Predation risk and the evolution of odours in island birds

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Abstract

It is only recently that studies have explored the use of olfaction in birds. Birds are now known to use odour cues for navigation, and locating food. Odours produced by the birds themselves can also function in nest recognition and even mate choice. The odours of most birds stem from the preen wax produced by the uropygial or preen gland. The wax is comprised of a complex mixture of esters and volatiles, and is known to vary in some species with age, sex, season, or environmental conditions. Its function has been associated with feather maintenance, but it may also play a role in sexual selection and chemical communication. In this thesis, I used the preen gland and its preen wax to perform comparative studies on the evolution of odours between island birds and their continental relatives. I used the birds of the Oceania region as a model system, where most passerines originated from continental Australia but have colonised numerous surrounding islands such as New Zealand and New Caledonia. As islands generally lack mammalian predators, and have less parasites and less interspecific competition than continents, these differences in environmental conditions likely shaped functional differences in the preen gland and its products. I measured the size of the preen gland and collected preen wax from a variety of forest passerines in Australia, New Zealand and New Caledonia. I found that island birds have larger preen glands and therefore likely produce more preen wax than their continental relatives. I also found that the preen wax composition differed among species, with a shift to birds on islands producing disproportionately lighter and more volatile compounds. I suggest that selection favoured the gain of more volatile molecules in island birds as they were released from the constraint to camouflage their odours that is imposed by mammalian predators on continental areas. It is possible that this also allowed greater communication through olfactory channels in island birds, and such communication is enhanced through the use of more volatile compounds. To support this hypothesis I showed that the South Island robin (*Petroica australis*) was able to detect and react to the odour of a conspecific (odours produced by preen wax) in the absence of any visual cues. From a conservation perspective, increased volatility of the preen waxes of island birds might place them at increased risk from introduced mammalian predators that use olfaction to locate their prey. However, in both laboratory tests using Norway rats (*Rattus norvegicus*), a common exotic predator, and in field trials using rodent tracking tunnels, I found only limited evidence to suggest the odour of island birds places them at greater risk,

and more experiments are needed to test this hypothesis. Finally, my findings of more conspicuous odours in island birds suggest new avenues of research for their conservation, including whether island species that seem especially prone to predation have preen waxes (and thus odours) that are also especially attractive to exotic mammalian predators. Conservation programmes to protect endangered island birds may even benefit from considering whether olfactory cues can be minimised as a method of reducing predation risk.

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Chapter 1

General Introduction

A sense of smell and bird are two notions that most people would not associate. Birds show such extravagance in colours and songs to communicate that it is easy to overlook another of their senses: olfaction. With such complex and elaborate singing abilities, and a visual acuity able to reach into the ultraviolet, one might be tempted to think that birds would not have a need for a third channel of communication. The lack of obvious "sniffing" behaviours also accounted for the general view of birds being anosmic (i.e., impaired sense of smell). The absence of obvious specialised olfactory organs (at least visible externally) and descriptions by anatomists of the small olfactory bulbs further fuelled the belief that most birds had a poorly developed sense of smell (Bang & Cobb, 1968).

There are of course special cases where it was known that birds use their sense of smell, but they were viewed as an evolutionary rarity. The kiwi (*Apteryx* spp.) are obvious examples as they have nostrils at the tip of their long beaks, nocturnal habits (limiting vision) and they produce audible and obvious sniffing noises when searching for food. Other examples comprise seabirds (e.g. Procellariiformes), which possess very large olfactory bulbs, and nostrils that are located in obvious tubes on top of the bill (Bang, 1966). They were long thought to use olfactory cues to find food, spending most of their lives flying over the oceans. As a result, seabirds were the focus of much research on olfactory abilities. We now know, for example, that Antarctic prions (*Pachiptila desolata*) use the dimethyl sulphide emitted by phytoplankton as an odour cue to forage and navigate (Nevitt & Bonadonna, 2005). Olfaction is not limited to locating food, as some Procellariiforms use olfaction for homing, and individual and nest recognition. The blue petrel (*Halobaena caerulea*), a colonial and nocturnal seabird, uses odour cues to find its burrow at night. When offered the options of entering a burrow with no odour cues, or burrows with either its own scent, its mate scent, or the scent of a conspecific, blue petrels significantly choose their own burrow (Bonadonna, Villafane, Bajzak, & Jouventin, 2004; Mardon & Bonadonna, 2009).

Unlike seabirds and kiwi, smaller birds such as passerines were not thought to show any of the behavioural or physical particularities associated with olfaction. Indeed, up until ~20 years ago, passerines and other small birds were considered devoid of a functional olfactory system. However, recent studies are changing this view and have shown evidence of an increased role of odours in the lives of passerines. For example, blue tits (*Parus caeruleus*) and European starlings (*Sturnus vulgaris*) maintain an aromatic environment for their nestlings by selecting plants as nest materials that are known and used by humans for their antiseptic, fungicidal and insect repellent qualities (Gwinner & Berger, 2008; Petit, Hossaert-McKey, Perret, Blondel, & Lambrechts, 2002). A second example is found in the great tit (*Parus major*), which uses odour cues to locate its prey. Great tits are able to differentiate between a tree infested with predatory herbivorous insects (lepidopteran larvae) and an uninfested tree using only the volatiles emitted by the plants (Amo, Jansen, van Dam, Dicke, & Visser, 2013). Such an ability suggests great tits may be using olfaction to at least locate the best sites to search for prey, and perhaps even locate prey that may not be directly visible.

Origin of odours in birds

There are two elements to the study of the role of odours in the lives of birds. The first I describe as "active olfaction" which is the action of "sniffing" and involves the detection of volatiles in air passing over olfactory tissues (the volatiles in the air are then interpreted and identified by the nervous system). The second element to odour I refer to as "passive olfaction," and this is the odour produced by the body of the bird, which may or may not be detected by the individual producing the odour but which may nevertheless be detected by other individuals (including predators). This thesis focuses primarily on passive olfaction, the way the bird's body smells and what it implies for its ecology, but I also test the ability of one species to detect the presence of a conspecific using just odour cues alone (i.e., "sniffing").

Odours in birds can have many different sources such as faeces, stomach oils or blood (Jones & Black, 1979; Jones & Roper, 1997; Jouventin, 1977). Birds also possess specialised scent-producing organs such as the cloacal gland, salt gland, salivary gland or uropygial gland (for review, see: Hagelin & Jones, 2007). It is the uropygial or preen gland that is thought to be the main source of body odour. The uropygial gland resembles a mammalian sebaceous gland and is present in most birds, although it is reduced or absent in

some species (e.g., herons, pigeons; Johnston, 1988). They instead use a process of feather degradation that produces a fine talcum-like powder thought to function in a similar manner to preen wax (Wetmore, 1920). Some birds also use their dietary sources to produce toxic (pitohuis) or a stench-like smell (hoatzins); both probably evolved as a chemical defence mechanism to dissuade potential predators (Dumbacher et al., 2004; Weldon & Rappole, 1997).

The preen gland is unique to birds and is the only sebaceous gland they possess. It produces and secretes preen wax, an oily substance that is gathered onto the bird's bill and is then preened regularly onto the plumage. As a consequence of the volatiles in the preen wax and the fact that it is spread over the entire plumage (surface area) of the bird, this gland is seen as the key source of avian odours. The preen gland is located just above the tail (figure 1.1). Its shape and size vary among species but in all species with a preen gland, it is comprised of two secretory lobes flowing into a single papilla where the preen wax secretion accumulates (figure 1.2; Jacob & Zisweiler 1982; Salibian & Montalti 2009). The papilla has a nipple-like shape and is often surrounded by a tuft of small feathers, which help the bird collect the oil on its bill. It is thought the preen wax is extruded from the preen gland by the bird gently pressing the sides of the nipple, a process which I mimicked to express and collect preen wax for my studies.

Preen wax composition

The preen wax is a complex and variable mixture of lipids, esters, fatty acids and alcohols (Jacob & Zisweiler, 1982). It is commonly separated into two parts: the non-volatile fraction made of mainly branched long-chain esters and the volatile fraction including compounds such as alkanes and their simple derivatives (Campagna, Mardon, Celerier, & Bonadonna, 2012). Wax composition is known to vary across species. Gas chromatography-mass spectrometry studies reveal that some compound classes (e.g., some esters) are highly conserved between species while a large diversity of molecules exist within the volatile fraction (Jacob & Zisweiler, 1982; Soini, Whittaker, Wiesler, Ketterson, & Novotny, 2013; Zhang, Du, & Zhang, 2013). Differences in the composition of preen wax have been found between populations (Whittaker et al., 2010) and sexes (Amo et al., 2012; Mardon, Saunders, Anderson, Couchoux, & Bonadonna, 2010; Soini et al., 2013). Some authors even refer to some specific compounds in the dark-eyed junco (*Junco hyemalis*) and the budgerigar (*Melopsittacus undulatus*) as male and "female-like" compounds or

"pheromones" (Whittaker, Gerlach, Soini, Novotny, & Ketterson, 2013; Zhang, Wei, Zhang, & Yang, 2010). Wax composition is also highly repeatable within an individual suggesting it is under genetic control (Whittaker et al., 2010).

The composition of preen wax can also be influenced by age or sexual maturity of the individual (Sandilands, Savory, & Powell, 2004; Shaw, Rutter, Austin, Garvin, & Whelan, 2011), environmental factors (Haribal, Dhondt, & Rodriguez, 2009) or migration (Shaw et al., 2011). For example, in the gray catbird (*Dumetella carolinensis*), which is a migrant bird, the volatile and semivolatile compounds of the preen wax varied both qualitatively and quantitatively between adults and juveniles but also between their summer breeding ground and their wintering ground (Shaw et al., 2011). In some species, changes in diet can also alter the composition of preen wax although this factor is not universal (Thomas et al. 2010; L. Azzani, *pers.comm*.). More recently the composition of the preen wax of great tits has been found to be influenced by the parasite microbiome present on the feathers, with the relative abundance of some compounds changing in response to experimentally modified bacterial loads on feathers (Jacob et al., 2014).

In the dark-eyed junco and some European shorebirds (Family Scolopacidae), the constitution of preen wax has been shown to change seasonally. During the breeding season, birds modify the composition of their preen wax, switching production from the low molecular weight monoester waxes at the start of breeding to high molecular weight diester waxes while incubating. The loss of low molecular weight esters reduces the volatiles produced by the bird, and appears to function as a form of "olfactory camouflage" from predators that use olfactory cues to locate their prey (e.g. mammals). This switch occurs at a time when birds are most vulnerable from predators, and only occurs in the sex that incubates in some uniparental species (Reneerkens, Piersma, & Damsté, 2005, 2006; Soini et al., 2007).

Preen wax functions

The importance of preen wax to birds is obvious when considering the large amount of time birds spend preening every day (Cotgreave & Clayton, 1994). Despite extensive research, the function of preen wax is still highly debated. It has been linked to plumage maintenance (Jacob & Zisweiler, 1982) and water-proofing. For example, mallard ducks (*Anas platyrhynchos*) whose long-term (3 months) access to their preen gland was experimentally

blocked showed a significant decrease in their plumage condition and an increase in water retention (Giraudeau et al., 2010).

Preen waxes may also play a role in chemical defence against ectoparasites. The crested auklet (*Aethia cristatella*) is a colonial seabird known for its tangerine scent and its allo-anointing behaviour during courtship. This social ritual involves one individual preening a second individual (usually its mate) and is suggested to help the transfer of waxes and chemicals that have ectoparasite repellent properties (Douglas, Co, Jones, & Conner, 2001; Douglas, 2008). Preen wax has also been shown to limit or inhibit the growth of detrimental feather-degrading bacteria in the red knot, *Calidris canutus*, and the house finch, *Carpodacus mexicanus* (Reneerkens, Versteegh, & Schneider, 2008; Shawkey, Pillai, & Hill, 2003).

Another important function of preen secretions is in chemosignalling (Caro, Balthazart, & Bonadonna, 2014; Hagelin & Jones, 2007). The ability of birds to use olfactory cues for recognition has now been found in a variety of species. Blue petrels, European starlings, waxwings (*Bombycilla* spp.) and budgerigars have all been shown to use preen waxes to recognise conspecifics, and to identify sex (mates) and specific individuals (Amo et al., 2012; Bonadonna et al., 2004; Mardon et al., 2010; Whittaker et al., 2010; Zhang et al., 2010; Zhang et al., 2013). A role in sexual selection is also suggested by the discovery of male and "female-like" compounds (or "pheromones") in the dark-eyed junco and budgerigar waxes (Whittaker et al., 2013; Zhang et al., 2010). In a study by Hirao, et al. (2009), roosters (*Gallus gallus domesticus*) were found to prefer females with unimpaired uropygial glands compared to females that had their uropygial glands experimentally removed, showing the importance of the gland (and the odour of the preen wax) in their sexual behaviour. Cosmetic coloration has also been proposed to play an important role in sexual selection in some species in which the preen waxes are thought to help enhance colours and plumage ornamentation, hence reinforcing the sexual signal (Delhey, Peters, & Kempenaers, 2007; Lopez-Rull, Pagan, & Macias Garcia, 2010). Finally, it is possible that preen waxes may play a role in territoriality, in a fashion analogous with the way scent marking behaviours are used to defend a territory in many species of mammals. Although this behaviour has never been formally identified in birds, some observations of the kiwi (*Apteryx spp.*), a species defending large territories, suggest it could exist (Taborsky & Taborsky, 1992).

Although the odour of a bird is the result of a complex mix of substances (e.g. skin oils, parasite excretions) and environmental compounds (e.g. dirt on the feathers), preen waxes are the most abundant substance being spread over the entire surface of a bird on a daily basis. These waxes have a number of volatile elements and sometimes are even detectable by our relatively insensitive human nose (e.g., the tangerine scent of crested auklets). Thus, understanding the function of preen waxes could provide information on the selective forces that have shaped the odours of birds. The different evolutionary histories of birds in the South Pacific region, with the avifauna in different locations exposed to differing levels of environmental pressure (e.g. predation, interspecific competition, etc.) provides the ideal system to study co-evolutionary changes in odour. Such a comprehensive comparative analysis is not possible for scientists restricted to study birds in areas like Europe or America. Oceania provides a unique opportunity to study birds originating from continental Australia where they co-evolved with a range of predators (mammals, snakes and birds of prey) and environmental pressures. However, once these birds colonised the surrounding Pacific islands, they found themselves in habitats with few or no predators, less interspecific competition, fewer parasites and very different environmental conditions. Such evolutionary differences during the speciation process are likely to have shaped both morphological and functional variations between insular and continental species of birds.

A suggestion that the insular environment could be having profound effects on the evolution of olfaction was highlighted in a preliminary study of six native species of New Zealand passerines which showed that birds did not switch to a less volatile preen wax during breeding season, with one species, the South Island robin, even doing the opposite and increasing its volatility (Fluen, 2008). This was dramatically different to that of continental species, but was consistent with the hypothesis that island birds, having evolved in environments relatively free of predators that use olfaction to locate their prey, had altered their preen wax composition accordingly. Perhaps freed from the constraints imposed by mammalian predators in continental avifaunas, birds on islands were “free” to use odours in ways not possible elsewhere. Understanding the role of odours in the lives of birds is not just of interest to evolutionary biologists, but has direct implications for understanding the conservation problems facing many island birds. If island birds have evolved differences in their odours, might this be one of the reasons they appear so vulnerable to introduced mammalian predators? With so many island birds critically endangered through the introduction of exotic mammalian predators such as rats, cats and mustelids (Blackburn,

Cassey, Duncan, Evans, & Gaston, 2004), perhaps an examination of bird odours, and the role they play in predation risk, is warranted. This thesis aims to improve our understanding of the evolution of bird odours and the functional role it plays in their lives, as well as the practical application of this understanding towards the conservation and management of endangered birds.

Thesis outline

The main chapters of this thesis (chapters 2 to 4) have been written as independent manuscripts in anticipation of submitting them for publication. As a consequence, some repetition and cross-referencing was inevitable.

Chapter 2 introduces this thesis by looking at the morphological variations of the uropygial gland, the main source of odours in birds, between passerines from continental Australia and their relatives, which colonised New Zealand and New Caledonia. I hypothesize that evolutionary differences were likely to be expressed morphologically and that comparing variation in uropygial gland size would be a good start to understanding some of the factors shaping odours in birds. The second part of this chapter further examines the differences in bird odour by comparing the composition of preen waxes between island birds and their relatives from the Australian continent. I compare the preen waxes of 30 different species using a gas chromatograph (GC). Note that this part of the chapter is a collaborative project with Laura Azzani. Both of us contributed to collecting preen wax samples, running the samples through the GC, and in analysing the data.

Chapter 3 follows by examining the potential problem of island birds being more "smelly" and thus at greater risk from predation by introduced mammals which are more likely to rely on olfaction to find their prey, than do the native avian predators with which island birds evolved. With simple choice experiments in a laboratory setting (Y-maze) and in the wild (tunnel tracks), I use the Norway rat as a model to determine if mammalian predators are more attracted to the odour (preen wax) of island birds or continental birds.

