BIOLOGICAL REVIEWS
Cambridge Philosophical Society

Biol. Rev. (2022), pp. 000–000.
doi: 10.1111/brv.12837

Olfactory camouflage and communication in birds

Leanne A. Grieves[1†*], Marc Gilles[2†*], Innes C. Cuthill[3], Tamás Székely[4,5], Elizabeth A. MacDougall-Shackleton[6] and Barbara A. Caspers[2]

[1]*Department of Psychology, Neuroscience and Behaviour, McMaster University, Hamilton, Ontario, L8S 4M4, Canada*
[2]*Department of Behavioural Ecology, Bielefeld University, Konsequenz 45, Bielefeld, 33615, Germany*
[3]*School of Biological Sciences, Life Sciences Building, 24 Tyndall Avenue, Bristol, BS8 1TQ, U.K.*
[4]*Milner Centre for Evolution, Department of Biology and Biochemistry, University of Bath, Bath, BA2 7AY, U.K.*
[5]*Department of Evolutionary Zoology and Human Biology, University of Debrecen, Egyetem ter 1, Debrecen, H-4032, Hungary*
[6]*Department of Biology, University of Western Ontario, 1151 Richmond Street North, London, Ontario, N6A 5B7, Canada*

ABSTRACT

Smell is a sensory modality that is rarely considered in birds, but evidence is mounting that olfaction is an important aspect of avian behaviour and ecology. The uropygial gland produces an odoriferous secretion (preen oil) that can differ seasonally and between the sexes. These differences are hypothesized to function in olfactory camouflage, i.e. minimizing detection by nest predators (olfactory crypsis hypothesis), and/or intraspecific olfactory communication, particularly during breeding (sex semiochemical hypothesis). However, evidence for seasonal and sex differences in preen oil is mixed, with some studies finding differences and others not, and direct evidence for the putative function(s) of seasonal variation and sex differences in preen oil remains limited. We conducted a systematic review of the evidence for such changes in preen oil chemical composition, finding seasonal differences in 95% of species (57/60 species in 35 studies) and sex differences in 47% of species (28/59 species in 46 studies). We then conducted phylogenetic comparative analyses using data from 59 bird species to evaluate evidence for both the olfactory crypsis and sex semiochemical hypotheses. Seasonal differences were more likely in the incubating than non-incubating sex in ground-nesting species, but were equally likely regardless of incubation strategy in non-ground-nesting species. This result supports the olfactory crypsis hypothesis, if ground nesters are more vulnerable to olfactorily searching predators than non-ground nesters. Sex differences were more likely in species with uniparental than biparental incubation and during breeding than non-breeding, consistent with both the olfactory crypsis and sex semiochemical hypotheses. At present, the data do not allow us to disentangle these two hypotheses, but we provide recommendations that will enable researchers to do so.

Key words: bird odour, chemical cues, infochemicals, mate recognition, olfaction, parental care, preen oil, scent, sexual selection, uropygial gland secretion

CONTENTS

I Introduction 2
II Methods 4
  (1) Literature review 4
  (2) Preen oil chemical differences 4
  (3) Seasonal differences 5
  (4) Sex differences 5
  (5) Statistical analyses 5

---

* Authors for correspondence (Tel.: +1905 525 9140 ext. 23194; E-mail: grievel@mcmaster.ca); (Tel.: +4915734777035; E-mail: marc.gilles@live.fr)
† Co-first authors (contributed equally to this work).

III Results 7
(1) Literature review 7
(2) Seasonal differences 7
(3) Sex differences 7
IV Discussion 7
(1) Olfactory crypsis 8
(2) Sex semiochemicals 10
(3) Mechanisms of seasonal and sex differences 11
(*a*) Diet 11
(*b*) Symbiotic microbes 11
(*c*) Hormones 11
V Recommendations for future research 11
(1) Sampling and study design 11
(2) Hypothesis testing 12
(*a*) Olfactory crypsis hypothesis 12
(*b*) Sex semiochemical hypothesis 13
VI Conclusions 13
VII Acknowledgements 13
VIII Data accessibility 13
IX. References 14
X. Supporting information 16

I INTRODUCTION

All animals produce odours, either as metabolic by-products or as chemicals secreted by specialised glands. These odours can provide information about the producer that can be used during interspecific interactions (e.g. to detect the presence of potential predators or prey) or during intraspecific interactions (e.g. to assess the age, sex, relatedness, or genetic compatibility of a potential mate). In birds, body odours can derive from various sources, including faeces, blood, stomach oils, powder down, plumage, and from secretions of the anal gland, salt gland, salivary gland, ear glands, sebokeratocytes, or skin (Hagelin & Jones, 2007). Recently, much attention has focused on the odour-producing role of the uropygial or preen gland (Moreno-Rueda, 2017; Whittaker & Hagelin, 2020). The preen gland, located near the base of the tail, is present in almost all bird species (Johnston, 1988; Moreno-Rueda, 2017). The gland secretes preen oil, a complex mixture of wax esters (monoesters, diesters, and triesters) and other compounds (e.g. alcohols, alkanes, aldehydes, carboxylic acids, ketones; reviewed in Campagna *et al.*, 2012). Early work on preen oil was primarily descriptive, but there has been a remarkable growth in preen oil research, particularly with respect to its putative functions (reviewed in Moreno-Rueda, 2017; summarized in Fig. 1).

Over the past 20 years, researchers have begun to explore preen oil from the perspectives of ecotoxicology [effects of environmental pollutants on preen oil composition, a role for preen oil in pollutant depuration (López-Perea & Mateo, 2019; Grieves *et al.*, 2020)]; chemical defence [antimicrobial/antiparasitic activity, predator repellence, olfactory crypsis (Burger *et al.*, 2004; Reneerkens *et al.*, 2007a; Martín-Vivaldi *et al.*, 2010)]; vector attraction (preen oil as an attractant to parasite vectors such as mosquitoes; reviewed in Martínez-de la Puente *et al.*, 2020); species recognition and speciation [testing for chemical signatures of preen oil useful for taxonomic classification (Zhang, Du & Zhang, 2013; Gabirot *et al.*, 2016)]; and intraspecific communication [reproductive and social signalling (reviewed in Caro, Balthazart & Bonadonna, 2015; Whittaker & Hagelin, 2020)]. Researchers have also continued to study the mechanisms underlying preen oil production and chemistry [e.g. diet, endocrine regulation, symbiotic microbes (Thomas *et al.*, 2010; Whelan *et al.*, 2010; Whittaker *et al.*, 2019b)]. Despite this growth in research, the mechanisms of preen oil production and variation – as well as the

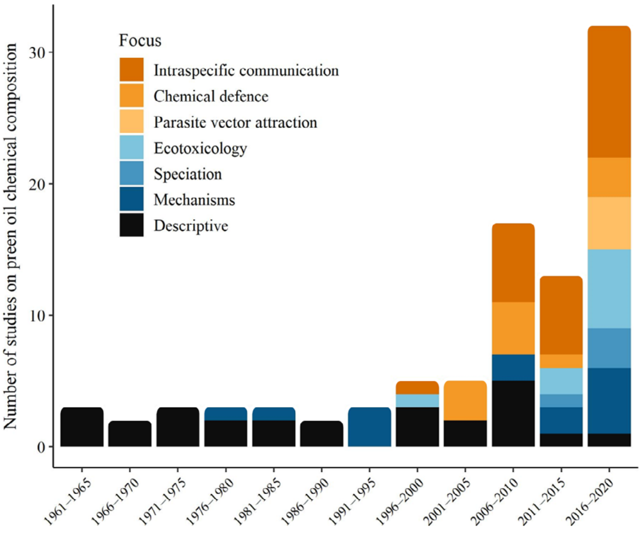


Fig. 1. Major study topics on preen oil chemical composition (97 studies). See Appendix S1 and Table S3 for further details.

putative functions of preen oil – are still poorly understood across all research areas. Thus, there is ample opportunity for researchers to make novel and valuable contributions to our understanding of preen oil production and its function in birds.

