support for olfaction mediating all of these behaviors in birds, but due to the relative infancy of this field, these findings are currently limited to a handful of species (for reviews see Bonadonna and Mardon 2013; Caro et al. 2015; Krause et al. 2018; Whittaker and Hagelin 2021). Thus, there is a need to determine how widespread chemical communication is across the avian group.

The primary objective of this dissertation was to expand our understanding of the role of chemical information in avian breeding behavior. Specifically, my research focused on the

Leach's storm-petrel (*Oceanodroma leucorhoa*), a pelagic seabird whose natural history points to a potentially important role for olfaction aiding multiple aspects of their breeding biology. Leach's storm-petrels are a member of the avian order Procellariiformes, which are known for their large olfactory apparatus and excellent sense of smell (Bonadonna and Mardon 2013). They are long-lived, monogamous breeders that remain with the same mate and reuse the same nest across multiple years (Pollet et al. 2020). They nest in a self-constructed, underground burrow and are nocturnally active at the colony (Warham 1990), which suggests a need for non-visual communication to facilitate social interactions. Furthermore, males and females are visually monomorphic and do not have seasonally variable plumage (Pollet et al. 2020). However, they do possess a strong, musky body odor, which may function to communicate information.

A second goal of this dissertation was to develop improved analytical methods for studying bird odors. A wide variety of methods have been used to analyze the chemicals produced by birds. Each method is associated with its own pros and cons, but perhaps most importantly, the methods vary based on the type of chemicals they target. Most of the compounds that make up bird odor are derived from the preen gland; a large sebaceous gland that produces a chemically-complex waxy secretion (Jacob 1992). The majority of previous studies have employed methods to identify the high molecular weight components of preen oil (Campagna et al. 2012). These compounds are typically non-volatile, which means they are not able to be picked up by the avian olfactory system that detects airborne, volatile chemicals. While these non-volatile chemicals may be precursors to scented compounds, analytical methods that target them are unable to identify the compounds that birds use in social

contexts. Furthermore, birds distribute preen oil throughout their feathers, which means the plumage, not the preen gland, is the substrate that emits odor compounds during social interactions. Oil directly from the preen gland shares chemical similarities with the plumage, but the feathers tend to contain more volatile compounds, which is likely due to preen oil chemistry being altered as it sits on the feathers (Mardon et al. 2011; Soini et al. 2007). I set out to develop methods that focused on the airborne chemicals available for detection by the avian olfactory system. To do this, I used headspace gas-chromatography mass-spectrometry methods to capture the scented compounds emitted from birds and their nests. Furthermore, I analyzed the compounds associated with the feathers, rather than the pure preen oil, to better approximate the odor present when one bird comes into close proximity with another.

With these two main goals in mind, I completed three projects to examine the chemical information that may underlie different aspects of breeding behavior in Leach's storm-petrels. In my first chapter, I investigated the chemical information present in their strong plumage odor. I collected feathers from a group of 30 individuals across two breeding seasons. Chemical profiles in other bird species are influenced by variable conditions such as diet (Thomas et al. 2010) and disease (Grieves et al. 2018; Kimball et al. 2013), so I predicted that the chemical profiles of Leach's storm-petrels would vary among years. However, if their strong odor does enable communication between individuals, I predicted that reliable information would be present in spite of annual variability. Specifically, I looked for the presence of a sex label that reflects whether an individual is a male or a female, which may be used during mate choice decisions, and the presence of an individual identity label that could enable long-term mates to recognize each other over multiple breeding seasons.

In the context of breeding, signals that indicate quality and enable individual recognition are particularly interesting because they may influence both mate choice decisions and social interactions between individuals such as long-term mates. The vertebrate immune genes of the Major Histocompatibility Complex (MHC) are highly polymorphic, with only close relatives sharing similar genotypes, indicating a potential role for these genes in recognition behavior (reviewed in Ruff et al. 2012). An organism's MHC genes are also tied to individual quality, with certain genotypes affording improved survival and reproductive success (Eizaguirre et al. 2009; Kalbe et al. 2012; Sepil et al. 2013; Worley et al. 2010). MHC genes are key determinants of personal odor in mammals, reptiles and fish, which use olfaction to assess the genetic makeup of other individuals (Milinski et al. 2005; Olsson et al. 2003; Potts et al. 1991; Reusch et al. 2001; Wedekind and Penn 2000; Yamazaki et al. 1976). However, it is currently unclear how widespread the link between MHC and odor is across vertebrates, and this connection has rarely been studied in birds. For the second chapter of this dissertation, I investigated whether the plumage odor of Leach's storm-petrels contains information about MHC genotype. This project was completed alongside a study into MHC mediated mate-choice in the same population, which revealed that MHC Class IIB genes are linked to reproductive success in female Leach's storm-petrels and that males breed less often than expected with females who have low quality genotypes (Hoover et al. 2018). Therefore, I was particularly interested in whether female odor reflects MHC genotype and may act as a mechanism for male storm-petrels to make MHC-based mate choice decisions.

Successful reproduction requires that birds make many trips to and from their nest, yet we have little understanding of how birds accomplish this crucial task. In colonial breeding

species, where many nests co-occur in small area, individuals are presented with an additional challenge because they must discriminate their nest from the nests of conspecifics. The third chapter of this dissertation investigated how chemical information might aid in this overlooked aspect of avian breeding behavior. Some of the earliest experiments on olfactory mediated behaviors in birds revealed that Leach's storm-petrels use olfaction to locate both their breeding island and their nest within the colony (Grubb 1974, 1979). More recently, O'Dwyer et al. (2008) showed that chicks are also able to discriminate the scent of their home burrow. Olfactory homing in other taxonomic groups (e.g., social insects, fish, mammals) has been linked to different sources of chemicals. Some organisms use chemicals associated with a particular landscape feature to home, while others make use of self- or conspecific-produced chemicals that are deposited in the environment (Gagliardo 2013; Jorge 2011; Steck 2012; Svensson et al. 2014). It is unclear what type(s) of chemical information Leach's storm-petrels use for homing. It has been suggested that procellarid seabirds use environmental chemicals to assist with homeward orientation towards the breeding colony (Gagliardo et al. 2013a; Padget et al. 2017; Pollonara et al. 2015), while the ability to locate their specific nest within the colony is aided by uniquely scented burrows that are infused with the occupants' body odor (Bonadonna et al. 2001). In this final chapter, I examined the chemical make-up of the breeding colony and its avian occupants to uncover the chemical information that may enable olfactory homing in Leach's storm-petrels.

CHAPTER 1

Individual Chemical Profiles in the Leach's storm-petrel

1.1 ABSTRACT

Avian chemical communication, once largely overlooked, is a growing field that has revealed the important role that olfaction plays in the social lives of some birds. Leach's storm-petrels (*Oceanodroma leucorhoa*) have a remarkable sense of smell and a strong, musky scent. This long-lived, monogamous seabird relies on olfaction for nest relocation and foraging, but whether they use scent for communication is less well studied. They are nocturnally active at the breeding colony and successfully reunite with their mate despite poor night-vision, indicating an important role for non-visual communication. We investigated the chemical profiles of Leach's storm-petrels to determine whether there is socially relevant information encoded in their plumage odor. To capture the compounds comprising their strong scent, we developed a method to study the compounds present in the air surrounding their feathers using headspace stir bar sorptive extraction coupled with gas-chromatography mass-spectrometry. We collected feathers from Leach's storm-petrels breeding on Bon Portage Island in Nova Scotia, Canada in both 2015 and 2016. Our method detected 142 commonly occurring compounds. We found interannual differences in chemical profiles between the two sampling years. Males and females had similar chemical profiles, while individuals had distinct chemical signatures across the two years. These findings suggest that the scent of the Leach's storm-petrel provides sociochemical information that could facilitate olfactory recognition of individuals and may inform mate choice decisions.

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1.2 INTRODUCTION

Olfaction is one of the oldest and most ubiquitous sensory modalities (Bradbury and Vehrencamp 2011; Wyatt 2003), yet in birds, it has often been overlooked in favor of their more obvious visual and acoustic capabilities. Over the last 60 years, however, findings from a small but detailed body of research have established that virtually all birds have a sense of smell (for reviews see: Balthazart and Taziaux 2009; Caro et al. 2015; Hagelin 2007; Roper 1999). In the growing field of avian chemical ecology, recent research has focused on uncovering the important role of olfaction in the lives of birds (reviewed in Balthazart and Taziaux 2009; Caro et al. 2015).

While olfactory-mediated behavior in birds is often not readily apparent to a human observer, the range of activities that birds accomplish using their sense of smell is extensive. Birds use olfaction in their environments for foraging (Graves 1992; Grubb 1972; Hutchison and Wenzel 1980; Nevitt 2000; Stager 1964), navigation (reviewed in: Gagliardo 2013; Papi 1990; Wallraff 2014), homing to their nest (Bonadonna et al. 2003, 2004; Bonadonna and Bretagnolle 2002; Grubb 1974) and the selection of nest materials (Clark and Mason 1985, 1987; Gwinner and Berger 2008; Petit et al. 2002). Olfactory capabilities also function in interspecific interactions for the detection of predators (Amo et al. 2008; Roth et al. 2008) and avoidance of parasites (see Hagelin and Jones 2007 for a review).

More recently, researchers have found that communication via chemicals can mediate interactions among individuals (reviewed in Campagna et al. 2012; Caro et al. 2015). Birds use scent to discriminate conspecifics from heterospecifics (Hagelin et al. 2003; Krause et al. 2014; Whittaker et al. 2011a), to identify mates and kin (Bonadonna and Nevitt 2004; Bonadonna and Sanz-Aguilar 2012; Caspers et al. 2017; Krause et al. 2012), and to recognize their eggs (Golüke et al. 2016; Leclaire et al. 2017a). In order for birds to perform these remarkable behaviors, information to facilitate these decisions must be present in the chemical profiles of birds. As the behavioral evidence suggests, avian chemical profiles differ at multiple levels: among species (Haribal et al. 2009; Soini et al. 2013), among populations (Gabirot et al. 2016; Grieves et al. 2019a; Whittaker et al. 2010), by sex (Amo et al. 2012a; Leclaire et al. 2011; Zhang et al. 2009, 2010), with breeding status (Reneerkens et al. 2002; Soini et al. 2007; Tuttle et al. 2014; Whittaker et al. 2019a), and even among individuals (Mardon et al. 2010, 2011). In some species, avian chemical profiles communicate information about genetic markers that are potential signs of individual quality, such as the immune genes of the major histocompatibility complex (Leclaire et al. 2014; Slade et al. 2016) and heterozygosity (Leclaire et al. 2012; Whittaker et al. 2019a). These findings imply a potentially important role for olfaction in both mate choice and individual recognition.

Studies of avian chemical profiles have primarily focused on the oil of the uropygial or preen gland as the main source of avian odor. This large holocrine gland is located at the dorsal base of the tail and is found exclusively in birds (Jacob and Ziswiler 1982). It produces a complex mixture of compounds including esters, fatty acids, alcohols, aldehydes, ketones, and hydrocarbons (reviewed in Campagna et al. 2012). Birds distribute the oil over their feathers to

maintain plumage condition and function (reviewed in Moreno-Rueda 2017). The chemical composition of preen oil has been investigated across a variety of different bird species. While there are many compounds and classes of compounds that are common across species (e.g., linear carboxylic acids, linear alcohols, aldehydes), to date no universal volatile chemicals have been found that are present in the preen oil of all birds (Soini et al. 2013; Whittaker et al. 2010). Rather, closely related species appear to be divergent in their chemical profiles (Bonadonna and Mardon 2013; Haribal et al. 2009; Soini et al. 2013). Moreover, within a species, chemical labels such as the sex or breeding status of an individual, are typically encoded by changes in the relative abundances of various ubiquitous compounds, rather than the presence or absence of unique compounds (Krause et al. 2018).

During preening, the scented compounds derived from the preen gland are distributed throughout the bird's plumage where they mix with the other molecules present on the feathers and skin. Some of these compounds readily evaporate, while other less volatile molecules get broken down through exposure to the both the bird's environment and its microbiota, contributing to the scent that is present when one individual smells another (discussed in Campagna et al. 2012; Nevitt and Prada 2015). Thus, feathers pose an alternative source of chemical information to the preen oil that is likely more reflective of the full range of compounds used in communication. In this study, we investigated the chemical profiles associated with the feathers of a previously unstudied species, the Leach's storm-petrel (*Oceanodroma leucorhoa*).

Scents are made up of volatile compounds that readily evaporate out of a solid or liquid matrix into the air where they can be picked up by the olfactory system. A wide variety of

analytical methods have been employed to investigate avian chemical profiles, which has made interspecies and intraspecies comparisons challenging. Most studies have relied on solvent-based extraction methods, which are effective for extracting moderate and high molecular weight analytes that occur in high concentrations (Amo et al. 2012b; Bonadonna et al. 2007; Leclaire et al. 2011; Mardon et al. 2010, 2011; Thomas et al. 2010; Zhang et al. 2009). In order to quantify the more volatile and/or trace compounds, solvent-less extraction methods that sample the air, or headspace, around a matrix are a better option and have been employed in a number of studies (Bertram et al. 2001; Burger et al. 2004; Douglas 2006; Hagelin et al. 2003; Syed and Leal 2009; Whelan et al. 2010). Stir bar sorptive extraction (SBSE) uses a stir bar coated in sorbent material to extract molecules. SBSE offers high reproducibility and sensitivity due to the large volume of extraction phase (reviewed in David and Sandra 2007; He et al. 2014; Sánchez-Rojas et al. 2009). Like all static headspace methods, SBSE relies on a stationary phase that has an affinity for certain compounds, so the blend of compounds collected by this method may differ somewhat from the actual blend associated with the sample. However, it is excellent at detecting compounds across a large range of molecular weights and boiling points using only a small quantity of sample. This technique has been successfully employed to measure volatile compounds in several songbird species by stirring the bar in a solution containing preen oil (Soini et al. 2007, 2013; Whittaker et al. 2019a), and by rolling the stir bar along the surface of a feather (Soini et al. 2006, 2007). We applied this technology in a new way to capture the compounds found in the headspace of feathers.

Among birds, the role of olfaction is particularly well understood in the Procellariiform seabirds, which are known for their large olfactory bulbs and exceptional sense of smell

(reviewed in Bonadonna and Mardon 2013). Members of this group are long-lived, sexually monomorphic, and have delayed sexual maturity (Warham 1990). Once they have secured a mate, the pair maintains a long-term, often life-long bond (Bried et al. 2003). During the non-breeding season individuals disperse across the ocean and members of mated pairs may go months without contact (Müller et al. 2015; Weimerskirch and Wilson 2000). This extreme life history calls for sensory mechanisms that aid in both mate selection and individual recognition to maintain their long-term bond. A number of procellariid species, namely the shearwaters, diving petrels, prions and storm-petrels, nest in underground burrows. They are nocturnally active at the colony and conduct their breeding activities under the cover of darkness (Warham 1990, 1996). This environment necessitates a mode of communication that works in low-to-no light conditions, which suggests an important role for non-visual modalities such as olfaction.

The Leach's storm-petrel is a small (45g) burrow-nesting species that is widespread in the northern hemisphere with breeding colonies in both the Pacific and Atlantic Oceans (Pollet et al. 2020). They use olfaction for locating their breeding island (Grubb 1979) and for homing to their burrow (Grubb 1974). At sea, they are attracted to odors that aid in foraging (Grubb 1972; Nevitt and Haberman 2003). Whether Leach's storm-petrels use their sense of smell in social interactions remains unstudied, although the importance of scent in the reproductive lives of their close relatives (reviewed in Bonadonna 2009; Bonadonna and Mardon 2013) suggests it likely plays a similar role in this species.

We investigated the chemical profiles of Leach's storm-petrels to determine whether they contain sociochemical information that could function in communication. We developed a method using stir bar sorptive extraction to sample the airborne compounds in the headspace

around Leach's storm-petrel feathers, which we analyzed using gas chromatography mass spectrometry (GC-MS) to quantify the chemicals that make up their scent. We collected samples from individuals during two breeding seasons and tested the hypothesis that the chemical profiles of Leach's storm-petrel feathers contain socially relevant information. Specifically, we predicted that we would find 1) Inter-annual differences, 2) A sex signal that differentiates males and females, and 3) An individual signature that allows for reliable identification of individuals over multiple years.

1.3 METHODS

Study Site. Fieldwork was carried out on Bon Portage island (43.46°N, -65.75°W), a low-lying 3 km x 0.5 km nearshore island, located off the southern tip of Nova Scotia, Canada. An estimated 39,000 breeding pairs of Leach's storm-petrels nest on Bon Portage each year (Pollet and Shutler 2018). We sampled storm-petrels at three sites spread across the southern end of the island where our research group has over 500 marked burrows that petrels have excavated on the forest floor amid balsam fir (*Abies balsamea*) and black spruce (*Picea mariana*) trees (Figure S1.1).

Field Methods. We collected feathers from 30 adult birds, 15 females and 15 males, across a 5-day period in the late incubation period (early July) in 2015 and 2016. Birds were removed from their burrow by hand and we confirmed their breeding status by verifying the presence of an egg. We plucked 6-8 small semiplume feathers (~3 cm in length, average mass of 2.8 mg per feather) from 5-10 cm above the preen gland at the base of the tail (Figure S1.2). We selected feathers from this area to capture compounds from the preen oil, as well as any other odorants

present on the feathers that might be contributing to storm-petrel scent. Each sample was stored in a clean 10 mL amber glass headspace vial sealed with PTFE/silicone lined metal screw cap (Restek Inc., Bellefonte, PA) and kept frozen at the field site. A new pair of clean nitrile gloves was worn while processing each bird to prevent the transfer of compounds from humans or other birds onto the sample. Leach's storm-petrels are sensitive to repeated investigator disturbance (Blackmer et al. 2004) and their population has recently declined worldwide (Pollet et al. 2020), so individuals were returned to their nest within five minutes and were only removed once during the season. Feather samples were transported from Nova Scotia to the University of California, Davis on dry ice and stored at -80 °C degrees prior to analysis.

All birds were fitted with unique metal identification bands issued by the Canadian Wildlife Service. Since Leach's storm-petrels are monomorphic, we collected a small (75 μL) blood sample from the brachial vein of each bird that was used to determine sex via the DNA extraction and Polymerase Chain Reaction (PCR) protocols described in Hoover et al. (2018).

Sample Preparation. We analyzed triplicate feather samples from each individual in a given year. Since feathers were collected from all 30 birds in both years, we analyzed a total of 6 samples per individual, for a final dataset that consisted of 180 samples. For nine individuals we were able to process an additional sample, which was used for model validation (6 individuals in 2015, 3 individuals in 2016). Each replicate consisted of two feathers that were placed into a new 10 mL amber glass headspace vial and sealed with a PTFE/Silicone lined metal screw cap. The pre-extraction mass of each feather sample was recorded with an average sample mass of 5.6 mg (range: 3.2 mg to 8.6 mg).

We extracted compounds from the headspace above the feathers using a 10 mm metal Twister™ stir bar (Gerstel Inc., Mülheim an der Ruhr, Germany) coated with 0.5 mm thickness of polydimethylsiloxane stationary phase. To suspend the stir bar above the feather sample we used two small magnets (8 mm diameter): we attached the stir bar to one magnet that was positioned inside the vial, while a second magnet on the outside of the vial held the internal magnet in place. The vials were set in a sand bath that was heated so that the temperature inside each vial reached 40 °C, the average body temperature of a storm-petrel (Ricklefs et al. 1986; Warham 1971). We chose this temperature to mimic the conditions the feathers would experience if they were still attached to the bird and thus provide a good approximation of the scent that is present when one bird smells the feathers of another. The stir bars were left inside the vials for 6 hours.

At the end of the extraction period, the stir bars were placed into glass desorption tubes (Gerstel Inc., Mülheim an der Ruhr, Germany) for analysis. An internal standard of 10 ppm (mg/L) naphthalene-d8 in 100% ethanol (Sigma Aldrich, St. Louis, MO) was added to each sample using a 0.5 μL glass microcapillary tube (Drummond Scientific, Broomall, PA). The capillary tube was placed inside a glass micro-vial (Gerstel Inc., Mülheim an der Ruhr, Germany) that was inserted into the desorption tube above the stir bar. Naphthalene-d8 elutes at approximately the mid-point in the chromatogram, approximating the boiling point and (non-polar) chemistry of many of the sample components, and is not found naturally in the samples.

Prior to use, all glassware (headspace vials, desorption tubes, and micro-vials) was baked for 24 hours at 100 °C. Magnets were cleaned with 100% methanol and baked for at least 12 hours between uses in the same oven as the glassware.

GC-MS Method. To separate and quantify the compounds in each sample, gas chromatography mass spectrometry (GC-MS) was performed using an Agilent Technologies, Inc. (Santa Clara, CA, USA) GC system (7890B) and mass spectrometer detector (5977A), equipped with a thermal desorption unit and cryo-cooled injection system (Gerstel Inc). The GC was fitted with a DB-5MS UI capillary column (30 m x 0.25 cm x 0.25 μm film thickness; Agilent Technologies, Inc., Santa Clara, CA USA). The MS was fitted with an Agilent Extractor Extra Inert (EI) electron source. To ensure optimal peak separation, the GC-MS was programmed as follows. The extracted compounds were thermally desorbed from the stir bar in the thermal desorption unit (TDU). The initial TDU temperature was 30 °C, it was heated to 250 °C at 720 °C/min and held for 5 min. The TDU was used in splitless mode and the transfer line was kept at 250 °C. The analytes were transferred from the TDU into the cooled injection system (CIS), which was cooled to -80 °C using liquid nitrogen. The CIS heated at a rate of 12 °C/s until it reached 280 °C and was held for 3 min. Analytes entered the GC inlet in solvent vent mode with vent pressure of 11.6 psi, a vent flow of 50 mL/min and a purge flow of 50 mL/min that turned on at 1.2 min. The GC had an initial oven temperature of 35 °C, which was held for 3 min followed by an increase of 5 °C/min to 220 °C with no hold; followed by a second increase at a rate of 15 °C/min to a final temperature of 280°C, which was held for 5 min. The GC was run in constant flow mode with helium as the carrier gas with a flow rate of 1.5 mL/min. The total GC run time was 49.00 min. MS parameters were set as follows: the transfer line from the GC to the MS was 280 °C; the electron source was 230 °C; the quadrupole temperature was 150 °C; the scan range was from 40 to 300 *m/z* at a rate of 2.8 scans/s. The EMV mode was set to gain factor, with the factor set to 1.0.

Twisters™ are reusable and were conditioned per manufacturer instructions after each use by heating them to 280°C in the TDU to remove previously sorbed analytes. A series of C7-C30 alkane standards (~40 mg/L each, Sigma Aldrich, St. Louis, MO) were analyzed using the same instrument settings as the feathers and used to calculate retention indices of the detected compounds. Blank samples that consisted of a clean Twister were run daily throughout the analysis and background noise was removed from the feather data by subtracting the signals observed in the blanks. We randomized the order in which samples were processed across the analysis period to ensure that samples from the same individual were analyzed on different days.

Data Analysis. We used Agilent MassHunter Unknowns Analysis Software Version B08.00 to find the analytes present in our samples. We restricted our search to compounds eluting before 41 minutes, which was the retention time of C23 in our alkane standard solution. We focused on this region of the chromatogram since we were primarily interested in the more volatile compounds. We retained all the analytes not observed in blank runs that were present in at least 25% of the samples. Compounds were tentatively identified through comparisons of their mass spectra and calculated retention indices to data in the NIST (National Institute of Standards and Technology) 14 Mass Spectral Library and other published sources (Pherobase and Flavornet).

Peak areas of the detected compounds were integrated using Agilent MassHunter Quantitative Analysis Software Version B.08.00 for GC-MS. The chemical mixtures associated with Leach's storm-petrel feathers were complex with some co-eluting compounds that exited the GC column simultaneously (Figure 1.1). To help resolve the co-eluting peaks, we integrated each compound using an extracted ion chromatogram (EIC), which was usually the most

abundant ion (see Table 1.1 for a complete list of ions used). Software-automated integrations of the peaks were visually inspected and manually corrected when needed. Certain compounds had poor peak shape or were unable to be resolved and were not retained in the dataset despite appearing in many samples (these compounds were not integrated and are noted in Table 1.1).

We standardized the data using a two-step process. First, we divided each compound peak by the area of the internal standard in the corresponding run to account for variation in instrument sensitivity across the analysis period. To account for differences in feather quantity, we performed a second standardization where we divided by the pre-extraction feather sample mass. Since our chromatograms consisted of a few highly abundant analytes and many low abundance analytes, we log(x+1) transformed the data to prevent the highly abundant compounds from having a disproportionate influence on the analysis (Clarke et al. 2014). We calculated the pairwise Euclidean distance between samples to generate a resemblance matrix, which served as the input for the following analyses. We felt that Euclidean distance was an appropriate distance measure since the compounds were on a similar scale after data transformation.

We used distance-measure based multivariate statistics to analyze our data, which are robust to departures from multivariate normality (Anderson et al. 2008; Clarke et al. 2014). First, to confirm that our analytical method was reliably measuring consistent differences between individuals, we examined the repeatability of the chemical profiles obtained in replicate samples from individual birds within each year. To do this, we used the Euclidean distance matrix and a two-sided permutation test with 10,000 permutations of the data to

compare the mean distance between replicate samples from the same bird to the mean distance between samples analyzed from different birds. We also visualized our data using Principal Coordinates Analysis (PCO; Gower 1966), which is an unconstrained ordination method. Since our dataset consisted of a large number of samples (n=180), we subdivided our data by year and sex (male 2015, female 2015, male 2016, female 2016) to more clearly display the patterns present.

After validating our analytical method, we used PERMANOVA (Anderson 2001; McArdle and Anderson 2001), a permutational distance-based equivalent of a traditional MANOVA analysis, to determine how the factors Year, Sex, and Individual identity contribute to variation in the multivariate data. This method is useful for uncovering separation between groups that differs from the main direction of variation in the dataset. We created two PERMANOVA tests, one using the matrix of Euclidean distance between all 180 feather samples. In the second test, we used a simplified Euclidean distance matrix where each bird had only two data points, one for each year (60 data points). This dataset was generated by averaging the across the three replicate samples within each year. The results from both tests were compared to ensure that the replicates in our larger dataset were not altering the model outputs. For both tests, we ran a three factor PERMANOVA including Year (fixed), Sex (fixed) and Individual (random, nested within Sex) using 10,000 permutations of the data and Type 1 sequential sum of squares. Due to the balanced design of our study, all types of sums of squares (Types I, II and III) produce the same results (Anderson et al. 2008) but we chose Type 1 due to the nested hierarchical nature of our data. To supplement the PERMANOVA analysis, we also examined the data using unconstrained PCO ordination plots to visualize the patterns associated with the factors Year,

Sex, and Individual identity. Additionally, we ran two single-factor PERMANOVAs within each year to examine the influence of the sample collection date using 10,000 permutations of the data, which confirmed there were no differences in chemical profiles related to the collection date across our short sampling period (Figure S1.3).

To further examine the individual chemical signature, we used Canonical Analysis of Principal Coordinates (CAP; Anderson and Willis 2003). CAP is a type of discriminant analysis that finds axes in the multivariate data that differentiate a priori groups of interest. CAP uses a leave-one-out cross-validation method to determine the number of PCO axes that should be retained. As a measure of model success, it calculates an allocation rate based on the samples that were correctly vs. incorrectly assigned to their respective groups. The model created by CAP can also be used for predictive modelling whereby new points are classified into the existing groups using the retained PCO axes.

In the search for a model that could discriminate between individuals, we focused on potential semiochemicals: substances derived from the bird that could play a role in communication. Our dataset consisted of many compounds that we suspected did not arise directly from the bird and were instead picked up on the feathers from the environment. We used two criteria to reduce the substances in our dataset to those likely coming from the storm-petrels (see Table 1.1 for classifications). As a first pass, we retained only analytes that were present in at least 50% of samples as potential semiochemicals. We used this criterion since previous studies that have found avian chemosignals are encoded by changes in the abundance of common compounds, rather the presence or absence of rare compounds (Krause et al. 2018). Secondly, we looked at each tentative identification and linked it to a probable source

(bird vs. not bird) based on findings from other studies that have detected these compounds or similar molecules in other bird species (Bonadonna et al. 2007; Campagna et al. 2012; Douglas et al. 2001; Gabirot et al. 2016, 2018; Hagelin et al. 2003; Haribal et al. 2009; Leclaire et al. 2011; Mardon et al. 2010; Soini et al. 2013, 2007; Whittaker et al. 2019a). For example, the aldehyde nonanal has been found in a number of avian species (Bonadonna et al. 2007; Gabirot et al. 2018; Hagelin et al. 2003; Soini et al. 2007; Williams et al. 2003), whereas the monoterpene, alpha-pinene, is a common plant-derived compound (Humphrey and Beale 2007) that likely originated from the vegetation surrounding the petrel burrow. Using this approach, we retained 80 compounds, of which 70 were found in 80% or more of the samples.

We ran CAP models to examine the individual chemical signature using 1) our entire set of 127 compounds, and 2) our reduced list of 80 bird-derived compounds. Due to the high number of individuals (n=30) in our dataset, we conducted three separate CAP analyses within the two sets of analytes to compare how the models varied in their discriminating ability with increasing sample size. Specifically, we looked at models using: 1) all birds (30 individuals), 2) males (15 individuals), and 3) females (15 individuals). The resulting six CAP analyses were performed using 10,000 permutations of the data in order to generate both a leave-one-out classification rate and a statistical probability, P-value. We validated the predictive ability of the CAP models using the 9 extra samples (n=4 for males, n= 5 for females) that were excluded from the model. The validation process treats the additional samples as unknowns and assigns them to a group, in this case to an individual bird, in the CAP model that was built using the original samples.

We examined the compounds associated with the CAP model axes to determine which analytes characterized the differences among individuals. Specifically, we calculated the

Pearson correlation coefficients (r) between the compounds and the axes retained by the two CAP models differentiating all 30 birds. For both models we considered the compounds with the largest correlations as these contribute more strongly to the differences among groups. We retained the top 40 analytes in both models, which had Pearson correlation coefficients of $r>0.45$ for the 127-compound model and $r>0.59$ for the 80-compound model. These correlation coefficients would be considered statistically significant in classical linear analysis with the same number of variables and samples. This approach is an exploratory step to describe the differences in chemical profiles between groups. It is not intended for assigning significance or inferring direct biological relationships, so no further tests were performed (Anderson et al. 2008).

All analyses were completed in R 3.5.3 (R Core Team 2019) and in the software program PRIMER v 7.0.13 (Clarke and Gorley 2015) with the PERMANOVA+ v1 add-on (Anderson et al. 2008). Figures were made in PRIMER or in R using the ggplot2 (Wickham 2016) and ggmap (Kahle and Wickham 2013) packages. Significance was assessed based on an alpha level of 0.05.

1.4 RESULTS

The headspace of Leach's storm-petrel feathers is chemically complex: using our method, we found 142 compounds that were present in at least one quarter of our samples (Table 1.1). We retained 127 of these compounds for use in our subsequent analyses, which included: aldehydes, ketones, terpenes, alkanes, hydrocarbons and alkylbenzenes. Certain compounds, specifically a number of straight chain fatty acids and linear fatty alcohols could not be integrated/measured due to poor peak shape and issues with co-elution despite being

observed in many of the samples. There were a number of compounds that eluted after C20 (retention index > 2000) that we were unable to confidently identify using the NIST 14 library. These molecules all had mass spectra similar to esterified long chain acids, so we labeled them as “unidentified long chain esters” and the primary ions in their mass spectra are listed in Table 1.1. The two most abundant compounds in most of the samples were pristane, a saturated terpenoid alkane, and the straight chain aldehyde nonanal. The vast majority of the 127 compounds (n=102) were present in 80% or more of the processed samples.

Method Validation. Both ordination PCO plots and permutation tests confirmed that replicate samples analyzed from a bird in a particular year had high repeatability. PCO plots, where the samples are grouped based on year and sex to allow for a smaller number of samples to be visualized at one time (n=45 vs 180), show that replicate samples from an individual bird group near each other (Figure 1.2). A two-sided permutation test comparing the mean Euclidean distances between samples from the same and different individuals further confirmed that the replicate samples in a given year were more similar to themselves than samples from other birds (Figure 1.3, $P < 0.001$ for both years).

Interannual Differences**.** The PERMANOVA test confirmed the presence of a year effect (Table 1.2, $P<0.001$). The influence of year was also clear in a PCO plot where the first two axes explained 52.38% of the total variation (Figure 1.4a). The samples appear to separate by year along PCO2. There were no compounds that were exclusively detected in one of the two sampling years. However, further examination of the chemicals associated with PCO2 showed that there were certain compounds that appeared to be primarily responsible for the interannual differences (Table S1.1). These compounds were all more common in 2016 than

2015. They included a number of monoterpenes (alpha-pinene, beta-pinene, o-cymene, limonene, gamma-terpinene), as well as a group of four unidentified compounds that all shared ions with *m/z* 88 and 115 in their mass spectra (Retention Times: 21.78, 23.51, 25.65, and 26.77 min), and several fatty acid ethyl esters.

***Sex Label*.** The PCO ordination of all samples did not show an obvious influence of sex (Figure 1.4b). The predicted sex-pattern was also missing when the data was broken into the two sampling years and visualized in two separate PCO plots (Figure S1.4). The fact that eight axes are required to explain more than 75% (76.49%) of the total variation indicates that the multivariate data cloud is complex and may contain patterns that are hard to visualize using two-dimensional plots. However, the PERMANOVA test, which is able to find patterns in the multivariate that differ from the main directions of variation, also suggested that the predicted sex label was missing (Table 1.2, P=0.2).

***Individual Signature*.** The PERMANOVA found strong evidence of an individual signature present in the feather profiles (Table 1.2, P<0.001). Additionally, we found a significant interaction of year*individual, which is evident in PCO plots where individuals separate along PCO1 with the samples from 2016 sitting above those from 2015 (Figure S1.5). While the direction of the year effect is the same for each individual, the ordination suggests that the magnitude of this effect differs between individuals since some birds have more distance between the two years of samples, leading to the significant interaction term.

Further investigation of the individual chemical signature produced CAP models that were successful at discriminating individuals using the entire suite of analytes, as well as the more limited dataset of bird-derived compounds (Table 1.3). The two CAP models using all 127

compounds required 15 PCO axes to discriminate individuals within the subsets of only males or only females, and correctly classified 87% and 89% of samples using the leave-one-out method ($P < 0.001$ for both models). The model for females assigned four out of the five validation samples to the correct individual, while the male model matched all 4 validation samples. A full model based on all 30 birds, used 17 axes for a leave-one-out classification rate of 82% ($P < 0.001$). This model assigned seven out of the nine validation samples to the correct bird.