The fourth chapter is dedicated to the understanding why some island birds would develop a "smellier" body. To do so, I focussed on a single New Zealand species, the South Island robin (*Petroica australis*), which produces an unusually volatile preen wax during the breeding season. The robin is an ideal candidate, being a tame, territorial, long-lived, monogamous bird living in dense and dark forests where visual cues are obscured and

where it would be advantageous to develop a different way of communicating, such as through olfaction.

This thesis ends by a general discussion (chapter 5) in which the results are summarised to give a broader picture of the evolution of avian odours. I also suggest future avenues of research, and whether what I discovered in my work can help in the conservation of endangered island birds.

This project was approved in New Zealand by the University of Canterbury Animal Ethics Committee (permit no. 2012/13R) and by the Department of Conservation (permit no. NM-34075-FAU), in New Caledonia by the "Direction de l'Environnement" (permit no. 20871/DENV/SCB) and in Australia by the Charles Sturt University Animal Care and Ethics Committee (permit no. 13/051) and by the NSW Government (permit no. APP-0000427487).

Figure 1.1. Uropygial gland of, on the left, a red-throated parrotfinch (*Erythrura psittacea*) and on the right, a song thrush (*Turdus philomelos*). Note the papilla and the bilobate shape of the gland.

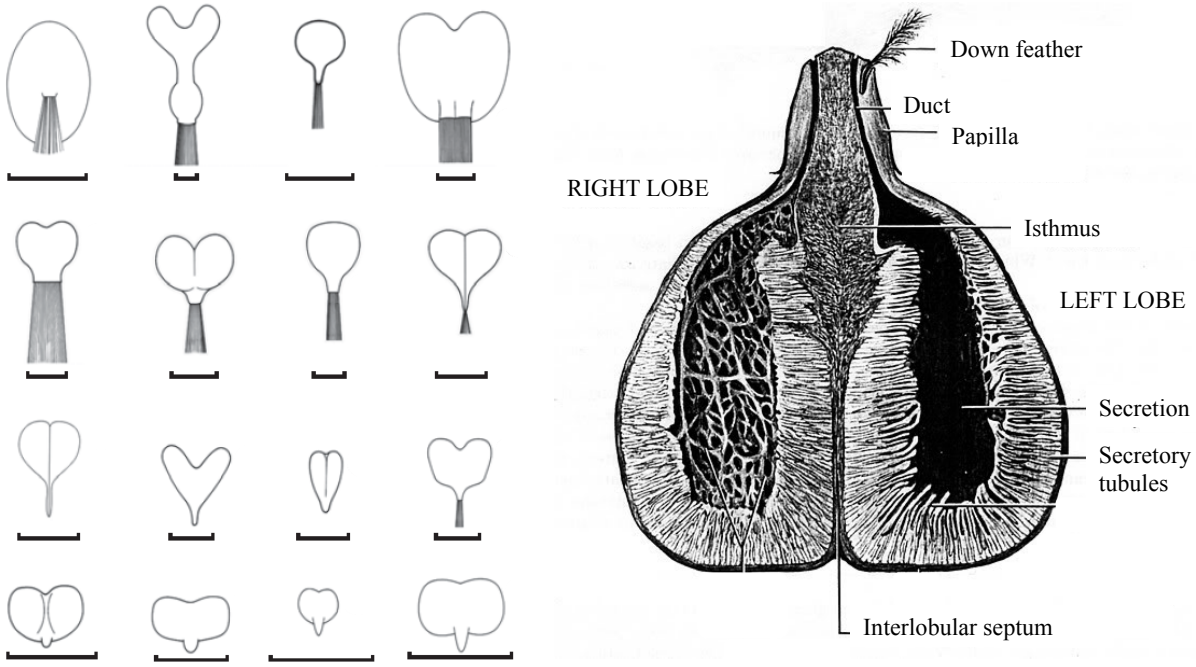

Figure 1.2. Morphological diversity of uropygial glands as shown in Salibian and Montalti (2009, linear scale equals 1 cm) and a modified cross section of a uropygial gland as in Jacob and Zisweiler (1982).

Chapter 2

A comparative analysis of uropygial gland size and preen wax composition between island and continental species of birds

Abstract: *The uropygial or preen gland is an organ unique to birds. It produces a waxy secretion that birds preen onto their feathers several times per day. Preen wax functions in feather maintenance, but may also play a role in sexual selection and chemical communication. It is comprised of a complex mixture of esters and volatiles, and is known to vary in some species with age, sex, season, or environmental conditions. Variation in gland size can be an indicator of the quantity of wax produced. In this study I compared differences in the relative size of the uropygial gland and the composition of preen waxes between island birds and their closest continental relatives. I used the birds of the Oceania region as a model system, where most passerines originate from continental Australia but have colonised numerous surrounding islands such as New Zealand and New Caledonia. I measured uropygial gland size in about 100 birds at each location, representing a total of 34 species sampled. I found that sex had no effect on variation in size of the gland but that there were significant differences due to body mass, species and the geographic region. Island birds had significantly larger uropygial glands than expected by their body mass. This difference remained significant when controlling for phylogenetic effects. I suggest that increased rates of preen wax production in island birds may be due to their increased use of olfactory modes of communication. I also collected preen waxes from a total of 162 birds, representing 30 species, and compared their profiles through a gas chromatograph. I found that island birds showed a significant loss of complexity in the non-volatile components but a significant increase in the diversity of compounds in the volatile part of their wax profile. This suggests birds on islands produce preen waxes, and thus odours, that are likely to be more conspicuous than those of continental species. I suggest that selection favoured the gain of more volatile molecules in island birds as they were released from the constraint to camouflage their odours that is imposed by mammalian predators on continental areas. It is possible that this also allowed greater communication through olfactory channels in island birds, and such communication is enhanced through the use of more volatile compounds.*

Introduction

The differences between insular and continental species are a recurrent topic of interest for evolutionary biologists. Islands differ strikingly with continents in their competitive environments and as a result favour species that evolve in different ways from that of the founding ancestral species. Furthermore, islands provide generally clearer patterns of diversity, providing means of correctly inferring causes of past evolution from its results. Perhaps the most famous example of evolution and adaptation in island birds is that of Darwin's finches on the Galápagos islands. Darwin thought that the differences in the beak sizes of different species of finches were the result of different food supplies between islands (Darwin, 1859). Later on, Mayr (1940) developed the idea that the reduction in the conspicuousness (colours and songs) of island birds, compared with continental birds, was due to a reduction in interspecific competition. Island birds are often freed from similarly-looking or similarly-singing sympatric species and thus face relaxed selection on maintaining isolating mechanisms (e.g., courtship events); in turn, it is expected island species should loose complexity in the traits that prevent hybridisation (Grant, 1965; Hill, Ji, Parker, Amiot, & Wells, 2013). Another major difference in environmental pressures on islands is the reduced risk of predation. Some predator guilds are either reduced or absent on islands by their reduced capacity of dispersion over large oceanic barriers. Mammalian predators in particular are often completely absent from islands (at least before introductions related to human colonisations). This absence of a major predatory guild has lead to differences in avian life histories such as the loss of flight, increased body masses, low reproductive rates and naïve behaviours, illustrated by emblematic species such as the moa, the dodo or the kiwi (Duncan & Blackburn, 2004). Islands also differ from continental areas in other respects such as climate, habitat, or diversity of parasites, which are also important selective forces driving differences in avian life histories (Dobson & McCallum, 1997).

Birds are a convenient model to study evolution. They are conspicuous animals and because they can fly, they have dispersed and undergone speciation on many islands. This chapter examines the evolution of odours in birds by comparing morphological differences and odorous secretions from island birds and their continental relatives. These secretions originate from the uropygial or preen gland (UG), which is located on the rump and secretes an oily substance called preen wax. Birds collect this oil by squeezing the gland with their bill and then spread it onto their plumage. They spend a considerable amount of time

preening everyday, suggesting preen wax has important functions (Cotgreave & Clayton, 1994). Although the exact functions of preen wax have been debated, a number of functions have been suggested, including plumage maintenance (Giraudeau et al., 2010; Jacob & Zisweiler, 1982), reducing parasite and bacterial loads (Douglas, 2008; Shawkey et al., 2003), colour enhancement (Lopez-Rull et al., 2010), predator avoidance (Reneerkens et al., 2005), sexual selection (Zhang et al., 2010), and individual and mate recognition (Whittaker et al., 2010). At least some of these functions are likely to vary in importance for island species, suggesting UG size and preen wax of insular birds are likely to be under differing selective pressures than that faced by continental species.

The uropygial gland is a sebaceous gland found only in birds but its shape and size can vary greatly among species (Salibian & Montalti, 2009). In all species it is comprised of two secretory lobes flowing into a papilla where the preen wax secretion accumulates (Jacob & Zisweiler, 1982). The papilla has a nipple-like shape and is often surrounded by a tuft of small feathers, which helps the bird collect the oil on its bill, which it then spreads onto its plumage.

In the house sparrow (*Passer domesticus*), variation in UG size has been positively correlated with body condition and feather quality, and negatively correlated with the number of feather holes (hence reducing chewing lice load; Moreno-Rueda 2010; Moreno-rueda 2011). The great tit (*Parus major*) shows a similar positive correlation with feather mite load (which in turn reduces bacterial load) and plumage brightness (Galván & Sanz, 2006). Intraspecific and interspecific variations in UG size have been shown to be independent of sex, positively correlated with the amount of secretion produced and to vary annually, with the gland becoming bigger during the breeding season (Amo et al., 2012; Elder, 1954; Martín-Vivaldi et al., 2009; Moreno-rueda, 2011; Pap, Vágási, Osváth, Mureşan, & Barta, 2010; Vincze, Vágási, Kovács, Galván, & Pap, 2013). Other sources of interspecific variation in UG size include habitat type and migratory habit, with aquatic and non-migratory species having larger UGs than terrestrial and migrant species (Vincze et al., 2013). However, variation in UG size is complex and still poorly understood.

Preen wax is a complex and variable mixture of lipids, esters, fatty acids and alcohols (Jacob & Zisweiler, 1982). It is commonly separated into two fractions, the non-volatile part made of mainly branched long-chain esters and the volatile part including compounds such as alkanes and their simple derivatives (Campagna et al., 2012). Wax composition is known

to vary across species (Jacob & Zisweiler, 1982; Soini et al., 2013; Zhang et al., 2013), populations (Whittaker et al., 2010), and sexes (Amo et al., 2012; Mardon et al., 2010; Soini et al., 2013; Whittaker et al., 2013; Zhang et al., 2010) but is highly repeatable within an individual suggesting a genetic control (Whittaker et al., 2010). It can also be influenced by age or degree of sexual maturity (Sandilands et al., 2004; Shaw et al., 2011), environmental factors (Haribal et al., 2009), and degree of migratory behaviour (Shaw et al., 2011). Some researchers have found preen wax is affected by diet, although this factor is disputed (Thomas et al. 2010; L. Azzani *pers.comm.*). More recently an effect of the parasite community has been suggested on the wax make-up of great tits, with the relative abundance of some compounds changing in response to experimentally modified bacterial loads on feathers (Jacob et al., 2014). The composition of preen wax has also been shown in European wading birds (Family Scolopacidae) and the dark-eyed junco (*Junco hyemalis*) to change from low molecular weight monoester waxes at the start of breeding to high molecular weight diester waxes. Diesters being less volatile than monoesters, this switch is proposed to reduce predation by olfactory-searching predators at a time when birds are most vulnerable (Reneerkens et al., 2005, 2006; Soini et al., 2007).

In contrast to continental species, once birds colonised oceanic islands they found themselves in habitats with few (or no) predators and less interspecific competition. This difference in evolutionary history between continental and island avifaunas provides an ideal opportunity to study functional differences. Comparative studies have also provided evidence that island birds generally have a larger body size or differ in morphology and colouration compared to their mainland counterparts (Dudaniec, Schlotfeldt, Bertozzi, Donnellan, & Kleindorfer, 2011; Myers, Brown, & Kleindorfer, 2009). My project is based in Oceania, where most birds originated from Australia where they evolved with a range of predatory and environmental pressures quite different from those on islands. Once established on islands, birds are likely to have developed and expressed evolutionary differences visible in the UG size and in the composition of their preen waxes. To address this question, I compared UG size and preen waxes in species pairs between Australia, and two island avifaunas from New Zealand and New Caledonia.

Methods

All data was collected during the breeding season in three different locations: in Kaikoura, New Zealand (173°37'E, 42°23'S) from July to December 2012, in Parc des Grandes

Fougères, Farino, New Caledonia (165°45'E, 21°37'S) in November 2012 and near Albury, New South Wales, Australia (146°50'E, 36°03'S) in September 2013. All birds were captured by mist-nets, apart from the South Island robin, which was captured by a pull-activated drop-trap. All species were native or endemic to their respective area. Each bird was banded or marked (to avoid resampling the same individual twice), weighed, and morphological measurements were taken. To reduce variation, all measurements were taken by one person (AT).

Uropygial gland

A total of 331 birds of 34 species were caught: 130 birds of 5 species in New Zealand, 104 birds of 12 species in New Caledonia and 97 individuals of 17 species in Australia (table 2.1). Preen gland measurements were taken after collection of the preen wax and therefore represent the volume of an empty gland. This limited the effect of daily variation in the UG size due to the volume of stored wax and whether birds had preened prior to capture.

Gland measurements were taken following the methodology of Galván and Sanz (2006). This consisted of taking the maximum length, maximum width and maximum height of the UG to the nearest 0.01 mm with IP54 electronic digital calipers. Length corresponded to the maximum distance between the two lobes, width was measured on the left lobe and height was measured from the base of the gland to the tip of the papilla. The three dimensions were multiplied to give an index of the gland volume. This UG size index has proven useful for comparative purposes (Galván et al., 2008; Moreno-Rueda, 2010). Repeated measures of the UG also allowed me to confirm the measurements had a relatively high level of repeatability of 0.86 (Lessells & Boag, 1987).

Preen wax collection

Preen wax was obtained by gently pressing the uropygial gland and collecting a small drop of wax with a sterile metal loop. This loop was immediately transferred into a sterile 100 µl glass insert and the insert was then placed inside a sterile glass vial. To minimise contamination, all glassware and loops had previously been cleaned with a sequence of 3 organic solvents, baked dry, and stored in clean vials until used. Samples were stored in a cooler for a few hours while in the field and then later frozen at -20°C until analysed. All birds and equipment were handled while wearing disposable rubber gloves to avoid

contaminating samples with human odours. A total of 162 birds of 30 species were captured and had their preen waxes collected and analysed by gas chromatography: 33 birds of 5 species in New Zealand, 63 birds of 12 species in New Caledonia and 66 individuals of 13 species in Australia (table 2.1).

Gas chromatography analysis

Each preen wax sample was mixed, within its glass insert, with 100 µl of ethyl acetate. This mix was then vortexed for 60 seconds at 700 rpm to ensure proper dissolution between the solvent and the wax. A sample of 1 µl was then injected in the gas chromatographer (GC) with a split ratio of 6:1. The GC was a Shimadzu GC-2010, equipped with a Shimadzu AOC-20i+s auto-injector and a Varian CP-SIL 5 CB capillary column (25 m length x 320 µm internal diameter x 0.12 µm film thickness). The injection port temperature was set at 250°C and the carrier gas was Nitrogen with a total flow of 19.0 ml/min and a linear velocity of 36.7 cm/sec. The FID detector operated at 320°C, with a sampling interval of 40 msec. Oven temperature was programmed with an initial temperature of 70°C and a hold time of 4 mins, then an increase to 130°C at a rate of 20°C/min, and finally an increase to 320°C at a rate of 4°C/min and a hold time of 15 minutes.

Chromatograms (figure 2.3) were obtained with the software Shimadzu's GCSolution, version 2.3 (©Shimadzu 2002-2009). I focused first on the nonvolatile fraction of the profile (wax esters) as described in Reneerkens, Piersma, & Damsté (2005). From each GC profile, I recorded 4 data types: mean retention time (RT), RT range, minimum RT and number of peaks. As I could not control for the original amount of wax collected from each bird, I used the relative area of each peak and calculated a weighed mean RT (RT * relative peak area), hereafter just stated as mean RT. The RT range corresponded to the time between the first peak (minimum RT) and the last peak of the wax ester fraction. Finally, the number of peaks was calculated for the wax ester part.

At this stage I could not identify the chemical nature of each peak as my samples still have to be processed through a mass spectrometer, however I did not want to ignore the peaks present before the wax esters just because I could not identify them. I visually counted the number of peaks present between the solvent peak and the start of the wax ester fraction; this is referred to as the "volatile fraction" (figure 2.3). I could not analyse as many

variables as for the ester fraction because of the GC settings being appropriate for the wax esters but not so refined to pick up on the more volatile molecules.

Statistical analysis

All statistical analyses were run in STATISTICA 6.0, ©StatSoft Inc. The statistical significance level was $p = 0.05$.

Uropygial gland

UG volume and mass were log10 transformed to normalise distributions. They were analysed using ANCOVAs with the UG volume as the dependent variable and body mass as a covariate. Species, sex and geographic region (island or continental species) were used as factors. The homogeneity of slopes was verified. I first determined whether sex had an effect on the UG size variation by using 10 species of island birds and 9 species of continental birds in which both sexes were sampled (table 2.1). As no effect of sex was found, I pooled male and female data together for all subsequent analyses. I then used the species in table 2.1 to estimate differences in UG size between island and continental species (figure 2.1).