Some of the functions of preen oil, including waterproofing, feather maintenance, and pollutant depuration, depend on its physical (i.e. oily, waxy) structure. In addition to these structural functions, preen oil is also odoriferous and considered to be a major source of avian body odour (Hagelin & Jones, 2007; Caro *et al.*, 2015). Accordingly, preen oil has been hypothesized also to act as an infochemical (Müller *et al.*, 2020) during intraspecific interactions (reviewed in Moreno-Rueda, 2017), or as a deleterious cue that reduces detection by predators, such that downregulation of its production, or volatility, would be indicative of olfactory crypsis.

Crypsis is the avoidance of detection through camouflage (Stevens & Merilaita, 2009). While most studies of crypsis involve vision, crypsis can also involve olfactory concealment (Ruxton, 2009). Birds in a nest can emit odours at all life stages (as eggs, chicks, and adults) and may be vulnerable to olfactorily searching nest predators such as mammals as a result. Birds should therefore benefit from olfactory crypsis at the nest (Shutler, 2019), especially since nest predation is a primary cause of reproductive failure (Martin, 1993). As such, birds might alter their odours to become less detectable to predators, especially during the critical period of nesting. By contrast, the use of sex semiochemicals for intraspecific chemical communication during breeding suggests that individuals might alter their odours to convey information to and/or modulate their detectability by conspecifics.

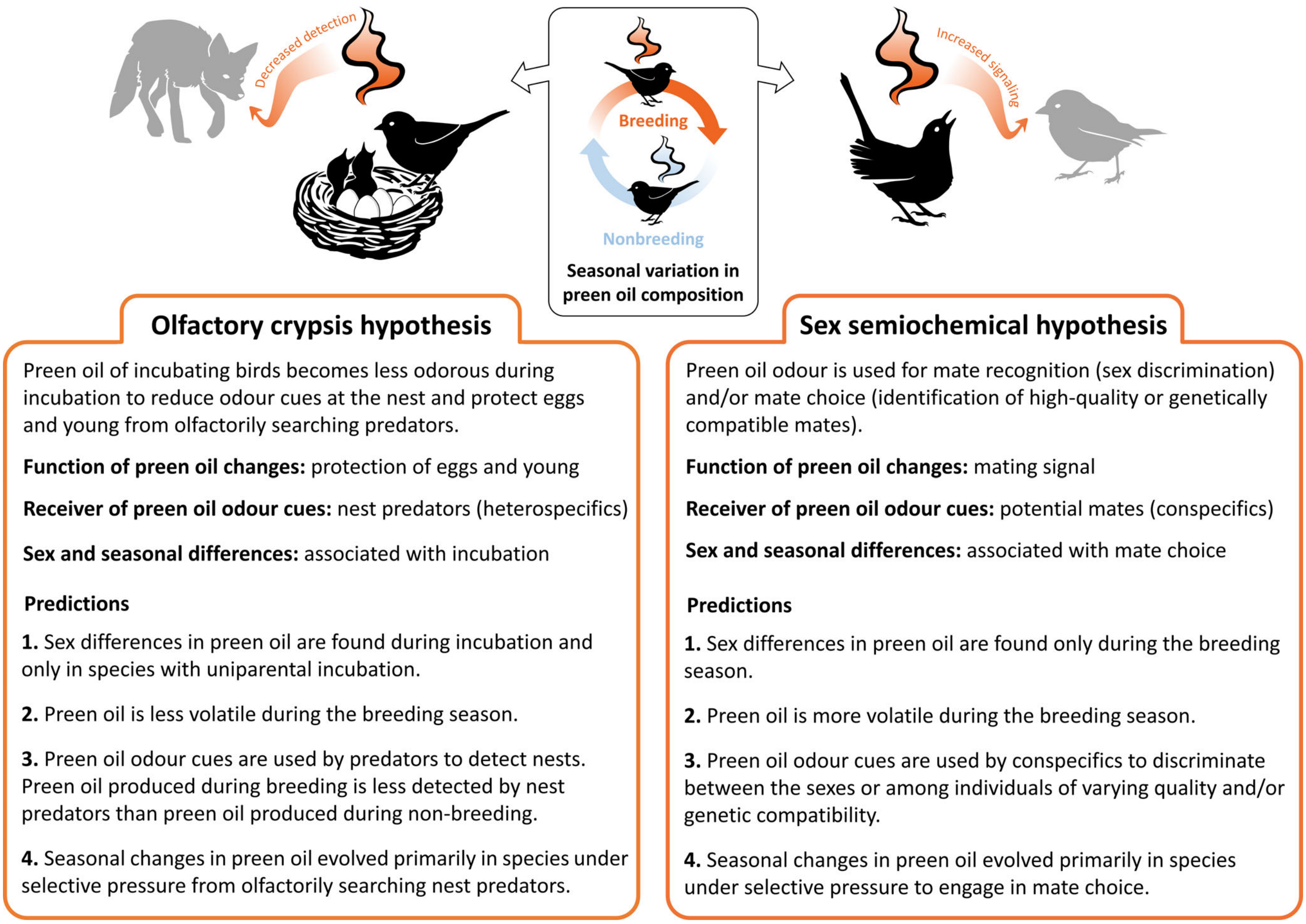
The chemical composition of preen oil is dynamic and can be affected by diverse factors, including diet (Thomas *et al.*, 2010; Leclaire *et al.*, 2019), food stress (Reneerkens, Piersma & Damsté, 2007b; Grieves *et al.*, 2020), infection status (Grieves *et al.*, 2018), plumage and preen gland microbiota (Jacob *et al.*, 2014; Whittaker *et al.*, 2019b), major histocompatibility complex (MHC) genotype, age (Shaw *et al.*, 2011; Grieves, Bernards & MacDougall-Shackleton, 2019b), hormone levels (Bohnet *et al.*, 1991; Whittaker *et al.*, 2018), season (Bhattacharyya & Chowdhury, 1995; Soini *et al.*, 2007), and sex (Jacob, Balthazart & Schoffeniels, 1979; Whittaker *et al.*, 2010). Seasonal and sex differences in preen oil composition may translate into seasonal and sex differences in odour, which could be linked to specific functions for olfactory crypsis and/or intraspecific communication. Avian preen oil thus has the potential to act as an infochemical that conveys a diversity of information to conspecifics, or as a deleterious cue that masks information from heterospecifics.

Avian chemical communication has been understudied because birds were historically believed to possess little to no sense of smell (Stager, 1967; Bang & Cobb, 1968). Fortunately, our understanding of avian chemical communication is growing rapidly. Indeed, birds use smell in intraspecific social contexts such as species discrimination (Zhang *et al.*, 2013; Krause *et al.*, 2014; Van Huynh & Rice, 2019), mate recognition (Bonadonna & Nevitt, 2004), kin recognition (Coffin, Watters & Mateo, 2011; Bonadonna & Sanz-Aguilar, 2012; Krause *et al.*, 2012; Caspers, Gagliardo & Krause, 2015; Caspers *et al.*, 2017), individual recognition (Bonadonna *et al.*, 2007; Bonadonna, Caro & Brooke, 2009; Fracasso *et al.*, 2018), distinguishing sex (Hirao, Aoyama & Sugita, 2009; Whittaker *et al.*, 2011a; Amo *et al.*, 2012; Grieves, Bernards & MacDougall-Shackleton, 2019a), and distinguishing the MHC genotype of potential mates (Leclaire *et al.*, 2017; Grieves *et al.*, 2019c).