The CAP models using only bird compounds produced similar results. Individuals within the 15 males and 15 females were differentiated using 15 PCO axes with a classification rate of 82% and 80% respectively ($P < 0.001$). The full model of 30 birds required 19 axes to discriminate individuals and classified 82% of samples correctly ($P < 0.001$). For these 3 models, all validation samples were assigned to the correct bird. CAP plots from these models based on only the first two axes also show good separation between individuals (Figure 1.5). With 30 individuals, as we had in our complete models, we would expect the correct classification of a sample based on chance alone 3.33% of the time, so the classification rates of greater than or equal to 80% that we observed for all of our models are very high. Together these results suggest that the individual chemical badge makes up a significant component of the variation within profiles of Leach's storm-petrel feathers.

For the two models based on our entire set of 30 birds, we inspected the top 40 compounds contributing to each model that also had a Pearson's correlation greater than $r=0.45$ (for 127 analyte dataset) and $r=0.59$ (for 80 analyte dataset). These levels would be considered significant in a classical correlation analysis based on the same number of samples and variables. Within the top 40 compounds, the two models shared 32 compounds in common,

showing that both models are primarily using the same compounds to discriminate individuals (Table S1.2). Only six of the compounds in the 127-compound model were not part of the "bird-derived" compound list, which indicates that both models primarily relied on compounds we had identified as potential semiochemicals to discriminate individuals. Most of the compounds selected by the models eluted in the later part of our chromatograms and had retention indices between 1900 to 2300.

1.5 DISCUSSION

In this study, we investigated the chemical profiles of Leach's storm-petrels, a long-lived, monogamous, seabird, with an excellent sense of smell. Specifically, we used headspace stir bar sorptive extraction (HS-SBSE) coupled with GC-MS to measure the compounds present in the headspace of their feathers. This high-sensitivity method allowed us to detect a large number of compounds across a wide range of boiling points and molecular weights using a small quantity of feathers. We tested for the presence of sociochemical information that could play a role in communication in this species. We found support for two of our three predictions; chemical profiles differed between the two sampling years and individuals had distinct chemical signatures across the study period. Males and females, however, had similar chemical profiles, opposite of what we predicted. These findings suggest that the scent of Leach's storm-petrel plumage may contain information that could facilitate individual recognition and inform mate choice.

Chemical Make-up of the Plumage Perfume. Leach's storm-petrels have been long recognized for their musky odor, which emanates from their plumage and perfumes their burrows (Gross

1935). Using our described method, we found 142 commonly occurring compounds in the headspace of Leach's storm-petrel feathers. Over half the compounds overlapped with what has been found in other studies examining the chemoprofiles of feathers and preen oil either as an exact match or a similar class of compounds (see Table 1.1 for a full list of references). Specifically, we observed alkanes, esters, aldehydes, ketones, linear alcohols, hydrocarbons and fatty acids. We also found a number of analytes that were likely from the bird's environment, including plant-derived compounds and pollutants.

Twelve straight chain aldehydes (C6 through C17) were present in all feathers, with nonanal (C9) appearing in the top three most abundant compounds of every sample. Aldehydes are known fragrance compounds that have been detected in the plumage of other bird species (Allan et al. 2006; Bonadonna et al. 2007; Hagelin et al. 2003; Mardon et al. 2011). One proposed function of these compounds is as an ectoparasite repellent (Douglas et al. 2001, 2005a, b; Douglas 2004). During incubation, Leach's storm-petrels spend multiple days at a time in their underground burrow, and yet curiously, they seem to carry very few ectoparasites. Moreover, mosquitoes, despite being abundant at the study site, are infrequently observed biting storm-petrels, which begs the question whether their musky scent acts as a natural repellent (Jennings pers. obs.). In humans, the aldehydes octanal, nonanal, and decanal were more abundant in people who were less attractive to *Aedes* mosquitos (Logan et al. 2008). However, nonanal, which is dominant in the chemical profiles of pigeons and chickens, as well as our study species, acted as an attractant for *Culex* mosquitoes, a finding which appears to conflict with the results from the *Aedes* mosquito (Syed et al. 2009). More recent research suggests that the ratio of aldehydes in the scent profiles of humans, rather than their individual

abundance, leads to the differential attraction of mosquitos, but this is an understudied topic in other vertebrates (Leal et al. 2017). The roles these compounds play in mediating interactions between birds and ectoparasites is an area warranting further study.

The other consistently high abundance compound was pristane (2, 6, 10, 14-tetramethylpentadecane), a saturated terpenoid alkane. This compound is found in the stomach oil of procellariform seabirds (Clarke and Prince 1976). While Leach's storm-petrels do occasionally regurgitate their stomach oil as a defensive behavior (Pollet et al. 2020), we avoided collecting feathers from birds with any evidence of recent regurgitation (determined through smell and/or visible residue on the beak), and yet this compound was still present in all of our samples. Whether the stomach oil is specifically used to perfume plumage, or if this compound reaches the feathers through a less direct pathway (e.g., via the cloaca) is currently unknown.

During the breeding season, seabirds occupy both the marine and terrestrial environments, where they encounter numerous sources of environmental contamination. We detected a number of probable pollutants in our samples. Feathers have been widely used as biomarkers of contaminant exposure, so the presence of these compounds was not unexpected (Rutkowska et al. 2018; but see Jaspers et al. 2019). Feathers may pick up pollutants by coming into direct contact with a substance, or the compound may get deposited indirectly via the preen oil after being excreted by the preen gland (Solheim et al. 2016). Seventeen of the compounds were alkylbenzenes that eluted from the GC column between 26 and 35 minutes (retention index 1534 to 1908). In blue petrels (*Halobaena caerulea*), a close-relative of the Leach's storm-petrel, the same type of benzene-derived compounds were detected by Mardon

et al. (2011), who suggested that these compounds came from oil slicks or ship fumes at sea. They also noted that these compounds were missing in the chemical profiles of the preen oil, supporting the hypothesis that they were from an exogenous source and not derived from the bird itself. Chemical profiles from other seabird species are needed to determine how ubiquitous these compounds are on their plumage.

Interannual Differences. We identified several groups of compounds that were primarily responsible for interannual differences in chemical profiles. Food availability is likely to vary temporally and dietary shifts can impact chemical profiles (in birds: Apandi and Edwards 1964; Grieves et al. 2020; Reneerkens et al. 2007b; Thomas et al. 2010, in non-avian vertebrates: Ferkin et al. 1997; Havlicek and Lenochova 2006; Kwak et al. 2008). Leach's storm-petrels in the Northwest Atlantic Ocean feed on fish, euphausiid and crustaceans (Hedd et al. 2009). Depending on the availability of their preferred prey sources, the composition of their diet could differ across years, leading to shifts in their chemical profiles. Physiological qualities such as the age of the birds and their overall health are also expected to change between years and these factors have been shown to influence chemoprofiles in birds (Age: Amo et al. 2012a; Shaw et al. 2011; Whittaker et al. 2019, Disease: Grieves et al. 2018; Kimball et al. 2013); however, we were unable to determine the influence of these factors in this study.

A number of compounds that were presumably environmentally derived varied between the two years; in particular, five terpenes were associated with PCO2, the axis separating years. Leach's storm-petrels breeding on Bon Portage Island encounter many sources of plant-derived volatiles as they navigate from the ocean to their burrow. Annual differences in these plant-derived analytes could be due to varying levels of contact with plants prior to sample collection,

but since all compounds were more abundant on average in 2016, climatic differences between the two years might also play a role. Finally, while we did our best to treat samples from both years identically, there is always a possibility that there were unintended discrepancies that led to some of the inter-annual differences described above.

Sex Label. One might expect that in a visually monomorphic species like the Leach's storm petrel, other sensory modalities, such as chemical signals, may be used to differentiate the sexes. However, we did not find evidence for a chemical sex label in the plumage of Leach's storm-petrels. There is mixed evidence for sexual dimorphism in the chemical profiles of other bird species. While many studies have detected differences between males and females (Amo et al. 2012a; Grieves et al. 2019a; Leclaire et al. 2011; Mardon et al. 2010, 2011; Reneerkens et al. 2002; Shaw et al. 2011; Whittaker et al. 2010, 2019a; Zhang et al. 2010, 2009), others have not (Bonadonna et al. 2007; Burger et al. 2004; Gabirot et al. 2016, 2018; Hagelin et al. 2003; Montalti et al. 2005). In the procellariid seabirds, sex-specific chemoprofiles have been found in the Antarctic prion (*Pachyptila desolata*) and the blue petrel. However, the sex badge in both species was weak relative to the individual signal, which was the main source of variation in their chemical profiles (Mardon et al. 2010, 2011). Our method, which relied on very small mass of feathers, may not have detected the compounds responsible for a chemical sex label, or if the sex signal is subtle, like in other related species, we may not have captured the quantitative differences between males and females. While chemical sex badges are present in blue petrels and Antarctic prions, neither species is able to discriminate sex using olfaction (Bonadonna et al. 2009; Mardon J unpublished data in Mardon et al. 2010). Whether Leach's storm-petrels also lack the ability to differentiate sex using scent remains unknown, but vocalizations are a

possible alternative mode of communication since Leach's storm-petrels possess sexually dimorphic calls (Taoka et al. 1989).

The feathers used in our analysis were collected during the late incubation period, which occurs weeks after the courtship phase when a sexually dimorphic chemical signal might be behaviorally useful for advertising readiness to mate. In seasonally breeding species like storm-petrels, multiple physiological and behavioral changes occur as they transition through the various breeding stages, many of which are mediated by steroid hormones (Farner and Wingfield 1980). Seasonally variable chemoprofiles have been reported in a number of species (Reneerkens et al. 2002, 2007a; Soini et al. 2007; Tuttle et al. 2014; Whittaker et al. 2019a), but how circulating levels of steroid hormones act to alter chemical profiles is not well understood. The covariation of steroid hormone levels and chemical profiles have only been documented in a handful of species (mallard duck *Anas platyrhynchos* Bohnet et al. 1991; crested auklet *Aethia cristatella* Douglas et al. 2008, 2018; grey catbird *Dumetella carolinensis* Whelan et al. 2010; dark eyed junco *Junco hyemali*s Whittaker et al. 2011b, 2018). If chemical profiles, like other sexual signals, are regulated by steroid hormones, then one possibility is that sex-labels are only seasonally present. Some evidence to support this hypothesis was recently shown in song sparrows (*Melospiza melodia*) where the wax ester composition of their preen oil was sex-specific during the breeding season, but not at the end of the breeding season (Grieves et al. 2019a). To determine whether chemical sex labels are seasonally present in Leach's storm-petrels, future studies should sample across the breeding season to better understand the relationship between sex, reproductive state and chemical profiles.

Individual Label. We found that Leach's storm-petrels have individual chemical signatures that are present across multiple breeding seasons. Chemical individuality has been investigated in a number of other bird species. Most studies have identified high individual repeatability in chemical profiles at multiple points within a single year (Gabirot et al. 2018; Karlsson et al. 2010; Potier et al. 2018; Whittaker et al. 2010; Zhang et al. 2009). To our knowledge, there are only three other species where the presence of individual chemical signatures has been shown across multiple years: Antarctic prion, black-legged kittiwake (*Rissa tridactyla)*, and blue petrel (Bonadonna et al. 2007; Leclaire et al. 2011; Mardon et al. 2011). Notably, all of these birds are long-lived seabirds that retain the same mate across several years and may benefit from individual signals to aid in mate recognition (Hatch et al. 2009; Warham 1996). In our study, the compounds associated with the individual signal were primarily higher molecular weight analytes with retention indices between 1900 and 2300. These compounds may contribute to olfactory signals if present at concentrations above sensory thresholds. Additionally, they could be broken down to other more volatile components that contribute to scent (e.g., degradation to shorter chain aldehydes and ketones) by the bird's microbiome or through environmental exposure. Further work is needed to fully understand which compounds directly carry socially-relevant information, since this study only provides correlations between the detected compounds and the variation between individuals.

The presence of an individual chemical label does not necessarily mean that an organism is able to use it for recognition. Several procellariid seabirds (Antarctic prion, blue petrel and Wilson's storm-petrel *Oceanites oceanicus*) perform olfactory partner recognition (Bonadonna and Nevitt 2004; Jouventin et al. 2007; Mardon and Bonadonna 2009). Antarctic

prions and blue petrels also practice self-odor avoidance such that they show preference for odors of conspecifics over their own scent in behavioral trials, which has been proposed as a mechanism for inbreeding avoidance (Bonadonna and Nevitt 2004; Mardon and Bonadonna 2009). The ability to recognize kin using olfaction has been demonstrated in the European storm-petrel (*Hydrobates pelagicus*; Bonadonna and Sanz-Aguilar 2012) and the zebra finch (*Taeniopygia guttata*; Krause et al. 2012; Caspers et al. 2017), but overall, the ability of birds to use odors for recognition is understudied and remains an area warranting further research. Leach's storm-petrel chicks are able to discriminate the scent of their burrow over that of conspecific, a finding that has been implicated in individual recognition behavior (O'Dwyer et al. 2008; O'Dwyer and Nevitt 2009). However, behavioral experiments specifically testing whether this species can use scent to discriminate individuals are needed to fully understand their olfactory capabilities.

Whether the individual-specific odors identified in this study broadcast signals of genetic quality remains an area for future research. The major histocompatibility complex, or MHC, is a family of highly polymorphic immune genes found in all vertebrates (Iwasaki and Medzhitov 2010). It is thought to be the primary genetic determinant of personal odor (Penn and Potts 1998; Yamazaki et al. 1976) and it has been implicated in facilitating individual discrimination in all classes of vertebrates (Fish: Milinski 2006; Reusch et al. 2001; Reptiles: Olsson et al. 2003; Birds: Grieves et al. 2019b; Leclaire et al. 2017b; Mammals: Potts et al. 1991; Wedekind and Penn 2000; Yamazaki et al. 1976). Furthermore, since MHC reflects the quality of the immune system, it is also expected to play a key role in mate choice (Boehm and Zufall 2006; Penn 2002; Penn and Potts 1999). While MHC-mediated mate choice is supported in a number of bird

species, including this population of Leach's storm-petrels (e.g. Bonneaud et al. 2006; Freeman-Gallant et al. 2003; Hoover et al. 2018; Knafler et al. 2012), the use of MHC-encoded odors for individual discrimination has only been tested in a few bird species so far. The blue petrel and the song sparrow have been shown to assess MHC similarity using olfactory cues (Grieves et al. 2019b; Leclaire et al. 2017c), and two species of birds, the song sparrow and the black-legged kittiwake, have chemical profiles that covary with MHC genetic distance (Leclaire et al. 2014; Slade et al. 2016). Further studies in additional species are needed, but these initial findings suggest that olfactory-facilitated identification of MHC could be common across birds.

Conclusion. This study employed headspace stir bar sorptive extraction with GC-MS to demonstrate that the chemical profiles of Leach's storm-petrel feathers contain social information. We found evidence of individual odor signatures that are present across multiple breeding seasons, which could have important implications for how this monogamous, nocturnal species conducts its cryptic breeding activities. These findings provide the foundation for future studies investigating whether these birds use these signatures to recognize individuals in social interactions and to choose their mates.

Funding and Ethics Statement: This research was supported by National Science Foundation (IOS 1258828). Research protocols were approved by UC Davis Animal Care and Use (protocol 17588 in 2013-2015, protocol 19288 in 2016) and the Canadian Wildlife Service (banding permit 10695, research permit SC2742). Samples were transported to the United States under USDA Veterinary Permit to Import Controlled Materials 125361 and Nova Scotia Permit to Export Wildlife.

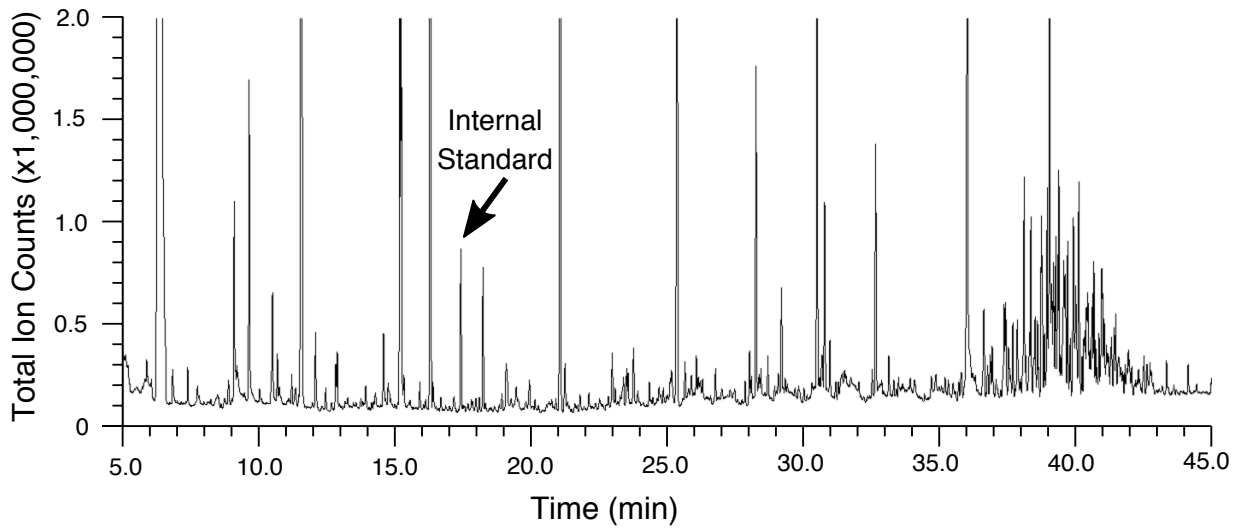

Figure 1.1. A representative GC-MS total ion chromatogram of the volatile profile extracted from Leach's storm-petrel feathers. The internal standard, Naphthalene-d8, is indicated with the arrow at 17.38 mins

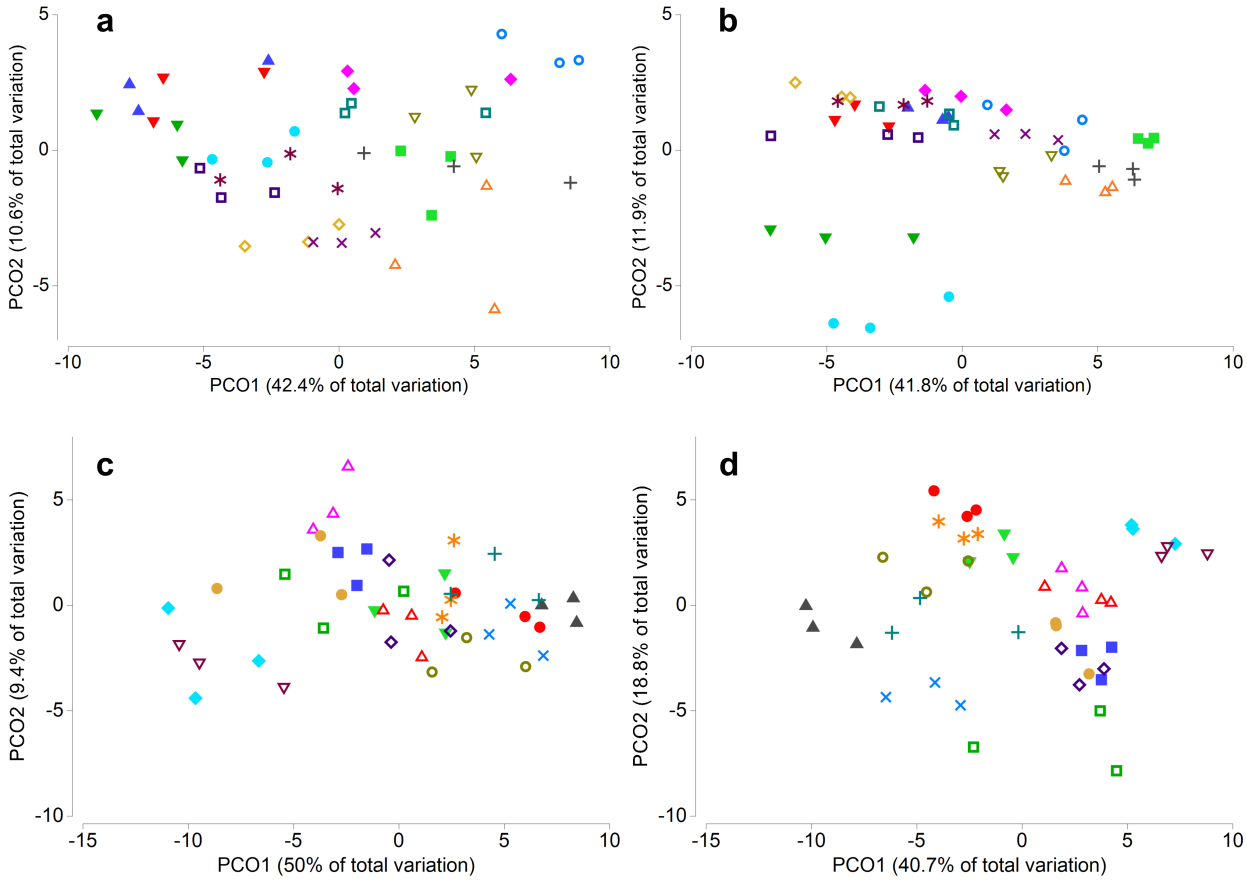

Figure 1.2. Two dimensional unconstrained PCO plots showing the relationships between triplicate samples from individual birds. To more easily visualize the patterns, the data has been plotted by sex and year, so that each plot displays only 45 of the 180 total samples. The upper plots show triplicate samples analyzed from females in a) 2015 and b) 2016. Triplicate samples from males are displayed in c) for 2015 and d) for 2016. Each symbol in the plot corresponds to a sample. The different shapes and colors represent an individual, such that each bird has three points per plot

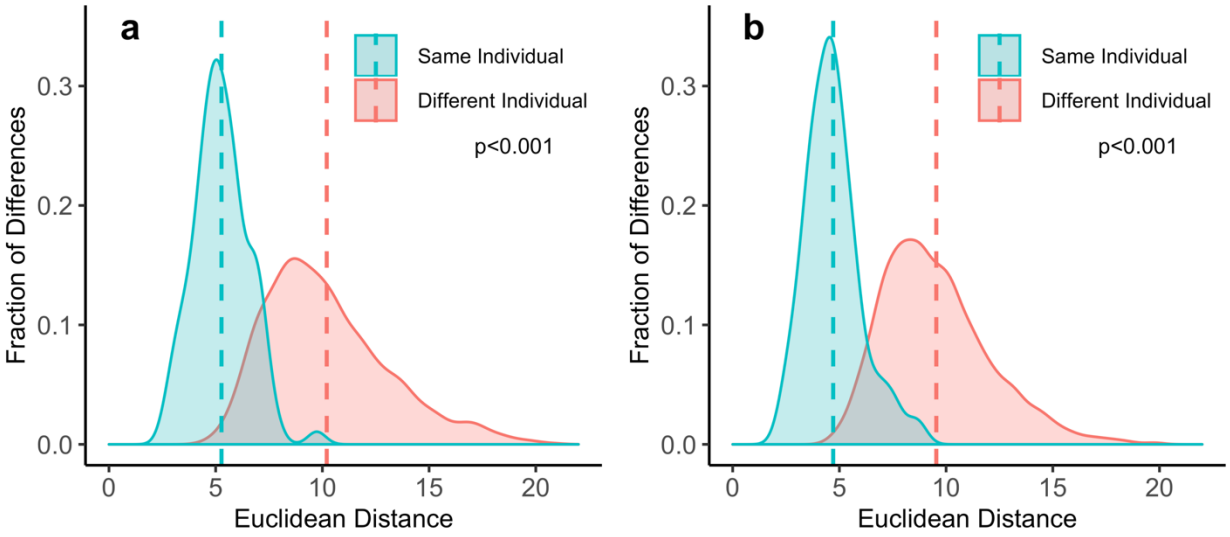

Figure 1.3. Comparison of chemical profiles between replicates from the same individual with samples from different individuals. Euclidean distances over 127 compounds were measured to compare samples from a) 2015 and b) 2016. P-values were calculated using a two-sample permutation test with 10,000 permutations. Dashed lines show group means

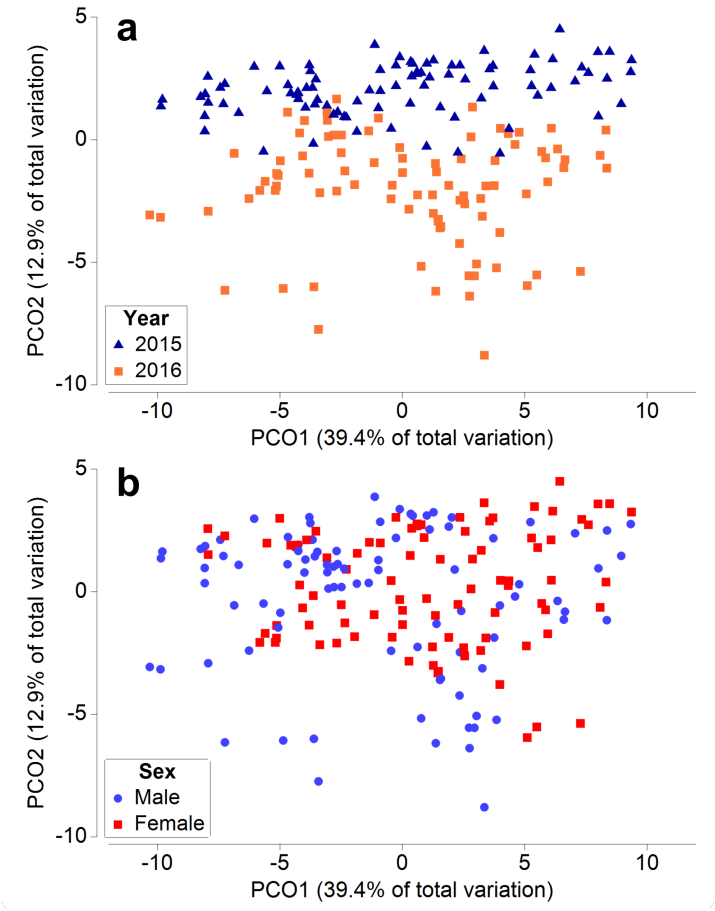

Figure 1.4. Two dimensional unconstrained PCO plots of the 180 samples used in our analysis. Each symbol in the plot corresponds to a sample. Both plots depict PCO1 and PCO2 but the data are represented using different symbols and colors to show the effect of a) Year and b) Sex

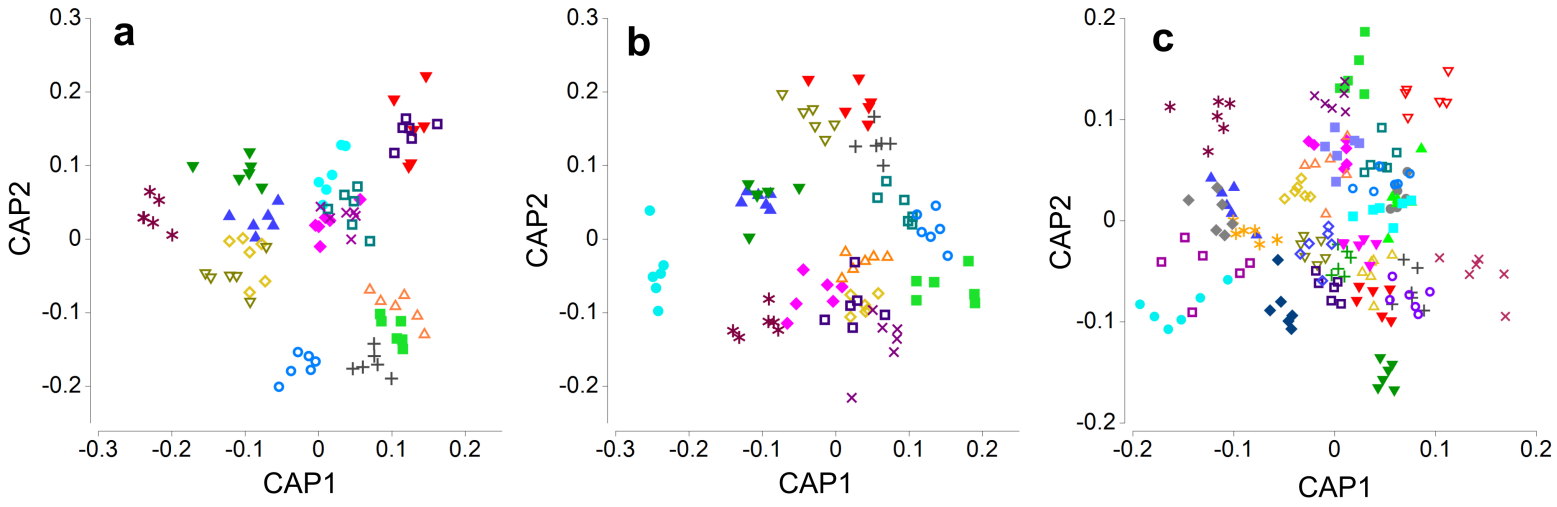

Figure 1.5. CAP analyses of the Individual chemical signature using 80 bird-derived compounds. Three models were compared, each using a different group of birds: a) Females (15 individuals,) b) Males (15 individuals), c) All Birds (30 individuals). Each data point corresponds to a sample and each color/symbol combination represents a different individual. The plots show only 2 CAP axes out of the 15 (for a and b) or 17 (c) retained by the models. The models correctly allocated 87% (a), 89% (b), and 82% (c) of samples using the leave-one-out classification method

Table 1.1. List of tentatively identified compounds detected in Leach's storm-petrel feathers

Retention Time	Compound Name	EIC	CAS Number	Calculated Retention Index	Published Retention Index	Reference Ions	Occurrence
5.99*	Hexanal	56	66-25-1	808	802	41, 43, 44, **56**, 57	100%
6.67*	Furfural	95	98-01-1	830	835	39, 67, **95**, 96, 97	100%
8.38*	Styrene	104	100-42-5	886	895	51, 77, 78, 103, **104**	100%
8.70	Nonane	N/A	11-84-2	897	899[a]	41, **43**, 57, 71, 85	N/A
8.77*	Heptanal	70	111-71-7	899	896	41, 43, 44, 55, **70**	100%
9.67	Alpha-pinene	93	80-56-8	928	931	77, 79, 91, 92, **93**	70%
10.33	2,4-Thujadiene	91	36262-09-6	950	956	77, **91**, 92, 119, 134	37%
10.64*	Benzaldehyde	106	100-52-7	960	961	50, 51, 77, 105, **106**	100%
11.08	(-) Beta-pinene	93	18172-67-3	974	975	41, 69, 79, 91, **93**	85%
11.43*	6-Methyl-5-hepten-2-one	108	110-93-0	986	988	41, **43**, 55, 69, 108	100%
11.59*	2-Octanone	58	111-13-7	991	992	43, **58**, 59, 71, 128	100%
11.91	Decane	N/A	124-18-5	1001	999[a]	41, 43, **57**, 71, 85	N/A
12.01*	Octanal	84	124-13-0	1005	1006	**43**, 44, 56, 57, **84**	100%
12.41	4-Cyanocyclohexene	107	100-45-8	1018		**54**, 79, 80, 106, 107	100%
12.61	o-Cymene	119	527-84-4	1024	1021	91, 117, **119**, 120, 134	42%
12.77	Limonene	93	138-86-3	1030	1030[b]	67, **68**, 79, 93, 136	90%
12.84*	2-Ethyl-1-hexanol	57	104-76-7	1032	1030	41, 43, **57**, 70, 83	100%
12.92*	6-Ethyl-2-methyloctane	71	62016-19-7	1034		43, **57**, 70, 71, 85	58%
13.23*	Benzeneacetaldehyde	91	122-78-1	1045	1043	65, **91**, 92, 120, 121	97%
13.69	Gamma-terpinene	93	99-85-4	1060	1062	77, 91, **93**, 121, 136	53%
13.83	1-Chlorooctane	91	111-85-3	1064	1064	43, 55, 69, **91**, 93	98%
13.90*	Acetophenone	105	98-86-2	1066	1062	51, 77, **105**, 106, 120	100%
14.18	1-Octanol	N/A	111-87-5	1076	1073	41, 43, 55, **56**, 70	N/A
14.76*	2-Nonanone	58	821-55-6	1095	1091	43, 57, **58**, 59, 71	100%
15.07	Undecane	N/A	1120-21-4	1105	1099[a]	41, 43, **57**, 71, 85	N/A
15.19*	Nonanal	57	124-19-6	1109	1102	41, 56, **57**, 70, 98	100%
16.87	1-(1-Tert-Butoxypropan-2-yloxy)propan-2-ol	59	132739-31-2	1163		41, 45, 57, **59**, 103	93%