To determine if any differences in UG size between island and continental birds were confounded by phylogenetic effects, I used the pair-wise method (Møller & Birkhead, 1992). I selected 9 closely related species pairs of which 7 were paired congeners (table 2.1 and figure 2.2). I used recent phylogenetic trees to select the closest related species (Arnaiz-Villena et al., 2009; Christidis, Irestedt, Rowe, Boles, & Norman, 2011; Nyári, Benz, Jønsson, Fjeldså, & Moyle, 2009; Nyári & Joseph, 2012). I then used the residuals of UG log10 (to control for body mass) and compared the paired species with a paired t-test. I did not pair and use any of the honeyeaters species as their phylogenetic relationships are distant and unclear (Joseph et al., 2014), however when paired at random and included in the analysis, none of the results changed.

Preen wax

All data followed a normal distribution. A two-way ANOVA was performed with sex and geographic location (island or continent) as factors. Dependent variables were mean RT, RT range, minimum RT and number of peaks. A one-way ANOVA, with male and female data pooled together was then performed with the geographic location as factor and the same

dependent variables.

To determine if any differences in the number of peaks between island and continental birds were confounded by phylogenetic effects, I used the pair-wise method (Møller & Birkhead, 1992). I selected 8 closely related species pairs of which 6 were paired congeners (table 2.1 and figure 2.5). I used recent phylogenetic trees to select the closest related species (Arnaiz-Villena et al., 2009; Christidis et al., 2011; Nyári et al., 2009; Nyári & Joseph, 2012), and compared them with a paired t-test.

Finally, I performed a one-way ANOVA with the visually counted number of peaks from the volatile fraction. Data for the pair-wise comparison was not normally distributed, even after transformation, so a Wilcoxon matched pairs test was used.

Results

Uropygial gland

No effect of sex (ANCOVA: $F(1) = 0.23$, $p = 0.64$) was found on variation in UG size. The interaction between sex and whether the species was from an island or the continent was also non-significant (ANCOVA: $F(1) = 0.01$, $p = 0.90$).

I found a significant effect of body mass (ANCOVA: $F(1) = 27.5$, $p < 0.001$), species (ANCOVA: $F(7) = 3.99$, $p < 0.001$) and geographic region (ANCOVA: $F(1) = 5.25$, $p = 0.035$) on variation in UG size (figure 2.1). The interaction between species and geographic region was non-significant (ANCOVA: $F(7) = 0.1$, $p = 0.46$). This analysis indicates that UG varied significantly between species, that larger species of birds had larger UG sizes, and that UG size varied between island and continental species.

There was a significant difference between the residuals of UG $\log_{10}$ of island and continental species when compared between close relatives (figure 2.2, paired t-test: $t(8) = 2.72$, $p = 0.03$). Thus, island species of birds had a larger UG size when controlled for both body mass and phylogenetic effects.

Preen wax

A multivariate test (all 4 variables analysed at the same time), showed no effect of sex (two-way ANOVA: $F(4, 40) = 0.14$, $p = 0.97$) and no significant interaction between sex and the

geographic location (F (4, 40) = 0.59, p = 0.67). However, a significant effect of the geographic location was found (F (4, 40) = 9.51, $p < 0.001$). I therefore divided this test into 4 separate two-way ANOVAs and found a significant effect of geographic location (island or continent) for the number of peaks (F (1, 43) = 14.79, $p < 0.001$) and the RT range (F (1, 43) = 12.18, $p < 0.01$). The mean RT (F (1, 43) = 0.10, p = 0.75) was non-significant but minimum RT showed a non-significant trend towards a slightly lower RT in the continental species (F (1, 43) = 3.63, p = 0.063). Overall, island species had fewer peaks and a narrower range of RT in the ester fraction.

Since there was no effect of sex, I pooled data from male and female together for each variable and confirmed that island birds had a significantly lower number of peaks in their wax ester fraction than continental species (one-way ANOVA: F (1, 29) = 6.18, p = 0.02; figures 2.3 and 2.4). The minimum RT (F (1, 29) = 1.49, p = 0.23) and mean RT (F (1, 29) = 0.09, p = 0.77) were non-significantly different between insular and continental birds, while RT range showed a non-significant trend (F (1, 29) = 2.96, p = 0.09) (figure 2.4). When controlled for phylogenetic effects, island species of birds had a lower number of peaks in their wax ester fraction compared with continental species (figure 2.5, paired t-test: t (7) = -2.42, p = 0.046).

On the other hand, island birds showed a significantly higher number of peaks in their volatile part of the GC profile compared with continental birds (one-way ANOVA: F (1, 28) = 5.48, p = 0.03; figures 2.3 and 2.4). When controlled for phylogenetic effects, island birds showed a non-significant trend towards a higher number of peaks in the volatile part compared with continental species (Wilcoxon test: Z = 1.86, p = 0.06).

Discussion

Uropygial gland size varied significantly with the body mass of a bird, across species and between geographic regions. Although variation in UG size has previously been found to vary across species and with body size (Kennedy, 1971; Vincze et al., 2013), my findings suggest that some variation in UG size is also due to whether the bird was an island or a continental species. I observed that island birds tended to have a bigger UG than expected for their body size, even when controlled for phylogeny. This suggests that island birds may produce a larger volume of preen wax secretions than their continental congeners. Why should selection have favoured this difference?

My comparison of preen wax composition in a range of passerine birds between continental Australia and the two island avifaunas on New Zealand and New Caledonia revealed consistent directional differences. In general, island species of birds produced preen waxes with fewer components in the ester fraction but more components in the volatile fraction. This overall shift towards lighter molecular weight compounds is likely to result in the preen waxes of island birds being more conspicuous because of their greater odour. This result held when I controlled for potential phylogenetic effects as the pattern remained when I only compared closely related species in which one member of a pair evolved in continental Australia while the other evolved on an island. Why should island birds produce preen wax that differs from that of continental birds?

The decreased number of peaks in the ester fraction in island birds indicates a loss of complexity in the less volatile lipidic components of the wax. One potential explanation could be the impoverished parasite communities found on islands (Dobson & McCallum, 1997; Dobson, Pacala, Roughgarden, Carper, & Harris, 1992). Parasitism is indeed recognised as a major selective force impacting avian life histories. Islands generally support impoverished parasite communities because hosts and parasites are subject to the same constraints limiting diversity (e.g. isolation, small population). If preen wax functions to inhibit ectoparasites, fewer parasites in island birds (in both numbers of individuals as well as diversity of species), may mean that birds require fewer different types of "long lasting" lipids to maintain their feathers. It is not clear whether a greater molecular diversity of esters is needed to control a broader range of ectoparasites, but it seems plausible that simpler parasite faunas may not need as many chemical "weapons." I was unable to survey the ectoparasite faunas of each species, although I did not find any difference in the prevalence of feather mites between island and continental species (unpubl. data). Interestingly, a lower diversity of ecto-parasites in insular population might be expected to lead to less preen wax production (less protection required) and thus smaller UG size than in continental species with a more diverse parasite fauna, a pattern that is opposite to my findings. Parasitism appears to be a plausible selective force for the composition of preen waxes but not for the UG size or amount of preen wax produced.

In the great tit, a continental forest passerine, increased UG size has been correlated to plumage brightness (Galván & Sanz, 2006), suggesting a link with intensity of sexual selection. If a similar process occurs in other species, then one might expect a positive relationship between UG size and plumage colour across species. However, differences in

plumage brightness between insular and continental species does not seem to explain the larger UG size of island birds. Indeed, islands birds typically show a loss of colours, suggesting less intense sexual selection (Doucet, Shawkey, Rathburn, Mays, & Montgomerie, 2004; Grant, 1965, 2001). New Zealand passerines in particular show a widespread loss of red colours from the plumage; this shift from their Australian red congeners to yellowish and black island forms remains unexplained (Thomas, McGraw, James, & Madden, 2014). Birds can synthesise melanin but must obtain carotenoids (precursor to red, orange and yellow colours) from dietary sources (Hill, 1991; McGraw, 2006). It is possible that dietary sources of carotenoids are less available on islands or alternatively that the role of plumage signals in sexual selection has been reduced. Whatever the reasons for loss of plumage brightness in island birds, changes in colouration cannot explain increased UG size in island birds.

Although there appears to be a general reduction in carotenoid-based colouration in island birds, melanin-based colours are common, with some species evolving increased melanism or even completely melanistic morphs (Atkinson, 2004; Doucet et al., 2004; Griffith, Parker, & Olson, 2006; Miller & Lambert, 2006). A generally darker plumage is known to reduce feather abrasion and protect against the damage from UV light (Burtt, 1986). Whether melanistic feathers require greater amounts of preen wax for their maintenance is unknown. It is possible that UV levels may differ between continental and island birds, although as my study sites were at similar altitudes and latitudes (with Australia in an intermediate position between the two islands), I can probably rule out this factor. On the other hand, if continental birds colonising Oceania faced increased abrasion of their feathers (e.g., due to more closed habitats on islands than the open eucalypt forests of Australia), this may have favoured melanism and increased wax production simply through the need to replace wax loss by increased contact with vegetation surfaces. Arguing against this hypothesis however is my finding that the preen waxes of island birds tend to have more volatile components. One might expect that if insular birds were trying to avoid the loss of preen wax from increased abrasion then less volatile (more “sticky”) preen waxes should evolve. At present, the rates at which preen waxes are lost from bird feathers is unknown but it would be worth investigating as it is possible that species with larger UGs simply need to produce more wax due to higher rates of loss once spread onto their feathers.

A potential explanation for the difference in preen wax between continental and island birds is that the intensity of sexual selection may be reduced in insular populations (Friedman et

al., 2009; Price et al., 2009). If preen wax functions as a sexual signal between individuals (see chapter 4 on robins), then fewer types of compounds may be needed by island birds. With island birds showing a general loss of colours and plumage brightness, it is possible that a smaller variety of lipids are needed to maintain feathers with duller colours. With a decrease in sexual selection on islands, birds may have a decreased need for preen waxes. However, island birds have bigger uropygial glands and as a result probably produce larger volumes of preen waxes than their continental counterparts. This argues against a decreased need for preen wax due to reduced plumage ornaments. A decrease in preen wax complexity due to a reduced signalling function also seems unlikely, as although it is true that the ester fraction is consistent with this hypothesis, the increase in the volatile fraction suggests the opposite, with island birds producing more volatile components in their preen wax and at least the potential for an increased signalling function.

As birds tend to loose colours and show reduced complexity of songs on islands (Friedman et al., 2009; Hamao & Ueda, 2000; Hill et al., 2013; Parker, Anderson, Jenkins, & Brunton, 2012; Price et al., 2009), sexual selection may favour a different pathway to assess potential mates, and therefore favour the use of olfactory signals as a channel of communication. This trade off between visual/auditory signals and olfactory signals might explain the presence of more volatile preen waxes, larger UGs and larger volumes of preen wax among island birds. For example, island species like the kiwi (*Apteryx spp.*) and the kakapo (*Strigops habroptila*) are known to emit strong odours (described as ammonia-like; Hagelin & Jones, 2007). The use of olfactory communication would have only been possible on islands because the predation risk by olfactory searching predators was non-existent or considerably reduced (at least until predatory mammals were introduced by humans). This trade off would have been particularly favoured for species living in a dense and dark forest where visual cues are obscured (e.g. South Island robin). For example, odours could be used to identify conspecifics trying to enter a territory, or even used to mark territorial boundaries. On continental areas, such a function of preen wax would be constrained by the increased risk of predation by predatory mammals using olfactory cues to locate their prey. Whether islands have "liberated" birds from the olfactory constraints faced by the continental relatives is not yet known, but would certainly be a worthy and interesting area of investigation. Although not island birds, females of the New World blackbirds (Icteridae) have concurrently lost their song complexity with almost every loss of plumage elaboration across species, suggesting a similar effect of sexual selection on both traits (Friedman,

Hofmann, Kondo, & Omland, 2009; Price, Lanyon, & Omland, 2009). Therefore, the loss of bright colours and song complexity may have driven the need in island birds for new ways of communicating: why not olfaction? This could explain the advantage in having a bigger gland and producing more preen wax, particularly during the breeding season. I examine the question of whether preen wax can be used in communication between individuals in chapter 4.

This study re-affirms that sex has no effect on the size of the UG. Although I found differences between island and continental species of birds, I found no significant difference in the composition of preen waxes between the sexes. This result contrasts to what has been found in some other studies in which males can have different compounds in their preen wax compared to females (Amo et al., 2012; Whittaker et al., 2013; Zhang et al., 2010). It is possible I did not find a difference as I only examined general features of the GC profile (mean retention time, RT range, minimum RT, number of peaks) and could not identify each of the individual compounds that comprised each peak. This issue will be examined once my samples are processed through a mass spectrometer. Nevertheless, the similarity and high degree of overlap in the peaks between males and females suggests sexual differences are likely to be small, especially in comparison to that observed between species and geographic locations.

I was careful to limit other factors confounding my results, such as degree of sexual maturity or season by only sampling adult birds during the breeding season. In some species UG size varies with season, becoming larger in the breeding season (Pap et al., 2010; Vincze et al., 2013). However, as I only measured birds during the breeding season, the differences in UG size that I observed between island and continental species could not be due to seasonal variation. I also controlled for migration or environmental effects by only sampling non-migratory forest passerines captured from locations with similar altitudes and latitudes. Our Australian field site is indeed situated in between the two islands with a difference of 15°S with New Caledonia and a difference of 6°S with New Zealand (see methods for exact GPS coordinates). Although some authors have found an effect of diet on the composition of preen waxes (Thomas et al., 2010), this influence is still debated. Laura Azzani, collaborator on this chapter, investigated this problem on New Zealand silvereyes and found no effect of diet on their preen wax composition (*pers. comm*.).

Since the intensity of an odour is controlled by its quantity, but the odour is created by the "bouquet" of compounds, I am still unable to say in practical terms what it means for the general odour signal of an island bird to gain or loose these wax compounds. It is very likely that the presence or absence of molecules within preen waxes are a reflection of life history changes and therefore some molecules could be specific to certain functions. The preen wax composition results, even if they should be viewed with caution until I can identify the compounds, are still valuable and lift a little bit of the veil on the evolution process of odours between island and continental birds.

Table 2.1. List of birds used in this study. The number of individuals sampled is given in the UG column for the uropygial gland measurements and in the PW column for the preen wax analysis. Species number follows those given in figures 2.1 to 2.5.

Species N°	Common name	Scientific name	UG	PW
New Zealand				
1	Bellbird	*Anthornis melanura*	36	6
2	Grey fantail	*Rhipidura fuliginosa*	6	7
3	Grey warbler	*Gerygone igata*	21	6
4	Silvereye	*Zosterops lateralis*	37	6
5	South Island robin	*Petroica australis*	30	8
New Caledonia				
6	Barred honeyeater	*Phylidonyris undulatus*	1	1
7	Dark-brown honeyeater	*Lichmera incana*	7	6
8	New Caledonian flycatcher	*Myiagra caledonica*	3	3
9	Fan-tailed gerygone	*Gerygone flavolateralis*	8	5
10	Grey fantail	*Rhipidura albiscapa bulgeri*	2	2
11	Streaked fantail	*Rhipidura verreauxi*	12	9
12	Red-throated parrotfinch	*Erythrura psittacea*	3	3
13	Rufous whistler	*Pachycephala rufiventris xanthetraea*	2	3
14	New Caledonian whistler	*Pachycephala caledonica*	16	6
15	Silvereye	*Zosterops lateralis griseonata*	9	11
16	Green-backed white eye	*Zosterops xanthochrous*	38	12
17	Yellow-bellied robin	*Microeca flaviventris*	3	2

Species N°	Common name	Scientific name	UG	PW
Australia				
18	Fuscous honeyeater	*Lichenostomus fuscus*	10	5
19	White-plumed honeyeater	*Lichenostomus penicillatus*	15	6
20	Yellow-tufted honeyeater	*Lichenostomus melanops*	10	6
21	Western gerygone	*Gerygone fusca*	3	1
22	Brown thornbill	*Acanthiza pusilla*	3	0
23	Yellow thornbill	*Acanthiza nana*	4	3
24	Buff-rumped thornbill	*Acanthiza reguloides*	1	0
25	Yellow-rumped thornbill	*Acanthiza chrysorrhoa*	2	0
26	White-browed scrubwren	*Sericornis frontalis*	8	7
27	Grey fantail	*Rhipidura albiscapa alisteri*	9	8
28	Willie wagtail	*Rhipidura leucophrys*	7	6
29	Rufous whistler	*Pachycephala rufiventris rufiventris*	3	2
30	Red-browed finch	*Neochmia temporalis*	4	6
31	Diamond firetail	*Stagonopleura guttata*	3	1
32	Silvereye	*Zosterops lateralis*	8	6
33	Eastern yellow robin	*Eopsaltria australis*	6	3
34	Restless flycatcher	*Myiagra inquieta*	1	0

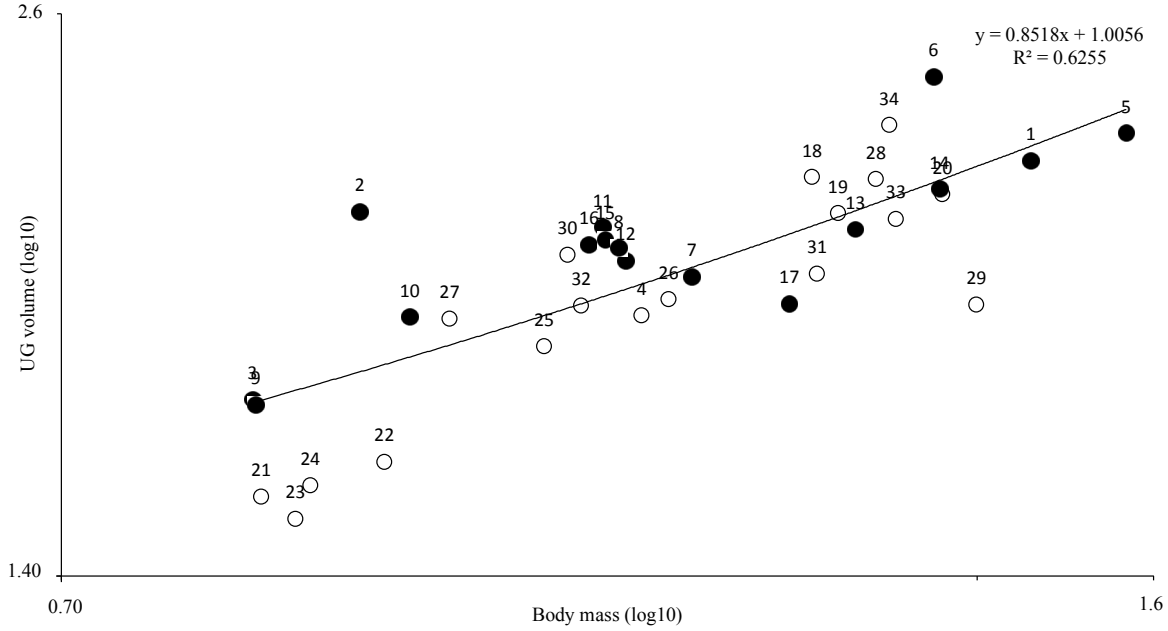

Figure 2.1. Relation between body mass and uropygial gland (UG) volume of island (closed circles, species N°: 1-3 and 5-17) and continental (open circles, species N°: 4 and 18-34) species of birds. Values are $\log_{10}$ transformed. Numbers represent species; names and sample sizes are detailed in table 2.1.