We systematically reviewed the literature on seasonal and sex differences in preen oil composition to investigate two non-mutually exclusive hypotheses. First, the 'olfactory crypsis hypothesis' posits that incubating birds switch from more odorous to less odorous preen oil during incubation as a means of reducing odour cues at the nest, thereby protecting eggs and young from olfactorily searching predators (Reneerkens, Piersma & Damsté, 2002; Reneerkens *et al.*, 2007a). Because less-odorous (higher molecular weight) preen oil is presumably more costly to produce, and perhaps also to apply (Reneerkens *et al.*, 2007b), it is predicted to be secreted only during incubation, when the benefits of crypsis outweigh the costs of production (Reneerkens, Piersma & Damsté, 2006). This hypothesis predicts an effect of both breeding stage and incubation type on the chemical composition of preen oil. Preen oil changes should occur specifically during incubation and only in the incubating sex, leading to sex differences in uniparentally incubating, but not biparentally incubating, species. Changes in preen oil composition specifically associated with incubation should have evolved primarily in species under strong selective pressure from olfactorily searching nest predators (Reneerkens *et al.*, 2006). Notably, this hypothesis assumes that nest predators should be better at detecting low molecular weight than high molecular weight preen oil (Reneerkens, Piersma & Damsté, 2005).

Next, we introduce the 'sex semiochemical hypothesis', which posits that sex differences in preen oil are associated with mate recognition (identifying the appropriate sex to mate with) and/or mate choice (identifying a suitable, e.g. genetically compatible, mate). The sex semiochemical hypothesis predicts that sex differences in the chemical composition of preen oil should be found only during breeding (particularly during mate pairing and egg laying), and that birds should use preen oil odour cues to discriminate between the sexes and/or among individuals. We expand on these two hypotheses further in Fig. 2.

The olfactory crypsis and sex semiochemical hypotheses are based on the odoriferous nature of preen oil. However, preen oil may also serve as a chemical defence against a range of parasites, including eggshell bacteria, feather-degrading bacteria, chewing lice, and mosquitoes (reviewed in Moreno-Rueda, 2017), and such antiparasitic defence does not require preen oil to be odoriferous (though chemical defences can indeed be odorous). Thus, the antiparasitic defence hypothesis is also non-mutually exclusive with the olfactory crypsis and sex semiochemical hypotheses. Due to a paucity of data, we were not able to conduct a comparative



Olfactory crypsis hypothesis

Preen oil of incubating birds becomes less odorous during incubation to reduce odour cues at the nest and protect eggs and young from olfactorily searching predators.

Function of preen oil changes: protection of eggs and young

Receiver of preen oil odour cues: nest predators (heterospecifics)

Sex and seasonal differences: associated with incubation

Predictions

1. Sex differences in preen oil are found during incubation and only in species with uniparental incubation.

2. Preen oil is less volatile during the breeding season.

3. Preen oil odour cues are used by predators to detect nests. Preen oil produced during breeding is less detected by nest predators than preen oil produced during non-breeding.

4. Seasonal changes in preen oil evolved primarily in species under selective pressure from olfactorily searching nest predators.

Sex semiochemical hypothesis

Preen oil odour is used for mate recognition (sex discrimination) and/or mate choice (identification of high-quality or genetically compatible mates).

Function of preen oil changes: mating signal

Receiver of preen oil odour cues: potential mates (conspecifics)

Sex and seasonal differences: associated with mate choice

Predictions

1. Sex differences in preen oil are found only during the breeding season.

2. Preen oil is more volatile during the breeding season.

3. Preen oil odour cues are used by conspecifics to discriminate between the sexes or among individuals of varying quality and/or genetic compatibility.

4. Seasonal changes in preen oil evolved primarily in species under selective pressure to engage in mate choice.

Fig. 2. Hypotheses and predictions to explain the function of seasonal and sex differences in the chemical composition of avian preen oil (a major source of avian body odour).

analysis to test for general support of this hypothesis, and therefore focused our analyses on the odour-based hypotheses.

Under the olfactory crypsis hypothesis, we predicted that, in uniparentally incubating species, only the incubating sex would show a shift in preen oil composition while in biparentally incubating species, both sexes would show shifts; thus, we expected that seasonal differences in preen oil chemical composition would be more common in the incubating sex. We also expected to find seasonal differences more commonly in species with nests more vulnerable to olfactorily searching predators (i.e. nests that are located on or near the ground compared to nests placed at height or in remote, inaccessible locations such as on cliffs). Similarly, we also predicted that sex differences in preen oil would be more likely in species with uniparental than biparental incubation. Under the sex semiochemical hypothesis, we predicted that sex differences in the chemical composition of preen oil would be more likely during breeding than non-breeding. To test these predictions, we conducted a comparative analysis of the available literature that tested for seasonal and sex differences in the preen oil of all bird species for which data were available.

II METHODS

(1) Literature review

We systematically reviewed studies that tested for an effect of season and/or sex on the chemical composition of preen oil. We screened the abstracts of 187 publications and the full text of 66 publications, retaining 55 publications (35 on seasonal differences and 46 on sex differences, including 26 publications addressing both seasonal and sex differences) that corresponded to our inclusion criteria. Details of the systematic review and the data used for analysis are available as online Supporting Information (Appendix S1, S2, Fig. S1, Tables S1 and S2).

(2) Preen oil chemical differences

Various analytical and statistical methods have been used to evaluate chemical differences in preen oil composition (Table S1). Given the diversity of methodologies used, if a significant chemical difference was observed at $\alpha = 0.05$, we recorded it as such. Thus, we created binary response variables of ‘sex difference’ and ‘seasonal difference’ (yes/no).

(3) Seasonal differences

We tested whether sex-specific seasonal changes are related to incubation and nest ecology. We obtained data on seasonal differences for 91 occurrences, defined as data on a given sex for a given species. For each occurrence, we recorded whether the sex exhibited a significant ($\alpha = 0.05$) seasonal change in preen oil composition (yes/no), whether the sex incubates (yes/no), whether the species nests on the ground (ground/non-ground; details below), and the timescale of the study (within breeding season/across breeding and non-breeding seasons; details below; Table S4). Thus, a species could be included multiple times in our analysis if it was included in multiple studies. Information about incubation and nest ecology was obtained from the *Handbook of the Birds of the World* (del Hoyo, Elliott & Christie, 2009). In some species, only one parent incubates, but the incubating parent can be of either sex (e.g. western sandpiper, *Calidris mauri*). Because a mix of both sexes would be incubating in any given study population for such species, we categorized these species as biparentally incubating. For studies on captive birds, we inspected the methods to confirm that seasonality was established using appropriate methods (e.g. by using artificial light cycles for birds kept indoors).

To estimate the vulnerability of different species to olfactorily searching nest predators, we described their nest ecology as 'ground nesting' (more vulnerable) or 'non-ground-nesting' (less vulnerable). Ground-nesting birds often suffer from higher nest predation rates than non-ground-nesting birds (Loiselle & Hoppes, 1983; Wilcove, 1985, but see Martin, 1995), notably by mammals (Söderström, Pärt & Rydén, 1998; Zuria, Gates & Castellanos, 2007; Macdonald & Bolton, 2008), which primarily rely on olfaction to detect nests (Reneerkens *et al.*, 2005, Whelan *et al.*, 2010). Species that nest in low shrubs (<2 m) were considered 'ground nesting' because they are likely more exposed to mammalian nest predators (e.g. Schaefer, 2004). Species that nest on cliffs were considered 'non-ground-nesting' because they are rarely exposed to such predators (Barros *et al.*, 2016).