17.17	Octanoic acid	N/A	124-07-2	1173	1191	**60**, 43, 73, 85, 101	N/A
17.38	Naphthalene-d8 (Internal Standard)	136	1146-65-2	1180		68, 108, 134, **136**, 137	N/A
17.79*	2-Decanone	58	693-54-9	1193	1193	43, 57, **58**, 59, 71	100%
18.07*	Dodecane	57	112-40-3	1203	1200	41, 43, **57**, 71, 85	100%
18.20*	Decanal	82	112-31-2	1207	1207	43, 55, **57**, 70, 82	100%
18.29	O-Ethyl sec-butylthiocarbamate	161	82360-12-1	1210		44, 72, 100, 132, **161**	85%
19.38	1,3-Ditert-butylbenzene	175	1014-60-4	1245		57, 65, **175**, 176, 190	65%
19.91	Nonanoic acid	N/A	112-05-0	1263	1272	55, 57, **60**, 115, 129	N/A
20.56	3-Tert-butylphenol	135	585-34-2	1284		95, 107, **135**, 136, 150	100%
20.64*	2-Undecanone	58	112-12-9	1286	1291	41, 43, **58**, 59, 71	100%
20.88*	Tridecane	71	629-50-5	1294	1299[b]	41, 43, **57**, 71, 85	100%
21.06*	Undecanal	82	112-44-7	1301	1308	41, 43, 55, 57, **82**	100%
21.78*	Unidentified	88		1330		70, **88**, 89, 115, 155	80%
22.54	n-Decanonic acid	N/A	334-48-5	1361	1387	41, 43, 57, **60**, 73	N/A
22.74	Texanol	89	77-68-9	1369	1380	43, 56, **71**, 89, 173	88%
23.34*	Unidentified	58		1394		43, 55, **58**, 71, 97	85%
23.51*	Unidentified	115		1401		70, 87, **88**, 89, 115	65%
23.55*	Tetradecane	57	629-59-4	1402	1399[b]	41, 43, **57**, 71, 85	100%
23.74	Longifolene	161	475-20-7	1410	1402	91, 93, 94, 107, **161**	97%
23.75*	Dodecanal	82	112-54-9	1411	1420	**41**, 43, 55, 57, 82	100%
23.91	Unidentified sesquiterpene	119		1417		93, 105, **119**, 161, 204	33%
24.34*	Ethyl decanoate	88	110-38-3	1435	1397	41, 43, 73, **88**, 101	53%
24.70*	Geranylacetone	69	3796-70-1	1449	1452	41, **43**, 69, 136, 151	100%
24.74	Beta-barbatene	108	72346-55-5	1451	1451	93. 94, 95, **96,** 108	27%
24.85	Unidentified	112		1455		57, 69, 70, 71, **112**	33%
25.31	1-Chloroundecane	91	2473-03-2	1474		43, 57, 69, 71, **91**	100%
25.65*	Unidentified	88		1488		43, 69, 70, 88, **115**	58%
26.07*	Pentadecane	57	629-62-9	1505	1500[b]	41, 43, **57**, 71, 85	100%
26.14	2,4-Ditert-butylphenyl 5-hydroxypentanoate	191	166273-38-7	1508		57, 163, **191**, 192, 206	100%
26.16	Alpha-chamigrene	136	19912-83-5	1509	1516	41, 93, 121, 133, **136**	38%
26.21	Beta-bisabolene	93	495-61-4	1511	1509	41, 67, **69, 93**, 204	38%
26.30*	Tridecanal	82	10486-19-8	1514	1510	41, 43, 55, **57**, 82	100%

26.45	Delta-cadinene	161	483-76-1	1520	1530[b]	105, 119, 134, **161**, 204	25%
26.50	cis-Calamenene	159	483-77-2	1522	1522	129, 131, **159**, 160, 202	75%
26.60	Methyl dodecanoate	N/A	111-82-0	1526	1526	41, 43, 55, **74**, 87	N/A
26.77*	Unidentified	88		1533		70, **88**, 89, 115, 183	52%
26.79	(1-Butylhexyl)benzene	91	4537-11-5	1534	1535	**91**, 105, 147, 161, 218	100%
27.03	(1-Propylheptyl)benzene	91	4537-12-6	1544	1534	**91**, 92, 105, 133, 175	100%
27.30*	n-Nonylcyclohexane	82	2883-02-5	1555	1556	41, 55, 67, 82, **83**	100%
27.44	Dodecanoic acid	N/A	143-07-7	1561	1562	41, 43, 57, 60, **73**	N/A
27.48	(1-Ethyloctyl) benzene	91	4621-36-7	1562	1553	**91**, 105, 119, 189, 218	100%
28.10	Txib	71	6846-50-0	1587	1587	43, **71**, 111, 159, 243	100%
28.27*	Ethyl dodecanoate	88	106-33-2	1594	1581	41, 43, 73, **88**, 101	100%
28.37	(1-Ethyloctyl) benzene	105	4537-13-7	1598	1588	91, 104, **105**, 106, 218	100%
28.44*	Hexadecane	57	544-76-3	1601	1600[b]	41, 43, **57**, 71, 85	100%
28.71*	Tetradecanal	82	124-25-4	1612	1615	41, 43, 55, **57**, 82	100%
29.02	(1-Pentylhexyl)benzene	91	4537-14-8	1625	1620	41, **91**, 92, 105, 161	100%
29.11	(1-Butylheptyl)benzene	91	4537-15-9	1629	1626	**91**, 92, 105, 147, 175	100%
29.35	(1-Propyloctyl)benzene	91	4536-86-1	1638	1636	**91**, 92, 105, 133, 189	100%
29.84	(1-Ethylnonyl)benzene	91	4536-87-2	1658	1656	41, **91**, 92, 105, 119	100%
30.03	Unidentified	195		1666		57, 165, 180, **195**, 210	48%
30.17	1-Tetradecanol	N/A	112-72-1	1672	1676	41, 43, **55,** 69, 83	N/A
30.62*	2-Pentadecanone	58	2345-28-0	1690	1698	43, 57, **58**, 59, 71	100%
30.69	(1-Methyldecyl)benzene	105	4536-88-3	1693	1692	79, 91, **105**, 106, 232	100%
30.70*	Heptadecane	57	629-78-7	1693	1700[b]	41, 43, **57**, 71, 85	100%
30.80*	Pristane	57	1921-70-6	1701	1703	41, 43, **57**, 71, 85	100%
30.90	Unidentified Sesquiterpene	175		1706		147, 160, **175**, 176, 218	32%
30.99*	Pentadecanal	82	2756-11-9	1710	1707	41, 43, 55, 57, **82**	100%
31.21	(1-Pentylheptyl)benzene	91	2719-62-2	1721	1719	**91**, 92, 105, 161, 175	100%
31.31	(1-Butyloctyl)benzene	91	2719-63-3	1726	1723	**91**, 105, 147, 189, 246	100%
31.59	(1-Propylnonyl)benzene	91	2719-64-4	1739	1735	**91**, 105, 133, 203, 246	100%
32.07	(1-Ethyldecyl)benzene	91	2400-00-2	1763	1735	**91**, 105, 119, 217, 246	100%
32.46	1-Pentadecanol	N/A	629-76-5	1783	1778	55, 57, 69, **83**, 97	N/A
32.68*	Ethyl tetradecanoate	88	124-06-1	1793	1790	41, 43, **88**, 89 101	83%

32.85	2-Ethylhexyl Salicylate	120		1801		57, 70, **120**, 121, 138	100%
32.86*	Octadecane	57	593-45-3	1802	1800[b]	41, 43, **57**, 71, 85	100%
32.91	(1-Methylundecyl)benzene	105	2719-61-1	1804	1791	77, 79 91, **105**, 106	100%
33.17*	Hexadecanal	82	629-80-1	1817	1818	43, 55, 57, **82**, 83	100%
33.31	(1-Pentyloctyl)benzene	91	4534-49-0	1824	1814	**91**, 105, 119, 161, 189	100%
33.45	(1-Butylnonyl)benzene	91	4534-50-3	1831	1821	**91**, 92, 105, 119, 147	100%
34.49	Homosalate	138	118-56-9	1882	1903	69, 109, 120, 121, **138**	67%
34.50	1-Hexadecanol	N/A	36653-82-4	1882	1879	41, 43, **55**, 69, 83	N/A
34.88*	2-Heptadecanone	58	2922-51-2	1901	1900	41, 43, **58**, 59, 71	100%
34.91*	Nonadecane	57	629-92-5	1902	1900[b]	41, 43, **57**, 71, 85	100%
35.02	1-Dodecyl-2-methylbenzene	105	4534-53-6	1908	1894	43, 91, 104, **105**, 106	100%
35.12	Unidentified long chain ester	141		1913		57, 71, 111, **141**, 159	25%
35.24*	Heptadecanal	82	629-90-3	1919	1920	43, 57, 68, **82**, 96	100%
35.37	Methyl hexadecanoate	N/A	112-39-0	1925	1927	41, 43, 55, **74**, 87	N/A
35.74	Unidentified long chain ester	141		1943		43, 57, 71, 140, **141**	37%
35.81*	Unidentified	70		1947		57, **70**, 71, 97, 111	63%
36.07	Hexadecanoic acid	N/A	57-10-3	1959	1964	41, **43**, 57, 60, 73	N/A
36.14*	Unidentified long chain ester	159		1963		57, 71, 84, **97**, 159	53%
36.62*	Unidentified long chain ester	140		1986		57, **70**, 71, 111, 140	52%
36.67*	Ethyl hexadecanoate	88	628-97-7	1989	1993	41, 43, 55, **88**, 101	100%
36.91*	Unidentified long chain ester	71		1998		57, 71, **111**, 155, 173	85%
36.98*	Unidentified long chain ester	125		2004		57, **70**, 71, 125, 159	90%
37.42*	Unidentified long chain ester	155		2037		**57**, 71, 85, 111, 155	92%
37.48*	Unidentified long chain ester	70		2042		57, **70**, 71, 111, 173	98%
37.58*	Unidentified long chain ester	112		2050		57, 84, 85, 112, **173**	95%
37.74*	Unidentified long chain ester	97		2062		57, 71, 84, **97**, 173	97%
37.90*	Unidentified long chain ester	111		2074		57, **70**, 71, 85, 111	95%
38.15*	Unidentified long chain ester	155		2094		57, 71, 84, **111**, 155	100%
38.35*	Unidentified long chain ester	140		2109		57, 85, 111, **140**, 187	95%
38.40*	Unidentified long chain ester	125		2113		57, **70**, 71, 125, 173	100%
38.53*	Unidentified long chain ester	111		2123		57, 84, 85, **111**, 187	90%
38.64*	Unidentified long chain ester	97		2131		57, 71, 85, **97**, 187	97%

38.79*	Unidentified long chain ester	155		2143		57, 71, 85, 154, **155**	100%
38.82*	Unidentified long chain ester	125		2145		57, **70**, 71, 125, 173	100%
38.90*	Unidentified long chain ester	173		2152		57, 71, 111, 126, **173**	95%
38.99*	Unidentified long chain ester	173		2158		43, 57, 71, **97, 173**	95%
39.00*	Unidentified long chain ester	111		2159		57, 71, 84, 85, **111**	98%
39.09	Octadecanoic acid	N/A	57-11-4	2166	2170	**43**, 55, 57, 60, 73	N/A
39.17*	Unidentified long chain ester	154		2172		**57**, 70, 85, 154, 187	93%
39.23*	Unidentified long chain ester	125		2177		57, 70, **125**, 154, 187	98%
39.32*	Unidentified long chain ester	111		2184		57, 71, **111**, 155, 173	97%
39.39*	Unidentified long chain ester	111		2189		57, **71**, 84, 111, 187	90%
39.43*	Unidentified long chain ester	84		2192		**70**, 71, 84, 125, 173	100%
39.61*	Unidentified long chain ester	125		2206		57, **70**, 71, 125, 187	100%
39.68*	Unidentified long chain ester	126		2212		57, **71**, 84, 85, 111	97%
39.76*	Unidentified long chain ester	187		2218		57, 70, 71, 97, **187**	98%
39.86*	Unidentified long chain ester	125		2226		57, 70, 71, **125**, 154	100%
39.99*	Unidentified long chain ester	139		2236		57, **70**, 71, 139, 173	100%
40.17*	Unidentified long chain ester	125		2249		**57**, 70, 71, 84, **125**	100%
40.29*	Unidentified long chain ester	168		2259		57, **70**, 71, 168, 187	83%
40.44*	Unidentified long chain ester	173		2270		57, 70, 71, 125, **173**	100%
40.51*	Unidentified long chain ester	125		2276		70, 71, 84, **125**, 187	100%

*Indicates the 80 compounds that were designated as “bird-derived”.

Compounds in grey were observed in many samples but were unable to be integrated due to poor peak shape or issues with co-elution.

EIC (extracted ion chromatogram) is the ion that was used to quantify the peak area of the compound.

All published retention indices were obtained from NIST Chemistry WebBook (webbook.nist.gov) except where marked. All values correspond to GC columns with similar properties to the DB-5MS used in this study, including DB-1, DB-5, HP-5, DB-5MS, and HP-5MS.

[a] designates indices from The Pherobase (pherobase.com)

[b] designates indices from Flavornet (flavornet.org).

Reference ions in bold indicate the most abundant ion in the mass spectrum of each compound

Table 1.2. PERMANOVA results for the analysis of sociochemical information in feather samples

Source	df	SS	MS	Pseudo-F	p (perm)
Using 180 Samples (6 per bird)					
Year	1	855.2	855.2	13.916	**<0.001**
Sex	1	268.8	268.8	1.359	0.2096
Individual (nested within Sex)	28	5538.7	197.8	14.733	**<0.001**
Year*Sex	1	68.6	68.6	1.0348	0.4081
Year*Individual	28	1720.6	61.5	4.5769	**<0.001**
Residuals	120	1611.2	13.4		
Using 60 Samples (2 per bird)					
Year	1	293.31	293.31	12.952	**<0.001**
Sex	1	92.332	92.332	1.357	0.213
Individual (nested within Sex)	28	1904.9	68.031	3.0042	**<0.001**
Year*Sex	1	23.888	23.888	1.0549	0.3775
Residuals	28	634.07			

df: degrees of freedom; SS: sum of squares; MS: mean square; significant effects are in bold. Significance assessed at α=5% using 10,000 permutations of the data

The second test using 60 samples lacks replication at the lowest level, so the model drops the highest order interaction effect of Year*Individual

Table 1.3. Results from CAP models investigating the individual chemical signature. We ran and compared 6 separate models using two set of analytes A) 127 compounds and B) 80 bird-derived compounds. Within each set of analytes, we examined models differentiating A) females, B) males and C) all birds

Original Group	Classified Group		Percent Correctly Allocated	m	Trace statistic	p (perm)	# of Validation Samples Correctly Assigned
	Correct Individual	Different Individual					
Using All 127 Compounds							
Females (n=15, 90 samples)	78	12	86.67	15	7.38762	**<0.001**	4 of 5
Males (n=15, 90 samples)	80	10	88.89	15	6.99706	**<0.001**	4 of 4
All Birds (n=30, 180 samples)	148	32	82.22	17	8.16728	**<0.001**	7 of 9
Using 80 Bird Compounds							
Females (n=15, 90 samples)	74	16	82.2	15	6.4572	**<0.001**	5 of 5
Males (n=15, 90 samples)	72	18	80.0	15	6.2034	**<0.001**	4 of 4
All Birds (n=30, 180 samples)	148	32	82.2	19	8.5349	**<0.001**	9 of 9

m: number of PCO axes retained by the CAP model. Significant effects are in bold. Significance assessed at α=5% using 10,000 permutations of the data

1.6 SUPPLEMENTARY MATERIALS

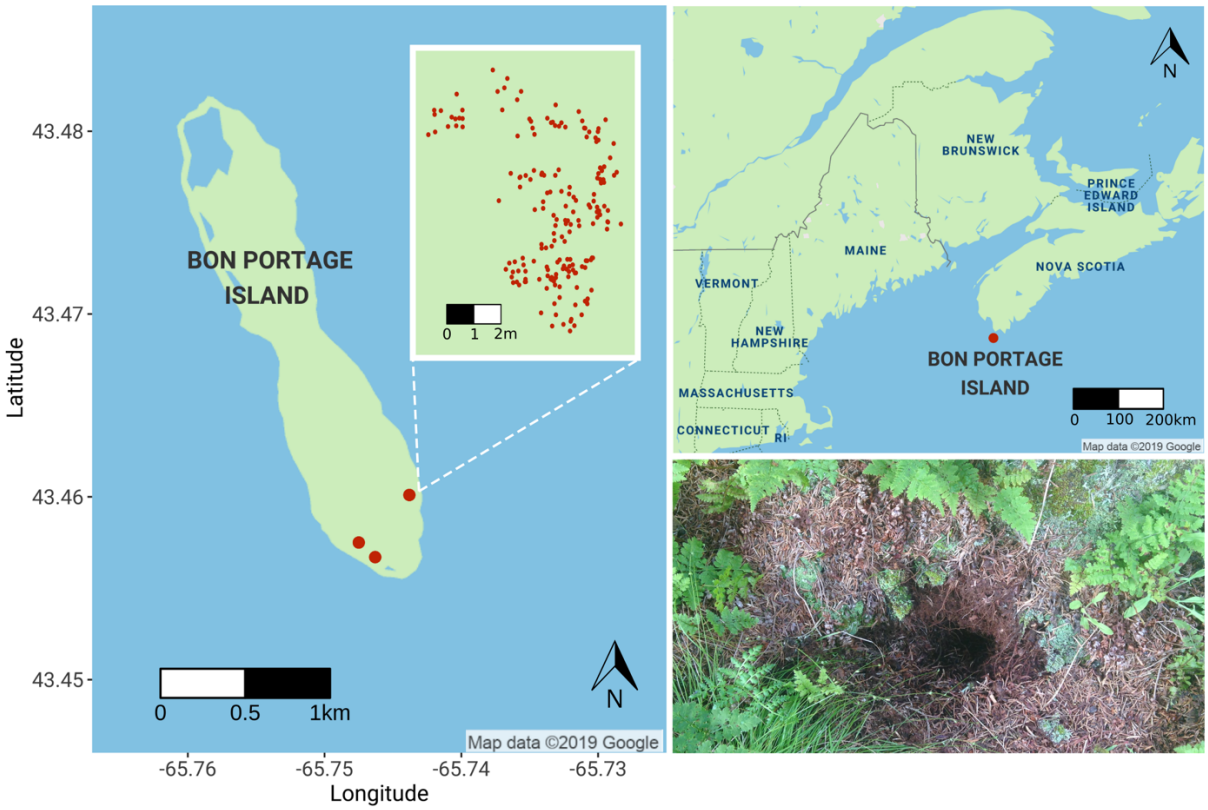

Figure S1.1. Map of Bon Portage Island, Nova Scotia, Canada. Our research group has 550 marked Leach's storm-petrel burrows on this island that are spread across 3 sites (marked by the circles). Burrow locations at one of the sites are depicted in the insert. A photo of a storm-petrel burrow is shown in the lower right

Figure S1.2. Feather collection method. Leach's storm-petrel feathers (6-8) were gently plucked from the base of bird's tail near the preen gland while wearing nitrile gloves

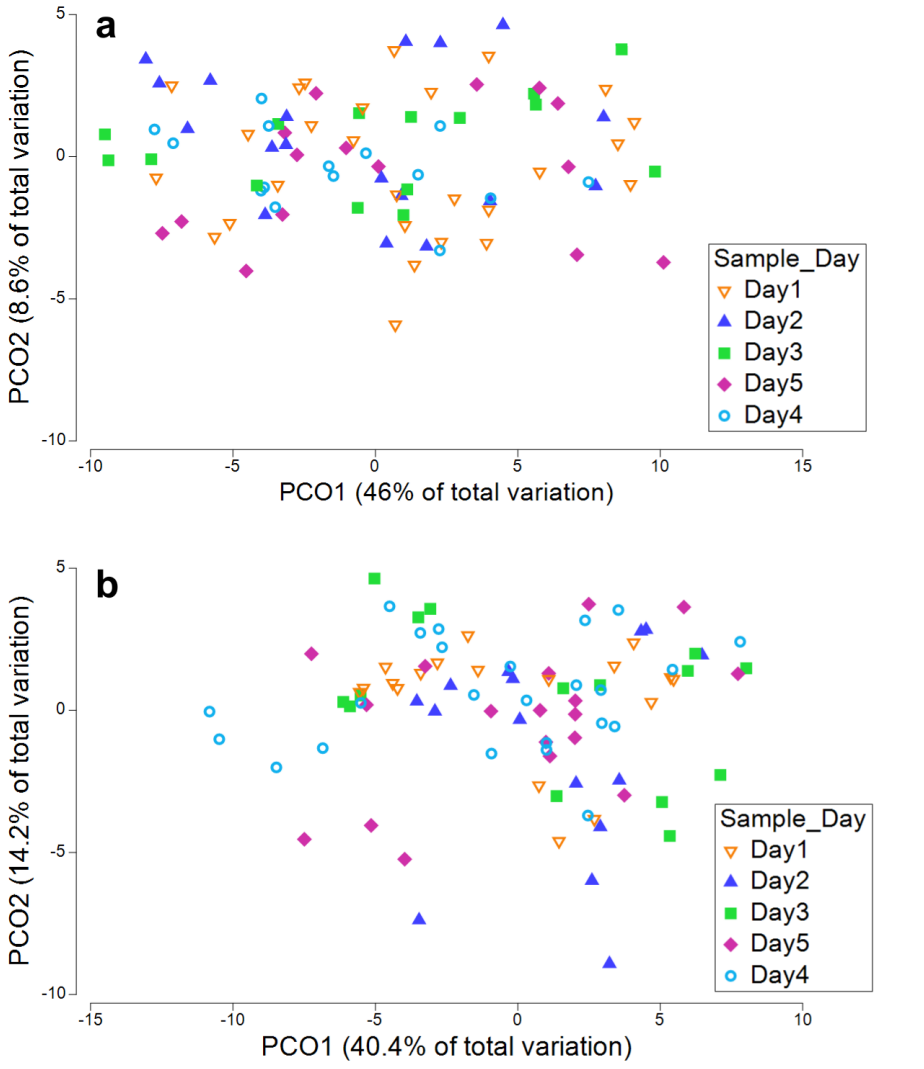

Figure S1.3. Two dimensional unconstrained PCO plots showing 90 samples from each of the two analysis years a) 2015 and b) 2016. Each symbol in the plot corresponds to a sample. The different shapes and colors represent the five different sample dates in each year. A single factor PERMANOVA test run separately on each year found no significant influence of sampling date (P=0.157 for 2015 and P=0.395 for 2016)

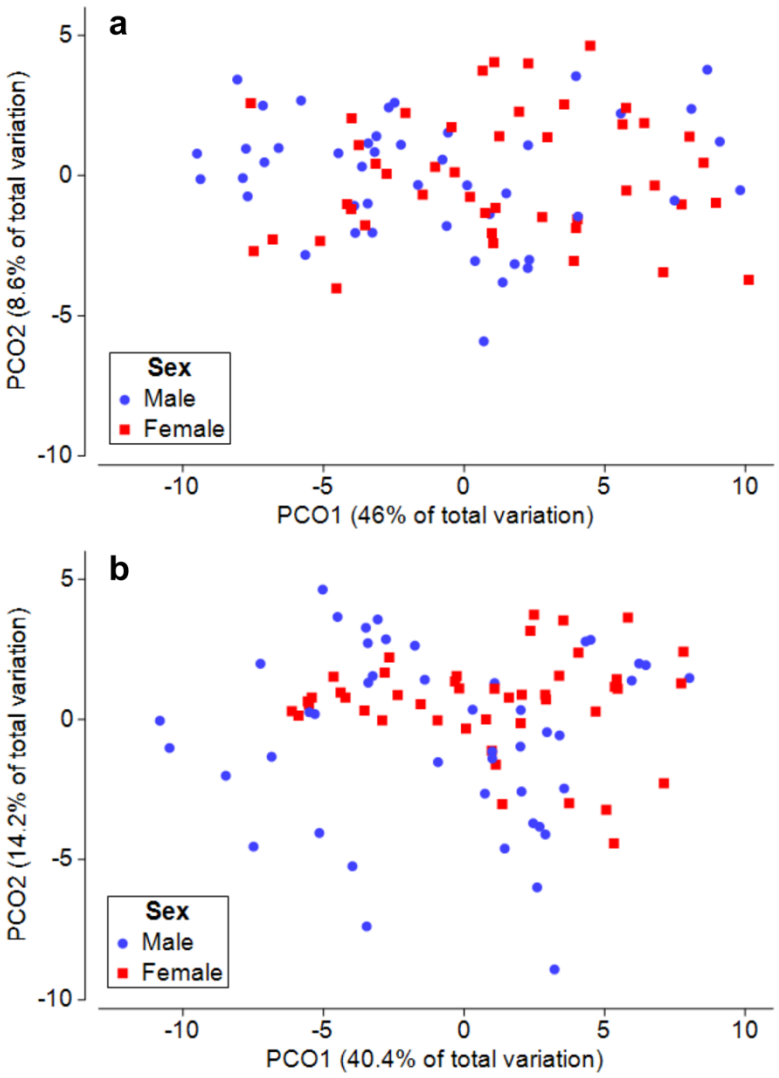

Figure S1.4. Two dimensional unconstrained PCO plots showing 90 samples from each of the two analysis years a) 2015 and b) 2016. Each symbol in the plot corresponds to a sample. The different shapes and colors represent the two sexes

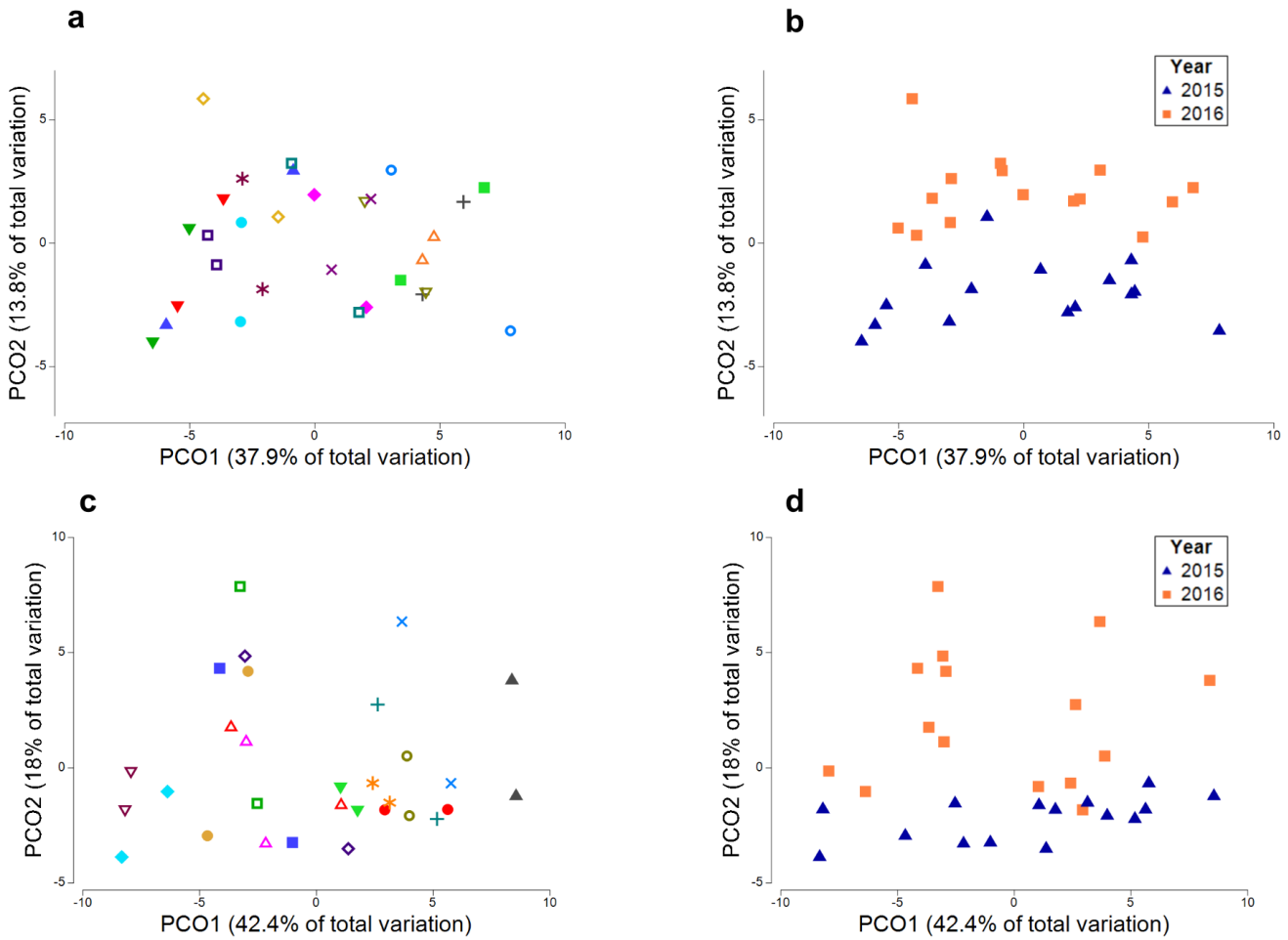

Figure S1.5. Two dimensional unconstrained PCO plots showing the relationships between samples from individual birds collected across both sampling years. To more easily visualize the patterns, the data has been divided by sex, so that each plot displays 15 individuals. Each symbol in the plot corresponds to an individual. Plots A and B both depict samples from females, while C and D

show samples from males. In plots A and C, the different shape and color combinations represent an individual, such that each bird has two points, one from each year. These two points were generated by averaging across the three replicates from each year. Plots B and D are colored by Year. These ordination plots help to explain the significant Year*Individual interaction detected in the PERMANOVA test (Table 3); they show that while the direction of the year effect is the same for each bird (2015 samples separate from 2016 samples along PCO2), the magnitude of this effect differs between individuals as some birds have more distance between the two years of samples

Table S1.1. Top compounds associated with PCO2 that have Pearson correlation coefficient r>0.4

Retention Time	Retention Index	Tentative Compound ID	EIC	Pearson Correlation (r)
11.08	974	(-) Beta-Pinene	93	-0.407
12.61	1024	o-Cymene	119	-0.692
12.77	1030	Limonene	93	-0.868
13.69	1060	Gamma-terpinene	93	-0.886
21.78	1330	Unidentified	88	-0.816
23.51	1401	Unidentified	115	-0.856
23.91	1417	Unidentified sesquiterpene	119	-0.631
24.34	1435	Ethyl decanoate	88	-0.900
24.85	1455	Unidentified	112	-0.550
25.65	1488	Unidentified	88	-0.886
26.14	1508	2,4-Ditert-butylphenyl-5-hydroxypenanoate	191	-0.482
26.77	1533	Unidentified	88	-0.915
28.27	1594	Ethyl dodecanoate	88	-0.822
32.68	1793	Ethyl tetradecanoate	88	-0.801
36.67	1989	Ethyl hexadecanoate	88	-0.471

Table S1.2. List of top 40 compounds associated with the individual chemical signature that were identified by the two CAP models discriminating 30 birds

		CAP using 80 compounds	CAP using 127 compounds
Retention Time	**Retention Index**	**r (Pearson correlation)**	**r (Pearson correlation)**
12.61*	1024		0.495
21.78	1330	0.682	0.554
23.51	1401	0.697	0.569
24.34	1435	0.684	0.571
24.74*	1451		0.619
24.85*	1455		0.453
25.65	1488	0.676	0.601
26.50*	1522		0.570
26.77	1533	0.671	0.591
28.27	1594		0.456
30.90*	1706		0.657
32.68	1793	0.590	0.461
32.86	1802		0.472
35.02*	1908		0.483
35.81	1947	0.796	0.473
36.14	1963	0.708	
36.62	1986	0.721	0.580
36.91	1998	0.741	0.617
36.98	2004	0.651	0.510
37.42	2037	0.741	0.606
37.48	2042	0.863	0.516
37.58	2050	0.875	0.461
37.74	2062	0.869	0.488
37.90	2074	0.776	0.558
38.15	2094	0.760	0.583
38.35	2109	0.714	0.564
38.40	2113	0.826	0.489
38.53	2123	0.621	0.597
38.64	2131	0.758	0.505
38.79	2143	0.826	0.508
38.82	2145		0.469
38.90	2152	0.838	0.586
38.99	2158	0.861	0.610
39.00	2159	0.648	0.607
39.17	2172	0.769	0.516
39.23	2177	0.706	

39.32	2184	0.809	0.552
39.39	2189	0.645	0.618
39.43	2192	0.771	
39.61	2206	0.722	
39.68	2212	0.752	0.488
39.76	2218	0.735	
39.86	2226	0.764	0.454
39.99	2236	0.801	0.507
40.17	2249	0.651	
40.29	2259	0.770	
40.44	2270	0.788	0.519
40.51	2276	0.656	

r is the Pearson correlation coefficient of a compound with one of the CAP axes retained in the corresponding models

*denotes analytes that are only found in the larger 127 compound list that are not also part of the smaller 80 bird-derived compound list

CHAPTER 2

Feather chemicals contain information about the major histocompatibility complex in a highly scented seabird

2.1 ABSTRACT

Mate choice informed by the polymorphic immune genes of the Major Histocompatibility Complex (MHC) may provide direct and indirect fitness benefits including offspring with increased immunocompetence. In many species, olfactory cues are considered the primary mechanism organisms use to evaluate the MHC genes of potential mates, yet this idea has received limited attention in birds. The oil produced by the avian preen gland contains scented compounds that birds spread on their plumage and the resulting chemical profile can reflect information that may facilitate mate choice decisions. Motivated by a finding of MHC-dependent mate choice in our study species, the Leach's storm-petrel (*Oceanodroma leucorhoa*), we examined whether the chemical profiles of this highly scented seabird contain information about MHC genes. We combined gas chromatography-mass spectrometry to measure the chemical profiles of feathers with locus-specific genotyping of MHC class IIB genes. Feather chemicals reflected individual MHC diversity through interactions with sex and breeding status. Furthermore, similarity in chemical profiles was correlated with similarity in MHC genotype within female-female and male-female dyads. We provide the first evidence that the scented compounds on bird feathers contain information about MHC genes. Our findings suggest that olfaction likely facilitates MHC-based mate choice in this species.

2.2 INTRODUCTION

The highly polymorphic genes of the Major Histocompatibility Complex (MHC) play a central role in the vertebrate adaptive immune system where they encode for cell surface receptors that detect foreign-derived peptides (Klein 1986). The range of pathogens an individual can respond to is determined by their MHC alleles, and thus different MHC genotypes are associated with differential survival (Sepil et al. 2013; Worley et al. 2010) and reproductive success (Eizaguirre et al. 2009; Kalbe et al. 2012; Thoß et al. 2011). Mating preferences for individuals with high quality or compatible MHC alleles can provide certain fitness advantages including direct benefits like parental care (Zelano and Edwards 2002) or indirect genetic benefits that enhance the pathogen-resistance of their offspring (Apanius et al. 1997; Potts and Wakeland 1990). Furthermore, as only close relatives are likely to carry similar genotypes, MHC may also facilitate inbreeding avoidance (Ruff et al. 2012). Because genes cannot be directly assessed, MHC-based mate choice requires individuals to detect and evaluate a phenotype that reflects the underlying genotype. Yet, in many species, it remains unclear exactly which physical trait organisms use to inform MHC-based mate choice.

Due to its important role in immune function and overall health, there are a wide range of phenotypes that can be influenced by MHC (Milinski 2006; Ruff et al. 2012). Correlations between MHC and condition-dependent visual and acoustic traits have been observed in a number of species (Ditchkoff et al. 2001; Dunn et al. 2013; Setchell et al. 2009; Slade et al. 2017; Von Schantz et al. 1996). However, olfactory cues present in bodily secretions are directly affected by MHC in some species and may be more reliable indicators of genotype (Boehm and Zufall 2006; Penn 2002). The use of olfaction to evaluate MHC has been implicated in all major

vertebrate groups (Bos et al. 2009; Grieves et al. 2019b; Grogan et al. 2019; Leclaire et al. 2017c; Milinski et al. 2005; Olsson et al. 2003; Reusch et al. 2001; Wedekind et al. 1995), but the vast majority of studies come from laboratory or captive mammals with well-studied olfactory abilities (Kwak et al. 2010).