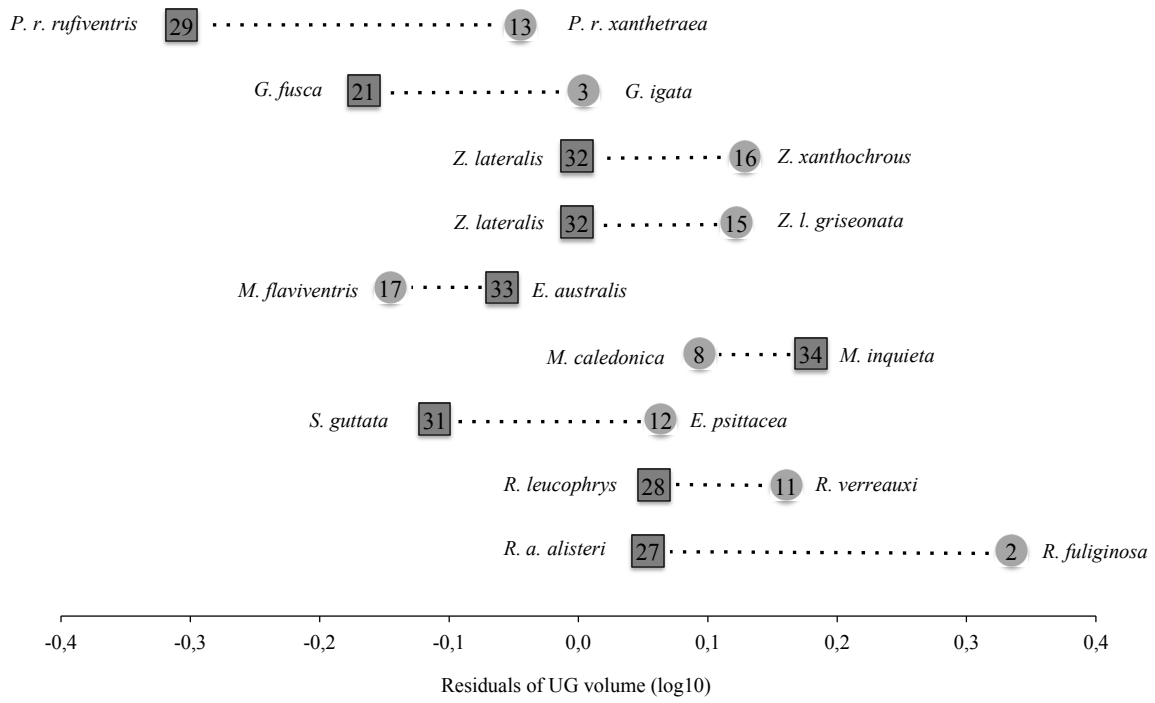

Figure 2.2. Paired comparison of uropygial gland (UG) size variations between 9 closely related species of island (circles) and continental (squares) birds. For clarity purposes, species are represented by numbers and full names are detailed in table 2.1.

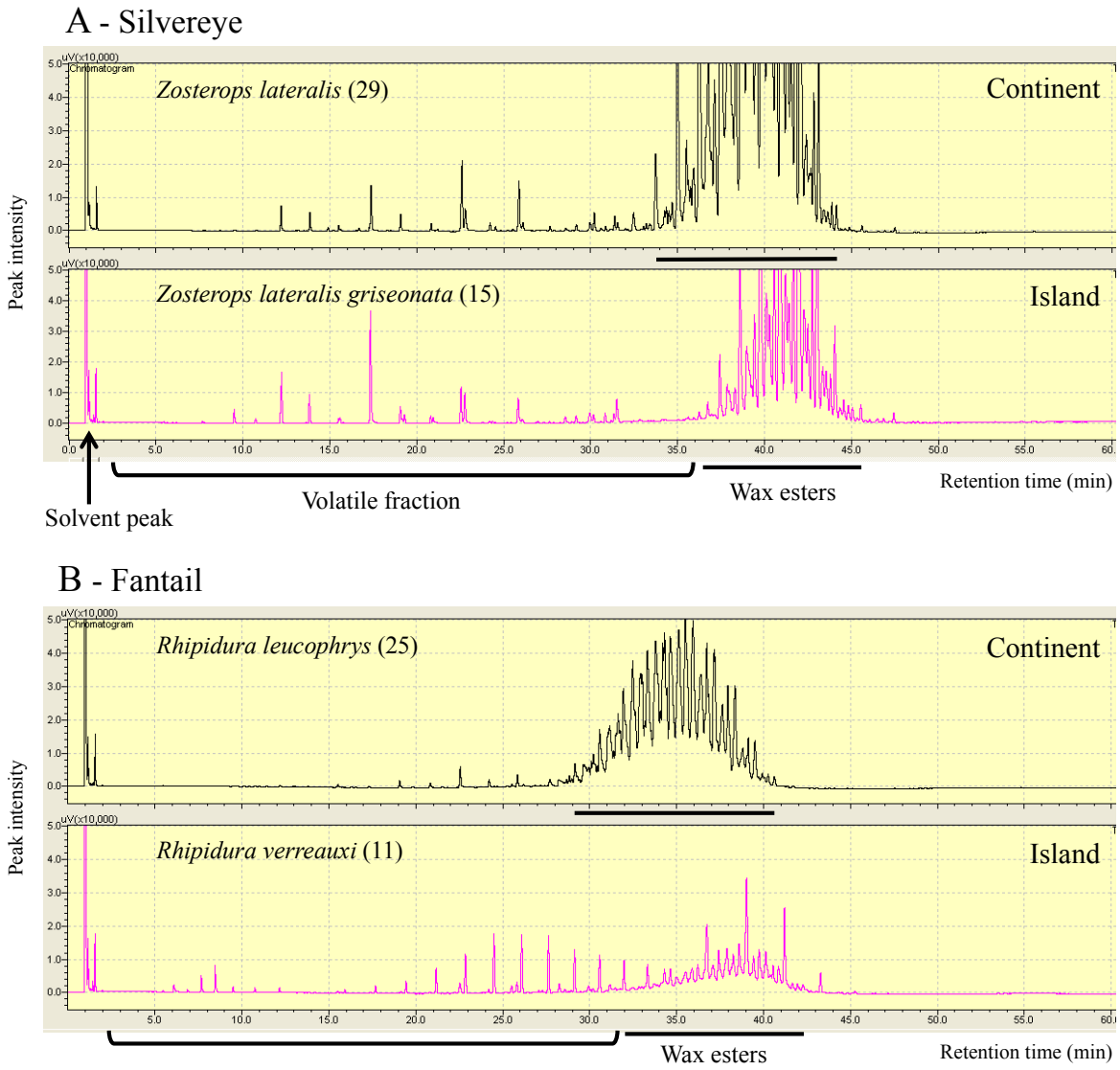

Figure 2.3. Example of chromatograms from two pairs of closely related bird species. Species number follows those given in table 2.1 and figure 2.5. In both species pairs, the island species has more peaks in the "volatile" region but fewer in the "wax ester" region.

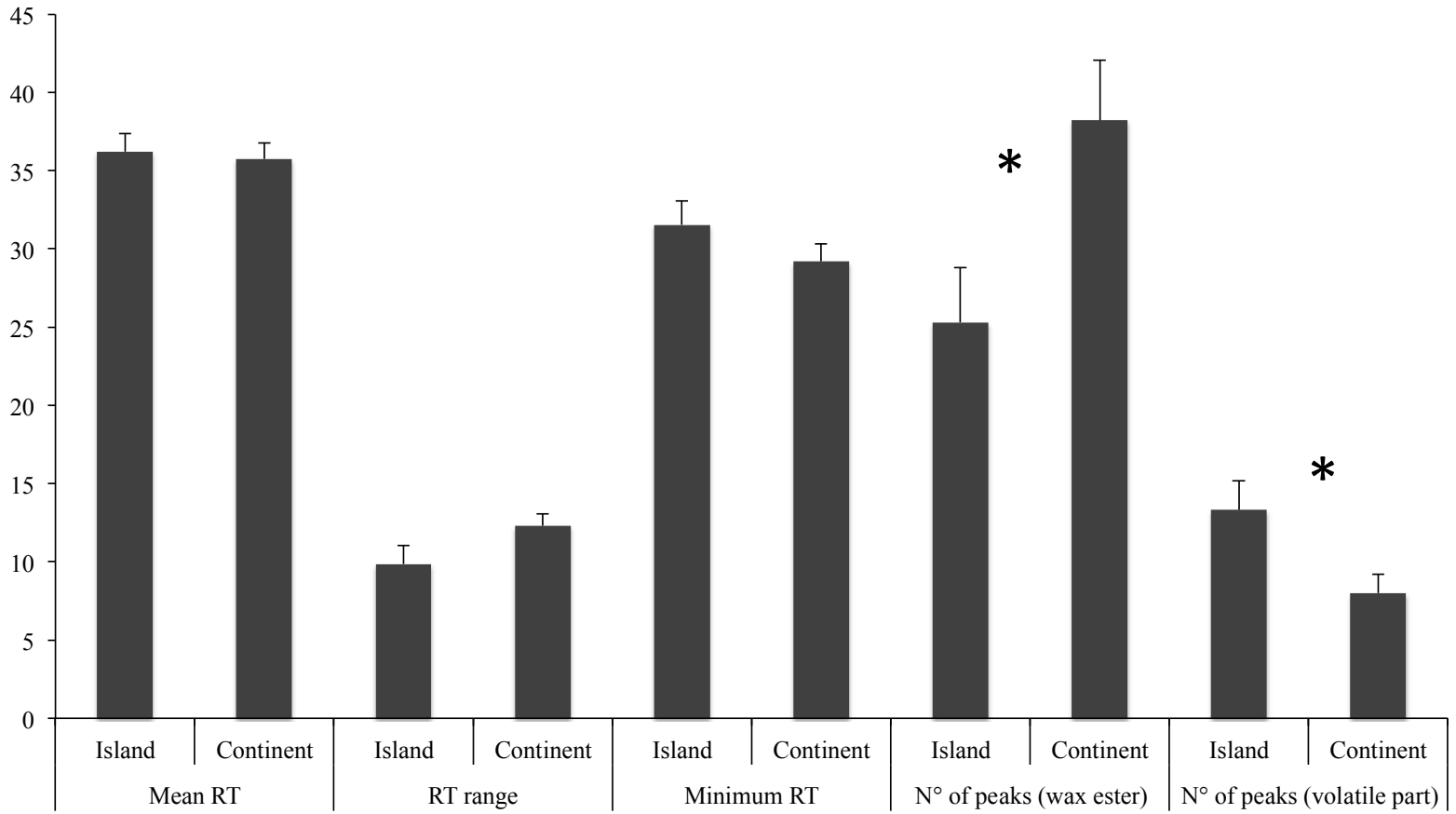

Figure 2.4. Averages of weighed mean retention time (RT), RT range, minimum RT and number of peaks obtained from chromatograms of 162 birds. Island birds show a significant lower number of peaks in their wax ester fraction and a significant higher number of peaks in their volatile fraction compared with continental species. RT is measured in minutes. Number of peaks is the average count of peaks in the two fractions of the gas chromatograph profile.

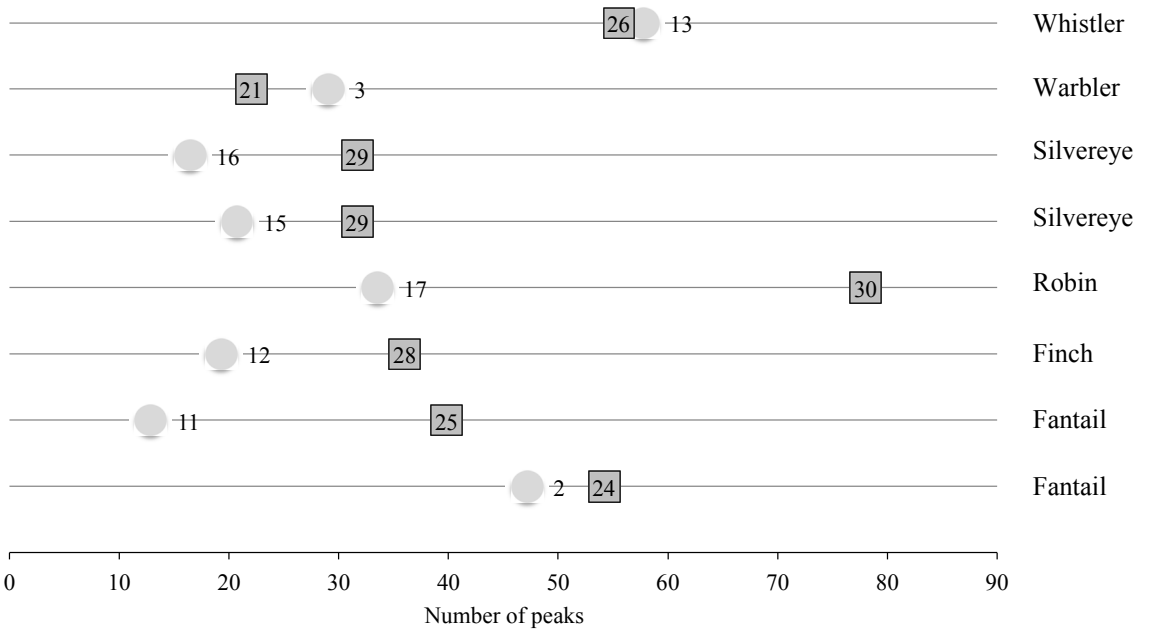

Figure 2.5. Paired comparison of the number of peaks from the wax ester fraction, between 8 closely related species of island (circles) and continental (squares) birds. For clarity purposes, species are represented by numbers and exact names are detailed in table 2.1.

Chapter 3

Do the odours of island birds increase their risk of predation from exotic mammalian predators?