Seasonal changes can occur at different timescales (within the breeding season, within the non-breeding season, and across the breeding and non-breeding seasons). To interpret any biological functions of preen oil changes, it is necessary to consider the timescale of the changes. We categorized timescale as 'within breeding season' (spanning nest building, egg laying, incubation, and brood care), and 'across breeding and non-breeding seasons' (where non-breeding encompasses fledging through winter, up to the start of nest building the following year). Studies conducted within the breeding season either compared samples from different periods within the breeding season (e.g. across mating, incubation, and brood care) or measured the effect of date on preen oil composition. Studies conducted across the breeding and non-breeding seasons either compared samples from the breeding and non-breeding season, or compared samples collected regularly throughout the year (e.g. monthly).

In total, our data set on seasonal differences comprised 91 occurrences (where one occurrence corresponds to one sex) from 43 species and 25 studies (Table S4). Effect sizes (Cohen's *d*) could be calculated for only three studies [using an online calculator (Lenhard & Lenhard, 2016), Table S4] and were therefore not used for analysis.

(4) Sex differences

We tested whether sex differences in the chemical composition of preen oil are related to season and incubation type. For each species, we recorded whether a significant ($\alpha = 0.05$) sex difference was detected in the composition of preen oil (yes/no), the season in which preen oil was sampled (breeding/non-breeding; where breeding includes nest building, egg laying, incubation, and brood care, and non-breeding encompasses fledging through winter, up to the start of nest building the following year), and the incubation type (uniparental/biparental; Table S5). Analysing sex differences during specific breeding periods (e.g. mate choice, incubation, chick rearing) would be more informative than distinguishing only breeding and non-breeding, but most studies sampled birds across multiple breeding stages, and we therefore could not conduct such an analysis. Also, in most cases, the nature and direction of sex differences were not explicitly recorded, so we could not include this information in our analyses. For studies on free-living birds, breeding stage dates and incubation type were verified using the *Handbook of the Birds of the World* (del Hoyo *et al.*, 2009). For studies on captive birds, we inspected the methods to confirm that birds were brought into breeding condition using appropriate methods (e.g. using natural light cycles for birds in outdoor aviaries or by using artificial light to photostimulate birds kept indoors).

In total, our data set on sex differences comprised 75 occurrences (where one occurrence corresponds to one season) from 49 species and 39 studies (Table S5). As with seasonal differences, because effect sizes could be calculated for only a limited number of studies (21, Table S5), we did not use effect sizes in our analysis.

(5) Statistical analyses

Our full data set included 59 species and 45 studies. We conducted comparative analyses for each model (seasonal differences, sex differences) using generalized linear mixed models (GLMMs) with Markov chain Monte Carlo techniques under a Bayesian statistical framework, using the package MCMCglmm (Hadfield, 2010) in R (R Development Core Team, 2017) that allowed us to control for phylogenetic dependency. The first model (seasonal differences) was run for 13×10^6 iterations, with a burn-in phase of 10000 and a thinning interval of 3500, resulting in a sample size of 3712. The second model (sex differences) was run for 10×10^6 iterations, with a burn-in phase of 5000 and a thinning interval of 2000, resulting in a sample size of 4998. These parameters were chosen to ensure model convergence

(Hadfield, 2010). Because we had no *a priori* predictions about the values of these parameters, both models were fit using a weakly informative inverse-gamma prior (Hadfield, 2010). We verified the absence of autocorrelation, verified convergence with the Gelman–Rubin diagnostic (Gelman & Rubin, 1992), and assessed the significance of fixed effects (at $\alpha = 0.05$) by checking whether their 95% credible interval spanned 0.

Our first model included *seasonal difference* as a binary response variable (yes/no) and the fixed effects *incubation* (sex incubates/sex does not incubate), *nest ecology* (ground/non-ground nesting), *timescale* (within breeding season/across breeding and non-breeding seasons), and the interaction term *incubation* × *nest ecology*. Our second model included *sex difference* as a binary response variable (yes/no) and the fixed effects *season* (breeding/non-breeding) and *incubation type* (uniparental/biparental).

For both models, we included *species* as a random effect because some species were used in multiple studies, and because some species were tested at two times of year (sex differences) or in both sexes (seasonal differences). We included *phylogeny* as a random effect to control for potential effects of phylogenetic relatedness. We calculated the phylogenetic relatedness between species using the consensus tree of 1000 phylogenetic trees (Stage2 MayrAll Hackett backbone) generated on birdtree.org (Jetz *et al.*, 2012). Finally, we verified that the inclusion of random effects improved the fit of the models, indicated by a lower deviance information