Birds produce a chemically rich, scented secretion called preen oil that may serve as a source of olfactory information about MHC. They spread this oil onto their feathers during preening, which allows the volatile chemicals within to be readily assessed by conspecifics. The chemical composition of preen oil can reflect breeding status (e.g. Whittaker et al. 2019a), sex (e.g. Whittaker et al. 2010), and individual identity (e.g. Mardon et al. 2010). However, the idea that birds use odors to evaluate mates has only recently gained traction because this group was widely considered to lack a sense of smell. As the number of bird species shown to detect and discriminate conspecific odors has grown (reviewed in Caro et al. 2015), a handful of studies have examined odor-based mechanisms of MHC assessment. This work has revealed that two species, a songbird (song sparrow *Melospiza melodia*; Grieves et al. 2019) and a seabird (blue petrel *Halobaena caerulea*; Leclaire et al. 2017), can use odor cues to judge MHC similarity, and that the non-volatile chemicals in preen oil, which may be precursors to odorants, can contain information about MHC (Leclaire et al. 2014; Slade et al. 2016). These findings indicate that olfactory discrimination of MHC may by far more common in birds that previously thought.

Our study species, the Leach's storm-petrel (*Oceanodroma leucorhoa*), is particularly well-suited for examining the role of MHC in avian social signaling. This small, pelagic seabird is known for its strongly scented plumage and excellent sense of smell (Grubb 1974; O'Dwyer et al. 2008). Leach's storm-petrels choose their mates based on the MHC class IIB gene (Hoover et

al. 2018). Individuals also possess unique odor profiles, a finding that is consistent with a genetic basis for personal odor (Jennings and Ebeler 2020). However, we do not yet know whether these individual scents are related to MHC genotype. Here, we tested the hypothesis that MHC genotype is reflected in the scent of Leach's storm-petrel plumage. To address this objective, we used locus-specific genotyping of MHC class IIB genes coupled with gas-chromatography mass spectrometry (GC-MS) to measure the chemical profiles of feathers. Unlike previous studies that have focused on the non-volatile components of the preen oil, we targeted the scented compounds associated with the feathers, which are more likely to reflect the chemicals available for detection by the avian olfactory system. We tested the following two predictions: 1) the chemical profiles of individuals contain information about their MHC genotypes; 2) individuals with functionally similar MHC genotypes have similar chemical profiles.

2.3 METHODS

Study Site and Field Methods. We sampled Leach's storm-petrels at a large breeding colony (~39,000 breeding pairs; Pollet and Shutler 2018) on Bon Portage Island in Nova Scotia, Canada (43.46°N, -65.75°W). As part of an earlier investigation into MHC-mediated mate choice in this population (2010 - 2015), blood was collected from a large number of birds and used to determine their MHC genotype and sex (see Hoover et al. 2018 for detailed methods). To measure chemical profiles, we collected feather samples from 60 incubating adults during the 2016 breeding season. By targeting previously genotyped birds, we were able to sample an

equal number of males and females (30 per sex) that encompassed the majority of common MHC class IIB genotypes in the study population. From each bird, we plucked 6 small body feathers from ~5 cm above the preen gland while wearing clean nitrile gloves. Each sample was placed in a 10 mL glass vial and kept frozen at -20 °C. We transported the feathers on dry ice to the University of California, Davis where they were stored at -80 °C prior to analysis.

We checked nests every 3 days to determine the hatch date of each chick. For each adult, we calculated the number of days between the sample date and the hatch date. This value, which we refer to as "breeding status", provided an estimate of how far into the ~45-day incubation period each individual was at the time of sampling.

Chemical Analyses. We used previously described methods to measure the chemical profiles associated with Leach's storm-petrel feathers (see Jennings and Ebeler 2020). We analyzed samples from each individual in triplicate. For each replicate, 2 feathers were weighed and placed into a 10 mL glass vial. Vials were heated to 40 °C and we extracted compounds from the headspace of the feathers over a 6 hour period using a 10 mm Twister® stir bar (Gerstel Inc, Mülheim an der Ruhr, Germany). We added an internal standard of 0.5 μL of 10 ppm (mg/L) naphthalene-d8 in 100% ethanol to each sample to account for variation in instrument sensitivity across the analysis period. The stir bars were analyzed using an Agilent 7890B gas chromatograph (GC) and 5977A mass spectrometer (MS) with a thermal desorption unit (TDU) and cryo-cooled injection system (CIS, Gerstel Inc). The instrument was programmed to optimize peak separation (see section 2.7 supplementary materials).

We quantified the peak areas of 80 feather compounds that were previously identified as bird-derived (versus from exogenous sources; see Jennings and Ebeler 2020). We standardized the data from each sample by dividing by the corresponding internal standard peak area and sample mass. We averaged across the three replicate samples to obtain one representative measure per bird. To prevent the few highly abundant compounds from disproportionately influencing our analysis, we log (X+1) transformed the data.

The information contained within complex chemical profiles is often encoded by a subset of the compounds present, rather than the entire suite of chemicals (Leclaire et al. 2012; Stoffel et al. 2015; Whittaker et al. 2019a). To examine whether certain groups of compounds are correlated with MHC in Leach's storm-petrels, we performed a Principal Components Analysis (PCA) to reduce the chemical information into several testable variables. To determine the number of principal components (PCs) to retain in our analysis, we compared the results from several statistical approaches (see section 2.7 supplementary materials), which indicated that we should proceed with two PCs. From the PCA, we extracted the PC1 and PC2 score for every individual bird. We also calculated the pairwise difference in PC scores between every dyad of individuals for PC1 and PC2 separately, creating two chemical distance matrices.

Genetic Analyses. We used PCR-based cloning and sequencing to determine the MHC genotype of each bird, focusing on the hypervariable β subunit of the MHC class II molecule (see Hoover et al. 2018). Specifically, we targeted the 300 bp gene fragments that extended from exon 1 through two-thirds of exon 2 in two MHC class II genes, Ocle-DAB1 and Ocle-DAB2, using previously developed locus-specific primers (OcleDAB1Fw 5'- AGAGGGAGGCACAGCAGGAG -3', OcleDAB2Fw.2 5'- GCTGAGAGCACCTTGAGG-3', OcleDAB12Rv 5'- AGGGAAATGCTCTGCCAAG-3').

We assessed functional differences between MHC alleles to measure the diversity of each individual's MHC genotype and to quantify the level of MHC similarity between individuals. We used five physicochemical properties to describe the amino acids encoded by the alleles: hydrophobicity (z1), steric bulk (z2), polarity (z3) and electronic effects (z4 and z5) (Sandberg et al. 1998). Using these five properties, we created a matrix of pairwise Euclidean distances between amino acids (Agbali et al. 2010; Sin et al. 2015). Next, to determine the functional distance between alleles, we calculated the average of the physicochemical differences across the amino acid sequence for every pair of alleles. The resulting matrix was used to assign MHC diversity and similarity values to the birds.

As a measure of each individual's MHC diversity, we determined the functional distance between the alleles that comprise their genotype. We also constructed pairwise distance matrices based on the maximum functional distance between the genotypes of every dyad of individuals. This provided a measure of MHC similarity between individuals, with lower values indicating more functionally similar MHC genotypes (see section 2.7 supplementary materials for additional details on both genetic measures). We determined the values for both individual MHC diversity and pairwise MHC similarity in three different ways: at each MHC IIB locus separately — Ocle-DAB1 and Ocle-DAB2 — and when considering both loci together. We used a locus-specific approach because our previous mate choice analysis had highlighted the importance of the Ocle-DAB2 locus in mate choice decisions (Hoover et al. 2018). However, the mechanisms by which MHC affects odor profiles are likely influenced by multiple MHC genes, and there is evidence to suggest both IIB loci are translated into proteins in this species (Dearborn et al. 2015), so we also calculated the genetic measures considering both loci.

Statistical Analyses. We used general linear models to determine whether the chemical profiles of individuals reflect their MHC diversity. In total, we examined 6 models that included either the PC1 or PC2 scores of individuals as the response variable and had one of the three MHC diversity measures as an explanatory variable: diversity at Ocle-DAB1, Ocle-DAB2, and at both MHC IIB loci. Other explanatory variables included in all models were sex, breeding status, and two-way interactions between sex, breeding status, and the measure of MHC diversity.

We assessed whether distance in chemical profiles, as described by pairwise differences for PC1 and PC2, is correlated with genetic distance, as described by pairwise MHC similarity at Ocle-DAB1, Ocle-DAB2, and both MHC IIB loci. Specifically, we looked for positive covariance between the chemical and genetic data to indicate that individuals with similar chemical profiles have similar MHC genotypes. We implemented partial Mantel tests, which allowed us to test the significance of each PC while controlling for the influence of the other, and generated p-values using 10,000 randomizations of the data (*mantel* in R package ecodist p; Goslee and Urban 2007). Gene-odor covariance may be limited to one sex (e.g. Leclaire et al. 2012), so we performed separate tests using Male-Male (M-M) dyads and Female-Female (F-F) dyads to test for relationships within males and within females respectively. A Mantel test was not possible on the matrix of Male-Female dyads (M-F), which was not square, so we used a Spearman's partial correlation test with 10,000 permutations (*pcor.test* in R package RVAideMemoire; Hervé 2020). A similar approach has been used to analyze mixed-sex dyads in several comparable studies (Grogan et al. 2019; Leclaire et al. 2012; Slade et al. 2016). The pairwise difference in breeding status between individuals was included as a covariate matrix in all the models.

For the Mantel tests where we found a significant positive relationship, we used the BIO-ENV procedure within PRIMER v7.0.13 to identify the specific compounds that best explain pairwise MHC similarity (Clarke and Ainsworth 1993). This process finds the combination of chemicals that maximizes the Spearman rank correlation between the chemical and genetic data. The user can specify a maximum number of variables to consider; we used groups of up to 10 compounds. This process offers an alternative approach to a PCA for determining which compounds in the chemical profile are potentially responsible for signaling MHC genotype.

All statistical analyses were performed using R 3.5.3 (R Core Team 2019) and PRIMER v 7.0.13 (Clarke and Gorley 2015). We assessed significance using two-tailed tests. For the linear models and Mantel tests, we applied Bonferroni corrections to account for multiple comparisons, so only very strong relationships remained significant (adjusted $p = 0.05/3 = 0.017$ to account for the three ways we measured genetic differences).

2.4 RESULTS

Chemical Profiles and MHC Genotypes. The first two principal components cumulatively explained 67% of the variation in the chemicals associated with Leach's storm-petrel feathers (Figure S2.1). PC1 was negatively correlated with a number of long chain esters (Table S2.1). PC2 was strongly positively correlated with eight compounds, which included four even-chain fatty acid ethyl esters (C10, C12, C14, and C16) and four unidentified compounds that contained m/z 88 and 115 as the most abundant ions in their mass spectra (Table S2.1).

PC1 scores of females were higher than males (Wilcoxon rank sum test: W = 592, p = 0.036), while PC2 scores did not differ between males and females (two sample t-test: t_{58} = 0.059, p = 0.952). In total, the 60 birds in our dataset represented 43 unique MHC IIB genotypes; females had 22 different genotypes and males had 24. The functional diversity of MHC genotypes did not differ between males and females (two sample t-test: t_{58} = 1.758, p = 0.084).

Chemical Profiles and Individual MHC Diversity. The PC1 scores of individuals were explained by a significant interaction between MHC diversity at Ocle-DAB1 and sex (R^2 = 0.248, Figure 2.1a, Table 2.1). The PC1 scores of males decreased with increasing functional diversity at the Ocle-DAB1 locus, while females showed the opposite pattern (Figure 2.1a). Removing two males with high Ocle-DAB1 diversity scores did not change this result (R^2 = 0.239, Table S2.2). We did not find support that the chemicals associated with PC1 reflected genetic diversity at the Ocle-DAB2 locus or when considering both MHC IIB loci (Table S2.3).

Individual chemical variation at PC2 was explained by a significant interaction between diversity across both MHC IIB loci and breeding status (R^2 = 0.223, Figure 2.1b, Table 2.1). To assist with the interpretation of this interaction effect, we plotted breeding status as a categorical variable with the mean breeding status ("mid-incubation"), +1SD above the mean ("late incubation"), and -1SD below the mean ("early incubation", Figure 1b). Birds in early and mid-incubation, which corresponded to individuals <36 days after egg laying, have PC2 scores that decrease with increasing diversity across both MHC IIB loci. Late incubation birds show the opposite relationship; their PC2 scores increase with increasing genetic diversity across both

loci. The chemicals associated with PC2 were not related to individual genetic diversity at either MHC locus when considered separately (Table S2.3).

Relationships Between Chemical and Genetic Distance. Chemical similarity was correlated with MHC similarity in both male-female and female-female dyads (Figure 2.2, Table 2.2). We found that chemical distance at PC1 was positively correlated with distance at Ocle-DAB 1 in male-female dyads (*rho* = 0.158, $p < 0.001$, Figure 2.2a, Table 2.2). There were no relationships between distance at PC2 and any of the genetic distance matrices in mixed-sex dyads.

Within females, chemical distance at PC2 positively covaried with MHC distance at the Ocle-DAB2 locus ($r = 0.313$, $p = 0.006$, Figure 2.2b, Table 2.2) and when considering both MHC IIB loci ($r = 0.334$, $p = 0.004$, Figure 2.2c, Table 2.2). Chemical distance at PC1, however, had no relationship with genetic distance in female-female dyads (Table 2.2).

Within males (M-M dyads), we found no evidence of positive covariation between chemical and genetic distance matrices. However, we did find a non-significant trend towards negative covariance between PC2 and Ocle-DAB2 ($r = -0.180$, $p = 0.034$), and PC2 and both MHC IIB loci ($r = -0.195$, $p = 0.032$), which suggests that there is an absence of useable information about MHC (from a behavioral standpoint) in male chemical profiles, equivalent to a finding of no correlation.

The BIO-ENV process identified the groups of compounds that best explained MHC similarity at Ocle-DAB2 and across both MHC loci in female-female dyads. Models for both genetic measures resulted in slightly higher correlations than the original models that used the chemical data represented by PC2 (Table 2.3, Table S2.4 for full results). At the Ocle-DAB2 locus, the best model used 7 compounds ($r = 0.377$), while the best model explaining MHC

similarity across both MHC IIB loci contained 6 compounds (r = 0.394). The BIO-ENV process selected several compounds that were highly correlated with PC2, but it also highlighted a possible role for a number of aldehydes and alkanes, which were not strongly associated with either of our retained PCs (Table 2.3 and Table S2.3).

2.5 DISCUSSION

We found support for our hypothesis that the chemical profiles of Leach's storm-petrels contain information about MHC genotype. Our analyses revealed that the feather-associated chemicals reflect individual MHC diversity in a sex-specific and breeding-status dependent manner. We also found that similarity in chemical profiles was correlated with MHC similarity in female-female and male-female dyads. These findings are consistent with olfaction as a mechanism for MHC-dependent mate choice in this species. This is only the third study to detect the presence of MHC chemosignals in birds, and while the previous two studies examined the non-volatile compounds of the preen oil (Leclaire et al. 2014; Slade et al. 2016), ours is the first to confirm that the volatile, scented feather compounds also reflect MHC genotype.

Our study was in part motivated by a finding that male Leach's storm-petrels make disassortative mate-choice decisions to breed less frequently than expected with females who are homozygous at the Ocle-DAB2 locus (Hoover et al. 2018). This study also found that these less-preferred, homozygous females are associated with lower reproductive success. Males may evaluate female MHC using one of two recognition mechanisms (reviewed in Ruff et al. 2012). If they use self-referent matching, their own phenotype would serve as a reference to assess the genotype of a potential mate. In our data, the best support for this mechanism would be a

correlation between chemical similarity and MHC similarity at Ocle-DAB2 in male-female dyads, which would suggest that males could gain information about this locus by comparing the odor of a female to their own scent. However, we only detected a correlation in male-female dyads at Ocle-DAB1. Alternatively, males could imprint on a female family member, such as their mother, and reference this template to discriminate potential mates. If this imprinting mechanism is at play, our finding that female chemical similarity at PC2 covaries with MHC similarity at Ocle-DAB2 strongly supports olfaction as a potential mechanism for choosy males to avoid mating with lower-quality, homozygous females. Behavioral experiments are needed to thoroughly explore whether male Leach's storm-petrels can use olfaction to discriminate female MHC, and if so, which of these two mechanisms they use. Olfactory trials could also help identify which compounds convey information about MHC. The compounds highlighted here, specifically the fatty acid ethyl esters with high loadings on PC2 and the aldehydes and alkanes selected by the BIO-ENV process are of particular interest in regard to the female MHC signal.

We detected covariance between MHC similarity and chemical similarity in females, but not in males. We also observed a sex-specific relationship between individual MHC diversity at the Ocle-DAB1 locus and the chemicals associated with PC1. There are known sex differences in the vertebrate immune response where females are associated with stronger immune responses than males (Foo et al. 2017; Roved et al. 2017). Furthermore, steroid sex hormones have important regulatory effects on the immune system (Ahmed et al. 1985; Foo et al. 2017). Testosterone, the primary male sex hormone, suppresses the immune system and has been shown to down regulate MHC class II expression (Hepworth et al. 2010; Koh et al. 2009). In comparison, estrogen and progesterone amplify certain immune responses in females, and

have been linked with increased expression of MHC class II (Hepworth et al. 2010; Yang et al. 2006; but see Relloso et al. 2012). The individuals in our study were in breeding condition, a phase associated with elevated levels of sex hormones (Farner and Wingfield 1980). If female storm-petrels had increased MHC expression compared with males at the time of sampling, the chemical profiles of females may have been more strongly influenced by MHC, enabling us to detect the signal in one sex but not the other.

In addition to only finding support for gene-odor covariance in certain dyads of individuals, the effect sizes associated with our positive findings were small. Both these results are consistent with other studies from mammals and birds, which found similar effect sizes and often only detected relationships in some dyads (Grogan et al. 2019; Leclaire et al. 2014; Setchell et al. 2011; Slade et al. 2016). The diverse array of factors that affect chemical profiles may explain these findings. In this study we targeted the hypervariable binding region of MHC class IIB, but storm-petrel odor profiles are likely also influenced by other MHC genes. Neutral markers like genome-wide heterozygosity (Leclaire et al. 2012; Stoffel et al. 2015), as well as interactions between MHC and background genes can also affect odor profiles (Willse et al. 2006). Moreover, avian chemical profiles vary with diet (Thomas et al. 2010) and disease (Grieves et al. 2018). Thus, there are a multitude of other genetic and non-genetic factors that could contribute variability to the data resulting in low effect sizes or non-significant findings. Studies using captive or MHC-congenic species where more of these confounding variables can be controlled may yield stronger results. However, we believe there is significant value in demonstrating support for odor-gene covariance in wild organisms—particularly in a context where birds may be making these discriminations to facilitate mate choice decisions.

Vertebrate chemical profiles change seasonally and may only reflect genetic markers during the breeding season (Boulet et al. 2009; Grogan et al. 2019; Milinski et al. 2010). The absence of genetic information in chemical profiles during the non-breeding season might be explained by energetic costs associated with producing chemical secretions (Johansson and Jones 2007b; Milinski et al. 2010), although there is currently limited support for this idea in birds (Moreno-Rueda 2017). Our results indicate that MHC diversity is reflected by chemical profiles in a way that changes within the breeding season. Individuals sampled earlier in the ~45-day incubation period had PC2 scores that decreased with increasing MHC diversity, but as they approached hatching, the relationship appears to switch. This suggests that there are likely complex interactions happening between steroid hormones, the immune system, and other aspects of an individual's physiology that alter the way chemical profiles reflect genetic markers over time, even within a single stage of the breeding cycle. Samples from courtship and provisioning would be particularly interesting to better explore how chemical signals shift in regard to MHC with changing reproductive state in this species.

The mechanisms that caused the observed relationships between feather chemicals and MHC are currently unknown. Both the MHC molecules and the peptides that bind to them can end up in bodily secretions where they may act as odorants or the precursors of odorants (Milinski et al. 2005; Singh et al. 1987). MHC also determines an organism's microbiome, so it may indirectly influence the scented compounds produced by the commensal microbiota (Penn et al. 2007; Schellinck et al. 1991; Singh et al. 1990). The microbiome presents a promising avenue for future research in birds because of its emphasis on the volatile compounds that can be detected by the avian olfactory system. Covariation between MHC and the avian

microbiome has been documented in this population of Leach's storm-petrels (Pearce et al. 2017), as well as a related species, the blue petrel (*Halobaena caerulea*, Bonadonna et al. 2018). An analysis incorporating all three factors —microbiome, chemical profiles and MHC — would be valuable to shed light on the mechanisms at play in this species.

This study adds to a growing body of work demonstrating that odor reflects information on MHC in wild vertebrates. Our findings highlight feather-associated chemicals as a potential source of olfactory information enabling MHC-based mate choice in Leach's storm-petrels. This species exhibits both high fidelity to their mate and nest site. Because they return to the same nest over many years, individuals also frequently breed next to the same neighboring birds. Thus, an exciting possibility for future research in this system is the role of MHC odortypes in facilitating social interactions beyond mate choice, such as the recognition of neighbors and kin. This system has numerous possibilities for further work that could expand our understanding of olfaction as a mechanism for social communication in birds, an area of research still in its infancy.

Funding and Ethics Statement: This research was supported by National Science Foundation (IOS 1258828). Research protocols were approved by UC Davis Animal Care and Use (protocol 17588 in 2013-2015, protocol 19288 in 2016) and the Canadian Wildlife Service (banding permit 10695, research permit SC2742). Samples were transported to the United States under USDA Veterinary Permit to Import Controlled Materials 125361 and Nova Scotia Permit to Export Wildlife.

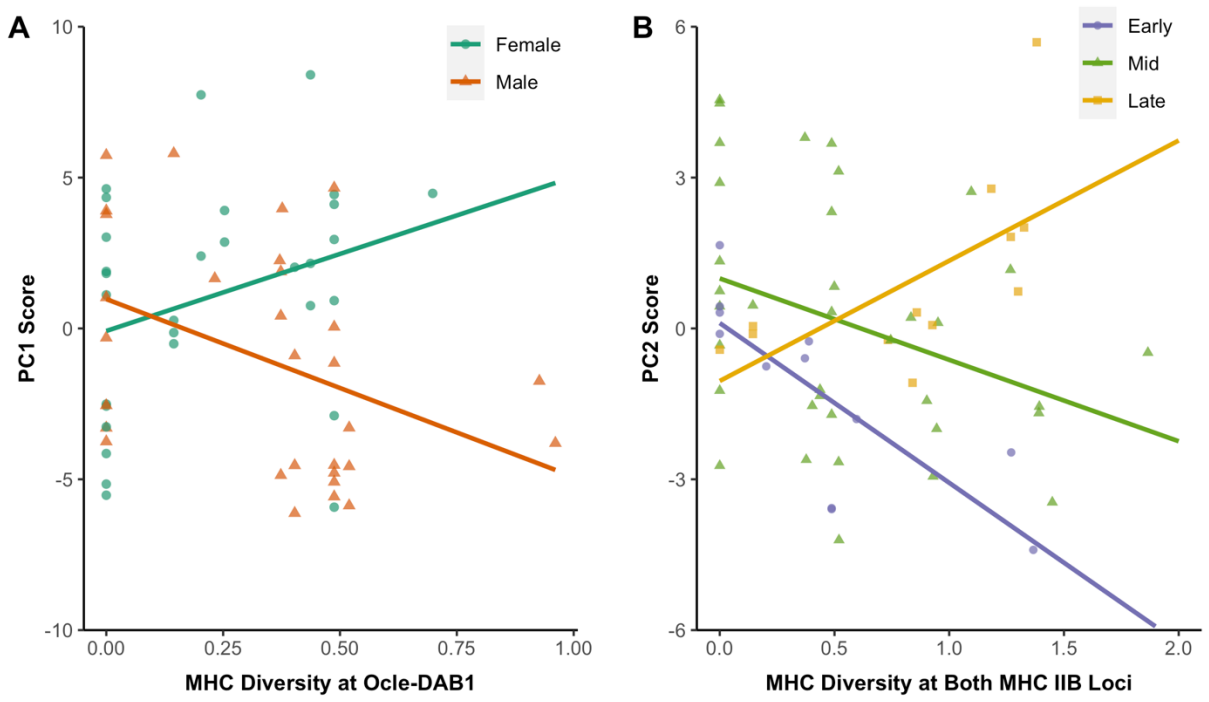

Figure 2.1. Linear relationship between chemical PC scores and MHC diversity. A) Individual PC1 score is explained by diversity at Ocle-DAB1 in a sex-specific way ($R^2 = 0.248$). B) Individual PC2 score is explained by diversity across both MHC IIB loci through an interaction with breeding status ($R^2 = 0.223$). Breeding status is represented by three categories with "mid" showing individuals in the mean breeding stage, "late" showing individuals +1SD above the mean, and "early" showing individuals -1SD below the mean. Solid lines show the least-squares regression for each group

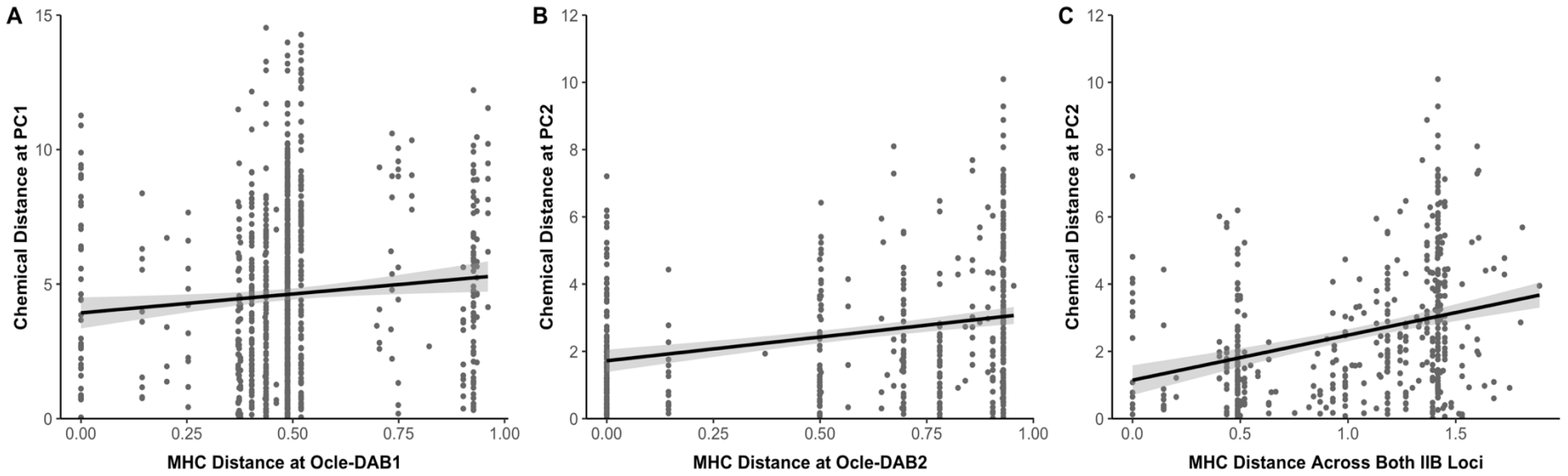

Figure 2.2. Relationships between pairwise MHC distance and pairwise chemical distance in dyads of Leach's storm-petrels. A) In Male-Female dyads there is a significant relationship between pairwise chemical differences in PC1 scores and MHC distance at Ocle-DAB1**.** In Female-Female dyads there is a significant positive correlation between pairwise chemical differences in PC2 scores and MHC distance at B) Ocle-DAB2 and C) both MHC IIB loci. Solid lines show the least-squares regression with 95% confidence interval

Table 2.1. Linear relationship between individual chemical profiles and MHC diversity.

Significant relationships are shown in bold (adjusted *p*-value for significance <0.017)

Chemical Variable	Explanatory Variables	Estimate	SE	95% CI	*p*-value
PC1	(*Intercept*)	-3.308	1.935	-7.189, 0.573	0.093
	Ocle-DAB1	9.760	5.435	-1.141, 20.662	0.078
	Sex	2.122	2.732	-3.358, 7.601	0.441
	Breeding Status	0.194	0.102	-0.011, 0.399	0.064
	Ocle-DAB1*Sex	-11.217	3.938	-19.115, -3.319	**0.006**
	Ocle-DAB1*Breeding Status	-0.271	0.277	-0.827, 0.286	0.334
	Sex*Breeding Status	-0.071	0.131	-0.333, 0.191	0.590
PC2	(*Intercept*)	0.0682	1.224	-2.387, 2.523	0.956
	Both IIB Loci	2.699	1.534	-0.277, 5.876	0.074
	Sex	-0.714	1.70	-4.118, 2.690	0.676
	Breeding Status	0.008	0.066	-0.123, 0.140	0.898
	Both IIB Loci*Sex	-0.454	1.163	-2.787, 1.879	0.698
	Both IIB Loci*Breeding Status	-0.203	0.081	-0.366, -0.041	**0.015**
	Sex*Breeding Status	0.060	0.077	-0.0945, 0.214	0.441

Table 2.2. Partial Mantel tests show the relationship between chemical distance (PC1 or PC2) and genetic distance (Ocle-DAB1, Ocle-DAB2 or both IIB loci) in Male-Male and Female-Female dyads. Spearman partial correlation permutation tests show the relationship between chemical and genetic distance in Male-Female dyads. Significant positive correlations are shown in bold (adjusted *p*-value for significance < 0.017)

Group of Dyads	Genetic Distance	# of Dyads	Test	Chemical Distance PC1		Chemical Distance PC2	
				Correlation Coefficient	*p*-value	Correlation Coefficient	*p*-value
Male-Male	Ocle-DAB1	435	partial Mantel	-0.070	0.325	-0.095	0.330
	Ocle-DAB2	435	partial Mantel	-0.012	0.851	-0.180	0.034
	Both IIB loci	435	partial Mantel	-0.051	0.451	-0.195	0.032
Female-Female	Ocle-DAB1	435	partial Mantel	-0.027	0.750	0.170	0.065
	Ocle-DAB2	435	partial Mantel	-0.101	0.295	0.313	**0.006**
	Both IIB loci	435	partial Mantel	-0.102	0.308	0.334	**0.004**
Male-Female	Ocle-DAB1	900	partial Spearman	0.158	**< 0.001**	-0.0102	0.611
	Ocle-DAB2	900	partial Spearman	-0.027	0.317	-0.054	0.066
	Both IIB loci	900	partial Spearman	0.031	0.461	-0.035	0.214

Correlation coefficient for F-F and M-M dyads is Mantel *r*, for M-F dyads it is rho

Table 2.3. The top three models from the BIO-ENV procedure that identified the subset of chemicals that maximized the correlation between chemical and genetic distance matrices in Female-Female dyads for genetic distance at Ocle-DAB2 and at both MHC IIB loci. Compounds listed in italics were also strongly correlated with PC2

Group of Dyads	Genetic Distance	Mantel *r*	# of Compounds	Compound Names
Female-Female	Ocle-DAB2	0.376	6	Styrene, Acetophenone, Pentadecane, *Unidentified 5*, Tetradecanal, Heptadecane
		0.377	7	Styrene, Benzaldehyde, Acetophenone, Pentadecane, *Unidentified 5*, Tetradecanal, Heptadecane
		0.376	8	Styrene, Benzaldehyde, Acetophenone, Decanal, Pentadecane, *Unidentified 5*, Tetradecanal, Heptadecane
Female-Female	Both IIB loci	0.391	5	Styrene, *Ethyl decanoate*, Tetradecanal, Heptadecane, Pentadecanal
		0.394	6	Styrene, Pentadecane, *Ethyl decanoate*, Tetradecanal, Heptadecane, Pentadecanal
		0.392	7	Styrene, Decanal, Pentadecane, *Ethyl decanoate*, Tetradecanal, Heptadecane, Pentadecanal

2.7 SUPPLEMENTARY MATERIALS

GC-MS Methods and Instrument Settings. We used 10 mm Twister® stir bars coated with 0.5 mm polydimethylsiloxane stationary phase to extract compounds from the headspace above the feathers (Gerstel Inc, Mülheim an der Ruhr, Germany). For each replicate sample, two feathers were placed into the bottom of a 10 mL deactivated amber glass headspace vial with a PTFE/Silicone lined metal screw cap (Restek Inc, Bellefonte, PA). A stir bar was affixed inside the vial above the feathers using two small 8 mm diameter magnets. We submerged the sealed vials into a sand bath and heated them using a hotplate so that the temperature inside the vials reached 40°C. The stir bars were left to extract compounds for 6 hours. Following extraction, we placed each stir bar into a glass thermal desorption tube and added a 0.5 μL glass microcapillary tube containing the internal standard (10 ppm (mg/L) naphthalene-d8 in 100% ethanol).

The stir bars were thermally desorbed in the thermal desorption unit (TDU), which was operated in spitless mode with an initial temperature of 30°C, then heated to 250°C at 720 °C/min and held for 5 min. The transfer line from the TDU was at 250 °C. The cryo-cooled Injection System (CIS) was cooled to -80°C using liquid nitrogen. After cryotrapping the analytes, the CIS heated at a rate of 12°C/s to 280°C (hold time: 3 mins). The CIS was operated in solvent vent mode, with a vent flow of 50 mL/min and a purge flow of 50 mL/min after 1.2 min. The GC was fitted with DB-5MS UI capillary column (30 m x 0.25 cm x 0.25 μm film thickness; Agilent Technologies, Inc., Santa Clara, CA) and used the following oven program: an initial temperature of 35 °C for 3 min, increasing at 5 °C/min to 220 °C (hold time: 0 mins), followed by a second increase at 15 °C/min to 280°C (hold time: 5 min). The GC was run in

constant flow mode at a rate of 1.5 mL/min with helium as the carrier gas. The MS was fitted with an Agilent Extractor Extra Inert (EI) electron source. The MS transfer line temperature was 280 °C; the electron source and the quadrupole were set to 230°C and 150°C respectively. The scan range was from 40 to 300 *m/z* at a rate of 2.8 scans/s. The EMV mode was set to gain factor, with the factor set to 1.0.