Abstract: *Many species of island birds became extinct shortly after the introduction of mammalian predators such as mice, rats and stoats. Exactly why island birds are so vulnerable to mammalian predators is not clear. In this study I tested whether the odours of island birds make them more vulnerable to introduced mammalian predators. Unlike native avian predators, introduced mammalian predators are more likely to use odour cues to locate their prey. It is possible the odours of native birds are more readily detected and this places them at greater risk than species that co-evolved with mammalian predators. To test this hypothesis I presented a model mammalian predator, the Norway rat (Rattus norvegicus) with the preen wax of birds in a series of laboratory experiments. I then used tracking tunnels in a field experiment to determine whether rodents visited the preen wax of native species more frequently. Preen wax contains a number of volatiles and is likely one of the main sources of odour in birds. I found that laboratory rats in a Y-maze selected the most volatile preen wax in one paired test. However, two additional paired tests in the lab and a series of field tests using tracking tunnels did not show any significant differences and more experiments are needed to confirm my results. Nevertheless, conservation programmes to protect endangered island birds may benefit from considering whether olfactory cues can be minimised as a method of reducing predation risk.*

Introduction

Recent discoveries have indicated that many birds use their sense of smell more than previously realised, whether searching for prey, recognising mates and nest sites, or even other conspecifics (Bonadonna et al. 2004; Mardon and Bonadonna 2009; Amo et al. 2013; chapter 4 of this thesis). Recognition of other individuals suggests that at least some species may even possess a personal olfactory signature (Whittaker et al., 2010). These odour cues most likely originate from the secretions of the uropygial gland. This structure, which resembles a mammalian sebaceous gland, is located above the base of the tail. It produces a waxy substance (preen wax) composed of a variety of esters, alcohols and fatty acids that birds use to preen onto their feathers (Jacob & Zisweiler, 1982).

The proposed functions of preen wax range from a role in plumage maintenance (Jacob & Zisweiler, 1982), in controlling bacteria and parasite loads (Douglas, 2008; Reneerkens et al., 2008), and to a role in sexual selection (Hirao, 2011; Zhang et al., 2010). It is also thought that changes in preen wax composition during the breeding season may provide olfactory camouflage to incubating birds from predators that use odours to locate their prey (Reneerkens et al., 2005; Soini et al., 2007). Indeed, as sitting on the nest can place birds in a vulnerable position, selection should favour the evolution of less detectable preen waxes in species most at risk from predators using olfactory cues to locate their prey. Viewed in this way, preen wax may be under selection to reduce detectability in a fashion analogous to how the cryptic colours of some birds or their nests act as camouflage against detection by predators using visual cues.

The Oceania region is an ideal place to study the role of preen waxes in relation to predation risk. Most passerines in Oceania originated from Australia where they co-evolved with a wide range of native mammalian predators that use olfaction to locate their prey and as a result would have been under selection to hide or minimise their odours. However, once they dispersed and colonised islands in the Pacific, most species of birds found themselves in habitats with no mammalian predators and few if any reptilian predators. This difference in evolutionary history has probably shaped the odours of island birds, for example, as they no longer needed to be camouflaged from mammalian predators. As expected, an analysis of preen wax composition from a variety of island birds and their continental relatives indicated significant differences in the range and

variety of ester components, suggesting insular environments have indeed led to changes in the preen wax of island birds (chapter 2, this thesis).

When humans arrived in New Zealand (and other oceanic islands), they typically introduced a variety of predatory mammals, both deliberate (e.g., cats) and accidental (e.g. rats). As a consequence, many native island birds became extinct and many of those that survived are now endangered. However, exactly why island birds are so vulnerable to introduced mammalian predators is not clear. In some cases island birds appear to lack the appropriate behavioural defences (Duncan & Blackburn, 2004; Lovegrove, 1996; Martin, 1995), and they tend to have life histories with low reproductive rates that mean their populations cannot cope with high levels of predation (e.g., kiwi; McLennan et al. 1996). The objective of this study is to assess if island birds have more detectable odours than continental birds, and thus if this difference is one of the factors that makes them more vulnerable to predation by introduced mammalian predators. To determine if the odour of a bird affects its potential risk of predation, I examined how an important mammalian predator, the Norway rat (*Rattus norvegicus*) responded to the preen waxes of island birds versus continental birds using laboratory experiments. My objective was to determine whether rats were more likely to approach the odour of a New Zealand native bird compared to that of a continental bird. I then carried out a field test using tracking tunnels to see if rodents were more likely to visit tunnels baited with the preen wax of island birds than tunnels baited with the preen wax of continental bird species.

Methods

Study species

I used the preen waxes of 5 species of native birds and 7 species of European introduced birds. Species are listed in table 3.1.

Preen wax collection

All samples were collected during the Southern Hemisphere breeding season between August and December 2012. Birds from New Zealand were captured at Kowhai and Waimangarara bushes, two patches of native forest near the town of Kaikoura (173°37’E, 42°23’S). The green-backed white-eyes, hereafter referred as white-eyes, were captured at

Parc des Grandes Fougères, Farino in New Caledonia (165°45'E, 21°37'S). Preen wax was obtained by gently pressing the uropygial gland and collecting a small drop of wax with a sterile metal loop. This loop was immediately transferred into a sterile 100 µl glass insert and the insert was then placed inside a sterile glass vial. To minimise contamination, all glassware and loops had previously been cleaned with a sequence of 3 organic solvents, baked dry, and stored in clean vials until used. Samples were stored in a cooler for a few hours while in the field and then later frozen at -20 C until used in experiments. All birds and equipment were handled while wearing disposable rubber gloves to avoid contaminating samples with human odours.

Laboratory experiment

This experiment used 20 laboratory-bred Long Evans rats to test their response to the preen wax samples, as wild caught rats may have had prior experience and preference with the bird species used in this study. The Long Evans strain was selected for this study as it appears to be an appropriate model for studying the behaviour of wild rats. For example, they are rapid learners, and an outbred strain (with high genetic variation) of the Norway rat (Harker & Whishaw, 2002; Lavenex & Schenk, 1997). This species of rat is a significant introduced predator of island birds (Atkinson, 1985; Moors, Atkinson, & Sherley, 1992). Females, 3 to 4 months old, were used as males can be aggressive (N. Harris, pers. comm.) and there was no documented difference between male and female performances in an olfactory behavioural test (Eade & Youngentob, 2009). Rats were kept at the Psychology Department of the University of Canterbury facilities. The room was maintained at 21°C with a 12h/12h light/dark cycle. Rats were housed by groups of 4, in plastic cages (62 x 40 x 22 cm) with wood shavings as bedding and provided with *ad libitum* water. They were fed daily (rat nuts, Reliance Feeds) but were maintained on a 90% of their free-feeding weight diet to encourage food-searching behaviour during the experiment. Cages were provided with retreats and new objects were added every week to stimulate and increase the well-being of the rats. While growing up, rats were handled at least 3 times per week by the author or the technicians in order to reduce the handling stress before the tests.

A Y shaped-maze, composed of three identical arms (50 cm long, 15 cm wide, 15 cm high), was used to test the ability of rats to detect preen wax samples. It was made out of

opaque grey PVC. The entry arm had a waiting compartment (25 x 15 x 15 cm) added and linked by a sliding opaque PVC door. The two other arms were called the choice arms and each had a scent compartment (10 x 15 x 15 cm) added with sliding mesh doors (figure 3.1). Each scent compartment had a device designed to heat each preen wax sample within its glass insert. The entire insert was heated *in situ* as this minimised the risk of contamination with human odours by any additional handling of the waxes. Heating devices were set to control the heating rate and maintain vials at a constant temperature of 40°C ± 2°C, similar to the body temperature of birds (Prinzinger, Pressmar, & Schleucher, 1991). This enabled rats to encounter the normal range of airborne volatiles from the preen wax, while the mesh doors allowed the odour cues to travel inside the Y-maze. As both arms were identical in appearance and the preen wax samples were housed in identical vials, there was no difference in visual cues between the two arms. The maze was also equipped with a Perspex lid to reduce the evaporation of the volatiles into the room, to prevent the rats from escaping and to be able to film the rat's reactions. The maze was placed in an empty, previously washed, controlled temperature room at 21°C.

Prior to the experiments, each rat was given two training sessions in the maze. These reduced their stress and allowed the rat to explore the set up prior to the introduction of any stimuli. Rats were also given one control run with peanut butter, a food that they had never encountered before. A drop of peanut butter was collected with a microbial loop and placed at random, within its insert, in one of the choice arms while the other arm served as control with an empty insert. The volume of the peanut butter was similar to that of the preen wax samples. These control trials helped to establish a baseline of the rat's behaviour to something they were known to be attracted to, and to confirm that the small quantities of stimuli used were sufficient to obtain a response.

After the training sessions and peanut butter tests, I then ran 3 paired comparisons in which the preen wax of one species was compared against that of another. In each case, I paired up an island species with a continental species. My expectation was that if there was something about the odour of preen wax from island birds that made them more detectable, the rats would first approach and spend more time in the arm with preen wax from an island species. In the first paired test, I compared preen waxes from a New Zealand population of silvereye and the white eye from New Caledonia. Despite samples being collected in New Zealand, the silvereye (3 females and 10 males) was classified as a

continental species as it has only recently colonised from Australia, where it has co-evolved with a variety of native mammalian predators. In contrast, the white-eye (13 males) is endemic to islands in the South Pacific and typical of many island species, did not co-evolve with mammalian predators. I used samples from male and female silvereyes and white-eyes as both sexes incubate (Desmoulins & Nicolas, 2005; Heather & Robertson, 2005). Additionally there was no obvious sex differences in the gas chromatogram profiles of these preen waxes (L. Azzani, *pers. comm.*). The second and third trials compared the response of rats to the preen waxes of South Island robin (n=4) and bellbird (n=4) compared to that of the common blackbird (n=8). All these species are similar in size. Both the robin and bellbird are endemic to New Zealand (and thus evolved in the absence of mammalian predators) while the common blackbird was introduced to New Zealand in the 19th century and co-evolved with mammalian predators in its native European range. For all 3 species I only used preen waxes collected from females as they are the only sex that incubates and therefore more at risk from a predator approaching its nest.

Experiments ran from June to July 2013, during the rat's light cycle. Frozen inserts containing the microbial loops with the preen wax samples were placed in the two scent compartments of the maze. A single rat was then placed in the waiting compartment in the entry arm and allowed 3 minutes to settle down. The waiting compartment was darkened to reduce stress. The 3 minutes was also enough time for the preen wax to be heated. After the settling in period, I switched on the camera, lifted the entry arm sliding door, and quietly left the room. Each trial ran for 10 minutes. These times were chosen after preliminary runs and adjusted to ensure that rats were not stressed by excessive time in the waiting box and still had time to explore the maze. The camera, a Sony Handycam DCR-SR85E, was mounted on a tripod, and placed on a table facing the Y-maze (which was placed on the ground). The camera was positioned to reduce light reflection from the Perspex lid while still allowing the entire maze to be recorded. At the end of the experiment, the rat was removed and I later transcribed the videos to record the response of the rat to the two stimuli.

The first arm entered, the total time spent in each arm and the total time spent scratching and sniffing the mesh door of the scent compartments were recorded as an indication of interest by the rat in the odour stimulus. It was assumed that if odour increases the risk of

predation, the rat would be attracted to and spend more time in the arm of the maze containing the preen wax of the island species. The number of replicates was dependent on the number of samples I could collect from the field and different rats were used in each experiment to avoid pseudo-replication. Stimuli were randomly alternated in the scent compartments to counteract any side effect of the Y maze. The different experiments were also presented to rats in a random order so there would be no exchange of information between rats within a cage. Brown paper was laid on the floor in each arm and changed in between trials. The maze was also thoroughly washed with 70% ethanol to remove any odours left by previous rats.

Field experiment

To determine whether the results of the laboratory experiment were consistent with the behaviour of wild predators, I used tracking tunnels to test if the preen waxes of island birds make them more vulnerable to mammalian predators. This experiment was carried out at Goose Bay (173°31'E, 42°27'S), near the town of Kaikoura, New Zealand, in a patch of native forest along the sea. The site was chosen for the tunnel tracking experiment as it was known that rodents, feral cats, and possums were present (DOC officer Mike Morrissey, *pers. comm*.).

Rectangular-shaped tunnels (59 x 10 x 10 cm) were made of a lightweight black corrugated plastic. The inside bottom of the tunnel was lined with a white cardboard card. At each end, near the entrance to the tunnel, I placed a 4 cm band of masking tape that I then covered with black long-lasting ink (purchased from Pest Control Research). The masking tape prevented the ink from soaking into the cardboard card underneath. The ink was placed at each entrance in order to record the potential visits of individuals that were otherwise too shy to enter except at the entrance-way.

Field trials of the tracking tunnels were carried out in February 2013, which coincided with the late part of the breeding season and when rats were expected to be actively foraging. Preen wax to bait each tunnel was collected in the same fashion as described above for the lab experiments. However, I used a wider range of species for the tracking tunnels. To test the attraction of rats to the odours of endemic species, I collected the preen waxes from bellbird and grey warbler. Both are endemic island species. To compare the response of

rats to continental species, I collected wax from a variety of introduced European species: blackbird, song thrush, chaffinch, goldfinch, greenfinch, redpoll and yellowhammer.

A total of 18 tracking tunnels (6 stations) were set along a walking path in Goose Bay. As recommended for small rodent species, each station was spaced 50 m apart (Cunningham & Moors, 1996; Jackson, 1982) and was composed of 3 tunnels: one tunnel baited with the preen wax from an island species, one tunnel baited with preen wax from a continental species and one tunnel acted as control with only an empty vial. Thus each tunnel appeared similar but differed in their potential odour cues. Within a station, tunnels were spaced 1.5 m from each other and all orientated in the same direction to control for wind (figure 3.2). Preen waxes were presented in their original glass vials hanging in the centre of the tunnels (figure 3.2). It was not possible to control for the volume of wax present in a vial, however as all samples were collected using the same sized metal inoculation loops, and the opening of the vial was the same among treatments, it is likely that the rate of diffusion was similar despite any slight difference in wax volume. Rubber gloves were worn when handling tunnels and vials to ensure human scent was not left as a cue. Tunnels were also thoroughly washed in between trials as possums and rats would sometimes defecate or leave strong scent marks.

Trials were replicated 6 times at 6 different locations within the study site (for a total of 108 tracking tunnels). The angle and position of the tunnels were changed between trials to avoid habituation from the rats. Each tunnel was left out for a 24 hour period and in the mornings I would collect card footprints, clean all tunnels, and renew waxes before placing the tunnels in a new position. Tunnels alone were used to ensure that rats were attracted to the preen wax in the absence of other cues that are associated with real nests (e.g. parental activity, smell of eggs, etc.).

The number of tunnels visited per night and the total number of prints were recorded as an indication of the rodent interest in the wax samples. The identification of prints was based on the guide from Gillies & Williams (2002) and from Ratz (1997).

Data was checked for normality and nonparametric statistics used when I could not transform data to ensure normality. Maze results were analysed using Chi-square, Fisher exact tests, paired t-tests and Wilcoxon tests. Tunnel results were analysed with Friedman tests. All statistics were run using the programme STATISTICA 6.0, ©StatSoft Inc. The

statistical significance level was $p = 0.05$.

Results

Laboratory experiment

During the peanut butter trial, 15 rats out of 20 spent more time in the arm with the peanut butter scent compared with the no-scent control (Chi square test, $\chi^2 = 5.0$, $p = 0.02$). This indicates that rats in the maze setup oriented and approached a stimulus based on odour cues alone.

When preen waxes were placed in the two arms of the maze, rats entered the arms randomly and showed no preference for any of the preen waxes: white-eye versus silvereye (Chi square test, n = 12, $\chi^2 = 0.33$, $p = 0.56$), robin versus blackbird (Fisher test, df = 1; $p = 1.00$) and bellbird versus blackbird (Fisher test, df = 1; $p = 1.00$). However, when compared with the preen wax of white-eyes, rats showed a significant preference for the preen wax of silvereyes in both the amount of time spent in the arm (paired t-test, n = 11, t (10) = -2.56, $p = 0.027$, figure 3.3) and in the amount of time spent scratching/smelling the mesh door close to the scent compartment (paired t-test, n = 12, t (11) = -3.28, $p = 0.007$, figure 3.4).

There was no significant difference in the time spent in each arm when rats were presented with the preen waxes of blackbird and robins (Wilcoxon test, n = 4, T = 5.0, Z = 0, $p = 1.0$, figure 3.3) nor in the time spent scratching the mesh door (Wilcoxon test, n = 4, T = 3.0, Z = 0.73, $p = 0.46$, figure 3.4). Similar results were found when the preen waxes of bellbirds and blackbirds were presented simultaneously to rats. No preference was observed in the time spent in each arm (Wilcoxon test, n = 4, T = 2.0, Z = 1.09, $p = 0.27$, figure 3.3) nor in the time spent scratching the mesh door (paired t-test, n = 4, t (3) = 0.75, $p = 0.50$, figure 3.4).

Field experiment

The number of tracking tunnels visited per night did not vary significantly with treatment (Friedman tests, $\chi^2 (16, 2) = 2.37$, $p = 0.30$, figure 3.5). The number of tunnels baited with the preen waxes of introduced birds showed the lowest number of visits but this was not

significantly less than tunnels baited with preen wax from native species nor the unbaited control tunnels. Similarly, the total number of foot-prints left in each tracking tunnel did not differ significantly among the three treatments (Friedman tests, χ^2 (16, 2) = 2.19, p = 0.33, figure 3.5). Although tunnel tracks baited with preen wax from native birds had the highest number of foot-prints, this did not differ from control tunnels or those baited with preen wax from introduced birds.