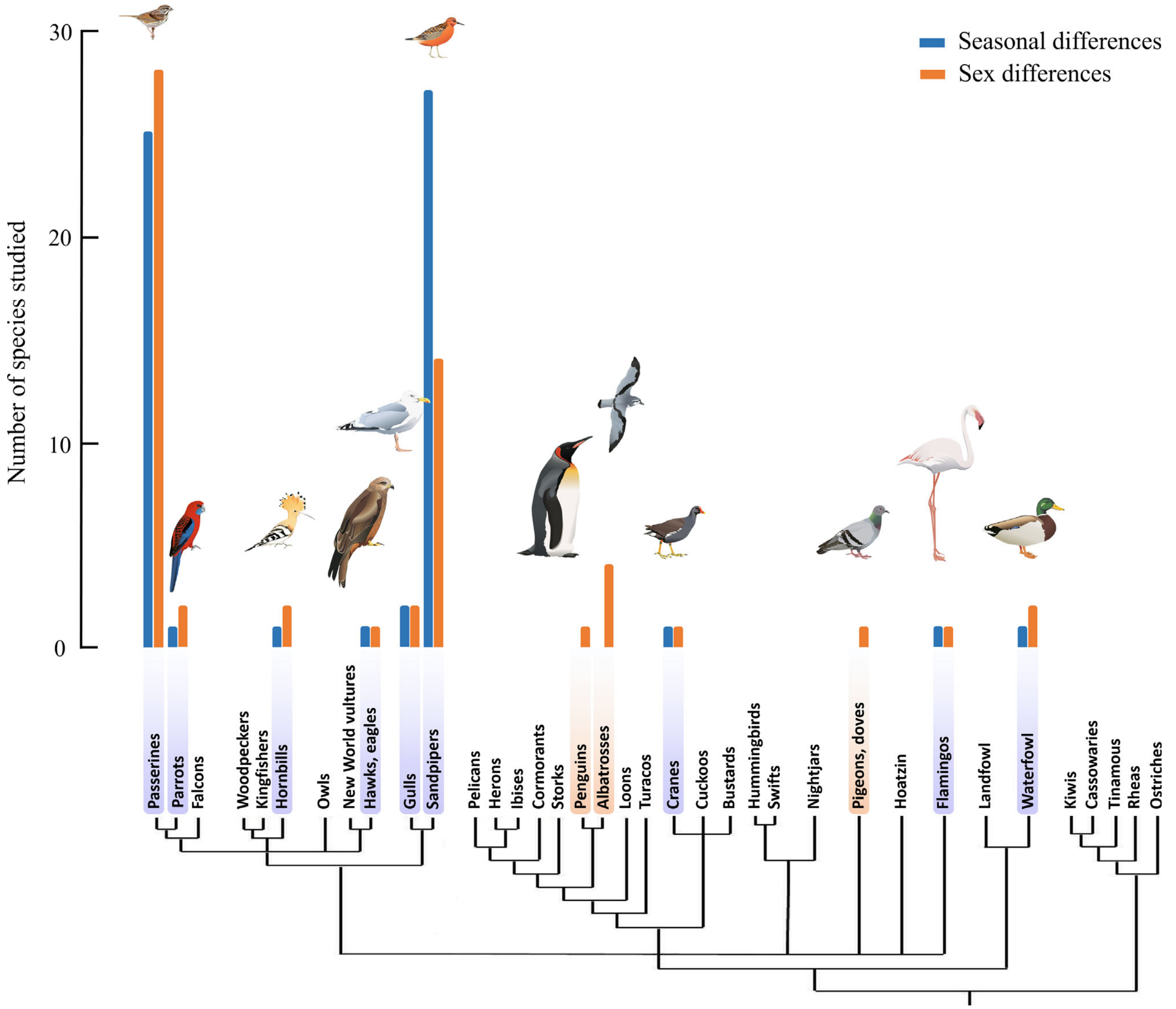


Fig. 3. Distribution of species studied with respect to seasonal (blue) and sex (orange) differences in preen oil chemical composition in birds. Orders highlighted in purple were studied with respect to both seasonal and sex differences. No order was studied with respect to seasonal differences only. Phylogeny is based on Hackett *et al.* (2008); gulls (family Laridae) and sandpipers (family Scolopacidae) belong to the order Charadriiformes. Illustrations created by M.G. using Microsoft PowerPoint.

Table 1. Summary of phylogenetically controlled Markov chain Monte Carlo generalized linear mixed effects models to investigate factors affecting seasonal and sex differences in preen oil chemical composition. The model on seasonal differences (91 occurrences) tests whether the occurrence of seasonal differences (no = 0; yes = 1) depends on incubation (sex does not incubate = 0; sex incubates = 1), nest ecology (non-ground-nesting = 0; ground-nesting = 1), the timescale of the study (within breeding season = 0; across breeding and non-breeding season = 1), and the interaction between incubation and nest ecology. The model on sex differences (75 occurrences) tests whether the occurrence of sex differences (no = 0; yes = 1) depends on the species' incubation type (uniparental = 0; biparental = 1) and the season (non-breeding = 0; breeding = 1)

Dependent variable	Effect	Independent variable	Posterior mean	Lower 95% CI	Upper 95% CI	P_{MCMC}
Seasonal difference	Fixed	Intercept	71.68	−90.48	239.04	0.370
		Incubation	2.87	−142.84	146.06	0.961
		Nest ecology	−163.19	−365.86	34.83	0.084
		Timescale	100.72	−18.76	232.14	0.099
		Incubation × Nest ecology	287.64	66.39	543.24	**0.010**
	Random	Phylogeny	2865	3e-04	16492	–
		Species	1478	2e-04	9524	–
		Residual	23546	5337	44760	–
Sex difference	Fixed	Intercept	−142.77	−364.12	33.30	0.096
		Incubation type	−221.20	−388.98	−43.52	**0.001**
		Season	339.49	108.42	586.84	**<0.001**
	Random	Phylogeny	5855	2e-04	33352	–
		Species	2116	2e-04	14095	–
		Residual	39544	1056	84827	–

CI, credible interval; bold, $P_{\text{MCMC}} < 0.05$.

criterion (DIC) score. These analyses are detailed in the Supporting information (Appendices S3 and S4). Detailed sample sizes used in each analysis are available in Table S6.

III RESULTS

(1) Literature review

Of the 55 studies included in our systematic review, 35 investigated seasonal differences (60 species) and 46 investigated sex differences (59 species) in preen oil composition, with 26 of these papers investigating both seasonal and sex differences. While 76 species have been investigated, most studies (61) involved just two phylogenetic orders, Passeriformes (songbirds, 32 species) and Charadriiformes (gulls and shorebirds, 29 species; Fig. 3). Seasonal differences were found in 95% (57/60) of species studied and sex differences were detected in 47% (28/59) of species studied.

(2) Seasonal differences

The probability of detecting a seasonal change in preen oil composition was related to the interaction between incubation and nest ecology (posterior mean = 287.64, 95% CI = [66.39, 543.24], $P_{\text{MCMC}} = 0.01$; Table 1, Fig. 4). To elucidate the direction of the interaction, we performed separate analyses for ground-nesting species (45 occurrences) and non-ground-nesting species (46 occurrences). For ground-nesting species, seasonal differences were more likely in the incubating than the non-incubating sex (posterior mean = 286.66; 95% CI = [98.27, 494.14], $P_{\text{MCMC}} < 0.001$), whereas for non-ground-nesting species, seasonal differences were apparent regardless of which sex incubated (posterior mean = 51.73, 95% CI = [−66.78, 182.74], $P_{\text{MCMC}} = 0.29$). Timescale had no effect on the probability of detecting seasonal changes (Table 1). Accounting for *phylogeny* and *species* increased the fit of the models slightly but had little effect overall (Table S7). *Phylogeny* and *species* explained 7 and 5% of the total variance, respectively (Table S8).

(3) Sex differences

The probability of detecting sex differences in preen oil composition was related to both breeding stage and incubation type (Table 1; Fig. 5). Sex differences were more likely during breeding than non-breeding (posterior mean = 339.49, 95% CI = [108.42, 586.84], $P_{\text{MCMC}} < 0.001$), and in species with uniparental than biparental incubation (posterior mean = −221.20; 95% CI = [−388.98, −43.52], $P_{\text{MCMC}} = 0.001$; Fig. 5). Accounting for *phylogeny* and *species* increased the fit of the models slightly but had little effect on the overall model results (Table S7). *Phylogeny* and *species* explained 9 and 5% of the total variance respectively (Table S8).

IV DISCUSSION

This study reviewed and analysed the literature on olfactory crypsis and sex semiochemicals and found support for both hypotheses. Seasonal changes in the chemical composition of preen oil were nearly ubiquitous. Consistent with predictions derived from the olfactory crypsis hypothesis, the

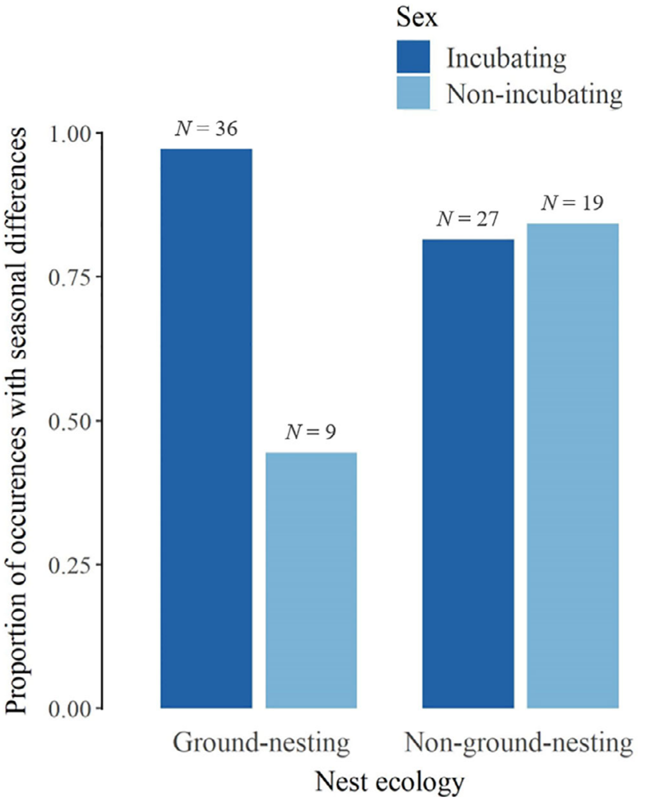


Fig. 4. Proportion of occurrences (i.e. sex within species) of seasonal differences in preen oil chemical composition in ground- *versus* non-ground-nesting species and if the sex incubates *versus* does not incubate. Sample size (91 occurrences) exceeds the number of species (43) because most studies sampled both sexes of a species, and some species were examined in multiple studies.