Determining the Number of Principal Components. To determine the appropriate number of principal components to retain in our analyses, we examined a scree plot of eigenvalues to look for an "elbow", a point in the graph where the amount of variation explained by additional PCs levels off. Since the interpretation of a scree plot is subjective, we also relied on three analyses to determine which PCs were statistically distinct: the broken-stick model (Legendre and Legendre 2012), a randomization test (Peres-Neto et al. 2005), and a Bayesian approach called the Auer Gervini Sensitivity Analysis (Auer and Gervini 2008). All three tests were performed using the PCDimension package v1.11.1 in R (Wang et al. 2018). The scree plot suggested we should retain either two or three PCs (Figure S2.1). Two out of three statistical tests (Auer-Gervini and the randomization test) indicated that PC1 and PC2 were significant, while PC3, which explained 6.16% of the total variation, was not significant. As such, we proceeded with two PCs to summarize the chemical data.

MHC Genetic Measures. *Individual MHC Diversity:* MHC diversity is often measured as the number of unique alleles in an individual's genotype. This value can be informative in species that have many MHC loci but is less useful for differentiating individuals in species like Leach's storm-petrels, which have only two MHC IIB loci and are characterized by low inter-individual variation in allelic richness (range: 2-4 alleles per bird). We used an alternative measure that

accounted for both the number of alleles possessed by an individual, as well as the degree of functional divergence between the amino acid sequences encoded by their alleles. This approach provided a more informative and variable measure to describe the MHC diversity of the individuals in our study. We determined this value for the two MHC IIB loci separately (Ocle-DAB1 and Ocle-DAB2) by taking the number that described the functional difference between an individual's two alleles. For homozygous birds, this resulted in a score of zero, while heterozygous birds could have a score that ranged from 0.058 to 0.96 at Ocle-DAB1, or 0.10 to 0.98 at Ocle-DAB2. A higher value corresponded to larger functional differences in peptide binding ability between the amino acid sequences produced by the two alleles. When considering both MHC IIB loci, we summed the scores from the two separate loci.

Pairwise MHC Similarity. We also used the same functional measures to compare the MHC alleles of each individual to every other individual in our dataset. We stored these comparisons in a distance matrix where each value estimated the maximum MHC distance between a dyad (or pair) of individuals. Low pairwise values corresponded to highly similar MHC genotypes, while larger values described greater functional difference between a dyad's genotypes. Like the MHC diversity values, we also assessed MHC similarity at each locus separately and with both IIB loci combined. For example, the maximum difference between a bird with the genotype Ocle-DAB1*02/Ocle-DAB1*03 and another individual with the genotype Ocle-DAB1*05/Ocle-DAB1*08 is 0.93. The comparisons made to obtain this value include: Ocle-DAB1*02 to Ocle-DAB1*05 =0.93; Ocle-DAB1*02 to Ocle-DAB1*08 = 0.52; Ocle-DAB1*03 to Ocle-DAB1*05 = 0.73; and finally, Ocle-DAB1*03-Ocle-DAB1*08 = 0.20. The largest or maximum difference is 0.93, so this is the value we retained. We applied to same process to the Ocle-

DAB2 locus. For the pairwise comparisons across both loci, we summed the two maximum values from each locus separately to determine the overall maximum functional difference.

Figure S2.1. Results from Principal Components Analysis (PCA) using 80 feather chemicals. A) Two-dimensional Principal Components Analysis (PCA) plot showing the chemical profiles of the 60 birds used in our analysis. Each symbol in the plot corresponds to an individual. B) Scree plot showing the percentage of total variation explained by the first 10 Principal Components

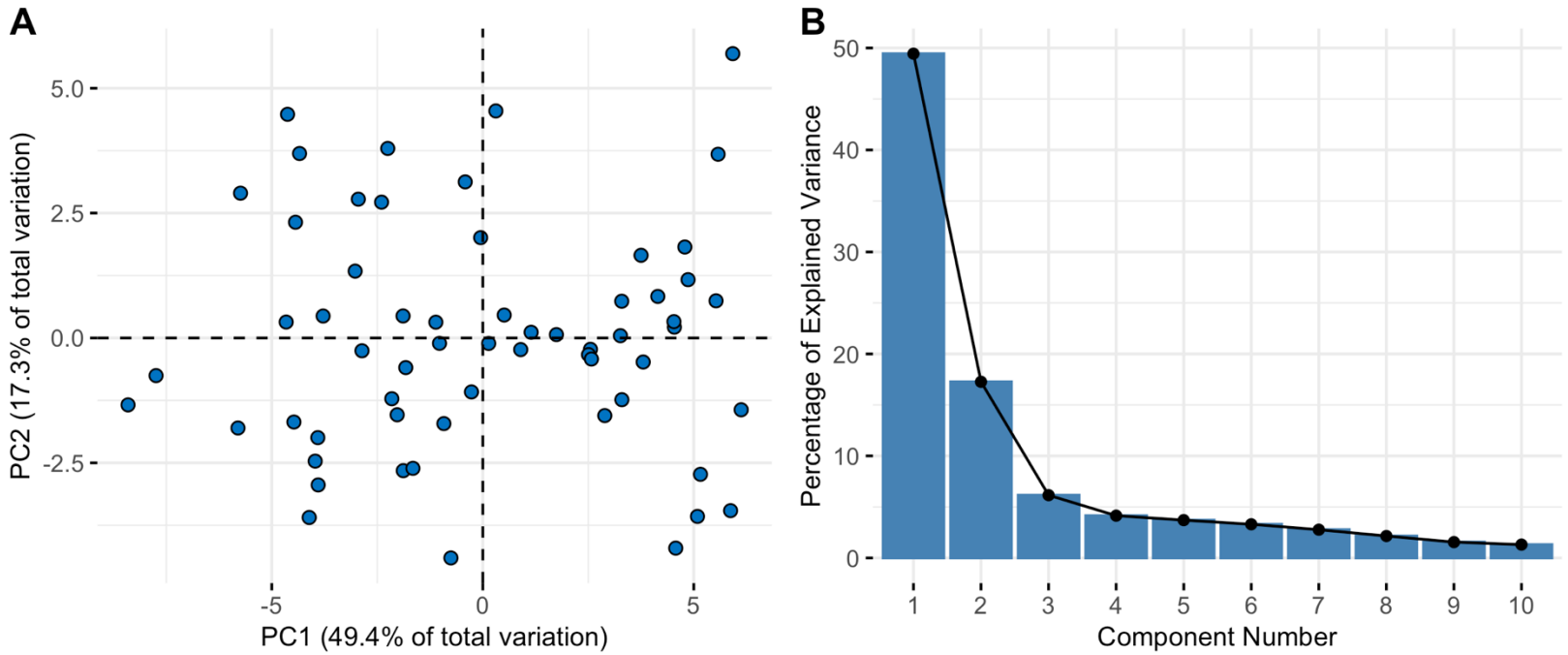

Table S2.1. Eighty odorants measured in Leach's storm-petrels feathers. Compounds that were highly correlated with PC1 and PC2 (r>|0.5|) are marked. The retention index and occurrence of each chemical across the 60 birds are also noted.

Retention Time	Compound Name	PC1 Compounds (r>|0.5|)	PC2 Compounds (r>|0.5|)	EIC	Calculated Retention Index	Occurrence
5.39	Hexanal			56	808	100%
6.20	Furfural			95	830	100%
7.75	Styrene			104	886	100%
8.23	Heptanal			70	899	100%
10.02	Benzaldehyde			106	960	100%
10.83	6-Methyl-5-hepten-2-one			108	986	100%
11.02	2-Octanone			58	991	100%
11.45	Octanal			84	1005	100%
12.26	2-Ethyl-1-hexanol			57	1032	100%
12.35	6-Ethyl-2-methyloctane			71	1034	43%
12.64	Benzeneacetaldehyde			91	1045	90%
13.33	Acetophenone			105	1066	100%
14.19	2-Nonanone			58	1095	100%
14.62	Nonanal			57	1109	100%
17.21	2-Decanone			58	1193	100%
17.49	Dodecane			57	1203	100%
17.63	Decanal			82	1207	100%
20.06	2-Undecanone			58	1286	100%
20.31	Tridecane			71	1294	100%
20.47	Undecanal			82	1301	100%
21.18	Unidentified 1		0.66	88	1330	90%
22.73	Unidentified 2			58	1394	90%
22.92	Unidentified 3		0.71	115	1401	90%
22.97	Tetradecane			57	1402	98%
23.16	Dodecanal			82	1411	98%
23.75	Ethyl decanoate		0.67	88	1435	87%
24.11	Geranylacetone			69	1449	98%
25.07	Unidentified 4		0.75	88	1488	90%
25.47	Pentadecane			57	1505	100%
25.70	Tridecanal			82	1514	100%
26.18	Unidentified 5		0.76	88	1533	88%
26.69	n-Nonylcyclohexane			82	1555	88%
27.67	Ethyl dodecanoate		0.93	88	1594	100%
27.85	Hexadecane			57	1601	100%
28.10	Tetradecanal			82	1612	98%
30.00	2-Pentadecanone			58	1690	100%

30.10	Heptadecane			57	1693	100%
30.19	Pristane			57	1701	100%
30.39	Pentadecanal			82	1710	100%
32.07	Ethyl tetradecanoate		0.97	88	1793	97%
32.24	Octadecane			57	1802	100%
32.55	Hexadecanal			82	1817	100%
34.26	2-Heptadecanone			58	1901	98%
34.28	Nonadecane			57	1902	100%
34.62	Heptadecanal			82	1919	93%
35.19	Unidentified 6	-0.54		70	1947	80%
35.54	Unidentified long chain ester			159	1963	68%
36.02	Unidentified long chain ester	-0.55		140	1986	75%
36.08	Ethyl hexadecanoate		0.54	88	1989	100%
36.33	Unidentified long chain ester	-0.63		71	1998	97%
36.39	Unidentified long chain ester	-0.50		125	2004	93%
36.88	Unidentified long chain ester	-0.66		155	2037	100%
36.94	Unidentified long chain ester	-0.71		70	2042	100%
37.05	Unidentified long chain ester	-0.67		112	2050	100%
37.22	Unidentified long chain ester	-0.80		97	2062	100%
37.39	Unidentified long chain ester	-0.68		111	2074	100%
37.66	Unidentified long chain ester	-0.78		155	2094	100%
37.86	Unidentified long chain ester	-0.56		140	2109	95%
37.91	Unidentified long chain ester	-0.71		125	2113	100%
38.08	Unidentified long chain ester	-0.74		111	2123	95%
38.16	Unidentified long chain ester	-0.67		97	2131	100%
38.31	Unidentified long chain ester	-0.72		155	2143	100%
38.35	Unidentified long chain ester	-0.60		125	2145	100%
38.43	Unidentified long chain ester	-0.66		173	2152	100%
38.52	Unidentified long chain ester	-0.77		173	2158	100%
38.53	Unidentified long chain ester	-0.69		111	2159	100%
38.71	Unidentified long chain ester	-0.57		154	2172	100%
38.77	Unidentified long chain ester	-0.60		125	2177	100%
38.87	Unidentified long chain ester	-0.75		111	2184	100%
38.94	Unidentified long chain ester	-0.60		111	2189	100%
38.98	Unidentified long chain ester	-0.68		84	2192	100%
39.16	Unidentified long chain ester	-0.61		125	2206	100%
39.24	Unidentified long chain ester	-0.61		126	2212	100%
39.32	Unidentified long chain ester	-0.70		187	2218	100%
39.42	Unidentified long chain ester	-0.61		125	2226	100%
39.54	Unidentified long chain ester	-0.63		139	2236	100%
39.73	Unidentified long chain ester	-0.64		125	2249	100%
39.86	Unidentified long chain ester			168	2259	97%
40.02	Unidentified long chain ester	-0.62		173	2270	100%
40.08	Unidentified long chain ester	-0.57		125	2276	100%

Table S2.2. Linear relationship between individual PC1 scores and MHC diversity at Ocle-DAB1 excluding two males with high diversity values (>0.90)

Chemical Variable	Explanatory Variables	Estimate	SE	95% CI	*p*-value
PC1	(*Intercept*)	-3.056	2.040	-7.151, 1.039	0.140
	Ocle-DAB1	8.449	6.222	-4.042, 20.941	0.181
	Sex	2.669	2.876	-3.105, 8.443	0.358
	Breeding Status	0.179	0.109	-0.040, 0.397	0.107
	Ocle-DAB1*Sex	-12.672	4.500	-21.707, -3.638	**0.007**
	Ocle-DAB1*Breeding Status	-0.191	0.332	-0.856, 0.475	0.567
	Sex*Breeding Status	-0.082	0.134	-0.350, 0.186	0.543

Table S2.3. Linear relationship between individual chemical profiles (as described by PC1 and PC2 scores) and MHC diversity. Findings from models that were not significant

Chemical Variable	Explanatory Variables	Estimate	SE	95% CI	*p*-value
PC1	(*Intercept*)	0.076	1.964	-3.863, 4.015	0.969
	Ocle-DAB2	-3.977	3.346	-10.689, 2.735	0.240
	Sex	-1.018	2.538	-6.110, 4.073	0.690
	Breeding Status	0.077	0.109	-0.141, 0.296	0.480
	Ocle-DAB2*Sex	1.332	2.736	-4.156, 6.820	0.629
	Ocle-DAB2*Breeding Status	0.180	0.166	-0.153, 0.513	0.283
	Sex*Breeding Status	-0.082	0.127	-0.339, 0.171	0.512
PC1	(*Intercept*)	-0.672	2.160	-5.005, 3.660	0.757
	Both IIB Loci	-1.019	2.706	-6.448, 4.409	0.708
	Sex	1.790	2.995	-4.216, 7.796	0.553
	Breeding Status	0.099	0.116	-0.133, 0.331	0.396
	Both IIB Loci*Sex	-2.470	2.053	-6.587, 1.647	0.234
	Both IIB Loci*Breeding Status	0.080	0.143	-0.206, 0.367	0.576
	Sex*Breeding Status	-0.134	0.136	-0.406, 0.138	0.328
PC2	(*Intercept*)	0.798	1.78	-1.564, 3.160	0.501
	Ocle-DAB1	0.889	3.308	-2.745, 10.523	0.245
	Sex	-1.312	1.662	-4.647, 2.022	0.434
	Breeding Status	-0.017	0.062	-0.142, 0.108	0.785
	Ocle-DAB1*Sex	0.544	2.396	-4.262, 5.351	0.821
	Ocle-DAB1*Breeding Status	-0.385	0.169	-0.724, -0.046	0.027
	Sex*Breeding Status	0.075	0.080	-0.084, 0.235	0.347
PC2	(*Intercept*)	0.441	1.142	-1.851, 2.732	0.701
	Ocle-DAB2	2.871	1.947	-1.034, 6.776	0.146
	Sex	-0.256	1.477	-3.218, 2.706	0.863
	Breeding Status	-0.016	0.063	-0.143, 0.111	0.799
	Ocle-DAB2*Sex	-0.312	1.592	-3.505, 2.881	0.846
	Ocle-DAB2*Breeding Status	-0.207	0.097	-0.401, -0.0137	0.036
	Sex*Breeding Status	0.013	0.074	-0.135, 0.161	0.858

Table S2.4. Complete results from the BIO-ENV procedure that identified the subsets of chemicals that maximized the Mantel's r correlation between chemical and genetic distance matrices in Female-Female dyads for A) genetic distance at Ocle-DAB2 and B) genetic distance at both IIB loci. Compounds that listed in italics are also strongly correlated with PC2

Number of Compounds	Mantel *r*	Compound Names
1	0.254	Acetophenone
2	0.348	Styrene, *Unidentified 5*
3	0.360	Styrene, *Unidentified 5***,** Heptadecane
4	0.370	Styrene, Pentadecane, *Unidentified 5***,** Heptadecane
5	0.374	Styrene, Acetophenone, Pentadecane, *Unidentified 5,* Heptadecane
6	0.376	Styrene, Acetophenone, Pentadecane, *Unidentified 5***,** Tetradecanal**,** Heptadecane
7	0.377	Styrene, Benzaldehyde, Acetophenone, Pentadecane, *Unidentified 5*, Tetradecanal, Heptadecane
8	0.376	Styrene, Benzaldehyde, Acetophenone, Decanal, Pentadecane, *Unidentified 5*, Tetradecanal, Heptadecane
9	0.371	Styrene, Benzaldehyde, Acetophenone, Decanal, Pentadecane, *Unidentified 5*, Tetradecanal, Heptadecane, Pentadecanal
10	0.366	Styrene, Benzaldehyde, Acetophenone, Decanal, Pentadecane, *Unidentified 5*, Tetradecanal, Heptadecane, Pentadecanal, *Ethyl hexadecanoate*

Number of Compounds	Mantel *r*	Compound Names
1	0.288	*Ethyl decanoate*
2	0.339	Styrene, *Unidentified 5*
3	0.363	Styrene, *Unidentified 5*, Heptadecane
4	0.382	Styrene, *Ethyl decanoate*, Tetradecanal, Heptadecane
5	0.391	Styrene, *Ethyl decanoate*, Tetradecanal, Heptadecane, Pentadecanal
6	0.394	Styrene, Pentadecane, *Ethyl decanoate*, Tetradecanal, Heptadecane, Pentadecanal

7	0.392	Styrene, Decanal, Pentadecane, *Ethyl decanoate*, Tetradecanal, Heptadecane, Pentadecanal
8	0.390	Styrene, Acetophenone, Decanal, Pentadecane, *Ethyl decanoate*, Tetradecanal, Heptadecane, Pentadecanal
9	0.384	Styrene, Benzaldehyde, Acetophenone, Decanal, Pentadecane, *Ethyl decanoate*, Tetradecanal, Heptadecane, Pentadecanal
10	0.376	Styrene, Benzaldehyde, Acetophenone, Decanal, Undecanal, Pentadecane, *Ethyl decanoate*, Tetradecanal, Heptadecane, Pentadecanal

CHAPTER 3

Smells Like Home: Bird-Scented Nests as a Mechanism for Olfactory Homing in a Burrow Nesting Seabird

3.1 ABSTRACT

Homing to and from a familiar site is a common behavior in the animal kingdom. Many species make use of chemical information to home either in the form of environmental chemicals associated with specific locations or chemicals deposited in the environment by themselves or their conspecifics. In birds, individuals regularly commute to and from their nest during the breeding season, but how they accomplish these trips is not well understood. Olfaction, which is an underappreciated sensory modality in birds, may facilitate homing in a range of species. This is best studied in nocturnal burrow nesting seabirds that rely on their excellent sense of smell to locate their breeding colony and to identify their specific nest. We examined the chemical information present at a breeding colony of Leach's storm-petrels (*Oceanodroma leucorhoa*) to determine whether environmental chemicals, bird-produced chemicals, or a combination of the two facilitate olfactory homing in this burrow-nesting species. We characterized the chemical profiles associated with the colony landscape and the storm-petrels occupants using gas-chromatography mass-spectrometry. We found that the colony contains gradients of environmental chemicals that could facilitate homeward navigation at multiple spatial scales. We also show that storm-petrel burrows are uniquely scented due to chemicals deposited by their avian occupants, which likely enables olfactory nest discrimination in this species. Moreover, our results reveal that sharing a burrow shapes the chemical profiles of the

storm-petrel occupants, such that mated pairs and their nest possess a common odor, which may further reinforce olfactory nest recognition. Our findings also have implications for avian chemical communication as bird scented nests may function to transfer information between conspecifics, analogous to scent marks in other taxonomic groups.

3.2 INTRODUCTION

Many animals maneuver around their worlds to locate food, avoid predators, attract mates, and find shelter. Movement that involves returning to a specific, familiar site is called homing (Able 2001; Papi 1992). Individuals rely on a wide variety of external and internal inputs to home. On short distance trips, they may continually receive cues that let them remain in contact with "home". When venturing further afield, some animals memorize their outbound trip and return by retracing their route (e.g., path integration in honeybees; Wehner and Srinivasan 2003), while others use a map or a positioning system to determine their current location relative to their final destination (e.g., magnetic compass in sea turtles; Lohmann and Lohmann 1993). Homing often relies on information gathered from the environment through an animal's sensory systems. Visual cues can allow organisms to identify landmarks or landscape features; they also enable celestial compasses (sun, stars, polarized light) that can aid navigation (reviewed in Hansson and Akesson 2014). Chemical information is also commonly used for homing (Gagliardo 2013; Steck 2012; Svensson et al. 2014). This can include place-specific environmental chemical cues that are fairly stable over times, which are used by both North African desert ants (*Cataglyphis fortis*) returning to their nest and Pacific salmon (*Oncorhynchus spp*.) returning to their natal stream (Dittman and Quinn 1996; Steck et al.

2009). Homing is also facilitated by chemicals produced or deposited in the environment by an individual or its conspecifics. Social insects (Vander Meer et al. 2019), rodents (Wallace et al. 2002), and molluscs (Chelazzi et al. 1990) all follow pheromone scent trails to make trips to and from foraging areas. Moreover, many organisms leave scent marks at the edge of their territory, which can facilitate the final stages of homing once they are in the vicinity of their destination (Hurst 2005; Steck 2012).

Homing often involves returning to a nest, or a location where an organism raises their offspring. Nest construction and usage is found across many taxonomic groups including fish, social insects, reptiles, mammals, but is most famously associated with birds (Hansell 2000). While bird nests primarily function to house eggs and eventually growing nestlings, they can also offer protection from predators, buffer occupants from environmental conditions, and even act as extended signals of phenotypic quality of the nest builder (Mainwaring et al. 2014). Adult birds frequently move between their nest and the surrounding environment. Failure to relocate the correct nest is associated with fitness consequences (i.e., reproductive failure) and yet we know very little about how birds accomplish this ubiquitous behavior.

While long considered an unused sense in birds, olfaction is increasingly recognized as an important mediator of navigation and other avian behaviors (Caro et al. 2015; Gagliardo 2013; Roper 1999). Birds use their sense of smell to find food (Nevitt et al. 1995; Stager 1964), select nest materials (Clark and Mason 1987; Petit et al. 2002), facilitate social interactions with conspecifics (Bonadonna and Nevitt 2004; Whittaker et al. 2011a), and navigate across long distances (Gagliardo et al. 2013b; Papi 1989). Furthermore, both songbirds and seabirds can discriminate the odor of their nest as adults (Bonadonna et al. 2003, 2004; Bonadonna and

Mardon 2010; Grubb 1974; Krause and Caspers 2012) and nestlings (Caspers et al. 2013; Caspers and Krause 2011; Mínguez 1997; O'Dwyer et al. 2008), indicating a potentially important role for olfaction in mediating homing across a range of avian species at multiple life stages. It is currently unknown whether birds use environmental odors to identify their nests, such as the materials that make-up their nest, or if they recognize bird-produced chemicals that are deposited on the nest by its occupants. Because homing involves a series of steps — orienting towards home, finding the area where the nest is located, and pinpointing the actual nest — olfactory information may function at multiple stages to aid in this larger process, with environmental and bird-produced chemicals facilitating different tasks.

Burrow nesting seabirds belonging to the order Procellariiformes are an ideal group to explore the chemical information underlying olfactory homing in birds. They are nocturnal at the breeding colony (Warham 1990) despite poor vision in low light conditions (Mitkus et al. 2016), but they possess a well-developed olfactory apparatus (Bang 1966). Several species fail to navigate to their breeding colony (Gagliardo et al. 2013b; Grubb 1979; Padget et al. 2017; Pollonara et al. 2015) or home to their nest when olfaction is impaired (Benvenuti et al. 1993; Bonadonna et al. 2001; Bonadonna and Bretagnolle 2002; Grubb 1974). Furthermore, many members of this group have strong-scented plumage, which contains chemical information about species (Gabirot et al. 2016), sex (Mardon et al. 2010), and individual identity (Jennings and Ebeler 2020). The ability to discriminate the scent of their nest over that of conspecifics is a commonly observed behavior (Bonadonna et al. 2003, 2004; Mínguez 1997; O'Dwyer et al. 2008) that is thought to be aided by their scent accumulating on their nest (Bonadonna et al. 2001). However, this idea has never been explicitly tested.

We conducted an in-depth exploration of the volatile chemical information present at the breeding colony of a burrow nesting seabird, the Leach's storm-petrel (*Oceanodroma leucorhoa*), to uncover the compounds that facilitate olfactory homing. We collected samples for odor analysis from the colony floor, inside occupied and unoccupied burrows, and from the storm-petrel inhabitants. We used headspace gas-chromatography mass spectrometry (GC-MS) to measure and describe the volatile chemical profiles of the various samples. With the resulting data, we examined three non-exclusive hypotheses relating to odor-mediated homing. First, we tested the hypothesis that Leach's storm-petrels use environmental odors for navigating to and moving around the colony. This hypothesis generated the following testable predictions: 1) There is an olfactory landscape present at the colony such that different geographic areas are associated with a specific chemical profile; 2) Samples from different landscape features within the colony (forest floor vs underground burrows) can be chemically discriminated. We also investigated the hypothesis that Leach's storm-petrels use self-deposited chemicals to identify their burrow. We tested three predictions associated with this hypothesis: 1) Storm-petrel chemicals are present and/or elevated in occupied burrows but absent or less abundant in other sample types; 2) Each burrow contains a unique blend of storm-petrel derived chemicals; 3) The types and quantities of storm-petrel produced chemicals in the burrow match with those on the plumage of the occupants. In addition to storm-petrels potentially altering the scent of their nest, chemicals may also be transferred from the burrow on to the birds, and/or between the two burrow occupants. We hypothesized that olfactory nest recognition is aided by the burrow and its storm-petrel occupants possessing a shared odor created by this multidirectional transfer of chemicals, which allows homing storm-petrels

use the scent of their own plumage as a template to recognize their burrow (i.e., a form of phenotype matching). To address this idea, we compared the chemical profiles of individuals in mated pairs and examined the overlap between the chemical profiles of storm-petrels and their burrow. We tested two predictions: 1) Paired birds have similar types and quantities of environmental chemicals on their plumage, which match the environmental compounds in the burrow; 2) Bird-produced chemicals contribute to a pair-specific odor that is reflected by the storm-petrel chemicals deposited inside the burrow.

3.3 METHODS

***Study Site*.** We collected samples on Bon Portage Island in Nova Scotia, Canada (43° 28'N, 65°, 44'W), which is a breeding colony for 39,000 pairs of Leach's storm-petrels (Pollet and Shutler 2018). There are over 500 marked burrows on the island that are distributed across three separate sites located in balsam fir (*Abies balseamea*) and black spruce (*Picea mariana*) forest (Figure 3.1). Sampling occurred during the incubation period after the burrows had been occupied by breeding birds for approximately one month.

Sample Collection.

Soil Samples: A storm-petrel burrow consists of a narrow entrance tunnel that leads to a roomier nest cavity (Warham 1990). We collected surface soil samples from two locations: 30 cm inside the burrow entrance and 30 cm directly outside the burrow entrance on the forest floor, hereafter referred to as the "background" (Figure 3.2). As the occupants enter or leave their burrow, they brush against the sides of the tunnel, which may cause their scent to be deposited in this area. In contrast, the background samples were taken from an area that has contact with

storm-petrels less often than the burrow tunnel. Within each burrow or background location, we collected 3 subsamples by scooping up the top layer of soil using a clean metal spatula (Figure 3.2). Each subsample was collected using a new pair of nitrile gloves to avoid cross contamination and was stored in a 20mL glass vial with a metal cap (Restek Inc, Bellefonte, PA). We collected the samples prior to other activities that disturb the soil, including checking the occupancy of the nest. In total, we sampled 44 burrows and 44 corresponding background locations. Thirty of the burrows were occupied by a breeding pair (confirmed at the time of sampling by the presence of an incubating adult or an egg), while 14 were unoccupied and remained empty for the duration of the breeding season. To preserve odor compounds, the samples were stored at -20°C degrees at the field site, shipped on dry ice, and kept at -80°C prior to analysis.

Feather Samples: Our feather collection protocol has been described in detail in Jennings and Ebeler (2020). Briefly, we took 6 small feathers from each individual, which were stored and transported at the same temperature as the soil to preserve their chemical make-up. In total, we obtained feathers from 56 birds.

Sample Preparation. We measured the chemical profiles of the soil and feather samples using gas chromatography mass spectrometry (GC-MS). We used headspace stir bar sorptive extraction (HS-SBSE), a solvent-less extraction technique where a 10 mm magnetic stir bar (commercially named Twister™, Gerstel Inc., Mülheim an der Ruhr, Germany) coated in polydimethylsiloxane is placed above a sample matrix and used to extract volatile compounds. This technique is highly sensitive, allows for the detection of trace compounds, and has been successfully employed in previous work on storm-petrel feathers (Jennings and Ebeler 2020) and other biological and environmental matrices (Kawaguchi et al. 2006; Lancas et al. 2009; Soini et al. 2005, 2006).

Soil Samples: We analyzed three soil samples for each location, one per subsample vial. For each sample, we placed approximately 2 g of soil into a 30 mL clean glass jar with a PTFE (polytetrafluoroethylene) lined lid (Qorpak, Clinton, PA) and recorded an accurate weight. A stir bar was suspended above the soil sample using two magnets and left to extract compounds from the headspace for 6 hrs. Samples were placed in a sand bath on top of a hotplate that was programmed so the temperature inside the vials remained at 24 °C.

Feather Samples: We analyzed three replicate samples per individual that consisted of two feathers per sample. Detailed methods for sample preparation can be found in Jennings and Ebeler (2020).

Chemical Analysis. Following extraction, we placed the stir bars into glass desorption tubes along with a micro-capillary tube containing 0.5 μL of internal standard (d_8-naphthalene in 100% ethanol, Sigma-Aldrich, St. Louis, MO). This compound compensated for variation in instrument response across the analysis period and assisted with quantifying the analytes. Because the soil samples produced larger chromatogram peaks than feathers, we used a higher concentration of the standard (25mg/L vs. 10mg/L). All stir bars were analyzed using the same instrument program.

We performed gas chromatography mass spectrometry (GC–MS) using an Agilent (Santa Clara, CA, USA) GC system (7890B) and mass spectrometer detector (5977A), with a thermal desorption unit (TDU) and cryo-cooled injection system (CIS) (Gerstel Inc., Mülheim an der Ruhr, Germany). The instrument was equipped with a DB-5MS column (30 m x 0.25 cm x 0.25 μm film thickness) and an Agilent Extractor Extra Inert electron source. The TDU thermally desorbed the

compounds collected by the stir bars using splitless mode with an initial temperature of 30 °C, heated to 250°C at 720 °C/min and held for 5 min. The analytes passed through the TDU transfer line, which was set to 250 °C, and were cryotrapped in the CIS, which was cooled to -80 °C using liquid nitrogen. The CIS then heated to 280 °C a rate of 12°C/s (hold time: 3 mins) and was operated in solvent vent mode with a vent flow of 50 mL/min and a purge flow of 50 mL/min after 1.2 min. Analytes entered the GC, which had an initial temperature of 35 °C for 3 min, increased at 5 °C/min to 220 °C (hold time: 0 mins), followed by a second increase at 15 °C/min to 280 °C (hold time: 5 min). The GC used helium as the carrier gas and was run in constant flow mode at a rate of 1.5 mL/min. The MS used the following temperature settings: the transfer line was at 280 °C, the electron source at 230 °C, and the quadrupole at 150 °C. The scan range was from 40 to 300 *m/z* at a rate of 2.8 scans/s and the EMV mode was set to gain factor, with the factor set to 1.0.

We used Agilent MassHunter Quantitative Analysis Software for GC-MS (version B.08.00) to quantify the detected compound peaks using an extracted ion chromatogram (EIC) for each compound. We ran two blank samples daily, which included a clean stir bar and a stir bar that underwent the extraction protocol inside an empty vial. Any compounds found in these blank samples were removed from the data. We analyzed a series of C8-C30 alkanes (Sigma Aldrich, St. Louis, MO) using the same settings as the samples to calculate retention indices for the analytes. We tentatively identified compounds by comparing their mass-spectral fragmentation pattern and measured retention index with those in NIST 14 Mass Spectral Database and other established sources (Pherobase, Flavornet).

Soil Dry Mass. To account for variation in soil moisture between sampling locations, we determined the water content of each soil subsample. We weighed out approximately 2 g of soil from each vial onto a watch glass and recorded an accurate mass. The soil was left to dry in a 105°C oven for 24 hours and was re-weighed to obtain the dry mass. We used the moisture content of these samples to convert the quantity of soil used in the chemical analysis into dry mass.

Data Analysis. The analysis of each chromatogram resulted in a list of detected compounds and their corresponding peak areas. We successively standardized the data from both sample types by dividing by the peak area of the internal standard and the sample mass. We applied a log (x+1) transformation to reduce the influence of a small number of highly abundant chemicals (Clarke et al. 2014). We averaged the standardized and transformed peak areas from the three replicates to make one representative chemical profile for each bird and colony location. We generated two pairwise resemblance matrices of Bray-Curtis dissimilarity between samples, one for soil and a second for feathers (Clarke et al. 2006). These chemical distance matrices served as the input for our subsequent analyses.

Olfactory Landscape at the Colony: To examine the olfactory landscape present at the colony, we used a two-way crossed PERMANOVA to test for differences among the three colony sites (site 1, 2, and 3: Figure 3.1) and soil sample types (background, occupied burrow, and unoccupied burrow). PERMANOVA is a permutational distance-based equivalent of a multivariate analysis of variance (Anderson 2001; McArdle and Anderson 2001). PERMANOVA indicates how the various model factors (e.g., colony site or sample type) contribute to the overall variation in the data.

We also used Canonical Analysis of Principal Coordinates (CAP), a type of discriminant analysis, to further explore the differences among colony sites and sample types (Anderson and Robinson 2003). CAP offers a complimentary approach to PERMANOVA; it identifies axes in the data that differentiate the groups of interest and determines how distinct the groups are using a leave-one-out classification process. We constructed two CAP models, one for colony location and second for sample type.

To further investigate whether soil chemical profiles varied across the island, we created a pairwise geographic distance matrix using the latitude and longitude coordinates for each burrow. We used Mantel tests to determine whether soil samples collected from nearby locations are more chemically similar to each other than samples from distant locations (Mantel 1967). We created one model for burrow samples and a second for background samples. We also used the BVStep procedure in PRIMER v7.0.13 to find the subset of soil chemicals that maximized the Spearman rank correlation between the chemical and geographic distance matrices (Clarke and Ainsworth 1993).