Discussion

I found only limited evidence that different preen waxes elicited a different response by rats in a maze setting. Although the trials with peanut butter confirmed that laboratory rats responded positively to the odour of something they were known to prefer, I did not find any preference for the first arm of the Y-maze that the rats chose to enter. It is possible that the rats could not detect any differences in the odour of each arm (and therefore did not make a choice) because the volatiles had not yet reached that part of the maze. However, rats did express a significant preference for the arm with preen wax of New Zealand silvereyes when compared with the preen wax of white-eyes. They spent both more time in the arm with silvereye preen wax and more time scratching the wire divider that separated the sample vial from the arm. Nevertheless, a similar difference was not found when rats were given the choice between two other species of island birds (bellbird and New Zealand robin) and a continental species (European blackbird). Likewise, there was no difference in visitation of wild rodents to tracking tunnels baited with the preen wax of either native or introduced species.

The preference for the maze arm containing the preen wax of silvereyes suggests the odour of this species was either more readily detected by rats or was preferred by them. When I analysed the preen wax samples through a gas chromatogram (GC), I observed that the profiles of white-eyes and silvereyes were quite similar, but that silvereye wax was slightly more volatile than the wax of white-eyes. For example, the region of the profile encompassing the wax esters show a mean weighed retention time (RT) of 38.26 min for silvereyes and a minimum RT of 32.26 min (data from chapter 2, this thesis). In comparison, whites eyes had a mean weighed RT of 38.60 min and a minimum RT of 35.21 min. It is possible the rats perceived this difference and were attracted to the more volatile preen wax of silvereyes. On the other hand, differences in volatility (as estimated

by mean RT) were also present in the species in the other paired maze tests yet they did not result in differences in the behaviour of the rats. The wax GC profiles of robins had the lowest RT with 29.51 min (indicating they were the most volatile) but the response of rats did not differ from that of blackbirds (which had the least volatile preen wax of all the species I studied with a RT of 46.9 min). A similar lack of difference was observed between paired tests with bellbirds (RT of 37.58 min) and blackbird (with again blackbirds having the less volatile preen wax). Although my results suggest a trend for rats having more interest in robin and bellbird waxes the sample sizes are too small for this difference to be significant.

Given differences in the wax profiles between island and continental birds (see chapter 2), I was expecting that visits by rodents to the tracking tunnels baited with preen wax should vary. Although wild rats tended to prefer tracking tunnels baited with the preen waxes of native species, compared with introduced bird waxes or the controls, these differences were not significant. The lack of a difference could be due to a number of factors. Firstly, wild rodents may have already had some experience with the odours of birds used in this study and so differed in levels of familiarity. The apparent low density of wild rodents at the time of study may also have affected the probability of a tunnel being visited, and given that the tunnels were in place for only one night at a time (before being moved and restocked with fresh preen wax), neophobic individuals may have been reluctant to visit tunnels with unfamiliar odours (whether from a native or introduced species). The difference between my laboratory and field results could also be due to differences in the temperature and thus amount of volatiles created. In the tunnel experiment, I was not able to heat the wax samples and wild rodents may not have been able to detect them from a distance as in a laboratory set up. Finally, it is possible that the most volatile components of the preen wax quickly dissipated in the field and so by the time rodents approached the tracking tunnels in the evening, only the least volatile compounds remained in all species, making them all relatively similar in detectability. This is a problem of using preen wax samples alone, as a live bird would be producing a more continuous stream of volatiles (and at a high temperature). Future field tests may need to more closely replicate the odour signals created by live birds sitting on a nest in order to determine whether risk of detection differs and if this difference is due to differences odours originating from the preen wax.

One potential reason that island birds are more vulnerable to introduced mammalian predators is that they are more readily detected because of their odour. This might be especially important at night, when many mammalian predators are active, and which use olfactory cues to help locate potential prey. A bird with a particular "strong" odour could thus be at increased risk while incubating at night. If this is the case, then this could mean that birds with the most volatile preen wax (e.g. robin) should be at greatest risk from mammalian predators. However, a comparison of preen waxes between island and continental birds suggests that the differences are not simply one of volatility (chapter 2). Instead, my hypothesis that island birds in general are "smellier", because they evolved without mammalian predators, might not be true. This does not rule out a role for odour in the vulnerability of island birds, but suggests that features other than volatility may be worth investigating. How rats might perceive differences in the preen waxes of different species is unknown and it is quite possible that the composition of some preen waxes makes them less conspicuous, regardless of their overall levels of volatility. The greater diversity of compounds in the preen waxes of continental species and smaller uropygial gland size (chapter 2) may even function in reducing predation risk in continental species by reducing the amount (and thus potentially detectable signal) of any given single compound.

In summary, my results suggest that rats may be attracted to at least some types of preen waxes, but not others. Whether such an attraction is species-specific needs further work, and it would be worthwhile investigating if some island species that seem especially prone to predation have preen waxes (and thus odours) that are also especially attractive to exotic mammalian predators. More experiments, with larger sample sizes, are needed to confirm this hypothesis but conservation projects may nonetheless benefit from considering odours in a species prone to predation when planning management actions. For example, olfactory decoys or olfactory pre-exposure techniques (Price & Banks, 2012) could be used next to nests of highly threatened species. It has also been shown that some island species are able to adapt behaviourally to increased predation pressure from introduced mammalian predators, by reducing parental activity next to the nest (Massaro, Starling-Windhof, Briskie, & Martin, 2008), but such adaptations might not be sufficient if birds are still emitting highly detectable odour cues.

Table 3.1. List of the species of birds used in this study.

Origin/Status	Common name	Scientific name
New Zealand		
native	Bellbird	*Anthornis melanura*
native	Grey warbler	*Gerygone igata*
native	Silvereye	*Zosterops lateralis*
native	South Island robin	*Petroica australis*
introduced	Blackbird	*Turdus merula*
introduced	Song Thrush	*Turdus philomelos*
introduced	Chaffinch	*Fringilla coelebs*
introduced	Goldfinch	*Carduelis carduelis*
introduced	Greenfinch	*Carduelis chloris*
introduced	Redpoll	*Carduelis flammea*
introduced	Yellowhammer	*Emberiza citrinella*
New Caledonia		
native	Green-backed white eye	*Zosterops xanthochrous*

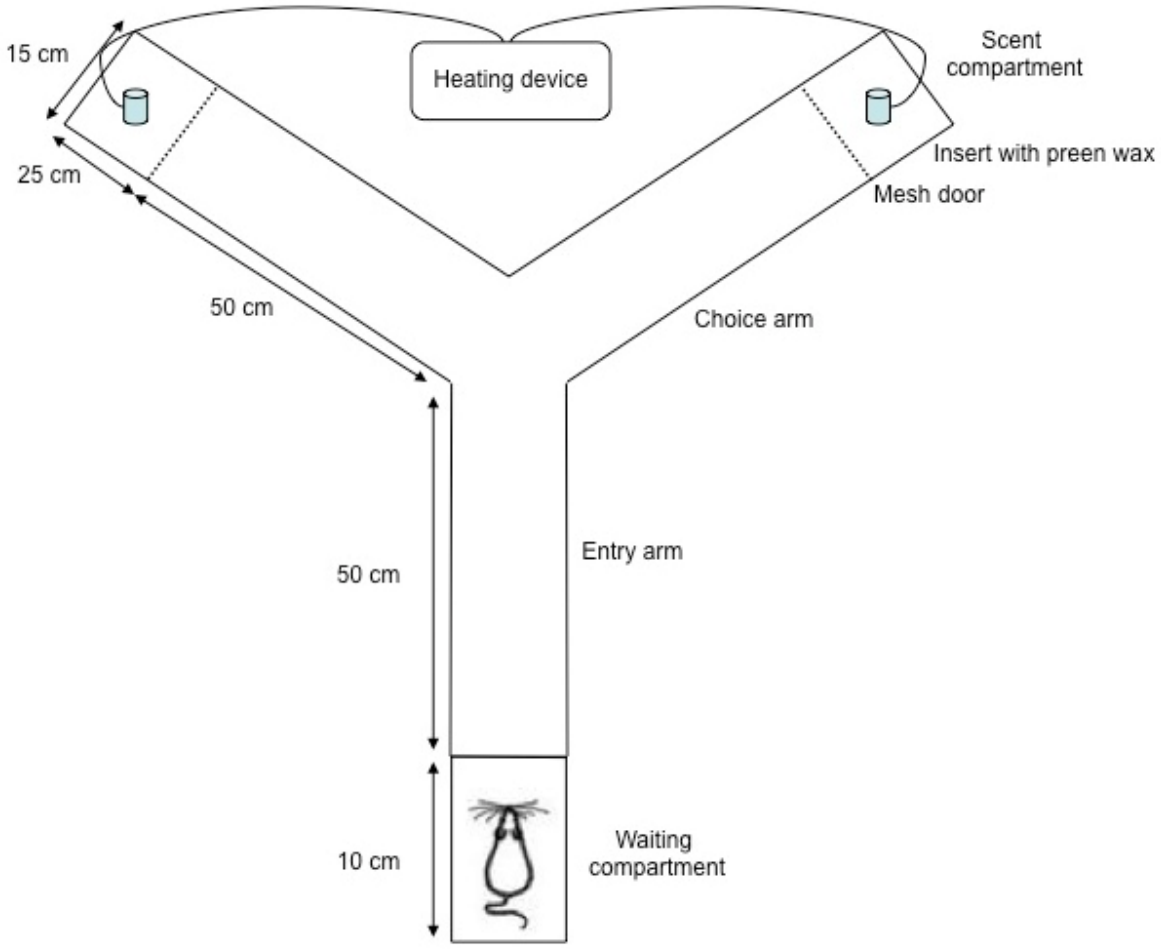

Figure 3.1. Outline of the PVC Y-maze composed of three identical arms. The scent compartments containing the preen waxes are at the end of the two choice arms. A Perspex lid prevented odours from evaporating in the room and the rats from escaping.

Figure 3.2. Left. Inside view of a tracking tunnel with a glass vial (stimulus) hanging from the top, a white card for recording prints of visitors to the tunnel, and a strip of ink at the entrance. Visitors were able to enter from either end of the tunnel. Both ends were fitted with ink strips and white cards. Right. Example of a station with 3 tunnels orientated in the same direction to control for wind direction.

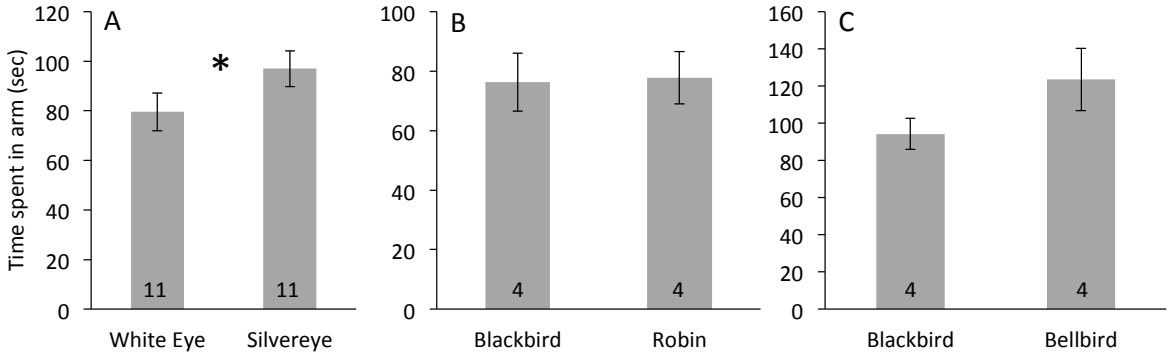

Figure 3.3. Mean time spent by Norway rats in each arm of the Y-maze, over a 10 min exposure to preen waxes from 5 species of birds. Preen waxes were presented in a paired design, with A, B and C referring to each pair of species tested. Numbers at the bottom represent sample size, * indicates a significant difference from expected ($p < 0.05$). Vertical bars indicate standard errors.

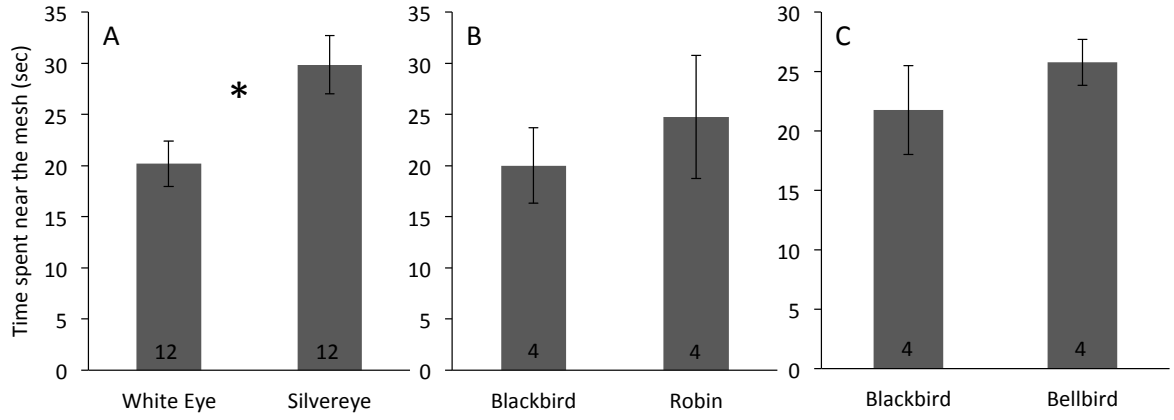

Figure 3.4. Mean time spent by Norway rats scratching the mesh door separating the scent compartment of the Y-maze. Experiments ran for 10 min and stimuli were preen waxes of 5 different species of birds. Numbers at the bottom represent sample size, * indicates a significant difference from expected ($p < 0.05$). Vertical bars indicate standard errors.

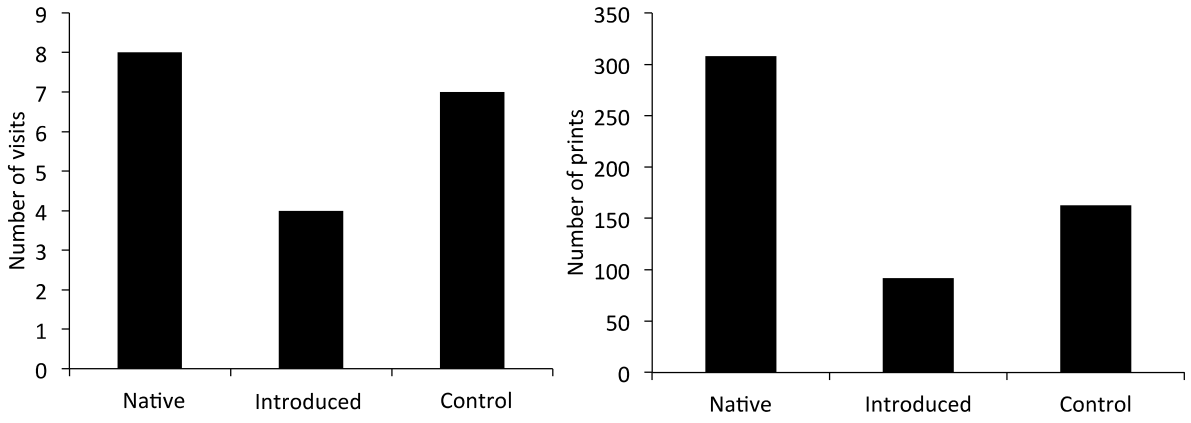

Figure 3.5. Total number of tunnels visited by wild rats, over 6 nights, and total number of prints they left behind.

Chapter 4

Conspecific odour recognition in the South Island robin: I can smell you before I even see you!

Abstract: *Most bird odours are thought to originate from preen waxes produced by the uropygial gland. Preen waxes are spread over the feathers by preening, and although their exact functions are debated, a role in social signalling has been suggested. In this study, I tested whether the preen wax from male South Island robins (Petroica australis) is recognised by conspecific males. Robins are unusual in that they switch to producing more volatile preen waxes during the breeding season. This pattern is opposite to that found in other species, and suggests that their preen wax may function in social chemo-signalling. Using a remote-controlled blind chamber that allowed me to expose free-living robins to an odour sample, I recorded how males reacted to the preen wax of conspecific males in comparison to a control sample. Robins exposed to a sample of conspecific preen wax were more likely to hop, wing flick and physically attack the chamber. This suggests that even in the absence of visual cues, the odours produced by preen wax could be detected by conspecifics and function in intraspecific communication.*

Introduction

The study of the role of olfaction in birds is still in its infancy. Some colonial seabirds use their sense of smell for finding their nests or for assessing and recognising mates (Bonadonna et al., 2004; Mardon & Bonadonna, 2009), but little is known about the importance of olfaction in other groups of birds such as passerines. The main reason for this gap in our knowledge might be that until recently, passerines have been considered relatively anosmic, because they possess the smallest olfactory bulb relative to brain size among all bird species (Bang & Cobb, 1968). However, recent studies have provided evidence that olfaction does play a role in passerine birds (Amo et al., 2013; Gwinner & Berger, 2008; Petit et al., 2002), and that odours can be used in social communication (Amo et al., 2012), even if their olfactory abilities may be less developed than in some seabirds.