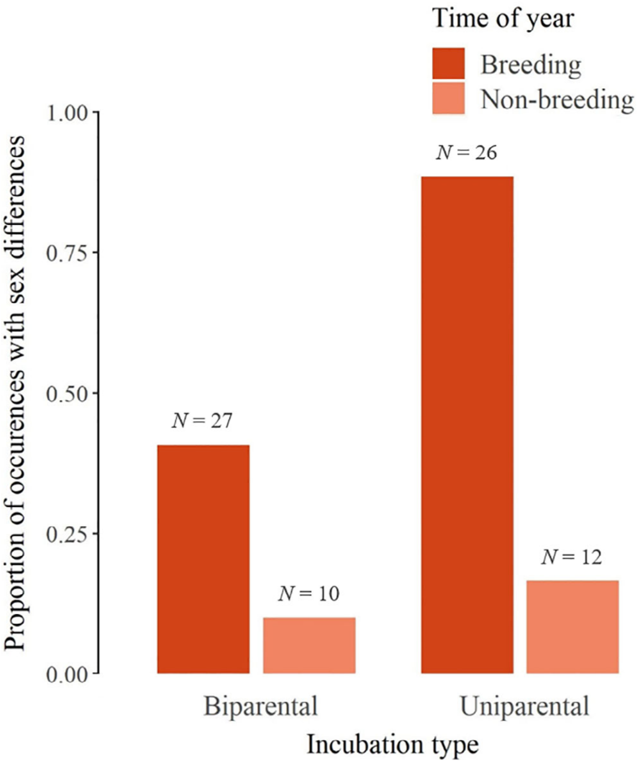


Fig. 5. Proportion of occurrences (i.e. season within species) of sex differences in preen oil chemical composition with biparental *versus* uniparental incubation and during breeding *versus* non-breeding. Sample size (75 occurrences) exceeds the number of species (49) because some species were tested during both breeding and non-breeding seasons, and some species were examined in multiple studies.

likelihood of detecting a seasonal change in preen oil composition was related to the interaction between incubation and nest ecology such that seasonal differences were more likely in the incubating sex, but only in ground-nesting species. For non-ground-nesting species, seasonal changes were equally likely, regardless of which sex incubated. By contrast, sex differences were less ubiquitous than seasonal differences, occurring in less than half of the species studied. Consistent with predictions of both the sex semiochemical and olfactory crypsis hypotheses, the likelihood of detecting sex differences in preen oil composition was related to both breeding stage and incubation type. Specifically, sex differences were more likely during breeding than non-breeding, and in species with uniparental than biparental incubation. It should be noted that our results on the probabilities of seasonal and sex differences may be overestimates if there is publication bias in favour of significant results. On the other hand, the probabilities of seasonal and sex differences may also be underestimated, since some studies were not designed specifically to test for such differences (e.g. in cases where studies sampled across breeding and/or non-breeding stages, and/or had small sample sizes), and as a result could not or did not detect any differences in preen oil composition. With a more appropriate design, such studies may have detected seasonal and/or sex differences in preen oil composition.

At present, there are insufficient data to disentangle these non-mutually exclusive hypotheses. Thus, our work is not the definitive test of these two hypotheses, but it is the best we can achieve to date. Below, we review current support for the olfactory crypsis and sex semiochemical hypotheses and offer recommendations for more direct hypothesis testing.

(1) Olfactory crypsis

Evidence for a role of preen oil in olfactory crypsis is currently limited. Studies on the preen oil composition of 27 ground-nesting shorebird species (order Charadriiformes) revealed a seasonal shift from monoesters to diesters at the onset of breeding (Piersma, Dekker & Sinninghe Damsté, 1999; Reneerkens *et al.*, 2002, 2006, 2007a), with diester secretion being maintained during incubation and chick-rearing (Reneerkens *et al.*, 2002, 2006). Remarkably, diesters were secreted equally in both sexes in species where both sexes incubate, only in males in species where only males incubate, and mainly in females in species where only females incubate (Reneerkens *et al.*, 2007a). Because diesters are less volatile than monoesters, these authors hypothesized that seasonal changes in the preen oil of incubating birds enhance olfactory crypsis by reducing olfactory cues at the nest, thereby limiting detection by olfactorily searching nest predators (Reneerkens *et al.*, 2002, 2007a). In support of this hypothesis, a trained dog was better at detecting preen oil composed of monoesters than diesters (Reneerkens *et al.*, 2005).

Other studies have also interpreted seasonal and sex differences in preen oil composition using the olfactory crypsis hypothesis. For example, the preen oil of Eurasian moorhens (*Gallinula chloropus*) is less volatile during breeding than non-breeding, and olfactory crypsis was proposed as an explanation (López-Perea & Mateo, 2019). In mallards (*Anas platyrhynchos*), a shift from monoesters to diesters at the onset of breeding was observed in females but not in males (Jacob *et al.*, 1979; Bohnet *et al.*, 1991). The shift was first thought to be involved in mate choice, by providing an olfactory cue that males might use to identify females in breeding condition (see Section IV.2). But given that diesters are less volatile than monoesters, that only females incubate in this species, and that mallard nests are exposed to mammalian predators (Johnson, Sargeant & Greenwood, 1989), this shift may be more relevant for olfactory crypsis than intraspecific communication and mate choice.

Finally, a New Zealand study on 13 non-ground-nesting passerine species compared the preen oil of introduced species that co-evolved with mammalian predators to that of native species that have a long evolutionary history without mammalian predators. Consistent with the olfactory crypsis hypothesis, preen oil was less volatile during breeding than non-breeding in introduced but not in native species (Fluen, 2008). However, sample sizes were low and preen oil volatiles were not lower in females for species with female-only incubation (Fluen, 2008), as would be predicted by the olfactory crypsis hypothesis. Overall, most studies speculate on the role of seasonal and sex variation in preen oil in maintaining olfactory crypsis without providing evidence. Additional studies on the ability of predators to detect preen oil secreted during various breeding and life-cycle stages are thus warranted.

Based on the olfactory crypsis hypothesis, we predicted that seasonal differences in preen oil composition would be more common in the incubating than non-incubating sex(es), especially in species with nests that are more vulnerable to olfactorily searching predators, such as ground nesters. We also predicted that sex differences in preen oil composition would be more common during breeding than non-breeding, and in uniparentally incubating than biparentally incubating species. We found support for all three predictions. Consistent with our first prediction, seasonal differences were more frequently detected in sexes that incubate than in sexes that do not incubate, but only in ground-nesting species (i.e. species more likely exposed to olfactorily searching predators). This suggests that preen oil changes are indeed associated with incubation in species that are under stronger selection pressure from olfactorily searching predators, supporting the olfactory crypsis hypothesis. However, our findings also highlight that olfactory crypsis cannot explain our findings for non-ground-nesting species. Birds exhibited seasonal changes in preen oil regardless of which sex(es) incubated, suggesting that there are also other explanations for seasonal changes – such as intraspecific chemical communication, as predicted under the sex semiochemical hypothesis.