We performed a series of one-way ANOVAs for each of the soil chemicals to determine which compounds differentiated occupied burrows, unoccupied burrows, and the background. This step identified seven compounds were elevated in occupied burrows that were important components of storm-petrel scent. To test whether each occupied burrow contained a distinct blend of these storm-petrel chemicals, we used the chemical data for the 3 subsamples from the 30 occupied burrows (n = 90), which provided replication at the level of the burrow. We generated a Bray-Curtis dissimilarity matrix for the occupied burrow subsamples using the

seven chemicals and performed a one-way PERMANOVA on this matrix to test whether occupied burrows differed in their chemical makeup.

Burrows and their Occupants: We examined the degree of concordance between the chemical profiles of burrows and their storm-petrel occupants using the 75 chemicals that were present in both sample types. We created Bray Curtis dissimilarity matrices for both the soil and feathers and performed a Procrustes analysis (least-squares orthogonal mapping) to measure the overlap between the soil and feather matrices (Gower 1971, 1975). The Procrustes analysis centered, scaled, and rotated the two data matrices until they were maximally superimposed. It provided a measure of fit (m^2) between the two data sets (lower values indicate higher overlap) and assessed the significance of this value using permutations (Jackson 1995). Because this analysis requires that the two data matrices have the same number of entries (one burrow that corresponds to one bird occupant), we used feathers from 22 birds that were sampled on the same day as the soil. To determine whether the detected overlap between burrows and their occupants was due to chemicals transferred from the burrow onto the bird and/or due to chemicals transferred from the bird onto the burrow, we performed additional Procrustes tests using subsets of the chemicals: 1) plant chemicals (n = 33), 2) contaminants (n = 18), 3) all bird chemicals (n = 24) the bird chemicals that were elevated in occupied burrows (n = 7).

Chemical Profiles of Mated Pairs: Using the feather chemical profiles from 22 mated pairs, we ran a one-way PERMANOVA to assess whether individuals in pairs were chemically similar and differed from other pairs. To determine whether the chemical similarity observed within a pair was driven by environmental compounds from their shared burrow or by bird-produced compounds, we performed four additional one-way PERMANOVA tests using the following

subsets of the total chemical suite: 1) plant chemicals (n = 41), 2) contaminants (n = 29), 3) all bird chemicals (n = 85), and 4) bird chemicals that were elevated in occupied burrows (n = 7).

All statistical analyses were performed in R 3.5.3 (R Core Team 2019) using the packages ecodist (Goslee and Urban 2007; 2020) and vegan (Oksanen et al. 2020) or in PRIMER v7.0.13 (Clarke and Gorley 2015). Permutation-based analyses (i.e., PERMANOVA, CAP, Mantel, and Procrustes) were performed using 10,000 randomizations of the data. PERMANOVA tests used Type III sums of squares, the most conservative approach for unbalanced designs. We visualized the relationships between samples using ordination plots that were either outputs of the CAP models or produced using nonmetric multidimensional scaling (nMDS; Clarke 1999).

3.4 RESULTS

Soil and Feather Chemical Profiles. To test our hypotheses about the types of chemical information facilitating olfactory homing in Leach's storm-petrels, we analyzed two types of samples: soils and feathers. The soil samples were chemically complex; we detected 257 different volatile compounds with an average of 235 $\pm$ 15 s.d. chemicals per sample (Table S3.1, Figure 3.3). The soil contained a large number of plant chemicals (monoterpenes, sesquiterpenes and diterpenes), environmental contaminants (plasticizers, pollutants, phthalates), as well as hydrocarbons, ketones, aldehydes, alcohols, esters, and lactones. The odor compounds associated with Leach's storm-petrel plumage included hydrocarbons, aldehydes, ketones, and esters produced by the bird, as well as plant and contaminant compounds from the environment (Table S3.2, see Jennings and Ebeler 2020 for full results). We identified 75 chemicals that were common to both sample types (Table S3.2).

Spatial Gradients of Environmental Chemicals. We used the soil samples to determine whether there is a landscape of volatile chemicals within the colony. We found that soil from the three colony sites varied in their chemical profiles (PERMANOVA Pseudo-F = 6.226. p = 0.0001, Table 3.1). Each site was chemically distinct, with the CAP model successfully matching 91% of the samples to the correct location using 11 multivariate axes (Table 3.2, p = 0.0001; Figure 3.4). The chemical profiles of the soil also varied with geographic distance; nearby locations had a more similar chemical makeup than distant locations for both the background (r = 0.14, p = 0.0257) and the burrows (r = 0.23, p = 0.003). These correlations were greatly strengthened using a subset of total chemicals identified by the BVStep analysis. In the background soil, a group of 14 compounds produced a correlation of r = 0.659, while 15 compounds in the burrow soil gave a correlation of r = 0.734. The chemicals identified by both models included plant chemicals, hydrocarbons, aldehydes, and a number of contaminants (Table 3.3).

Chemically Distinct Landscape Features. We compared the three types of soil samples – from unoccupied burrows, occupied burrows, and the background – and found that each had significantly different chemical profiles (Pseudo-F = 2.008, p = 0.0423, Table 3.1), which provided support for our prediction that the colony landscape contains chemically distinct features. The CAP model that differentiated the sample types used 9 multivariate axes and correctly identified 78% of samples (p = 0.0001, Table 3.4, Figure 3.5). Overall, the model had greater success classifying the samples from the background and occupied burrows than those from unoccupied burrows, which were less distinct.

Occupied Burrows are Uniquely Scented by Storm-petrel Chemicals. We performed a series of one-way ANOVAs on the soil compounds, which identified 9 plant chemicals and 12 other

compounds (either ketones, hydrocarbons or aldehydes) that were elevated in the background soil compared with inside the burrows (occupied or unoccupied), as well as 5 different plant chemicals that were more abundant in burrows relative to the background (Table 3.5). Eight compounds were elevated inside occupied burrows (Table 3.5), 7 of which were major constituents of storm-petrel odor where they accounted for an average of 38% of the total peak area in the feather chromatograms. These compounds were on average 8 times more abundant in occupied burrows, which supported our prediction that active nests contain storm-petrel chemicals. The compounds included five aldehydes (C6, C7, C9-C11) that are also detected in other birds (Allan et al. 2006; Bonadonna et al. 2007; Hagelin et al. 2003; Mardon et al. 2011), as well as pristane, a saturated terpenoid alkane found in the stomach oil of this species (Clarke and Prince 1976), and styrene, a compound that is naturally occurring in plants, animals, and bacteria (Lafeuille et al. 2009; Pinches and Apps 2007; Steele et al. 1994). Moreover, each occupied burrow contained a unique blend of these seven storm-petrel chemicals (Pseudo-F = 42.765, $p = 0.001$, Table 3.6, Figure 3.6).

Burrows and Their Occupants are Chemically Matched. We measured the degree of overlap between burrows and occupants, which revealed that the chemical relationships among the burrows matched the relationships among their storm-petrel occupants (Procrustes $m^2 = 0.532$, $r = 0.684$, $p = 0.00039$, Figure 3.7). There was a high degree of overlap between the data matrices for burrows and occupants based on the plant-derived chemicals ($m^2 = 0.259$, $r = 0.861$, $p < 0.0001$, Figure 3.7), but not for contaminants ($m^2 = 0.880$, $r = 0.347$, $p = 0.368$). We also found a match between the burrows and their occupants using the bird-produced chemicals ($m^2 = 0.540$, $r = 0.678$, $p = 0.0021$), and this relationship was highly significant when

considering only the seven storm-petrel compounds that were elevated in occupied nests (m^2 = 0.362, r = 0.799, p < 0.0001, Figure 3.7). Together these findings supported the transfer of chemicals from the birds onto the burrow and vice versa.

Pair-specific odors. We found that individuals in mated pairs had similar chemical profiles that were distinct from other pairs (Pseudo-F = 2.131, p = 0.0001, Table 3.7, Figure 3.8A). This finding was driven primarily by paired birds being alike in the types and abundances of environmental chemicals, including both contaminants (Pseudo-F = 2.190, p = 0.0002, Table 3.7, Figure 3.8B) and plants (Pseudo-F = 7.917, p = 0.0001, Table 3.7, Figure 3.8C). The chemical profiles of paired birds were not similar when using all bird-produced compounds (Pseudo-F= 1.441, p = 0.0608, Table 3.7). However, they were highly similar when considering only the seven storm-petrel chemicals that were elevated inside occupied burrows (Pseudo-F= 8.775, p = 0.0001, Table 3.7, Figure 3.8D).

3.5 DISCUSSION

All birds make trips to and from their nest and olfaction may play an important role facilitating multiple steps within this task. Leach's storm-petrels rely on chemical information to locate their breeding colony and home to their burrow (Grubb 1974, 1979; O'Dwyer et al. 2008). We explored the olfactory information present at a Leach's storm-petrel colony using GC-MS. We compared the chemical profiles of the colony floor, the storm-petrel burrows, and the birds that occupy them to test three non-exclusive hypotheses about olfactory homing. We found gradients of environmental chemicals across the landscape, which supported the hypothesis that navigation to and around the colony relies on environmental odors. We also show for the

first time that occupied nests contain unique combinations of bird-produced chemicals that reflect the individuals that inhabit them, which provided support for the hypothesis that storm-petrels use self-deposited chemicals to identify their nest. Finally, we revealed that there is a multidirectional transfer of chemicals between the burrow and its occupants that creates a shared odor carried by both the birds and their nest, which we hypothesized aids olfactory nest recognition in this species.

***Olfactory Landscape of Environmental Chemicals*.** We found that environmental odors formed a landscape of chemicals within the colony, which may function at multiple spatial scales to assist homing Leach's storm-petrels. Three colony sites were associated with different chemicals and certain compounds varied with distance across the island. Both homing pigeons (Benvenuti and Wallraff 1985; Gagliardo et al. 2016, 2018; Wallraff et al. 1984) and seabirds (Gagliardo et al. 2013b; Padget et al. 2017; Pollonara et al. 2015) rely on olfaction for homeward navigation and spatial gradients in environmental chemicals, including plant derived compounds, may function as an olfactory map for navigating birds (Zannoni et al. 2020). A number of bird species are able to smell plant chemicals (Amo et al. 2013; Clark and Mason 1987; Gwinner and Berger 2008; Petit et al. 2002), and the forested storm-petrel colony is rich in these compounds. If these plant chemicals are also present in the air above the colony, they may help individuals returning from the ocean to locate the island. Furthermore, many birds fly over the forest and drop through the canopy in the vicinity of their burrow (S. Jennings personal observation; Warham 1990). Because different areas of the colony are associated with different scents, environmental chemicals may assist with identifying the right place to descend to the

forest floor. Once on the ground, environmental compounds differentiated underground burrows from the colony floor, which could help with the final stages of homing.

In addition to naturally occurring chemicals, we detected spatial gradients in several synthetic environmental contaminants. A large number of these compounds were present in every feather and soil sample. Paired individuals contained similar types and abundances of contaminants, but the compounds on the birds were not correlated with those found in their burrow, likely because storm-petrels encounter these chemicals while at sea, as well as at the breeding site. It is unknown whether storm-petrels can smell any of these molecules, but their ubiquitous presence in a relatively remote habitat emphasizes a broader need to study how contaminants impact the chemical senses, particularly in species that rely heavily on olfaction.

Burrow and Pair Odors. We found that storm-petrel burrows were scented like their occupants. Seven key components of storm-petrel odor were more abundant inside occupied burrows than on the colony floor or in unoccupied nests. Each occupied burrow contained a unique blend of these chemicals, which was highly correlated with the blend found on the occupants' plumage. These bird-derived volatiles are strong candidates for how Leach's storm-petrels identify their burrow within the colony and behavioral trials testing the responsiveness of storm-petrels to them are an important next step. Procellarid seabirds have historically occupied predator-free breeding sites where there was little risk associated with pungent nests. This is not the case for most bird species, which are highly vulnerable to olfactory predators and may favor camouflaged over conspicuously scented nests (Shutler 2019). Nest odors have also been studied in zebra finches (*Taeniopygia guttata*), a passerine, where nests contained chemicals that were not found on unused nest material and family members had similarly scented nests

(Kohlwey et al. 2016). These findings suggest that the presence of bird-derived compounds on nests is not restricted to burrow-nesting seabirds. However, just how widespread this phenomenon is across the avian group warrants further investigation and will likely vary based on the selection pressures experienced by different species.

There was clear transfer of chemicals from the burrow onto the bird and from the bird onto the burrow. We found that paired individuals were similar in the types and abundances of plant compounds on their plumage, and that there was a strong correlation between the plant compounds in the burrow and those found on the occupant, indicating that the burrow was a direct contributor of these chemicals. Carrying a scent of "home" may assist in olfactory burrow discrimination if birds compare their own odor to the odor of the burrow (i.e., a type of phenotype matching). Additionally, shared environmental chemicals between paired individuals could aid or reinforce odor-mediated mate recognition, which is observed in a number of species in this group (Bonadonna and Nevitt 2004; Jouventin et al. 2007; Mardon and Bonadonna 2009).

We found that the chemical profiles of birds within a pair differed when considering the entire suite of bird-produced compounds, which was expected because Leach's storm-petrels have unique individual odors (Jennings and Ebeler 2020). However, pairs were highly similar in the subset of 7 compounds that were elevated inside their burrow, which suggests that the chemicals deposited on the burrow reflect the identity of the pair and not just the individual who most recently entered the nest. Many social insects have colony-specific chemical blends that they use to mark the area surrounding their nest and to recognize nest-mates. These colony labels primary consist of hydrocarbons found on the outside their bodies, which are

transferred both directly among colony members during social interactions (e.g., allogrooming and mouth-to-mouth feeding) and indirectly via the nest material (Blomquist and Bagnères 2010; van Zweden and d'Ettorre 2010; Vander Meer et al. 2019). Analogous mechanisms could explain the chemical similarity between mates in Leach's storm-petrels who could exchange chemicals through bodily contact and/or could pick up chemicals deposited on the burrow soil by their mate.

Scent-producing microbes, which create group-specific odors in some mammals (Leclaire et al. 2017b; Theis et al. 2012), may also contribute to pair-specific chemical profiles in Leach's storm-petrels. Bacteria produce chemical cues in birds (Whittaker et al. 2019b), and a shared nest often yields similar microbiomes (Lucas et al. 2005; Martínez-García et al. 2016; Ruiz-Rodríguez et al. 2014; van Veelen et al. 2017; Whittaker et al. 2016). The high degree of overlap between paired Leach's storm-petrels for certain chemicals could be due to pairs harboring similar microbes. Previous research in this species failed to find overlap in the microbiomes of paired birds, but the sample size was small (n= 5 pairs), and the relationships were tested using the entire microbial community (Pearce et al. 2017). It is possible that microbial similarity between paired Leach's storm-petrels mirrors the pattern we observed in the bird-derived chemicals and only occurs for specific types of microbes.

Scent as Social Communication. In a colonial species like Leach's storm-petrels, the bird-derived chemicals on the burrow could also function as a mode of chemical communication between colony members. Avian chemical profiles contain a variety of socially relevant information such as sex, species, age and breeding status (reviewed in Campagna et al. 2012). In Leach's storm-petrels, the plumage odor reflects both individual identity and individual

quality (Jennings et al. *in prep* (Chapter 2 of this dissertation); Jennings and Ebeler 2020). Male Leach's storm-petrels are thought to construct the burrow and occupy it while courting a mate (Gross 1935; Pollet et al. 2020). If burrow-deposited chemicals broadcast the same information as the feather chemicals, they may function to attract potential mates, analogous to scent marks in mammals (Brennan and Kendrick 2006; Gosling and Roberts 2001; Roberts et al. 2014) and reptiles (Martín and López 2006; Olsson et al. 2003).

Moreover, scent acts as a marker of territory in many species (reviewed in Wyatt 2014), and the bird chemicals we detected inside storm-petrel burrows may function similarly. While some Leach's storm-petrels dig their own burrow, other birds take over an existing nest. Prospective breeders looking to acquire a burrow for the following year regularly visit empty nests but infrequently enter burrows occupied by breeding birds (S. Jennings personal observation; Pollet et al. 2020). Scented burrows may allow individuals to differentiate vacant versus active nests and may help them avoid territorial incursions. Further support that nest-odors communicate information about burrow ownership comes from two related species, blue petrels (*Halobaena caerulea*) and Antarctic prions (*Pachuptila desolata*). Blue petrels use olfaction to preferentially occupy the empty burrows of conspecifics rather than the similar-sized burrows of Antarctic prions, who often eject blue petrels that they find squatting in their nests (Bonadonna and Mardon 2010). Taken together, these findings suggest that bird odors inside nests may function not only as olfactory cues for homing but as signals that enable communication between senders and receivers.

Limitations and Future Directions. The bird chemicals we detected inside occupied nests were volatile, making them ideal for detection by the avian olfactory system but prone to dissipate

rapidly from the burrow. However, the scent of Leach's storm-petrels is enduring, with field equipment retaining the smell for years (S. Jennings personal observation), which suggests a role for more persistent non-volatile chemicals. The primary source of scented compounds in birds is oil produced by the preen gland, which birds spread onto the plumage, and contains both volatile and non-volatile compounds (Campagna et al. 2012). The compounds we found in occupied burrows could be produced through the breakdown of higher molecular weight molecules deposited by the bird, but our analytical method, which sampled volatiles, was not well suited for detecting them. Solvent extraction methods that are better for measuring non-volatile compounds may yield different results. Additionally, we only examined the chemical profiles at one time during the incubation period. Determining which environmental and bird-produced chemicals are consistent through time could help refine which source(s) of olfactory information would be most useful for homing birds. Other areas for future study include measuring how long the bird scent persists inside the burrow, which is particularly relevant in Leach's storm-petrels that reuse the same nest over multiple years.

Funding and Ethics Statement: This research was supported by National Science Foundation (IOS 1258828). Research protocols were approved by UC Davis Animal Care and Use (protocol 17588 in 2013-2015, protocol 19288 in 2016) and the Canadian Wildlife Service (banding permit 10695, research permit SC2742). Samples were transported to the United States under USDA Veterinary Permit to Import Controlled Materials 125361 and Nova Scotia Permit to Export Wildlife.

3.6 FIGURES AND TABLES

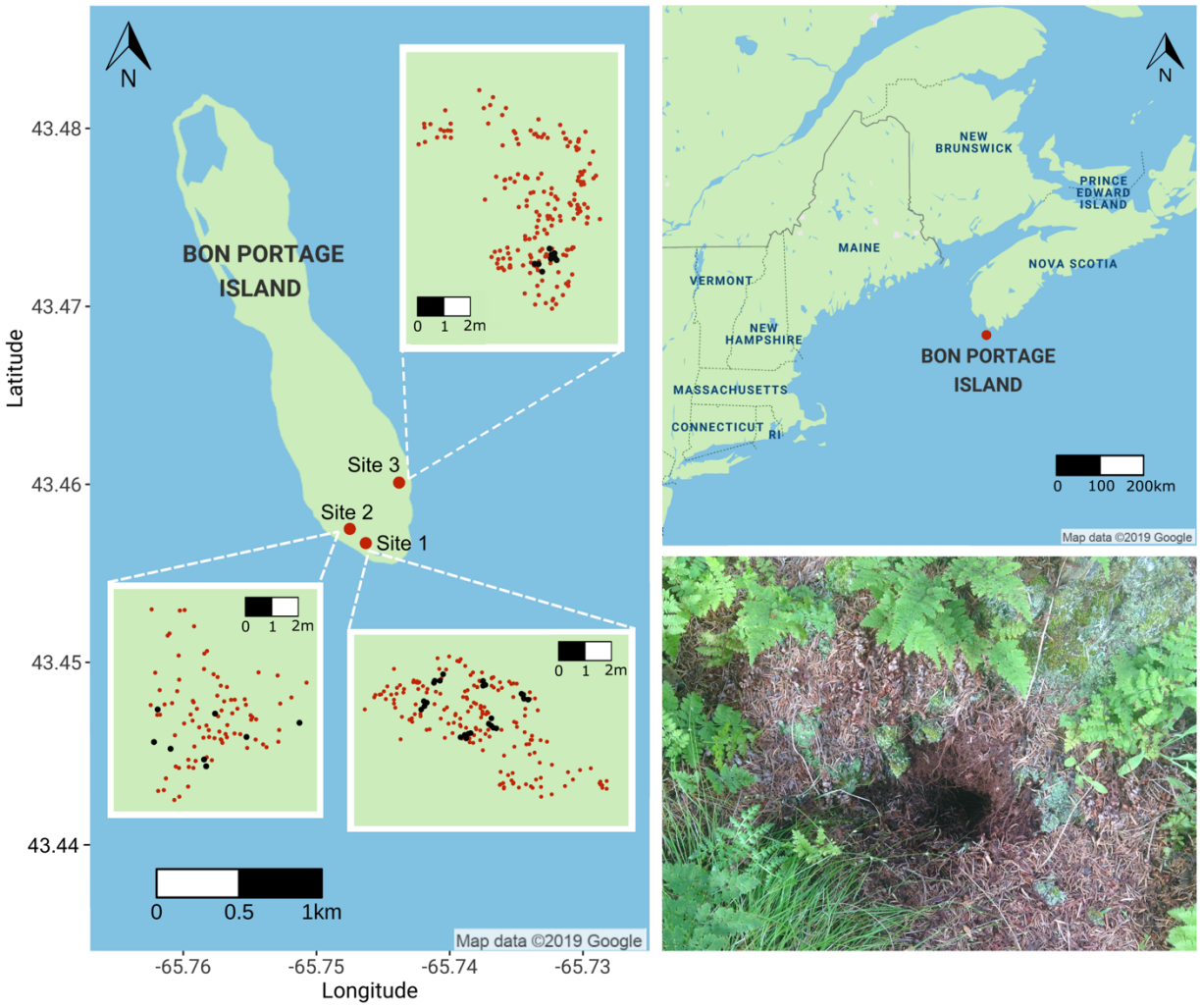

Figure 3.1. Map of Bon Portage Island, Nova Scotia, Canada. The three colony sites where sampling took place are marked. The specific locations of the burrows at each site are depicted in the inserts and the locations marked in black show the burrows where soil was collected. The photo in the lower right shows the entrance to a Leach's storm-petrel burrow

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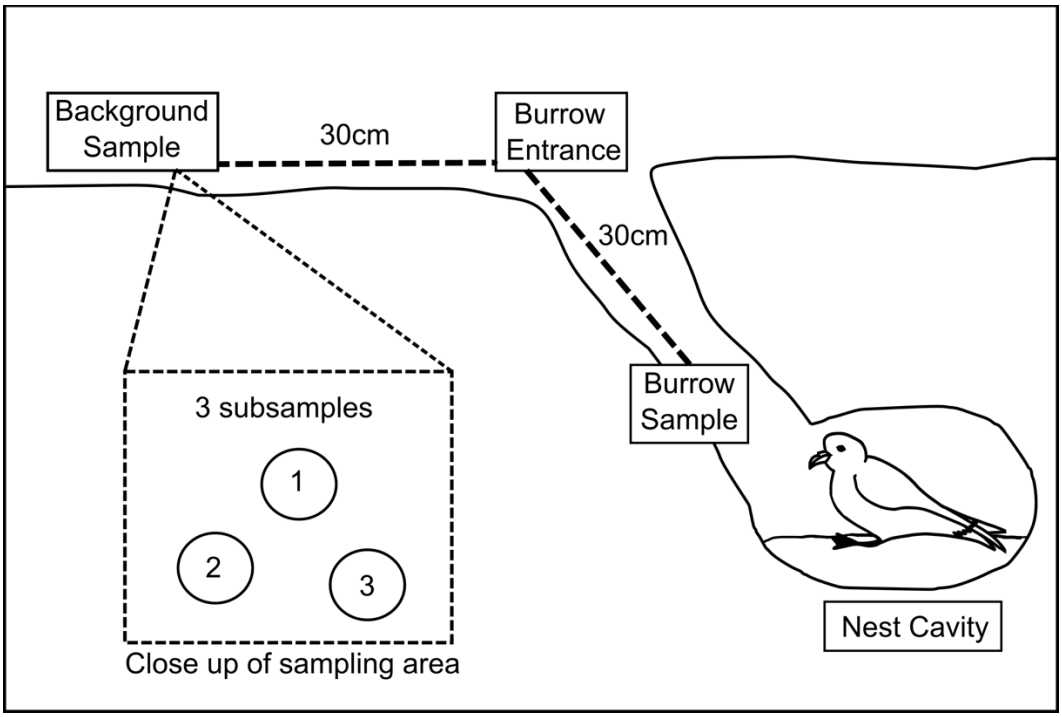

Figure 3.2. Soil collection method. Samples were collected in two locations: inside the burrow tunnel (burrow sample) and outside the burrow on the colony floor (background sample). We took three subsamples in each location that were stored in separate vials

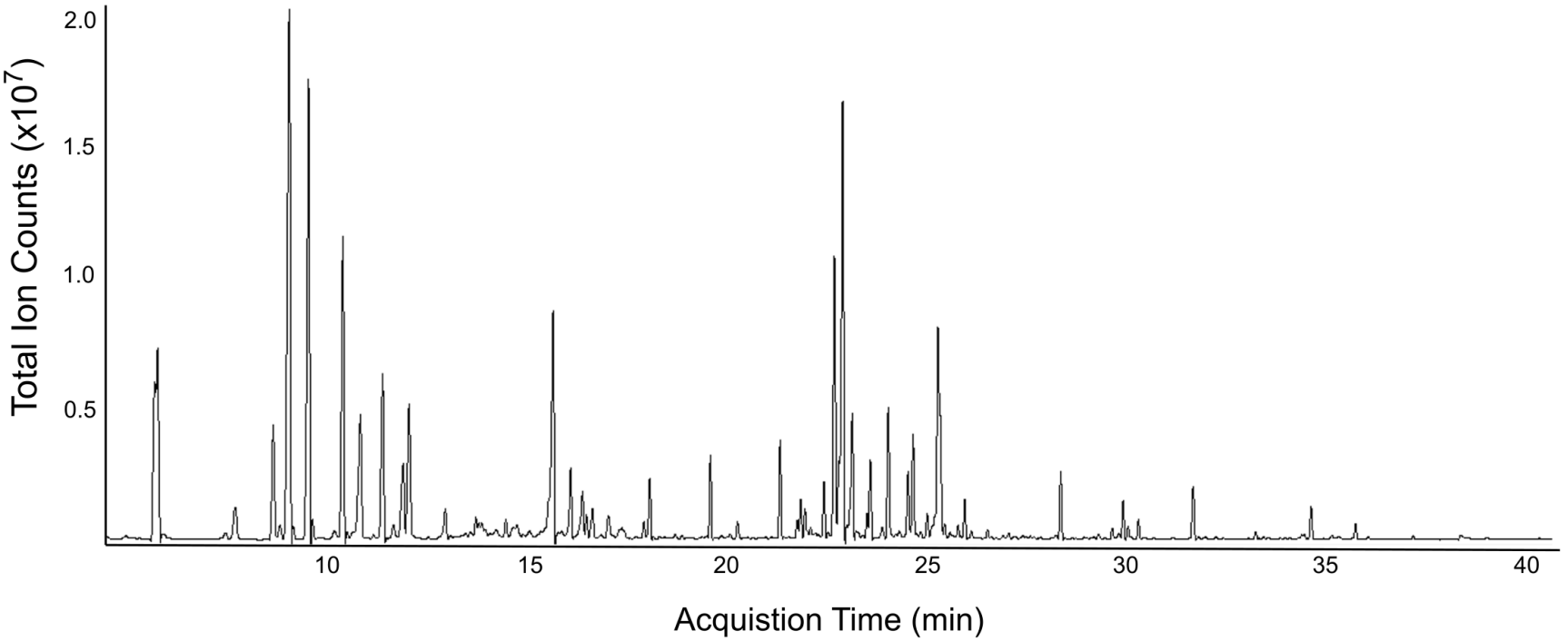

Figure 3.3. Representative GC-MS total ion chromatogram for volatiles extracted from a Leach's storm-petrel burrow

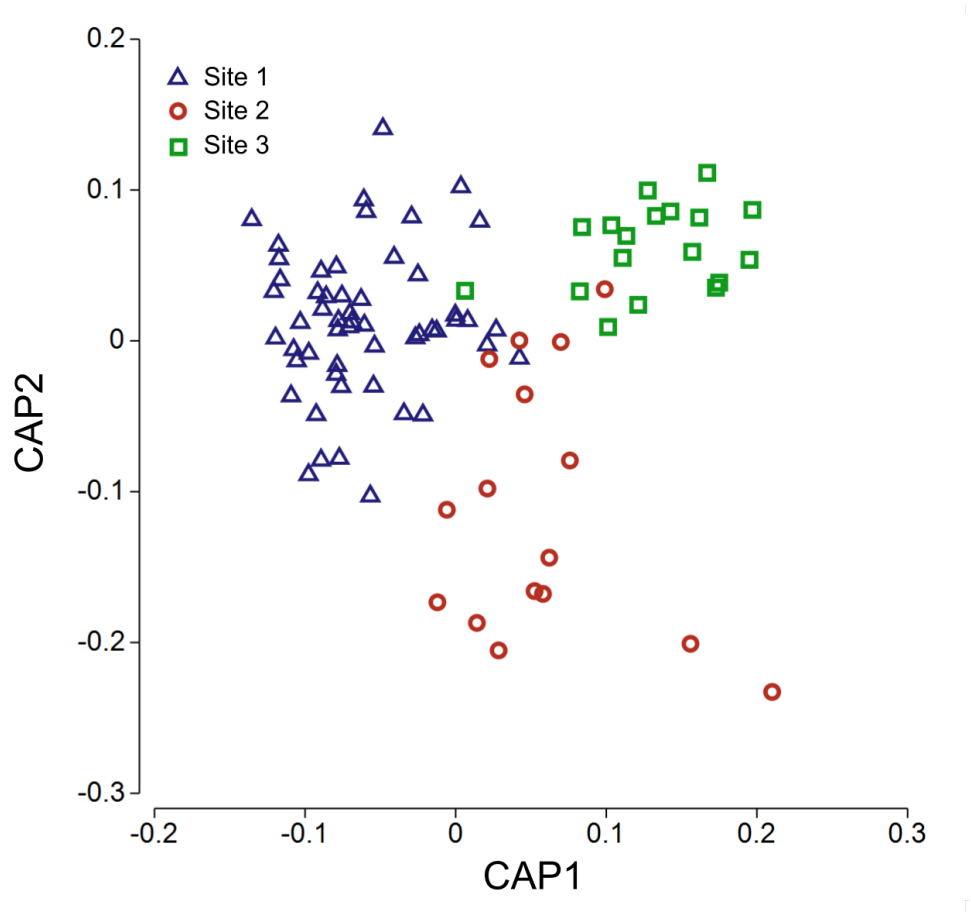

Figure 3.4. CAP analysis to examine the chemical profiles of soil samples collected from three different colony sites. Each symbol represents a different sample, and the different colors/symbols show the three colony sites. The model assigned 91% of samples to the correct colony site

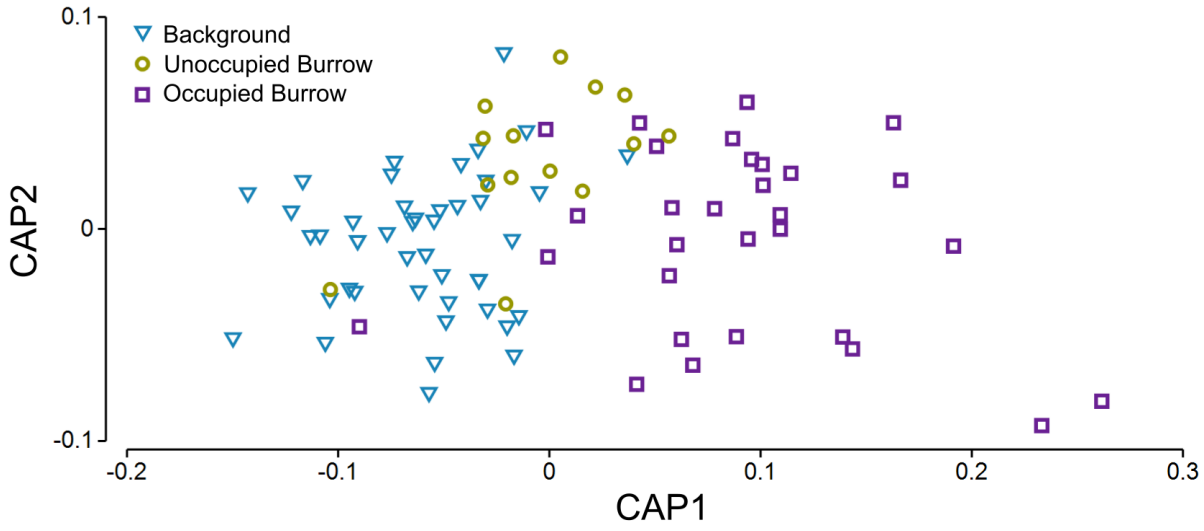

Figure 3.5. CAP analysis to examine the chemical profiles of soil samples collected from three different colony features (background, unoccupied burrows, occupied burrows). Each symbol represents a different sample, and the different colors/symbols show the three types of samples. The model assigned 78% of samples to the correct sample type