The main source of body odours in birds likely comes from the uropygial gland, which secretes preen wax, an oily excretion that is collected with the beak and preened onto the feathers. This secretion is composed of a variety of esters, alcohols and fatty acids (Jacob & Zisweiler, 1982). The chemical composition of preen waxes varies both within and between species, with intraspecific variation related to e.g. sex, time of year and geographic location (Soini et al., 2007; Whittaker et al., 2010). Several functions of preen wax have been proposed, including a role in plumage maintenance (Jacob & Zisweiler, 1982), reducing predation risk (Reneerkens et al., 2005), reducing ectoparasites (Moreno-Rueda, 2010), and sexual selection (Hirao et al., 2009; Lopez-Rull et al., 2010; Zhang et al., 2010). Recent studies have also indicated that preen wax odours may signal species identity and thus allow the recognition of individual conspecifics, including mates and nests (Bonadonna et al., 2004; Mardon et al., 2010; Whittaker et al., 2010; Zhang et al., 2013).

The South Island robin (*Petroica australis*) is an ideal species in which to study the potential role of preen wax odours in social communication. Robins have an unusually volatile preen wax and show shifts to even more volatile components during the breeding season (Fluen, 2008). To determine whether male robins are able to detect the presence of another male robin, based solely on the odour of its preen wax, I developed a method to present preen wax to free-living birds in the absence of any visual cues. As robins are

highly territorial, but inhabit the dense understorey of forests in which visual signals are often obscured, it would be advantageous for them to recognise the presence of conspecifics in their territory based on olfactory cues, especially if males do not vocalise and act clandestinely when intruding in a territory. Such olfactory cues could be left e.g. by an intruding bird brushing its feathers against vegetation or by rubbing their bills on vegetation (which may be covered with preen wax from previous preening bouts).

My results show that, for the first time, wild living birds (robins) appear to recognise the preen wax of rival conspecifics solely by the sense of smell. This adds to the growing body of evidence that songbirds can use chemo-signalling.

Methods

Study site and species

This study was conducted in April and June 2013 at Kowhai Bush and Waimangarara Bush, two patches of native forest near the town of Kaikoura, New Zealand (173°37'E, 42°23'S). During this period male South Island robins established and defended territories prior to the start of breeding in July. Robins are medium-sized (30-40 g), ground-feeding insectivorous forest passerines. They are socially monogamous and territorial year round (Higgins & Peter, 2002; Powlesland, 1980). Robins are naturally tame and curious which makes it easy to approach them and to conduct odour recognition experiments on free-living birds.

Preen wax sampling

Birds were captured and colour banded (if not already banded) from April to June 2013. Preen wax was obtained by gently pressing the uropygial gland and collecting a small drop of wax with a sterile metal loop. This loop was immediately transferred into a 100 µl glass insert and the insert was then placed inside a glass vial. To minimise contamination, all glassware and loops had previously been cleaned with a sequence of 3 organic solvents, baked dry, and stored in clean vials until used. Preen wax samples were stored in a cooler for a few hours while in the field and then later frozen at -20°C until used in the presentation experiments. All preen wax samples were obtained from adult male robins,

and all birds and equipment were handled while wearing disposable rubber gloves to avoid contaminating samples with human odours.

Experimental procedure

I tested a total of 14 adult male robins that were resident on a territory, both with a control treatment (n = 12) and an experimental treatment (n = 13; preen wax of a non-neighbouring conspecific male). The difference in sample size is due to failure to attract all individuals to the experimental setup. All experiments involved the use of a presentation box (figure 4.1). In the experimental treatment, the box contained a preen wax sample within a glass insert and could be heated. The chamber was sealed but equipped with an automatic door that was remotely controlled. This allowed me to activate the opening to the chamber (and thus release the odour of the preen wax) while I was positioned at a distance of 3 m from the focal bird. As the volatility of the preen wax likely depends on temperature (i.e., higher temperatures lead to increased release of volatiles), the preen wax sample was heated to mimic the volatiles (and hence odour) that might be detected in an encounter between two live robins. To this end, a thermocouple was attached to the insert holder to control the heating rate and ensure the temperature was similar to the body temperature of an active bird during the entire duration of the experiment (~42°C; Prinzinger, Pressmar, & Schleucher, 1991). Heating the sample also allowed me to control for any variation in response that might be due to ambient temperature. As the preen wax within the presentation box was not visible, the only cue presented to the robin was the volatiles arising from the preen wax when the chamber was opened. To further camouflage the presentation box, it was covered with a black papier-mâché structure, which itself was camouflaged with leaves and twigs. These were changed for each experiment to cover any scent that could have been left by the previous bird. The control treatment consisted of the presentation of an empty glass insert with no preen wax.

To conduct an odour recognition experiment, I first located a male robin. I quickly set up the box on the ground in the male's territory, placed a frozen insert containing the preen wax inside the chamber and started the heating process. Less than a minute was required for the preen wax sample (or control) to be heated to 42°C. To attract the bird to the box, I then placed a total of 5 mealworms (*Tenebrio molitor*) in a cup on top of the box but on the opposite side of the opening to the chamber (figure 4.1). I also positioned a wooden stick,

about 10-15 cm high, on the side of the box to encourage the bird to perch on it while reaching for the mealworms. In this position, the robin would be placed immediately above the opening of the chamber and thus exposed to the volatiles from the preen wax sample when the chamber door was opened. The wooden stick and the black paper on top of the presentation box were changed between each experiment. The plastic dish holding the mealworms and the chamber were wiped cleaned with ethanol between tests, thus minimising any odour cue left by a previous bird. Recording started when the bird landed on the box and lasted for 5 minutes. As soon as the male landed on the box, the door to the chamber was opened, exposing the bird to the odour cue. The chamber remained open for the duration of the experiment. Each male was randomly presented with either a control or an experimental treatment, on average 26 days apart (1-66 days). Variation in timing between trials was due either to poor weather or to the time needed to relocate a previously tested bird. All experiments were filmed with a digital Canon Powershot SX210 camera mounted on a tripod. During all trials, the camera remained in one spot 3 m from the box.

Data extraction and analysis

From the video recordings I noted five behavioural responses and calculated their frequencies. I categorised the responses of robins into three types of signals: visual, vocal and tactile. The visual signals were (1) raising the forehead feathers, (2) wing flicking, and (3) hopping. The feathers on the forehead of South Island robins are white and erection of these feathers may function in agonistic displays between conspecifics (Powlesland, 1980). Wing flicking involved the bird rapidly opening and closing its wings. Hopping was defined as the bird making a large sidewise hop away from the presentation box. Both wing flicking and hopping appear associated with a heightened state of alertness, and are often seen in encounters between two birds. Vocal signals consisted of (4) short alarm calls and bill snapping. An alarm call comprised a single note given in sequence; its use has been linked with aggressive situations (Higgins & Peter, 2002). Bill snapping is a dominance sign (Powlesland, 1980) and is typically given when a male chases away another bird. For analysis, I combined both types of vocal signals and refer to them as "vocalization". Finally, tactile behaviours were defined as the bird directly (5) pecking at and tearing apart the chamber.

Each type of behaviour was scored as a binomial response (presence or absence of a behaviour per bird) and this was analysed using the Freeman-Halton extension of the Fisher exact probability test (Freeman & Halton, 1951). The frequency of each of the five behaviours during the 10 min observation period was also compared among treatments with Mann-Whitney tests, using the programme STATISTICA 6.0, ©StatSoft Inc. I used unpaired tests and the whole range of our available data (including results for 3 unpaired males) to maximise my results.

Results

Male robins were more likely to peck the presentation box (Fisher test: df = 1, $p = 0.039$), wing flick (Fisher test: df = 1, $p = 0.03$) and hop (Fisher test: df = 1, $p = 0.041$) when presented with the preen wax of a non-neighbouring conspecific male than when presented with a control (figure 4.2). On the other hand, whether a male vocalised (Fisher test: df = 1, $p = 0.16$) or raised his forehead feathers (Fisher test: df = 1, $p = 1$) did not vary between exposure to the preen wax or to the control (figure 4.2).

The frequency of pecking (Mann-Whitney test, U = 48, Z = 1.63, $p = 0.019$) was significantly higher in the preen wax treatment than in the control treatment (figure 4.3). Although the frequency of wing flicking (Mann-Whitney test, U = 44.5, Z = 1.82, $p = 0.059$) and hopping (Mann-Whitney test, U = 46.5, Z = 1.71, $p = 0.069$) were also higher in the preen wax treatment than in the control, this difference was not quite significant. The frequency of the feather raise display (Mann-Whitney test, U = 66.5, Z = 0.62, $p = 0.51$) and vocalization (Mann-Whitney test, U = 56, Z = 1.20, $p = 0.11$) did not differ with the type of treatment (figure 4.3).

Discussion

Robins reacted aggressively to the preen wax of a conspecific male. The apparent odour of a conspecific in their territory resulted in males being more likely to physically attack the presentation box and increase levels of activity such as hopping and wing flicking. My results suggest that male robins can recognise the presence of another robin solely by olfactory cues and in the absence of any visual or auditory cue from a conspecific. Thus,

my results add to a growing body of literature suggesting that odours - and specifically preen wax odours - can function in intraspecific communication in birds.

Most of the evidence for chemo-signalling in other birds has came from studies on Procellariiformes; as these are nocturnal seabirds living in big colonies, the role of olfaction may be especially important in conspecific communication as other modes of signalling may be less effective. For example, researchers have shown that seabirds use their sense of smell for homing to their burrow in large colonies, finding their nest and recognising their partner (Bonadonna et al., 2004; Mardon & Bonadonna, 2009). However, the evidence for olfactory communication in other groups of birds is limited. Hirao et al. (2009) and Zhang et al. (2010) found some evidence that preen waxes may be used in mate choice in chickens (*Gallus domesticus*) and budgerigars (*Melopsittacus undulatus*), respectively. The first evidence of intraspecific chemical communication in a passerine bird was found by Whittaker et al. (2011). Using 2-way choice tests, they showed that dark-eyed juncos (*Junco hyemalis*) could discriminate the sex of conspecifics solely on the basis of uropygial gland secretions. Although the number of studies is still limited, the existing evidence (including this study) suggests that chemical communication in birds is probably more widespread than currently appreciated.

Robins responded to the odours of preen waxes by increased movements (hopping and wing flicking) and by directly attacking the source of the odour by pecking the opening of the chamber. The increased expression of wing flicks, in which the wings are rapidly opened and closed, appears to be a signal associated with aggressive interactions between birds and is sometimes seen when two birds encounter each other at close range (pers. obs.; Powlesland 1980). As no other robins were present during our experiments, the increased wing flicking appears to have been stimulated by the presence of odour alone. Similarly, increased levels of hopping may indicate a heightened state of readiness to engage in interactions with a rival, and the direct attacks on the chamber are clearly a strong indication of aggressiveness directed toward the source of the odour. However, I failed to observe a significant increase in other aggression-related behaviours (e.g., raising of white forehead feathers, increased alarm calls). Perhaps some behaviours are only elicited in the presence of visual and/or auditory signals from a conspecific and not by odour alone.

Although I found evidence that South Island robins responded to the odour of preen wax from conspecifics, some individuals did not react differently to the experimental and the control treatment. I can think of several explanations for this lack of difference in response. First, it is possible that some birds could not perceive any odour as they were positioned outside the stream of volatiles coming from the chamber. Such an event could occur if the wind carried the volatiles away from the chamber and the perched robin. Although I tried to avoid this by sampling only on days with low wind, the invisibility of volatiles meant I could not confirm they were always projected directly onto the perched bird as I assumed. Second, variation in the amount of wax collected and in the chamber may explain some of the variation in response. If the volume of wax was too small, there may not have been enough to be detected by the perched robin. However, this explanation seems unlikely as I used samples of similar size (at least half of the end of the wire loop had to be filled). Third, I do not have information about aggressive interactions prior to the experiment. Some robins may have been involved in aggressive behaviour or exposed to a conspecific intruder before the experiment started, and this might have influenced their response during the experiment. Finally, individuals may differ intrinsically in their aggressiveness. Some birds might not have reacted because they might not have felt threatened by the odour of an intruder alone. The chemical composition of the preen wax might provide information about the status of the individual. For example, if the preen wax I used was collected from a subordinate bird or a juvenile from the previous breeding season, it might not pose enough of a threat to the focal male to initiate energetically costly behaviours such as attacks or vocal signals. Unfortunately, I do not have information about the dominance status of the individuals from which I collected preen wax. None were direct neighbours, so I can rule out that some of the focal males were familiar with the odours of some birds.

I conducted my experiments just prior to the start of the breeding season, which is a time when males are strongly defending their territories and when the risk of intruding males (seeking either territories of their own, or extra-pair copulations) was expected to be high. At such times it could be advantageous for males to be able to detect the presence of an intruder that behaves stealthily and is thus unlikely detected by visual or auditory cues. Although my results clearly suggest that males can detect the presence of a conspecific male based on just the odour of his preen wax, more work is needed to determine if such

recognition plays a role in conspecific territorial interactions. Whether intruding males leave an olfactory "trail" and whether resident males can detect such a signal remains unknown. However, it is interesting to note that on a number of occasions I saw robins wiping their bill on branches throughout their territory (often after preening or eating) and this could function in part to leave an olfactory "scent mark" that either advertises (territorial male) or betrays (intruding male) a bird's presence.

My experiment was conducted with free-living birds in their natural environment. As robins are naturally curious and tame, they readily approached the chamber on their own and were free to leave. There was no "training period" to use the chamber because birds readily approached and perched to retrieve the mealworms provided. All experiments were conducted on the ground and in an individual's territory. Although this likely reduced any potential artefacts and stress that could result from a similar study in captivity, it meant I could not control some variables, such as the time spent by the birds on the perch, the period between the control and experimental treatment, the birds' hunger level, their mating status, or their recent experience before the treatment. Despite such confounding factors, the responses I found suggest that olfactory signals can be detected by birds in their own environment.

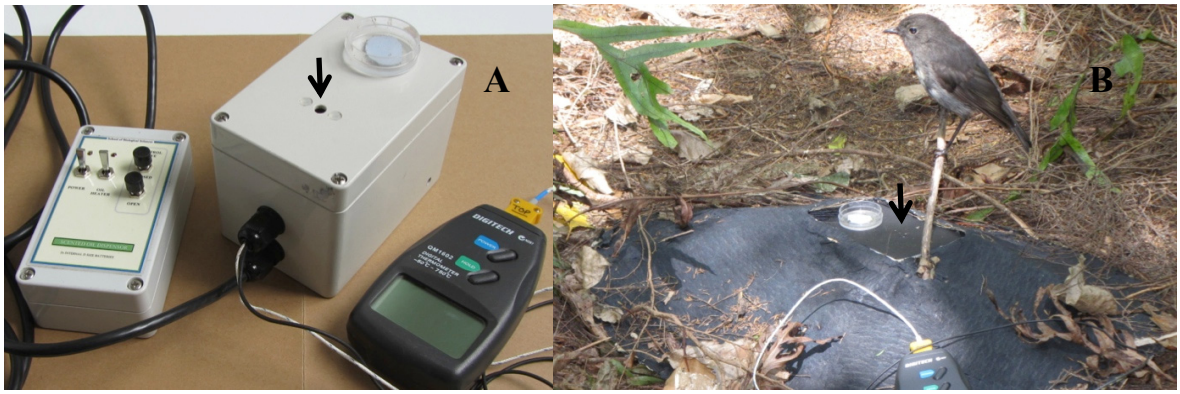

Figure 4.1. **A**. Preen wax presentation box, with the remote control on the left and the thermocouple on the right. Note the chamber door (pointed out by the arrow) and the plastic dish for worms. **B**. South Island robin (*Petroica australis*) perched on the camouflaged preen wax presentation box. As the bird leans forward to grab a worm in the small plastic dish, it is exposed to the odour of a conspecific male coming out by the chamber door (arrow).