Consistent with our second and third predictions, sex differences in preen oil composition were more common during breeding and in species with uniparental incubation. This is consistent with both the olfactory crypsis and sex semiochemical hypotheses. Additional information about the nature of sex differences could allow us to disentangle these two hypotheses. For example, less-volatile preen oil during breeding compared to non-breeding could corroborate a cryptic function, while more volatile preen oil during breeding compared to non-breeding could corroborate a signalling function.

We used a comparative analysis to re-evaluate Reneerkens' olfactory crypsis hypothesis, which predicts that seasonal changes in the preen oil of incubating birds is primarily due to mammalian predation (Reneerkens *et al.*, 2002, 2007a). Based on our results, we propose expanding the definition of olfactory crypsis to consider other biologically relevant factors. First, nest predation can be as high during the nestling stage as during the incubation stage (Pietz & Granfors, 2000; although we note this is not always the case), so olfactory crypsis could be important during both breeding stages. This is consistent with diesters being secreted until the end of the chick-rearing period in shorebirds (Reneerkens *et al.*, 2006). Second, preen oil could reduce the detectability of nests in two main ways: preen oil could enhance the crypsis of brooding (i.e. incubating and chick rearing) parents, thereby masking nest odours while adults are on the nest, but preen oil could also enhance crypsis of the eggs and chicks directly, if preen oil is transferred from parents to the offspring. Evidence that preen oil is transferred directly from parents to eggs and chicks is limited (but see Soler *et al.*, 2014), so the mechanisms and efficacy of preen oil transfer at nests are worth exploring further. Third, olfactory crypsis may be applicable not only to non-volatile (e.g. diesters) but also to volatile compounds. For example, certain volatile compounds could enhance crypsis if they blend in with the olfactory background of the nest (i.e. background matching; Soini *et al.*, 2007). Comparing preen oil compounds secreted during incubation and brooding with background odours of the nest and surrounding environment could help determine whether olfactory background matching is occurring. Finally, the olfactory crypsis hypothesis could apply not just to mammals, but to any olfactorily searching nest predators, regardless of taxon (e.g. insects, snakes, and even birds; Shutler, 2019).

In this study, we estimated the vulnerability to olfactorily searching nest predators by describing the nest ecology of the species and distinguishing ground-nesting and non-ground-nesting species. We assumed that ground nests are more vulnerable to olfactorily searching predators, because ground nests are more commonly depredated by mammals

(Söderström *et al.*, 1998; Zuria *et al.*, 2007; but see Angelstam, 1986; Mallord *et al.*, 2012) and because mammalian nest predation usually occurs at night and is mostly olfactorily based (Whelan *et al.*, 2010; Cox, Thompson & Reidy, 2013). However, this assumption is simplistic. Both ground nests and non-ground nests are susceptible to predation by three taxa in particular: mammals, snakes, and birds (and, to a lesser extent, insects; Thompson, 2007). The prevalence of nest predation by each taxon may depend more on habitat characteristics than nest ecology (Martin, 1995; Thompson, 2007; Reidy & Thompson, 2012). Moreover, each predator taxon can use multiple cues to detect nests. Mammals and snakes depredate nests mostly at night (e.g. Cox *et al.*, 2013; DeGregorio *et al.*, 2014) using olfactory cues (Ford & Burghardt, 1993; Whelan *et al.*, 2010) but may also use other cues [e.g. visual cues (Mullin & Cooper, 1998; Stake *et al.*, 2005; Dawson *et al.*, 2014)]. Birds commonly depredate nests during the day (Reidy & Thompson, 2012) and rely mainly on visual cues, but may also use olfactory cues (e.g. Buitron & Nuechterlein, 1985; Molina-Morales *et al.*, 2020). A more accurate proxy of vulnerability to olfactorily searching nest predators would be, for example, the incidence of such predators weighted by the likelihood of nest detection by olfaction, but such a measure was impossible to obtain for the species and populations included in our analyses. Although simplistic, we consider that nest ecology is a reasonable proxy of vulnerability to olfactorily searching predators in the absence of site-specific information on predation dynamics. Furthermore, if olfactory crypsis prevents some (although not all) predator detections, it could still be sufficiently beneficial to have evolved. Overall, although evidence from experimental studies with natural predators and from studies on taxa other than shorebirds are still lacking, our results and literature review provide compelling support for a role of preen oil in olfactory crypsis.

(2) Sex semiochemicals

Preen oil chemical cues are increasingly thought to play a role in avian mate choice and reproduction (Balthazart & Taziaux, 2009; Caro & Balthazart, 2010; Caro *et al.*, 2015; Whittaker & Hagelin, 2020). Reproductive signals or cues should differ between the sexes and reflect aspects of quality or condition (Johansson & Jones, 2007), and there is growing evidence that preen oil provides odour cues of sex that at least some bird species respond to. Thus, we proposed the sex semiochemical hypothesis, positing that sex differences in preen oil are associated with reproduction and preen oil odour cues are involved in mate recognition and/or mate choice. The sex semiochemical hypothesis predicts that there should be an effect of breeding stage (breeding *versus* non-breeding) on preen oil. Indeed, sex differences were more common in breeding birds, suggesting a role for preen oil in reproductive chemical signalling.

The preen oil of several passerine species becomes more volatile during the breeding season [e.g. white-throated sparrows, *Zonotrichia albicollis* (Tuttle *et al.*, 2014), gray catbirds, *Dumetella carolinensis* (Shaw *et al.*, 2011), dark-eyed juncos, *Junco hyemalis* (Soini *et al.*, 2007)], and birds may use these preen oil odour cues to attract mates and compete with same-sex conspecifics (Whittaker & Hagelin, 2020). Such findings argue against the chemical crypsis hypothesis, at least for some species. An increased volatility of preen oil chemical cues could serve to advertise for mates and/or to compete with same-sex conspecifics (e.g. *via* territorial scent marking), and such signals might reinforce or enhance other indicators of sex, breeding status, or dominance, such as song characteristics, plumage traits, and other sexually selected ornaments. However, sex differences in preen oil are often, but not always, associated with a greater abundance and/or diversity of chemical compounds in the preen oil of females (Whittaker & Hagelin, 2020), who often display fewer sexually selected ornaments than males. This apparent female emphasis on chemical differences in preen oil may be driven by three main factors: intersexual advertisement (e.g. of female receptivity and/or quality) and physiological priming effects on males; intrasexual competition (e.g. territorial scent marking, dominance, and reproductive suppression); and maternal behaviours (e.g. maternal care, mother–offspring recognition, chemical protection of eggs and nestlings) (Whittaker & Hagelin, 2020). Additional experiments testing for evidence of a role for preen oil in intersexual advertisement, intrasexual competition, and parental behaviours are warranted.

Shifts in the preen oil composition of breeding birds may also act as indicators of quality (Whittaker & Hagelin, 2020). In dark-eyed juncos, females with more ‘female-like’ odour and males with more ‘male-like’ odour both produce more offspring (Whittaker *et al.*, 2013). Further, males with more ‘male-like’ odour have more surviving nestlings (regardless of nestling paternity) while males with more ‘female-like’ odour have more extrapair young in their home nest (Whittaker *et al.*, 2013). In the lance-tailed manakin (*Chiroxiphia lanceolata*), the likelihood that offspring survive to fledging increases with male microsatellite heterozygosity (a proxy for genome-wide heterozygosity), and this almost certainly reflects genetic quality, because male manakins do not provide parental care (Sardell, Kempenaers & Duval, 2014). Furthermore, some preen oil components are correlated with increased heterozygosity in males, suggesting that females could use preen oil odour cues to evaluate male heterozygosity (Whittaker *et al.*, 2019a).