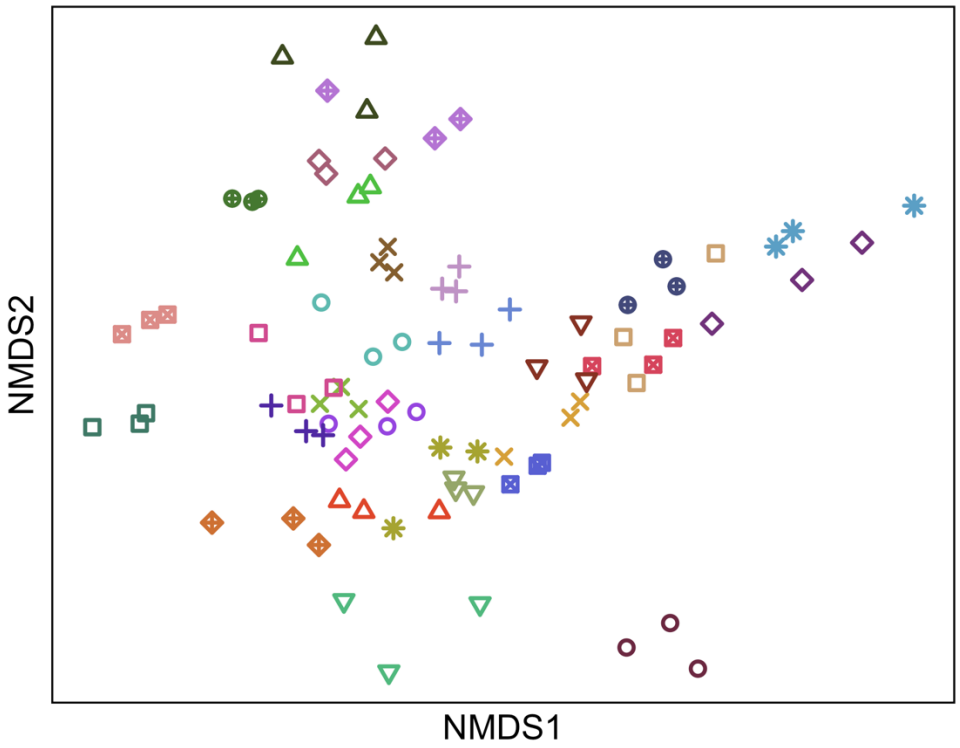

Figure 3.6. Non-metric multidimensional scaling plot (nMDS) visualizing the chemical profiles of occupied burrows based on seven storm-petrel chemicals. The color/symbol combinations depict the 30 occupied burrows, and each point represents a different soil subsample, such that each burrow has three points. 2D Stress = 0.15

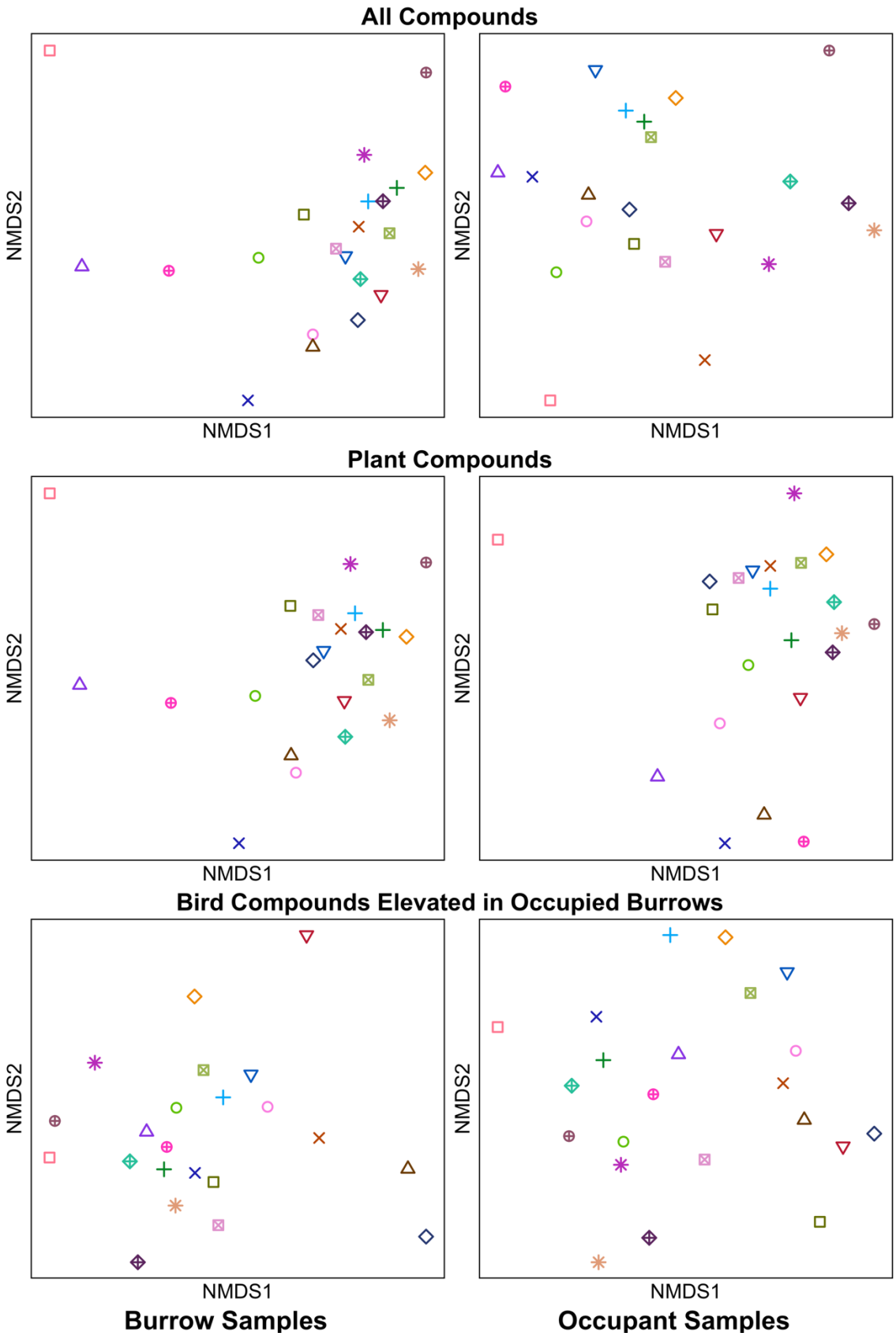
All Compounds
NMDS2
NMDS1
NMDS2
NMDS1
Plant Compounds
NMDS2
NMDS1
NMDS2
NMDS1
Bird Compounds Elevated in Occupied Burrows
NMDS2
NMDS1
NMDS2
NMDS1
Burrow Samples
Occupant Samples

Figure 3.7. Non-metric multidimensional scaling plots (nMDS) visualizing the chemical profiles of 22 burrows (left column) and 22 storm-petrel occupants (right column). The colors/symbols match so that the burrow (left plot) and the corresponding occupant (right plot) are shown using the same combination. We tested the degree of overlap between the burrow and occupant data matrices using a Procrustes analysis. The three pairs of nMDS plots (rows) show the different suites of chemicals where there was a significant correlation between burrows and birds. The overlap detected by the Procrustes analysis is visible in these unmanipulated plots because the burrow sample (left plot) occupies a similar location in the nMDS as the corresponding occupant sample (right plot). 2D Stress values for the plots from top left to bottom right = 0.07, 0.17, 0.05, 0.13, 0.12, 0.15

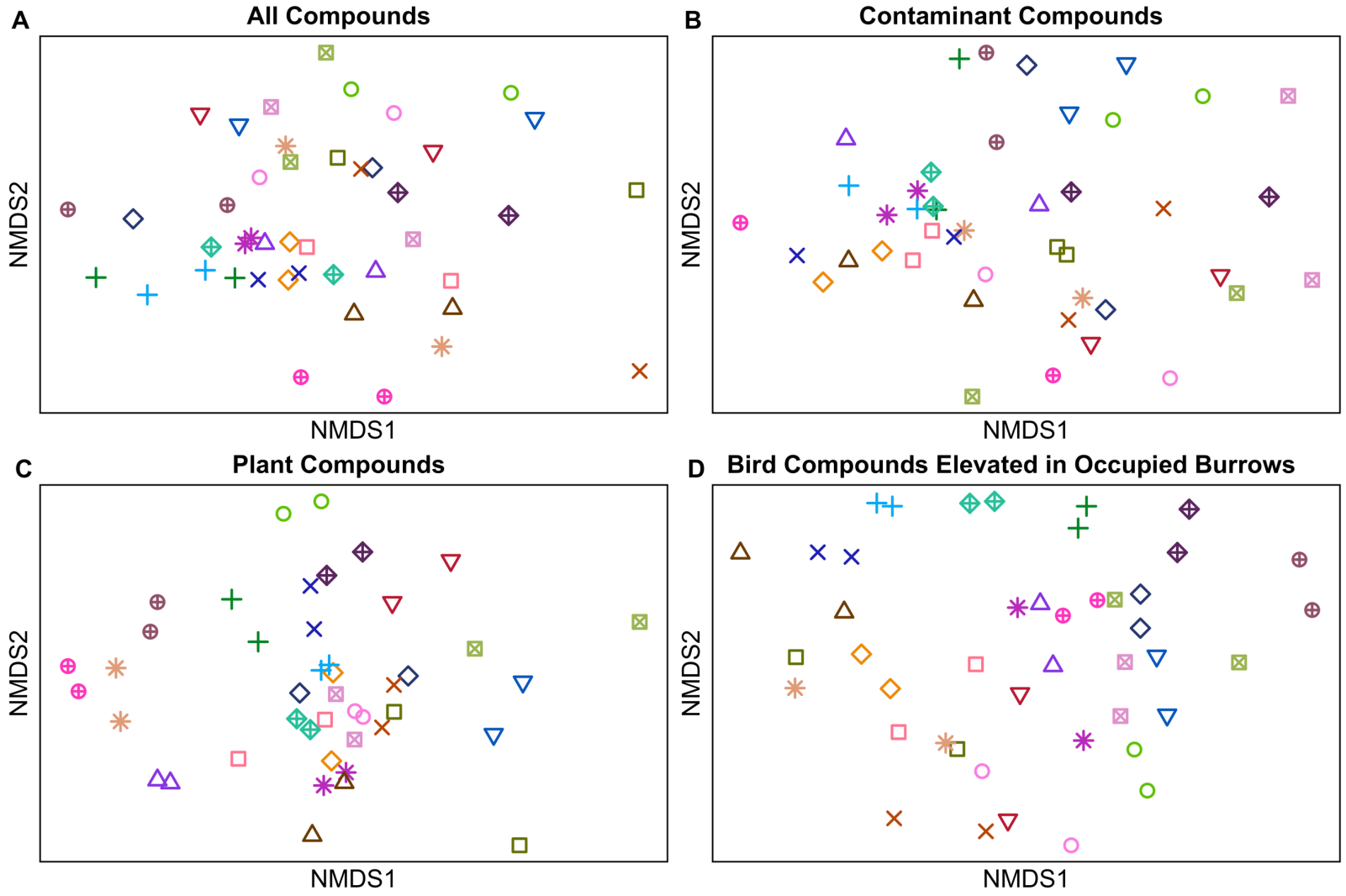

Figure 3.8. Non-metric multidimensional scaling plots (nMDS) visualizing the chemical profiles of 22 mated pairs of Leach's storm-petrels. Each color/symbol combination represents a different pair. The four plots depict the different suites of chemicals where

paired birds were similar to each other and distinct from other pairs: A) All compounds, B) Contaminants, C) Plant compounds, D) Storm-petrel chemicals that were elevated inside occupied burrows. 2D Stress values for the plots: A) 0.15, B) 0.16, C) 0.18, D) 0.16

Table 3.1. PERMANOVA analysis to test for the effect of colony site and sample type on the chemical profiles of soil

Source	df	SS	MS	Pseudo-F	*p* (perm)
Colony Site	2	8719	4360	6.226	**0.0001**
Sample Type	2	2910	1455	2.008	**0.0423**
Site*Type	4	3597	89	1.284	0.1992
Residuals	79	55321	700		

df: degrees of freedom, SS: sum of squares, MS: mean squares. Significant effects are in bold. Significance assessed at α=5% using 10,000 permutations of the data

Table 3.2. Results from CAP analysis examining the effect of colony site on the soil chemical profiles

Original Group	Classified Group		% Correctly Classified	m	Trace statistic	*p* (perm)
	Correct Group	Incorrect Group				
Colony Site (n=88)	80	8	91%	11	1.274	**0.0001**
Classification rate by group						
Site 1 (n=54)	51	3	94%			
Site 2 (n=16)	12	4	75%			
Site 3 (n=18)	17	1	94%			

m: number of multivariate axes used in the model. Significant effects are in bold. Significance assessed at α=5% using 10,000 permutations of the data

Table 3.3. The subset of chemicals identified by the BVSTEP process that maximized the correlation between chemical and geographic distance for burrow and background soil samples

Sample Type	Correlation (*r*)	Number of Compounds	Compound Retention Times	Compound Names
Burrow	0.734	15	4.33, 5.39, 12.29, 13.52, 14.27, 16.45, 19.02, 19.07, 19.27, 20.41, 25.83, 26.33, 27.24, 27.41, 33.26	Tolulene, Hexanal, 6-Ethyl-2-methyloctane, Unidentified, p-Mentha-1,5,8-triene, 5-Pentylcyclohex-1,3-diene, 2-n-Ocytlfuran, 1,4,p-Menthadien-7-al, Citral, Undecanal, Unidentified, (1-Propylheptyl)benzene, Caryophyllene oxide, 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate, Unidentified phthalate
Background	0.659	14	4.33, 4.85, 13.20, 13.52, 14.27, 17.54, 17.86, 19.07, 22.69, 25.83, 27.41, 29.49, 30.30, 39.28	Tolulene, 1-Octene, Unidentified branched chain alkane, Unidentified, p-Mentha 1,5,8 triene, Levoverbenone, Fenchyl acetate, 1,4,p-Menthadien-7-al, 1-Tetradecene, Unidentified, 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate, 1-Tetradecanol, Pentadecanal, Sandaracopimaral

Table 3.4. Results from CAP analysis examining the effect of sample type on the soil chemical profiles

Original Group	Classified Group		% Correctly Classified	m	Trace statistic	*p* (perm)
	Correct Group	Incorrect Group				
Sample Type (n=88)	69	88	78%	9	0.776	**0.0001**
Classification rate by group						
Background (n=44)	35	9	79%			
Occupied Burrow (n=30)	25	5	83%			
Unoccupied Burrow (n=14)	9	5	64%			

m: number of multivariate axes used in the model. Significant effects are in bold. Significance assessed at α=5% using 10,000 permutations of the data

Table 3.5. Compounds that differentiated the chemical profiles of the background, occupied burrows, and unoccupied burrows. Significance determined using a series of one-way ANOVAs on the average abundance of each chemical in the three sample types

Retention Time	Retention Index	Compound Name	m/z	Compound Type	Average Compound Abundance			*p*-value
					Background	Unoccupied Burrows	Occupied Burrows	
Elevated in background								
7.44	876	2-Norbornene	94	Hydrocarbon	1.899	0.785	0.872	0.0004
7.87	890	2-Heptanone	58	Ketone	0.253	0.081	0.075	< 0.0001
9.44	941	(-)-Frontalin	72	Cyclic ketal	0.020	0.013	0.009	< 0.0001
9.85	954	6-Methylheptan-2-one	58	Ketone	0.168	0.108	0.090	< 0.0001
10.82	986	3-Octanone	99	Ketone	7.050	2.960	2.830	0.0002
11.56	1010	2,5,6-Trimethyloctane	57	Hydrocarbon	0.199	0.123	0.128	0.0016
13.80	1082	2-Norbornanone	69	Ketone	0.205	0.116	0.132	0.0039
14.10	1092	5-Ethyldecane	57	Hydrocarbon	0.668	0.311	0.378	0.0073
17.14	1191	2-Decanone	58	Ketone	0.463	0.313	0.316	< 0.0001
19.02	1258	2-n-Octylfuran	81	Hydrocarbon	0.213	0.128	0.121	0.0035
21.69	1358	Unidentified sesquiterpene	119	Sesquiterpene	0.079	0.044	0.045	0.0002
22.12	1374	Longicyclene	94	Sesquiterpene	1.087	0.586	0.502	< 0.0001
22.63	1393	(+)-Satviene	108	Sesquiterpene	2.798	1.594	1.403	< 0.0001
23.08	1410	Longifolene	161	Sesquiterpene	15.867	8.791	7.491	< 0.0001
23.25	1417	beta-Cedrene	161	Sesquiterpene	3.829	1.918	2.077	0.0004
23.67	1432	cis-Thujopsene	119	Sesquiterpene	1.945	0.896	1.001	< 0.0001
25.52	1503	beta-Bisabolene	93	Sesquiterpene	1.658	1.044	1.091	0.0005
26.91	1564	2-Methylpentadecane	71	Hydrocarbon	0.032	0.023	0.022	0.0004
27.17	1576	Unidentified hydrocarbon	82	Hydrocarbon	0.024	0.006	0.006	< 0.0001
28.02	1613	Tetradecanal	82	Aldehyde	0.022	0.014	0.018	0.0015

32.16	1800	Octadecane	57	Hydrocarbon	0.041	0.032	0.034	0.0121
33.65	1874	Unidentified	134	Contaminant	0.117	0.068	0.075	0.0004
Elevated in burrows								
15.64	1142	(-)-trans-Pinocarveol	92	Monoterpene	0.023	0.085	0.090	0.0008
16.17	1159	trans-Pinocamphone	83	Monoterpene	0.277	0.507	0.408	0.0016
16.64	1174	cis-Pinocamphone	83	Monoterpene	0.562	0.909	0.821	0.0107
17.17	1192	Myrtenal	107	Monoterpene	0.243	0.703	0.524	< 0.0001
19.73	1284	L-Bornyl Acetate	136	Monoterpene	1.094	2.664	2.210	0.0001
Elevated in occupied burrows								
5.39	809	Hexanal	56	Aldehyde	0.061	0.056	0.203	< 0.0001
7.71	885	Styrene	104	Hydrocarbon	0.174	0.208	0.376	0.0013
8.10	900	Nonane	85	Hydrocarbon	0.060	0.048	0.329	< 0.0001
8.23	901	Heptanal	70	Aldehyde	0.042	0.035	0.119	< 0.0001
14.61	1108	Nonanal	57	Aldehyde	0.139	0.120	1.764	< 0.0001
17.56	1203	Decanal	82	Aldehyde	0.179	0.142	0.661	< 0.0001
20.41	1310	Undecanal	82	Aldehyde	0.032	0.029	0.230	< 0.0001
30.11	1706	Pristane	57	Hydrocarbon	0.095	0.074	2.684	< 0.0001
33.75	1879	Homosalate	138	Contaminant	0.021	0.017	0.030	< 0.0001
Elevated in unoccupied burrows								
13.98	1088	Fenchone	81	Monoterpene	1.265	2.265	1.248	0.0018
14.95	1120	Fenchol	81	Monoterpene	0.103	0.218	0.128	0.0005
18.29	1230	Unidentified phthalate	149	Contaminant	0.267	1.833	0.232	0.0003
21.92	1367	2-Undecenal	70	Aldehyde	0.125	0.329	0.113	0.0009
30.50	1723	Oxacyclopentadecan-2-one	55	Lactone	0.092	0.189	0.121	0.0005
34.60	1924	Oxacycloheptadecan-2-one	97	Lactone	0.027	0.045	0.029	0.0057
35.33	1963	Sandaracopimaradiene	137	Diterpene	0.054	0.094	0.051	0.0014

Table 3.6. PERMANOVA analysis for the effect of burrow ID on the chemical profiles of occupied burrows using seven storm-petrel chemicals

Source	df	SS	MS	Pseudo-F	*p* (perm)
Occupied Burrow ID	29	76333	2632	42.765	**0.0001**
Residuals	60	3693	62		

df: degrees of freedom, SS: sum of squares, MS: mean squares. Significant effects are in bold. Significance assessed at α=5% using 10,000 permutations of the data

Table 3.7. PERMANOVA analyses for the effect of pair ID on the chemical profiles of Leach's storm-petrels using different groups of chemicals (all compounds, plant compounds, contaminants, bird compounds and bird compounds elevated in occupied burrows)

Source	df	SS	MS	Pseudo-F	*p* (perm)
Using All Compounds (n = 155)					
Pair ID	21	5793	276	2.131	**0.0001**
Residuals	22	2848	129		
Using Plant Compounds (n = 41)					
Pair ID	21	41977	1999	7.917	**0.0001**
Residuals	22	5555	252		
Using Contaminants (n = 29)					
Pair ID	21	2824	135	2.190	**0.0002**
Residuals	22	1351	61		
Using Bird Compounds (n = 85)					
Pair ID	21	4801	229	1.441	0.0608
Residuals	22	3490	159		
Using Elevated Bird Compounds (n = 7)					
Pair ID	21	1217	58	8.775	**0.0001**
Residuals	22	145	7		

df: degrees of freedom, SS: sum of squares, MS: mean squares. Significant effects are in bold. Significance assessed at α=5% using 10,000 permutations of the data

3.7 SUPPLEMENTARY MATERIALS

Table S3.1. Tentatively identified compounds in soil samples

Retention Time	Compound Name	EIC	Reference Ions	CAS Number	Calculated Retention Index	Published Retention Index	Feather Retention Time	Occurrence
4.33	Toluene	91	63, 65, **91**, 92, 93	108-88-3	775	763		100%
4.64	Methyl isovalerate	74	43, 57, 59, **74**, 85	556-24-1	785	765		88%
4.85	1-Octene	55	41, **43, 55**, 56 70	111-66-0	792	796		100%
5.16	Octane	85	**43**, 56, 57, 71, 85	111-65-9	800	800		100%
5.39	Hexanal	56	41, 43, 44, **56**, 57	66-25-1	809	802	5.39	100%
5.83	1,3-Octadiene	54	41, **54**, 67, 81, 110	1002-33-1	824	826		100%
6.22	2,4-Dimethyl-1-heptene	70	**43**, 55, 56, 57, 70	19549-87-2	836	842		100%
6.95	4-Methyloctane	85	**43**, 57, 70, 71, 85	2216-34-4	860	858		100%
7.07	m-Xylene	91	77, **91**, 92, 105, 106	108-38-3	864	865		100%
7.26	1-Hexanol	56	42, 43, 55, **56**, 69	111-27-3	870	869		80%
7.44	2-Norbornene	94	77, 79, 91, **94**, 122	529-16-8	876	887		99%
7.71	Styrene	104	51, 77, 78, 103, **104**	100-42-5	885	895	7.75	100%
7.87	2-Heptanone	58	41, **43**, 58, 59, 71	110-43-0	890	889		100%
8.10	Nonane	85	**43**, 56, 57, 71, 85	111-84-2	900	900		100%
8.23	Heptanal	70	41, 43, 44, 55, **70**	111-71-7	901	896	8.23	100%
8.24	1-Ethyl-3-methylcyclohexane	97	55, 69, 96, **97**, 126	3728-55-0	902	931		82%
8.55	Unidentified	133	**133**, 134, 135, 151, 152	1000222-86-6	912			100%
8.67	Tricyclene	93	91, 92, **93**, 121, 136	508-32-7	916	914		99%
9.08	alpha-Pinene	93	77, 79, 91, 92, **93**	80-56-8	929	931	9.10	100%
9.44	(-)-Frontalin	72	**43**, 71, 72, 100, 142	28401-39-0	941	949		89%
9.65	Camphene	121	79, 91, **93**, 107, 121	79-92-5	948	952	9.59	100%
9.71	2,4-Thujadiene	91	77, **91**, 92, 119, 134	36262-09-6	949	956	9.73	99%
9.85	6-Methylheptan-2-one	58	**43**, 57, 58, 70, 71	928-68-7	954	954		100%

9.96	Benzaldehyde	106	50, 51, 77, 105, **106**	100-52-7	958	961	10.02	95%
10.19	Mesitylene	105	77, **105**, 106, 119, 120	108-67-8	965	962		98%
10.25	Unidentified monoterpene	119	91, 117, **119,** 120. 134		967			100%
10.44	(-)-beta-Pinene	93	41, 69, 79, 91, **93**	18172-67-3	973	975	10.50	100%
10.72	1-Octen-3-ol	57	41, 43, 55, **57**, 72	3391-86-4	982	978		92%
10.77	2-Methylenebornane	107	79, 93, **107**, 121,150		984	964		100%
10.82	3-Octanone	99	**43**, 57, 71, 72, 99	106-68-3	986	985		100%
10.93	beta-Myrcene	93	**41**, 67, 69, 79, 93	123-35-3	989	993		99%
10.95	Pseudocumene	105	91, **105**, 106, 119, 120	95-63-6	990	994		100%
11.18	2-Carene	121	79, 91, **93**, **121**, 136	554-61-0	997	995		65%
11.20	3-Octanol	59	41, 55, **59**, 83, 101	589-98-0	998	994		100%
11.37	Octanal	84	**43**, 44, 56, 57, **84**	124-13-0	1003	1006	11.45	100%
11.47	3-Carene	93	77, 79, 91, 92, **93**	13466-78-9	1007	1007	11.50	100%
11.56	2,5,6-Trimethyloctane	57	41, 43, 56, **57**, 70	62016-14-2	1010			91%
11.64	Unidentified	57	43, **57**, 71, 85, 98		1012			95%
11.76	4-Carene	121	79, 91, **93**, 121, 136	29050-33-7	1016	1018		100%
11.77	2-Methyl-2-Bornene	107	79, 91, 93, 94, **107**	72540-93-3	1016	1021	11.84	100%
11.84	m-Cymene	119	115, 117, **119**, 120, 134	535-77-3	1019	1023		99%
11.87	4,5-Dimethylnonane	57	43, **57**, 70, 71, 85	17302-23-7	1020	1035		91%
11.98	o-Cymene	119	91, 117, **119**, 120, 134	527-84-4	1023	1021	12.04	100%
12.16	Limonene	93	67, **68**, 79, 93, 136	138-86-3	1029	1030	12.19	100%
12.23	Eucalyptol	108	**43**, 81, 84, 108, 111	470-82-6	1031	1035		85%
12.29	6-Ethyl-2-methyoctane	71	41, **43**, 57, 71, 85	62016-19-7	1033			90%
12.41	trans-beta-Ocimene	93	77, 79, 91, 92, **93**	3779-61-1	1037	1042		90%
12.44	Unidentified branched alkane	57	43, 57, 71, 85, 112		1038			99%
12.81	p-Propyltoluene	105	77, 91, **105,** 106, 134	1074-55-1	1050	1048		99%
12.88	Unidentified branched alkane	57	43, 56, **57**, 71, 85		1052			100%
13.05	Gamma-Terpinene	93	77, 91, **93**, 121, 136	99-85-4	1058	1062	13.12	100%
13.20	Unidentified branched alkane	71	43, **57,** 70, 71, 85		1063			100%

13.28	Unidentified branched alkane	57	43, 56, **57**, 71, 113		1065			100%
13.33	cis-3-Butyl-4-vinyl-cyclopentene	79	77, **79**, 80, 91, 93,	93779-52-3	1067			97%
13.38	Unidentified branched alkane	57	43, 56, **57**, 71, 85		1069			100%
13.52	Unidentified	121	93, 107, **121**, 135, 150		1073			73%
13.57	Unidentified branched alkane	57	43, 56, **57**, 70, 71		1075			100%
13.67	Unidentified	85	43, 71, 84, **85**, 127		1078			100%
13.76	m-Cymenene	132	91, 92, 115, 117, **132**	1124-20-5	1081	1082		95%
13.80	2-Norbornanone	69	41, 66, 67, **69**, 138	13211-15-9	1082	1083		98%
13.84	5-Propylnonane	71	43, **57**, 71, 112, 126	998-35-6	1084			100%
13.88	alpha-Terpinolene	136	79, 91, **93**, 121, 136	586-62-9	1085	1083		100%
13.98	Fenchone	81	41, 69, 80, **81**, 152	1195-79-5	1088	1096		100%
14.06	3,4-Dimethylstyrene	132	91, 115, 117, 131, **132**	27831-13-6	1091	1100		100%
14.10	5-Ethyldecane	57	43, **57**, 70, 71, 85	17302-36-2	1092	1123		100%
14.12	2-Nonanone	58	43, 57, **58**, 59, 71	821-55-6	1093	1091	14.19	100%
14.17	Unidentified branched alkane	57	43, **57**, 58, 70, 71		1094			99%
14.23	Unidentified branched alkane	57	43, 56, **57**, 98, 99		1096			93%
14.27	p-Mentha-1,5,8-triene	134	91, 92, 105, 119, **134**		1097	1108		73%
14.40	cis-2-p-Menthen-1-ol	93	**43**, 69, 71, 93, 139	29803-82-5	1102	1110		91%
14.61	Nonanal	57	41, 56, **57**, 70, 98	124-19-6	1108	1102	14.62	95%
14.95	Fenchol	81	43, 69, 80, **81**, 84	1632-73-1	1120	1110		97%
14.99	Chrysanthenone	107	79, 80, 91, **107**, 150	473-06-3	1121	1123		81%
15.03	5-Butylnonane	71	43, 57, **71**, 85, 126		1122	1204		100%
15.14	3,4,5,6-tetramethyloctane	85	43, 57, 71, 84, **85**,	62185-21-1	1126	1116		100%
15.18	alpha-Campholenal	108	67, 93, 95, **108**, 109	91819-58-8	1127	1125		97%
15.21	Unidentified branched alkane	57	43, **57**, 71, 85, 112		1128			100%
15.26	(4E,6Z)-allo-Ocimene	121	79, 91, 105, **121**, 136	7216-56-0	1130	1131		94%
15.28	4-Acetyl-1-methylcyclohexene	138	43, 67, 95, 123, **138**	6090-09-1	1130	1131		90%
15.38	Unidentified branched alkane	57	43, **57**, 70, 71, 85		1133			100%
15.46	Unidentified branched alkane	57	43, **57**, 70, 71, 85		1136			100%

15.49	(1R)-(+)-Nopinone	83	55, 81, **83**, 95, 109	38651-65-9	1137	1142		100%
15.58	Unidentified branched alkane	57	43, **57**, 70, 71, 98		1140			100%
15.64	(-)-trans-Pinocarveol	92	55, 70, 83, 91, **92**	547-61-5	1142	1141	15.63	80%
15.74	(+)-2-Bornanone	95	81, 83, **95**, 108, 152	464-49-3	1145	1141		100%
15.90	2,3-Dimethylnonane	71	43, **57**, 70, 71, 112	2884-06-2	1150	1054		98%
15.98	Camphenilanol	96	43, 69, **71**, 86, 96	465-31-6	1153	1148		91%
16.17	trans-Pinocamphone	83	41, **55**, 69, **83**, 95	547-60-4	1159	1159		98%
16.20	Pinocarvone	81	53, 81, **108**, 135, 150	30460-92-5	1160	1164	16.24	100%
16.38	Unidentified branched alkane	71	43, 57, 70, **71**, 85		1166			100%
16.45	5-Pentylcyclohex-1,3-diene	79	77, **79**, 80, 91, 93	56318-84-4	1168	1161		98%
16.52	Borneol	95	**95**, 110, 121, 136, 139	507-70-0	1170	1168	16.55	100%
16.55	Unidentified branched alkane	71	43, 57, 70, **71**, 85		1171			100%
16.64	cis-Pinocamphone	83	41, 55, 69, **83**, 95	15358-88-0	1174	1175		99%
16.79	Terpinen-4-ol	111	43, **71**, 86, 93, 111	562-74-3	1179	1178		99%
16.98	p-Cymen-8-ol	135	43, 91, 117, 132, **135**	1197-01-9	1185	1186		100%
16.99	2-Methylisoborneol	95	43, **95**, 107, 108, 110	2371-42-8	1186	1180		100%
17.04	Methoxymesitylene	135	79, 91, **135**, 136, 150	4028-66-4	1187	1170		24%
17.14	2-Decanone	58	43, 57, **58**, 59, 71	693-54-9	1191	1193	17.21	100%
17.17	Myrtenal	107	79, 105, 106, 107, 108	564-94-3	1192	1197		99%
17.20	alpha-Terpineol	93	**59**, 81, 93, 121, 136	98-55-5	1193	1190	17.27	47%
17.28	trans-Dihydrocarvone	95	**67**, 68, 82, 95, 109	5948-04-9	1195	1199		76%
17.43	Dodecane	57	41, 43, **57**, 71, 85	112-40-3	1200	1200	17.49	100%
17.45	(+)-Dihydrocarvone	95	67, 68, 82, **95**, 152	7764-50-3	1201	1200		89%
17.54	Levoverbenone	107	80, 91, **107**, 135, 150	1196-01-6	1202	1204		100%
17.56	Decanal	82	43, 55, **57**, 70, 82	112-31-2	1203	1207	17.63	100%
17.86	Fenchyl acetate	81	43, 80, **81**, 93, 136	13851-11-1	1214	1223		94%
17.91	4-Methyleneisophorone	108	79, 91, 107, **108**, 150	20548-00-0	1216	1224		82%
18.05	Isothymol methyl ether	149	91, 119, **149**, 150, 164	31574-44-4	1221	1215		91%
18.17	Unidentified	109	81, 93, 108, **109**, 123		1226			100%

18.24	Thymol methyl ether	149	91, 119, 134, **149**, 164	1076-56-8	1228	1233		100%
18.29	Unidentified phthalate	149	93, 108, 121, **149**, 164		1230			100%
18.45	Isothymol methyl ether	149	91, 119, **149**, 150, 164	31574-44-4	1236	1244		97%
18.58	D-Carvone	82	54, **82**, 93, 107, 108	2244-16-8	1241	1234		99%
18.59	Unidentified phthalate	149	81, 93, 107, **149**, 164		1242			100%
18.65	(-)-Car-3-en-2-one	150	67, 107, 108, 135, **150**	53585-45-8	1244	1254		42%
18.67	Unidentified	93	67, 69, **93**, 111, 121		1245			78%
18.85	2-Isopropyl-5-methylcyclohex-3-en-1-one	82	82, 95, 109, **110**, 137	1000155-47-0	1251	1251		98%
19.02	2-n-Octylfuran	81	53, **81**, 82, 95, 180		1258	1281		86%
19.07	1,4,p-menthadien-7-al	107	77, 79. 91, **107**, 121	22580-90-1	1260	1267		61%
19.20	2,5-Bornanedione	166	41, **69**, 83, 109, **166**	4230-32-4	1264	1264		88%
19.27	Citral	69	41, 53, **69**, 84, 94	5392-40-5	1267	1247		65%
19.58	Unidentified	57	55, **57**, 70, 71, 80		1279			93%
19.73	L-Bornyl Acetate	136	43, 93, **95**, 121, 136	5655-61-8	1284	1280	19.83	100%
19.95	3-tert-Butylphenol	135	95, 107, **135**, 136, 150	585-34-2	1293	1295	19.99	86%
19.99	2-Undecanone	58	41, 43, **58**, 59, 71	112-12-9	1294	1291	20.06	100%
20.01	Methyl myrtenate	105	91, 93, **105**, 136, 137		1295	1301		73%
20.23	Tridecane	71	41, 43, **57**, 71, 85	629-50-5	1300	1300	20.31	100%
20.41	Undecanal	82	41, 43, 55, 57, **82**	112-44-7	1310	1308	20.47	100%
20.44	Unidentified	69	43, 57, **69**, 85, 111		1311			100%
20.60	4-Methylpentyl 4-methylpentanoate	117	43, 56, 84, 99, **117**	35852-42-7	1317	1315		32%
20.67	Unidentified	69	57, **69**, 85, 70, 111		1320			100%
20.80	4,6-Dimethyldodecane	57	41, 43, **57**, 71, 85	61141-72-8	1325	1325		100%
21.03	Unidentified sesquiterpene	189	105, 133, **161**, 189, 204		1333			81%
21.12	delta-EIemene	121	91, 93, **121**, 136, 161	20307-84-0	1337	1338		66%
21.27	Unidentified branched alkane	71	43, 57, **71**, 85, 141		1342			85%
21.49	alpha-Longipinene	119	93, 105, 107, **119**, 133	5989-08-2	1350	1342	21.58	100%