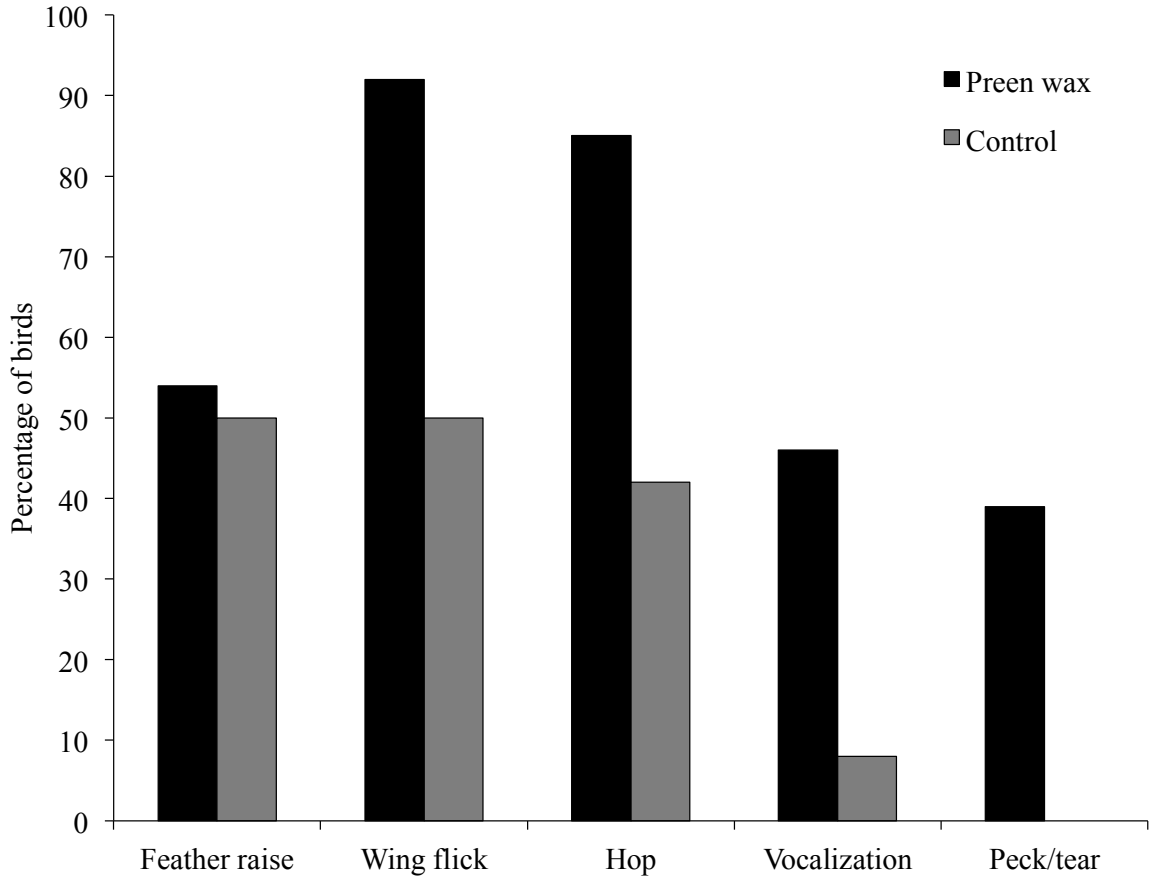

Figure 4.2. Proportion of male South Island robins (n = 14) and the type of behaviour they expressed when presented to either the preen wax of a conspecific or a control. Males reacted to the preen wax of a conspecific male by pecking the presentation box and by an increase of visual signals such as hopping and wing flicking.

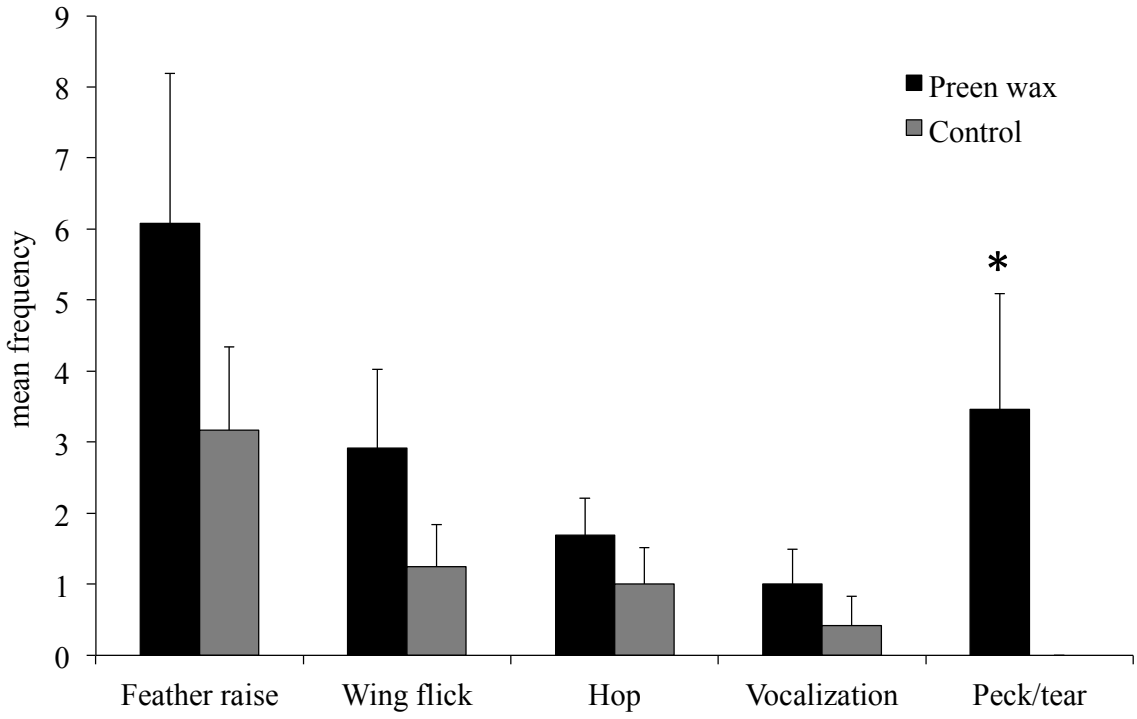

Figure 4.3. Mean frequencies of behaviours expressed by male South Island robins (n = 14) exposed to either the preen wax of a conspecific male or a control. * indicates a significant difference from expected ($p < 0.05$). Vertical bars indicate standard error.

Chapter 5

General Discussion

Until recently, research on olfaction in birds has been relatively overlooked and therefore the field is still very much in its infancy. While we know much about the visual abilities of birds, the functions of their beautiful colours and the complexity of their songs, in comparison we know little about their sense of smell, nor how olfaction might be used (if at all) in communication. For example, a quick search for scientific papers on bird song published in the last 50 years using Web of Science identified >1000 entries, while the same search engine found only 73 papers that examined any aspect of avian olfaction. This asymmetry in attention however provides a unique opportunity to take a second look at birds, and ask whether key concepts that were developed from studies of avian vocal and visual communication might be worth revisiting from a different angle, namely in terms of olfactory channels. It also means that for now, there are often more questions than answers. The aim of this thesis was to highlight as well as answer some of these questions.

I chose to examine odours in birds by first using a broad-scale comparison between Pacific island passerines and their phylogenetic relatives on the Australian continent. This allowed me to determine if the evolutionary processes that shaped the odours in birds were different between continental and insular birds. My first data chapter (chapter 2) approached this question from 2 angles, a morphological and a structural point of view. First, I compared the uropygial gland size of 34 different species and discovered that island birds on average had a significantly larger uropygial gland than would be expected by their body mass. This result remained significant when controlling for phylogenetic effects. Although variation in uropygial gland size has been noted before, and correlated with factors such as body size, and migratory behaviour (Vincze et al., 2013), my study is the first to provide evidence of a significant morphological difference in this structure between island and continental birds. This raises new questions on why island birds have larger preen glands, and assuming larger preen glands lead to increased rates of production, why would they need to produce more preen wax?

The second part of chapter 2 examines how preen waxes of different species differ in composition. When compared with the preen waxes of continental species, island birds

showed a contrasting pattern with a loss of complexity in the non-volatile fraction of their preen wax, comprised of the "long-lasting" type of molecules (e.g. lipids), while at the same time showing a significant gain of diversity in the volatile fraction of their preen wax. This result was confirmed when controlled for phylogeny. The simultaneous loss of heavy esters and the increase in lighter volatiles suggests a general shift towards lighter and more volatile preen waxes in island birds. As mentioned earlier, selection could favour this trend as island birds were released from the constraint to camouflage their odours that is imposed by mammalian predators on continental areas. It is then possible that this in turn allowed greater communication through olfactory channels, and such communication is enhanced through the use of more volatile compounds.

One consequence of having a larger uropygial gland that is producing more preen wax could be an increase in the odour of an individual bird and perhaps its detectability by predators that use olfaction to locate their prey. In other words, preen wax production is likely to have both benefits and costs, and it is differences in these between insular and continental birds that could lead to changes in uropygial gland size, and differences in preen wax composition and rates of production. Changes in preen wax production due to shifts in the risk of predation (i.e., reduced costs) could be particularly plausible on remote and oceanic islands where birds largely evolved in the absence of predatory pressure from mammals and snakes (which use olfactory cues to find prey). Chapter 3 address this hypothesis by comparing the attraction of Norway rats (a common introduced predator on islands) to the preen waxes (odours) of island and continental species of birds. I used tests, both in a Y-maze laboratory setting and in choice experiments in the field, to determine if preen wax can be detected by a potential mammalian predator and if this differs among species of birds. The laboratory results showed that rats were indeed attracted to the most volatile preen wax, but other tests were less conclusive and highlight the need for more experiments in order to confirm (or disprove) the hypothesis of "smellier" island birds being more at risk from introduced predators.

The key to interpreting the evolution of odours in birds lies in understanding the functions of the preen wax. What are they? Why and how do they differ on islands? Although addressing all possible functions was beyond the scope of a single thesis, in chapter 4, I test one possible function of preen wax and conclude that in at least some species it may play a role in communication and signalling. The South Island robin (*Petroica australis*) is

a tame and territorial bird from New Zealand that produces unusually volatile preen waxes during breeding. When free-living males were experimentally presented with the preen wax (and thus odour) of conspecific males, they responded aggressively, suggesting that even in the absence of visual cues, the odours produced by preen wax could be detected by conspecifics and could function in intraspecific communication. Island birds may have developed larger preen glands because they had the need or an increased opportunity to develop this channel of communication. On continental areas, birds are exposed to harsher environmental pressures (more parasites, more predators, more interspecific competition) and therefore may be more limited in using olfactory cues for communication. At present, this is just conjecture as more experiments need to be done on both island birds as well as their continental counterparts, to determine the relative importance of olfaction in communication. Although there is some evidence for olfaction being used for communication in some continental birds (e.g. dark-eyed junco, *Junco hyemalis*; Whittaker et al., 2011), it is interesting to note that those species most noted for the use of olfaction (e.g. in recognition of mates and burrows) are seabirds confined to islands free of mammalian predators (Bonadonna et al., 2004; Mardon et al., 2010).

To summarise my findings, I found that island birds have larger uropygial glands, which suggests they produce more preen wax than their continental relatives. Preen wax composition also differed between species with a shift on islands to lighter and more volatile compounds, possibly to function for increased chemical communication by insular birds, as shown with the South Island robin. Whether this gain of "volatility" in insular birds induces an increased risk of predation by exotic mammals is probable but still unknown. It is possible that the level of conspicuousness is species-specific and some island species have preen waxes (and thus odours) that are also especially attractive to exotic mammalian predators.

Further work

As is common in any scientific study, especially one in the early stages of a discipline, my results raised more questions than they answered. For example, my suggestion of increased chemical communication in island birds could be explored further by, for example, testing the type of information preen waxes give about the status of an individual. Can birds recognise by odour alone their own kin, and if so, is this learnt (e.g., in the nest) or is it

innate? For example, robins tolerate their juveniles on their territory for almost a year (pers. obs.) but do they recognise their own young just by visual or auditory cues (which change as birds mature, moult and learn new songs) or is it based on olfactory cues? Could birds also use olfactory cues to differentiate between dominance status of other individuals in the population, including their neighbours, or even in mate choice (including whether a male could determine whether a female has engaged in an extra-pair copulation by detecting the odour of a rival male's preen wax on his mate's feathers)? Could birds use odour cues as a mean to mark their territories, as is common in most species of mammals but absent from birds, except a suggestion that it could occur in the North Island kiwi, *Apteryx australis mantelli* (Taborsky & Taborsky, 1992)? The possibility is not too far fetched, even for passerines, as I observed on a number of occasions robins wiping their bill on branches throughout their territory after a preening session, and this could function in conspecific territorial interactions, either by advertising or betraying a bird's presence. These are just a few examples, and there is much more that can be tested and explored.

Another field worth exploring is the functions of preen waxes on plumage maintenance and whether this differs across species and in different environments. An enhancement in colours and plumage ornamentation by the use of preen waxes has been shown in some continental species (Delhey et al., 2007; Lopez-Rull et al., 2010). Since island birds have a general trend of being duller coloured, does having less conspicuous plumage means there is a similarly diminished requirement for preen waxes, or perhaps a diminished need for certain compounds in preen wax? Does a generally darker plumage require less or more preen wax for its maintenance? At present, we have no information that would answer such questions. Similarly, it would be interesting to study the rate at which preen waxes are degraded and lost from the feathers and compare this between birds living in open or closed habitats. I earlier suggested that feathers could be more damaged in the generally denser forests of New Zealand and New Caledonia compared with the more open eucalypt forests of Australia. It is possible that species produce more preen wax simply due to higher rates of loss once spread onto their feathers.

Another important selective force impacting avian life histories is parasitism, including ecto-parasites that live in the feathers and some of which are known to feed on preen wax (Dobson & McCallum, 1997). However, parasite communities are thought to be smaller on islands (at least in diversity of species; Dobson et al., 1992), and the consequence of this

difference on the evolution of avian preen waxes is unknown. Studies of continental bird species showed that uropygial secretion can function to repel ectoparasites (e.g. lice, flies) and limit or inhibit the growth of detrimental feather-degrading bacteria (Douglas, 2008; Shawkey et al., 2003). It has also been discovered that the great tit (*Parus major*) can alter the relative abundance of some wax compounds in response to experimentally modified bacterial loads on feathers (Jacob et al., 2014). This kind of knowledge is lacking for island birds. However, with impoverished parasite communities, it might be easier to use island rather than continental birds to search for specific preen wax compounds which act on a specific parasitic species or family. With my finding that island birds have a different preen wax than their continental relatives, odour-parasite interactions might be a particular productive area of future investigation.

Finally, the idea that character evolution may differ on islands from that on the mainland, and that there may be trade-offs between visual, auditory and olfactory signals deserves more attention. Such work is currently underway, as I am carrying out a collaborative study with A. Kearns, N. Friedman and K. Omland, on character evolution using a phylogenetic approach. Our project is examining character evolution simultaneously in colours, songs and odours using a single family of birds, the Australasian robins (Family Petroicidae) as a model system. This family is particularly good for such a study as it includes a number of species that have independently colonised (and speciated on) islands in the South Pacific from source populations on mainland Australia. Our objective is to determine if island birds show a loss of complexity in colours and songs and whether the degree of loss relates to increased changes in their preen wax and use of olfaction. This project is expected to bring a synthesis of the avian sensory modality, biogeography and sexual selection.

Implications for conservation

One of the aims of my thesis was to contribute not only to understanding the evolution of odours in birds, and learning something about their function, but also whether what I could learn would assist in the conservation of the birds I studied. It is the sad truth, that extinction has affected island birds disproportionately to their numbers, and both island avifaunas I studied have a long list of extinct species, plus many more that are endangered

or declining as the result of the accidental and misguided introductions of mammalian predators to places they do not belong.

Although I did not focus my thesis on a particular endangered species in terms of conservation management, my findings highlight the potential value of investigating further the link between odour and predation risk in island birds. Firstly, I confirmed that island birds in general appear to produce more wax and they also produce more volatile molecules compared to continental birds. Thus, it is likely that island species vary in the amount or in the volatility of their preen waxes, which in turn could increase the risk they are detected by introduced mammalian predators using olfactory cues to locate their prey. The first step in determining whether odour is a "problem" for a particular endangered species is relatively simple. The process of identifying a species with a strong "smell" would only require a researcher to collect and analyse the preen waxes through a GC-MS, a relatively easy and affordable method. It would then be straightforward to classify species into two groups, birds with relatively low proportion of volatiles and "not at risk" from their odours, and birds with a high volatility preen wax and at greater risk from their odours. Coupled with the endangered status of a bird, this method could identify those species particularly at risk from their conspicuous body odours. The next step would be to devise ways to minimise the risk to a bird despite its more conspicuous odour. It is possible that with time, selection would favour a decrease of the uropygial gland size and the production of less volatile molecules, however it is unlikely that this change would happen quickly and therefore might not be the best option for highly endangered species. Another option would be to somehow artificially mask the odour of these "stinky" birds, particularly around their nests when they are incubating and most vulnerable. Some chemicals or materials could be used to absorb the odours of the bird. Other chemicals could be used to repel the predators in a similar manner to what starlings (*Sturnus vulgaris*), hoopoes (*Upupa epops*) or hoatzins (*Opisthocomus hoazin*) are already known to do, producing such strong odours that even humans avoid them or their nests (Stanbury, 2010; Weldon & Rappole, 1997). A third option could be to confuse potential predators by overwhelming the surroundings with a kaleidoscope of odours (Price & Banks, 2012), a little like when we shop in a perfumery, we are unable to find the perfume we want by smell as we are overwhelmed by the scent of the other perfumes. However, we should be careful in manipulating the olfactory environment, as it is possible that a bird which is

particularly “smelly” is for a reason, as I described in chapter 4 with the robins. Caution should be taken as to not disrupt any chemical signals emitted by the birds as they may be critical for communication. Finally, just as light and noise pollution have become recognised as major issues in avian conservation, including disrupting sexually selected signals (Kempenaers, Borgström, Loës, Schlicht, & Valcu, 2010), it is possible that humans may be similarly impacting on olfactory channels of communication in birds through air pollution (e.g. car exhaust), at least at a local level.

The study of odours in birds clearly opens new opportunities to study evolution as well as to apply this new knowledge to the conservation challenges facing island birds. This is a new and exciting field that could re-define what we know about birds and how they perceive the world they live in. Odours have clearly evolved in birds, they are being used for communication, but also may be intercepted by unintended recipients such as introduced predators, to the detriment of the signaller. With so many new areas of research opening up by the realisation that birds do have a sense of smell, the next few decades should lead to many exciting discoveries.

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