21.69	Unidentified sesquiterpene	119	91, 105, **119**, 133, 204		1358			93%
21.74	gamma-Nonalactone	85	43, 55, **85**, 86, 99	104-61-0	1360	1362		99%
21.89	Unidentified	159	105, 131, 145, **159**, 202		1365			30%
21.92	2-Undecanal	70	41, 43, 55, 57, **70**	2463-77-6	1367	1376		90%
22.00	(+)-Cyclosativene	161	105, 119, 120, **161**, 204	22469-52-9	1370			100%
22.12	Longicyclene	94	91, **94**, 95, 105, 119	1137-12-8	1374	1374		100%
22.17	alpha-Copaene	119	91, 93, 105, 119, **161**	3856-25-5	1376	1375		86%
22.25	Unidentified sesquiterpene	121	105, 119, **121**, 123, 132		1379			84%
22.37	(-)-beta-Bourbonene	81	79, 80, **81**, 123, 161	5208-59-3	1384	1384		86%
22.45	Cedr-9-ene	119	105, **119**, 130, 161, 204	21996-77-0	1387	1388		56%
22.46	alpha-Isocomene	147	119, **147**, 161, 162, 204	65372-78-3	1387	1388		86%
22.63	(+)-Satviene	108	93, 105, **108**, 133, 161	3650-28-0	1393	1396	22.66	100%
22.69	1-Tetradecene	83	**43**, 55, 57, 70, 83	1120-36-1	1396	1389		99%
22.83	(-)-Cycloseychellene	123	93, 121, **123**, 133, 161	52617-34-2	1399	1417	22.87	100%
22.88	Tetradecane	57	41, 43, **57**, 71, 85	629-59-4	1400	1400	22.97	100%
22.94	4H-1,4a-Methanonaphthalene	69	**69**, 93, 105, 111, 119	79562-96-2	1405	1405		91%
23.08	Longifolene	161	91, 93, 94, 107, **161**	475-20-7	1410	1402	23.10	100%
23.09	Geosmin	112	43, 55, 111, **112**, 125	19700-21-1	1411	1417		47%
23.17	Isosativene	94	93, **94,** 105, 161, 204	24959-83-9	1414	1417		92%
23.25	beta-Cedrene	161	41, 69, 93, **161**, 204	546-28-1	1417	1418		97%
23.33	Caryophyllene	133	69, 79, 91, **93**, 133	87-44-5	1420	1420		98%
23.41	Unidentified sesquiterpene	161	119, 121, **161**, 162, 189		1423			93%
23.47	Unidentified sesquiterpene	119	105, **119**, 121, 123, 204		1425			77%
23.56	beta-Gurjenene	161	91, 93, 105, 107, **161**	17334-55-3	1428	1432		99%
23.67	cis-Thujopsene	119	93, 105, **119**, 121, 123	470-40-6	1432	1429	23.76	100%
23.76	Piceol	121	65, 93, **121**, 122, 136	99-93-4	1436	1442	23.83	100%
24.03	beta-Barbatene	108	93. 94, 95, **96,** 108	72346-55-5	1446	1451	24.10	97%
24.21	Humulene	93	41, 80, **93**, 121, 147	6753-98-6	1453	1454		99%
24.38	Unidentified sesquiterpene	161	91, 105, 119, **161**, 204		1459			93%

24.39	Unidentified sesquiterpene	207	123, 125, **207**, 208, 222	117591-80-7	1459			100%
24.50	Spiro[4,5]dec-7-ene	119	79, 93, 105, **119**, 121	729602-94-2	1463	1475	24.54	100%
24.65	Unidentified sesquiterpene	189	91, 105, 133, **189**, 204		1469			99%
24.74	1-Dodecanol	97	43, **55**, 56, 69, 70	112-53-8	1472	1473		100%
24.89	beta-Chamigrene	189	41, 93, 105, 107, **189**	18431-82-8	1478	1478		100%
25.03	beta-Cadinene	105	91, 105, **161**, 189, 204	523-47-7	1483	1481		100%
25.10	Bicyclosesquiphellandrene	161	91, 105, 119, **161**, 204	54324-03-7	1486	1488	25.16	67%
25.18	Unidentified sesquiterpene	121	93, **121**, 122, 136, 204		1489			100%
25.20	4,11- Selinadiene	189	93, 107, 133, **189**, 204	1000193-57-0	1490	1485	25.34	98%
25.28	alpha-Muurolene	161	93, 94, **105**, 161, 204	31983-22-9	1493	1497	25.36	77%
25.39	Pentadecane	57	41, 43, **57**, 71, 85	629-62-9	1500	1500	25.47	100%
25.45	alpha-Chamigrene	136	41, 93, 121, 133, **136**	19912-83-5	1501	1500	25.55	100%
25.50	(+)-Cuparene	132	119, 131, **132**, 145, 202	16982-00-6	1502	1504		100%
25.52	beta-Bisabolene	93	41, 67, **69, 93**, 204	495-61-4	1503	1509	25.60	100%
25.56	Unidentified sesquiterpene	132	119, 131, **132**, 145, 202		1505			100%
25.62	gamma-Cadinene	161	91, 105. 119, **161**, 204	39029-41-9	1507	1512	25.66	92%
25.77	delta-Cadinene	161	105, 119, 134, **161**, 204	483-76-1	1514	1530	25.84	97%
25.81	cis-Calamenene	159	129, 131, **159**, 160, 202	483-77-2	1516	1522	25.90	100%
25.83	Unidentified	109	67, 93, 96, 108, **109**		1517			75%
25.85	Epizonarene	161	81, 105, **161**, 189, 204	41702-63-0	1517	1501		45%
25.91	gamma-Dehydro-ar-himachalene	185	157, 170, 171, **185**, 200	51766-65-5	1520	1537		92%
25.96	(Z)-gamma-Bisabolene	119	91, 93, 107, **119**, 121	13062-00-5	1522	1519		82%
26.04	(-)-Calamenene	159	129, 131, **159**, 160, 202	438-77-2	1526	1522		99%
26.14	beta-Himachalene	119	93, 105, **119**, 134, 204	1461-03-6	1530	1516		78%
26.28	alpha-Calacorene	157	141, 142, 156, **157**, 200	21391-99-1	1536	1542	26.32	100%
26.30	trans-alpha-Bisabolene	93	80, **93**, 109, 119, 121	25532-79-0	1537	1540	26.38	95%
26.33	(1-Propylheptyl)benzene	91	**91**, 92, 105, 133, 175	4537-12-6	1539	1534	26.42	98%
26.42	delta-Cuprenene	111	55, 69, 91, 94, **111**	98093-94-8	1543	1549	26.54	95%

26.64	Unidentified branched alkane	71	43, 57, 69, **71**, 85		1552			86%
26.72	Unidentified	56	43, 56, 57, 69, 83		1556			20%
26.76	beta-Calacorene	157	91, 119, 142, **157**, 200	50277-34-4	1558	1564		88%
26.78	(1-Ethyloctyl)benzene	91	**91**, 105, 119, 189, 218	4621-36-7	1559	1553	26.87	100%
26.82	(+)-Longicamphenylone	206	107, 109, 123, 145, **206**	38647-26-6	1560	1559		30%
26.91	2-Methylpentadecane	71	41, **43**, 57, 71, 85	1560-93-6	1564			97%
27.01	Unidentified sesquiterpene	161	69, 91, **134**, 135, 161		1569			44%
27.07	3-Methylpentadecane	85	41, 43, **57**, 71, 85	2882-96-4	1571	1570		94%
27.17	Unidentified	82	43, **57,** 56, 82, 96		1576			67%
27.24	Caryophyllene oxide	93	41, **43**, 79, 91, 93	1139-30-6	1579	1578		86%
27.36	Diethyl phthalate	149	105, **149**, 150, 176, 177	84-66-2	1584	1585		100%
27.41	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	71	43, **71**, 111, 159, 243	6846-50-0	1586	1587	27.48	100%
27.58	1-Tridecanol	97	41, 43, **55**, 69, 83		1594	1585		94%
27.67	(1-Methylnonyl)benzene	105	91, 104, **105**, 106, 218	4537-13-7	1598	1588	27.75	100%
27.77	Hexadecane	57	41, 43, **57**, 71, 85	544-76-3	1600	1600	27.85	100%
27.88	Humulene-1,2-epoxide	138	43, 67, 96, **109**, 138	19888-34-7	1607	1606		82%
28.02	Tetradecanal	82	41, 43, 55, **57**, 82	124-25-4	1613	1615	28.10	97%
28.05	alpha-Corocalene	185	143, 157, **185**, 186, 200	20129-39-9	1615	1623		60%
28.27	Benzophenone	182	51, 77, **105**, 181, 182	119-61-9	1625	1625		99%
28.32	(1-Pentylhexyl)benzene	91	41, **91**, 92, 105, 161	4537-14-8	1627	1620	28.41	100%
28.41	(1-Butylheptyl)benzene	91	**91**, 92, 105, 147, 175	4537-15-9	1631	1626	28.49	100%
28.61	Tau-Cadinol	161	43, 95, 105, **161**, 204	5937-11-1	1640	1639		30%
28.64	(1-Propyloctyl)benzene	91	**91**, 92, 105, 133, 189	4536-86-1	1641	1636	28.72	100%
28.77	Himachalol	119	93, 107, 109, **119**, 121	1891-45-8	1647	1656		44%
29.13	(1-Ethylnonyl)benzene	91	41, **91**, 92, 105, 119	4536-87-2	1663	1656	29.21	100%
29.30	Cadalene	183	165, 168, **183**, 184, 198	483-78-3	1670	1671		97%
29.32	6,9-Heptadecadiene	82	54, **67**, 68, 82, 96,	81265-03-4	1671	1667		65%
29.49	1-Tetradecanol	97	41, **43**, 55, 69, 83	112-72-1	1679	1676		100%

29.83	Dodecyl acrylate	73	**55**, 69, 73, 83, 97	2156-97-0	1694	1675		100%
29.98	(1-Methyldecyl)benzene	105	79, 91, **105**, 106, 232	4536-88-3	1698	1692	30.06	100%
30.02	Heptadecane	57	41, 43, **57**, 71, 85	629-78-7	1700	1700	30.10	100%
30.11	Pristane	57	41, 43, **57**, 71, 85	1921-70-6	1706	1703	30.19	100%
30.30	Pentadecanal	82	41, 43, 55, 57, **82**	2756-11-9	1714	1707	30.39	100%
30.50	Oxacyclopentadecan-2-one	55	41, **55**, 69, 83, 96	3537-83-5	1723	1727		97%
30.51	(1-Pentylheptyl)benzene	91	**91**, 92, 105, 161, 175	2719-62-2	1724	1719	30.59	100%
30.61	(1-Butyloctyl)benzene	91	**91**, 105, 147, 189, 246	2719-63-3	1728	1723	30.69	100%
30.88	(1-Propylnonyl)benzene	91	**91**, 105, 133, 203, 246	2719-64-4	1740	1735	30.96	100%
31.36	(1-Ethyldecyl)benzene	91	**91**, 105, 119, 217, 246	2400-00-2	1761	1735	31.44	100%
32.12	2-Ethylhexyl Salicylate	120	57, 70, **120**, 121, 138	118-60-5	1795	1807	32.19	100%
32.16	Octadecane	57	41, 43, **57**, 71, 85	593-45-3	1800	1800	32.24	100%
32.19	(1-Methylundecyl)benzene	105	77, 79 91, **105**, 106	2719-61-1	1801	1791	32.28	100%
32.47	Hexadecanal	82	43, 55, 57, **82**, 83	629-80-1	1811	1818	32.55	100%
32.97	6,10,14-Trimethyl-2-Pentadecanone	58	**43**, 57, 58, 59, 71	502-69-2	1838	1844		100%
33.26	Unidentified phthalate	149	57, **149**, 150, 223		1853			100%
33.65	Unidentified	134	91, 92, 133, **134**, 258		1874			99%
33.75	Homosalate	138	69, 109, 120, 121, **138**	118-56-9	1879	1903	33.87	100%
33.79	1-Hexadecanol	97	41, 43, **55**, 69, 83	36653-82-4	1881	1883		82%
34.07	Rimuene	257	41, 55, 80, **257**, 272	1686-67-5	1896	1894		93%
34.18	2-Heptadecanone	58	41, 43, **58**, 59, 71	2922-51-2	1902	1900	34.26	100%
34.30	(1-Methydodecyl)benzene	105	43, 91, 104, **105**, 106	4534-53-6	1908	1894	34.39	100%
34.60	Oxacycloheptadecan-2-one	97	41, 43, **55**, 69, 83	109-29-5	1924	1932		84%
34.67	Methyl hexadecanoate	74	43, 55, **74**, 87, 143	112-93-0	1928	1927		100%
34.75	Unidentified diterpene	257	91, 94, 106, 257, **272**	5939-62-8	1932			68%
35.15	Dibutyl phthalate	149	76, **149**, 150, 205, 223	84-74-2	1953	1959		100%
35.33	Sandaracopimaradiene	137	81, 91, 136, **137**, 257	1686-56-2	1963	1961		99%
35.96	Unidentified diterpene	257	105, **109**, 119, 257, 272		1996			92%

36.23	Unidentified diterpene	257	91, 149, **257**, 258, 272		2011			84%
36.28	Unidentified	241	159, 185, **241**, 242, 256		2013			99%
36.39	(-)-Phyllocladene	229	69, 91, 229, 257, **272**	20070-61-5	2019	2017		41%
36.95	8,11,13-Abietatriene	255	159, 173, **255**, 256, 270	19407-28-4	2049	2054		84%
37.45	7,13-Abietadiene	272	105, 133, 136, 229, **272**	35241-40-8	2076	2080		55%
39.28	Sandaracopimaral	135	93, 105, 107, 119, **135**	3855-14-9	2224	2213	39.34	52%
41.08	Bis(2-ethylhexyl) adipate	129	57, 70, 71, 112, **129**	103-23-1	2382	2398		98%

EIC (extracted ion chromatogram) is the ion that was used to quantify the peak area of the compound.
All published retention indices were obtained from NIST (webbook.nist.gov), The Pherobase (pherobase.com) or Flavornet (flavornet.org).
All published retention indices correspond to GC columns with similar properties to the DB-5MS used in this study, including DB-1, DB-5, HP-5, DB-5MS, and HP-5MS.

Table S3.2. Tentatively identified compounds in Leach's storm-petrel feather samples

Retention Time	Compound Name	EIC	Reference Ions	CAS Number	Calculated Retention Index	Published Retention Index	In Soil?	Occurrence	Compound Source
5.39	Hexanal	56	41, 43, 44, **56**, 57	66-25-1	808	802	Yes	100%	Bird
6.20	Furfural	95	39, 67, **95**, 96, 97	98-01-1	830	835	No	100%	Bird
7.75	Styrene	104	51, 77, 78, 103, **104**	100-42-5	886	895	Yes	100%	Bird
8.23	Heptanal	70	41, 43, 44, 55, **70**	111-71-7	899	896	Yes	100%	Bird
9.10	alpha-Pinene	93	77, 79, 91, 92, **93**	80-56-8	928	931	Yes	96%	Plant
9.59	Camphene	121	79, 91, **93**, 107, 121	79-92-5	946	952	Yes	39%	Plant
9.73	2,4-Thujadiene	91	77, **91**, 92, 119, 134	36262-09-6	950	956	Yes	32%	Plant
10.02	Benzaldehyde	106	50, 51, 77, 105, **106**	100-52-7	960	961	Yes	100%	Bird
10.50	(-)-beta-Pinene	93	41, 69, 79, 91, **93**	18172-67-3	974	975	Yes	84%	Plant
10.83	6-Methyl-5-hepten-2-one	108	41, **43**, 55, 69, 108	110-93-0	986	987	No	100%	Bird
11.02	2-Octanone	58	43, **58**, 59, 71, 128	111-13-7	991	992	No	100%	Bird
11.45	Octanal	84	**43**, 44, 56, 57, **84**	124-13-0	1005	1006	Yes	100%	Bird
11.50	3-Carene	93	77, 79, 91, 92, **93**	13466-78-9	1008	1007	Yes	41%	Plant
11.84	2-Methyl-2-bornene	107	79, 91, 93, 94, **107**	72540-93-3	1018	1021	Yes	100%	Plant
12.04	o-Cymene	119	91, 117, **119**, 120, 134	527-84-4	1024	1021	Yes	93%	Plant
12.19	Limonene	93	67, **68**, 79, 93, 136	138-86-3	1030	1030	Yes	96%	Plant
12.26	2-Ethyl-1-hexanol	57	41, 43, **57**, 70, 83	104-76-7	1032	1030	No	100%	Bird
12.35	6-Ethyl-2-methyloctane	71	43, **57**, 70, 71, 85	62016-19-7	1034		No	80%	Bird
12.64	Benzeneacetaldehyde	91	65, **91**, 92, 120, 121	122-78-1	1045	1043	No	95%	Bird
13.12	Gamma-Terpinene	93	77, 91, **93**, 121, 136	99-85-4	1060	1062	Yes	39%	Plant
13.26	1-Chlorooctane	91	43, 55, 69, **91**, 93	111-85-3	1064	1064	No	100%	Contaminant
13.33	Acetophenone	105	51, 77, **105**, 106, 120	98-86-2	1066	1062	No	100%	Bird
14.19	2-Nonanone	58	43, 57, **58**, 59, 71	821-55-6	1095	1091	Yes	100%	Bird
14.62	Nonanal	57	41, 56, **57**, 70, 98	124-19-6	1109	1102	Yes	100%	Bird

15.63	E-Pinocarveol	92	55, 70, 83, 91, **92**	547-61-5	1140	1141	Yes	14%	Plant
16.24	Pinocarvone	81	53, 81, **108**, 135, 150	30460-92-5	1162	1164	Yes	45%	Plant
16.30	1-(1-Tert-butoxypropan-2-yloxy)propan-2-ol	59	41, 45, 57, **59**, 103	132739-31-2	1163		No	98%	Contaminant
16.55	Borneol	95	**95**, 110, 121, 136, 139	507-70-0	1172	1168	Yes	98%	Plant
17.21	2-Decanone	58	43, 57, **58**, 59, 71	693-54-9	1193	1193	Yes	100%	Bird
17.27	alpha-Terpineol	93	**59**, 81, 93, 121, 136	98-55-5	1196	1190	Yes	16%	Plant
17.49	Dodecane	57	41, 43, **57**, 71, 85	112-40-3	1203	1200	Yes	100%	Bird
17.63	Decanal	82	43, 55, **57**, 70, 82	112-31-2	1207	1207	Yes	100%	Bird
18.81	1,3-Ditert-butylbenzene	175	57, 65, **175**, 176, 190	1014-60-4	1245		No	48%	Contaminant
19.83	L-Bornyl acetate	136	43, 93, **95**, 121, 136	5655-61-8	1279	1280	Yes	29%	Plant
19.99	3-Tert-butylphenol	135	95, 107, **135**, 136, 150	585-34-2	1284	1295	Yes	100%	Contaminant
20.06	2-Undecanone	58	41, 43, **58**, 59, 71	112-12-9	1286	1291	Yes	100%	Bird
20.31	Tridecane	71	41, 43, **57**, 71, 85	629-50-5	1294	1299	Yes	100%	Bird
20.47	Undecanal	82	41, 43, 55, 57, **82**	112-44-7	1301	1308	Yes	100%	Bird
21.18	Unidentified	88	70, **88**, 89, 115, 155		1330		No	71%	Bird
21.50	Unidentified	97	55, 57, 71, 85, **97**		1343		No	66%	Bird
21.58	alpha-Longipinene	119	93, 105, 107, **119**, 133	5989-08-2	1347	1342	Yes	34%	Bird
22.14	Texanol	89	43, 56, **71**, 89, 173	77-68-9	1369	1380	No	96%	Contaminant
22.66	(+)-Satviene	108	93, 105, **108**, 133, 161	3650-28-0	1392	1396	Yes	23%	Plant
22.73	Unidentified	58	43, 55, **58**, 71, 97		1394		No	95%	Bird
22.87	(-)-Cycloseychellene	123	93, 121, **123**, 133, 161	52617-34-2	1399	1417	Yes	66%	Plant
22.92	Unidentified	115	70, 87, **88**, 89, 115		1401		No	50%	Bird
22.97	Tetradecane	57	41, 43, **57**, 71, 85	629-59-4	1402	1399	Yes	100%	Bird
23.10	Longifolene	161	91, 93, 94, 107, **161**	475-20-7	1410	1402	Yes	95%	Plant
23.16	Dodecanal	82	**41**, 43, 55, 57, 82	112-54-9	1411	1420	No	100%	Bird
23.28	Unidentified sesquiterpene	119	93, 105, **119**, 161, 204		1417		No	11%	Plant
23.39	beta-Cedrene	161	41, 69, 93, **161**, 204	546-28-1	1419	1424	No	11%	Plant
23.75	Ethyl decanoate	88	41, 43, 73, **88**, 101	110-38-3	1435	1397	No	50%	Bird

23.76	cis-Thujopsene	119	93, 105, **119**, 121, 123	470-40-6	1437	1429	Yes	27%	Plant
23.83	Piceol	121	65, 93, **121**, 122, 136	99-93-4	1439	1442	Yes	48%	Plant
24.10	beta-Barbatene	108	93. 94, 95, **96,** 108	72346-55-5	1448	1451	Yes	38%	Plant
24.11	Geranylacetone	69	41, **43**, 69, 136, 151	3796-70-1	1449	1452	No	100%	Bird
24.25	Unidentified	112	57, 69, 70, 71, **112**		1455		No	48%	Bird
24.54	Spiro[4,5]dec-7-ene	119	79, 93, 105, **119**, 121	729602-94-2	1468	1475	Yes	18%	Plant
24.71	1-Chloroundecane	91	43, 57, 69, 71, **91**	2473-03-2	1474		No	100%	Contaminant
25.07	Unidentified	88	43, 69, 70, 88, **115**		1488		No	48%	Bird
25.16	Bicyclosesquiphellandrene	161	91, 105, 119, **161**, 204	54324-03-7	1492	1488	Yes	9%	Plant
25.34	4,11- Selinadiene	189	93, 107, 133, **189**, 204	1000193-57-0	1498	1485	Yes	14%	Plant
25.36	alpha-Muurolene	161	93, 94, **105**, 161, 204	31983-22-9	1501	1497	Yes	20%	Plant
25.47	Pentadecane	57	41, 43, **57**, 71, 85	629-62-9	1505	1500	Yes	100%	Bird
25.52	2,4-Ditert-butylphenyl 5-hydroxypentanoate	191	57, 163, **191**, 192, 206	166273-38-7	1508		No	77%	Contaminant
25.55	alpha-Chamigrene	136	41, 93, 121, 133, **136**	19912-83-5	1509	1500	Yes	86%	Plant
25.60	beta-Bisabolene	93	41, 67, **69, 93**, 204	495-61-4	1511	1509	Yes	57%	Plant
25.66	gamma-Cadinene	161	91, 105. 119, **161**, 204	39029-41-9	1513	1512	Yes	39%	Plant
25.70	Tridecanal	82	41, 43, 55, **57**, 82	10486-19-8	1514	1510	No	100%	Bird
25.84	delta-Cadinene	161	105, 119, 134, **161**, 204	483-76-1	1520	1530	Yes	34%	Plant
25.90	cis-Calamenene	159	129, 131, **159**, 160, 202	483-77-2	1522	1522	Yes	88%	Plant
26.18	Unidentified	88	70, **88**, 89, 115, 183		1533		No	46%	Bird
26.19	(1-Butylhexyl)benzene	91	**91**, 105, 147, 161, 218	4537-11-5	1534	1535	No	100%	Contaminant
26.32	alpha-Calacorene	157	141, 142, 156, **157**, 200	21391-99-1	1540	1542	Yes	34%	Plant
26.38	trans-alpha-Bisabolene	93	80, **93**, 109, 119, 121	25532-79-0	1542	1540	Yes	27%	Plant
26.42	(1-Propylheptyl)benzene	91	**91**, 92, 105, 133, 175	4537-12-6	1544	1534	Yes	100%	Contaminant
26.54	delta-Cuprenene	111	55, 69, 91, 94, **111**	98093-94-8	1548	1549	Yes	16%	Plant
26.87	(1-Ethyloctyl)benzene	91	**91**, 105, 119, 189, 218	4621-36-7	1562	1553	Yes	100%	Contaminant
27.27	Unidentified	115	69, 87, 88, 89, **115**		1578		No	39%	Bird

27.48	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	71	43, **71**, 111, 159, 243	6846-50-0	1587	1587	Yes	100%	Contaminant
27.67	Ethyl dodecanoate	88	41, 43, 73, **88**, 101	106-33-2	1594	1581	No	95%	Bird
27.75	(1-Methylnonyl)benzene	105	91, 104, **105**, 106, 218	4537-13-7	1598	1588	Yes	100%	Contaminant
27.85	Hexadecane	57	41, 43, **57**, 71, 85	544-76-3	1601	1600	Yes	100%	Bird
28.10	Tetradecanal	82	41, 43, 55, **57**, 82	124-25-4	1612	1615	Yes	100%	Bird
28.41	(1-Pentylhexyl)benzene	91	41, **91**, 92, 105, 161	4537-14-8	1625	1620	Yes	100%	Contaminant
28.49	(1-Butylheptyl)benzene	91	**91**, 92, 105, 147, 175	4537-15-9	1629	1626	Yes	100%	Contaminant
28.72	(1-Propyloctyl)benzene	91	**91**, 92, 105, 133, 189	4536-86-1	1638	1636	Yes	100%	Contaminant
28.98	Unidentified Sesquiterpene	161	81, 105, **161**, 162, 204		1649		No	5%	Plant
29.21	(1-Ethylnonyl)benzene	91	41, **91**, 92, 105, 119	4536-87-2	1658	1656	Yes	100%	Contaminant
29.33	Unidentified sesquiterpene	108	81, **95**, 107, **108**, 109		1664		No	13%	Plant
29.39	Unidentified	195	57, 165, 180, **195**, 210		1666		No	71%	Contaminant
29.75	alpha-Bisabolol	119	41, 43, 69, **109**, 119	515-69-5	1680	1680	No	5%	Plant
30.00	2-Pentadecanone	58	43, 57, **58**, 59, 71	2345-28-0	1690	1698	No	100%	Bird
30.06	(1-Methyldecyl)benzene	105	79, 91, **105**, 106, 232	4536-88-3	1693	1692	Yes	100%	Contaminant
30.10	Heptadecane	57	41, 43, **57**, 71, 85	629-78-7	1693	1700	Yes	100%	Bird
30.19	Pristane	57	41, 43, **57**, 71, 85	1921-70-6	1701	1703	Yes	100%	Bird
30.27	Unidentified sesquiterpene	175	147, 160, **175**, 176, 218		1706		No	27%	Plant
30.39	Pentadecanal	82	41, 43, 55, 57, **82**	2756-11-9	1710	1707	Yes	100%	Bird
30.59	(1-Pentylheptyl)benzene	91	**91**, 92, 105, 161, 175	2719-62-2	1721	1719	Yes	100%	Contaminant
30.69	(1-Butyloctyl)benzene	91	**91**, 105, 147, 189, 246	2719-63-3	1726	1723	Yes	100%	Contaminant
30.96	(1-Propylnonyl)benzene	91	**91**, 105, 133, 203, 246	2719-64-4	1739	1735	Yes	100%	Contaminant
31.44	(1-Ethyldecyl)benzene	91	**91**, 105, 119, 217, 246	2400-00-2	1763	1735	Yes	100%	Contaminant
31.72	Dicumene	119	91, 103, **119**, 120, 236	1889-67-4	1778		No	66%	Plant
32.07	Ethyl tetradecanoate	88	41, 43, **88**, 89 101	124-06-1	1793	1790	No	68%	Bird
32.19	2-Ethylhexyl Salicylate	120	57, 70, **120**, 121, 138	118-60-5	1801	1807	Yes	100%	Contaminant
32.24	Octadecane	57	41, 43, **57**, 71, 85	593-45-3	1802	1800	Yes	100%	Bird
32.28	(1-Methylundecyl)benzene	105	77, 79 91, **105**, 106	2719-61-1	1804	1791	Yes	100%	Contaminant

32.55	Hexadecanal	82	43, 55, 57, **82**, 83	629-80-1	1817	1818	Yes	100%	Bird
32.69	(1-Pentyloctyl)benzene	91	**91**, 105, 119, 161, 189	4534-49-0	1824	1814	No	100%	Contaminant
32.82	(1-Butylnonyl)benzene	91	**91**, 92, 105, 119, 147	4534-50-3	1831	1821	No	100%	Contaminant
33.74	Unidentified	134	91, 92, 133, **134**, 258		1876		No	36%	Contaminant
33.79	Homosalate	138	69, 109, 120, 121, **138**	118-56-9	1882	1903	Yes	88%	Contaminant
34.26	2-Heptadecanone	58	41, 43, **58**, 59, 71	2922-51-2	1901	1900	Yes	100%	Bird
34.28	Nonadecane	57	41, 43, **57**, 71, 85	629-92-5	1902	1900	No	100%	Bird
34.39	(1-Methydodecyl)benzene	105	43, 91, 104, **105**, 106	4534-53-6	1908	1894	Yes	100%	Contaminant
34.50	Unidentified long chain ester	141	57, 71, 111, **141**, 159		1913		No	27%	Bird
34.62	Heptadecanal	82	43, 57, 68, **82**, 96	629-90-3	1919	1920	No	100%	Bird
35.14	Unidentified long chain ester	141	43, 57, 71, 140, **141**		1943		No	57%	Bird
35.19	Unidentified long chain ester	70	57, **70**, 71, 97, 111		1947		No	70%	Bird
35.54	Unidentified long chain ester	159	57, 71, 84, **97**, 159		1963		No	70%	Bird
36.02	Unidentified long chain ester	140	57, **70**, 71, 111, 140		1986		No	73%	Bird
36.08	Ethyl hexadecanoate	88	41, 43, 55, **88**, 101	628-97-7	1989	1993	No	86%	Bird
36.33	Unidentified long chain ester	71	57, 71, **111**, 155, 173		1998		No	98%	Bird
36.39	Unidentified long chain ester	125	57, **70**, 71, 125, 159		2004		No	96%	Bird
36.88	Unidentified long chain ester	155	**57**, 71, 85, 111, 155		2037		No	98%	Bird
36.94	Unidentified long chain ester	70	57, **70**, 71, 111, 173		2042		No	98%	Bird
37.05	Unidentified long chain ester	112	57, 84, 85, 112, **173**		2050		No	98%	Bird
37.22	Unidentified long chain ester	97	57, 71, 84, **97**, 173		2062		No	100%	Bird
37.39	Unidentified long chain ester	111	57, **70**, 71, 85, 111		2074		No	98%	Bird
37.66	Unidentified long chain ester	155	57, 71, 84, **111**, 155		2094		No	100%	Bird
37.86	Unidentified long chain ester	140	57, 85, 111, **140**, 187		2109		No	98%	Bird
37.91	Unidentified long chain ester	125	57, **70**, 71, 125, 173		2113		No	100%	Bird
38.08	Unidentified long chain ester	111	57, 84, 85, **111**, 187		2123		No	96%	Bird
38.16	Unidentified long chain ester	97	57, 71, 85, **97**, 187		2131		No	100%	Bird
38.22	Unidentified long chain ester	173	57, 71, 97, 168, **173**		2136		No	82%	Bird
38.31	Unidentified long chain ester	155	57, 71, 85, 154, **155**		2143		No	100%	Bird

38.35	Unidentified long chain ester	125	57, **70**, 71, 125, 173		2145		No	100%	Bird
38.43	Unidentified long chain ester	173	57, 71, 111, 126, **173**		2152		No	98%	Bird
38.52	Unidentified long chain ester	173	43, 57, 71, **97, 173**		2158		No	96%	Bird
38.53	Unidentified long chain ester	111	57, 71, 84, 85, **111**		2159		No	100%	Bird
38.71	Unidentified long chain ester	154	**57**, 70, 85, 154, 187		2172		No	96%	Bird
38.77	Unidentified long chain ester	125	57, 70, **125**, 154, 187		2177		No	100%	Bird
38.87	Unidentified long chain ester	111	57, 71, **111**, 155, 173		2184		No	100%	Bird
38.94	Unidentified long chain ester	111	57, **71**, 84, 111, 187		2189		No	98%	Bird
38.98	Unidentified long chain ester	84	**70**, 71, 84, 125, 173		2192		No	100%	Bird
39.16	Unidentified long chain ester	125	57, **70**, 71, 125, 187		2206		No	100%	Bird
39.24	Unidentified long chain ester	126	57, **71**, 84, 85, 111		2212		No	98%	Bird
39.32	Unidentified long chain ester	187	57, 70, 71, 97, **187**		2218		No	100%	Bird
39.34	Sandaracopimaral	135	93, 105, 107, 119, **135**	3855-14-9	2222	2213	Yes	21%	Plant
39.42	Unidentified long chain ester	125	57, 70, 71, **125**, 154		2226		No	98%	Bird
39.54	Unidentified long chain ester	139	57, **70**, 71, 139, 173		2236		No	100%	Bird
39.73	Unidentified long chain ester	125	**57**, 70, 71, 84, **125**		2249		No	100%	Bird
39.86	Unidentified long chain ester	168	57, **70**, 71, 168, 187		2259		No	95%	Bird
40.02	Unidentified long chain ester	173	57, 70, 71, 125, **173**		2270		No	100%	Bird
40.08	Unidentified long chain ester	125	70, 71, 84, **125**, 187		2276		No	100%	Bird

EIC (extracted ion chromatogram) is the ion that was used to quantify the peak area of the compound.
All published retention indices were obtained from NIST (webbook.nist.gov), The Pherobase (pherobase.com) or Flavornet (flavornet.org).
All published retention indices correspond to GC columns with similar properties to the DB-5MS used in this study, including DB-1, DB-5, HP-5, DB-5MS, and HP-5MS.

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