In species where it has been investigated, the chemical composition of preen oil is associated with MHC genotype, part of the adaptive immune system, such that individuals with more similar preen oil composition are more similar at MHC [e.g. in black-legged kittiwake, *Rissa tridactyla* (Leclaire *et al.*, 2014) and song sparrows, *Melospiza melodia* (Slade *et al.*, 2016; Grieves *et al.*, 2019c)]. This suggests that preen oil may provide cues of relatedness and/or genetic compatibility. Notably, such cues are detectable to at least some bird species [blue petrels, *Hydrobates caerulea* (Leclaire *et al.*, 2017) and song sparrows (Grieves *et al.*, 2019c)]. To understand better the role of preen oil chemical cues in avian reproduction, more information is needed on which sexes exhibit changes in preen oil and in what directions, whether the volatility of preen oil compounds increases or decreases in each sex, and at what breeding stages such changes occur.

Most of the studies included in our comparative analysis did not test birds' ability to discriminate between the sexes, but evidence for sex discrimination was found in all six of the studies that did (Zhang *et al.*, 2010; Whittaker *et al.*, 2011a; Amo *et al.*, 2012; Mihailova, 2014; Grieves *et al.*, 2019a; Van Huynh & Rice, 2019). In breeding-condition Passeriformes, both sexes spent more time with male odour in dark-eyed juncos (Whittaker *et al.*, 2011a) and spotless starlings (*Sturnus unicolor*; Amo *et al.*, 2012). By contrast, both sexes spent more time with opposite sex odour in black-capped chickadees (*Poecile atricapillus*), Carolina chickadees (*Poecile carolinensis*; Van Huynh & Rice, 2019), and song sparrows (Grieves *et al.*, 2019a). In Psittaciformes, female budgerigars (*Melopsittacus undulatus*) spent more time with male odour (Zhang *et al.*, 2010), and female crimson rosellas (*Platycercus elegans*) spent more time on nest boxes treated with male odour than female odour (Mihailova, 2014), suggesting a preference for these odour types.

Evidence for sex discrimination was also found in studies that were not included in our analysis (because sex differences in preen oil composition were not measured). In Galliformes, male domestic chickens (*Gallus gallus*) more frequently mount and copulate with females that have an intact preen gland, but this preference is abolished in anosmic males (Hirao *et al.*, 2009). In Charadriiformes, crested auklets (*Aethia cristatella*) of both sexes approached model birds treated with male odour more closely than they approached models treated with female odour; this study used a synthetic odour mimicking two major components of auklet odour (Jones *et al.*, 2004). Importantly, these sex-discrimination tests were all performed on birds in breeding condition. Taken together, these results suggest that the ability to use odour cues to discriminate conspecific sex is widespread in birds.

(3) Mechanisms of seasonal and sex differences

Seasonal and sex differences in preen oil composition may be related to changes in diet, preen gland microbes, and circulating hormone levels. Such shifts may provide both protection from predators (*via* olfactory crypsis) and indirect cues of reproductive status (i.e. readiness to breed) that play a role in both intersexual signalling and intrasexual competition (*via* sex semiochemicals; Whittaker & Hagelin, 2020).

(*a*) *Diet*

Many avian species change their diet at the onset of the breeding season (Bairlein & Gwinner, 1994). As such, seasonal differences in preen oil composition may also be affected by changes in diet. To our knowledge, no studies have tested whether natural seasonal dietary changes affect preen oil composition, but laboratory studies have shown that diet affects preen oil composition in captive birds (Apandi & Edwards, 1964; Thomas *et al.*, 2010, p. 201; Kanakri *et al.*, 2016). However, captive birds fed a constant diet still exhibit seasonal changes in preen oil (Reneerkens *et al.*, 2007b; Whelan *et al.*, 2010; Tuttle *et al.*, 2014; Potier *et al.*, 2018; Grieves *et al.*, 2020), demonstrating that differences in diet can only partly explain seasonal changes in preen oil composition. Sex differences in the chemical composition of preen oil may be driven partly by sex differences in diet, which is common in species with size dimorphism [e.g. seabirds (Phillips *et al.*, 2011), raptors (Catry *et al.*, 2016)] or with spatial segregation during foraging (e.g. shorebirds; Catry *et al.*, 2012).

(*b*) *Symbiotic microbes*

Preen gland microbes can also influence the chemical composition of preen oil (Martín-Vivaldi *et al.*, 2009, 2010; Whittaker *et al.*, 2019b). Gland microbiota can differ seasonally, which may be associated with seasonal changes in bacterial loads (e.g. an increase during the breeding season; Rodríguez-Ruano *et al.*, 2018) that can then affect preen oil composition. Preen gland microbiota can also differ between the sexes (Pearce *et al.*, 2017; Rodríguez-Ruano *et al.*, 2018, but see Whittaker *et al.*, 2019b; Grieves *et al.*, 2021). Given that nests can harbour unique microbial communities (Jacob *et al.*, 2014; van Veelen, Salles & Tieleman, 2017), sex differences in symbiotic microbes may be driven by sex differences in time spent at the nest (Saag *et al.*, 2011; Goodenough *et al.*, 2017). Seasonal changes in diet could also contribute to changes in preen gland microbes, but to our knowledge this has only been explored in avian gut microbiota (Grond *et al.*, 2018).

(*c*) *Hormones*

Seasonal and sex differences in the chemical composition of preen oil may be driven at least partly by endogenous changes in circulating levels of sex steroid hormones such as oestradiol and testosterone. Oestradiol injections trigger a shift from monoesters to diesters in mallard preen oil (Bohnet *et al.*, 1991). Testosterone implants have variable effects on preen oil composition across species, triggering increases in some compounds (Abalain *et al.*, 1984; Whittaker *et al.*, 2011b) and decreases in others (Whelan *et al.*, 2010). Thus, seasonal and sex differences in preen oil are likely at least partly related to physiological changes associated with reproduction.

V RECOMMENDATIONS FOR FUTURE RESEARCH

(1) Sampling and study design

Based on our comparative analysis, we found support for both the olfactory crypsis and sex semiochemical hypotheses. In most cases, the studies we reviewed do not consider the nature of seasonal and sex differences; that is, information on which sex(es) exhibited changes, and details on which chemicals changed (and how they changed) are rarely reported.

Seasonal changes in preen oil composition have been detected at fine timescales [e.g. less than a week in red knots, *Calidris canutus* (Reneerkens *et al.*, 2007b), less than 2 weeks in dark-eyed juncos and song sparrows (Whittaker *et al.*, 2011b; Grieves *et al.*, 2018)], and preen oil appears to be more subject to these finer scale seasonal changes during the breeding than the non-breeding season (e.g. Reneerkens *et al.*, 2002). Preen oil composition may thus be more stable (i.e. less variable) during the non-breeding season. That said, if there is selection on a specific mix of preen oil compounds during breeding, one might predict that preen oil should be less, not more, variable during the breeding season, or at least during specific stages of breeding; these contrasting possibilities are worth further study. Interestingly, the speed of seasonal changes in preen oil can be altered in captivity, as shown in red knots, where the shift to diesters was two times slower in captive (4 weeks) than wild individuals (2 weeks; Reneerkens *et al.*, 2007b). Our comparative analysis revealed that seasonal changes in preen oil were detected independently of the timescale of the study (within breeding or across the breeding and non-breeding seasons). Seasonal changes may have occurred before incubation, supporting olfactory crypsis, but may also have occurred at other times. Sampling preen oil at regular intervals across breeding and non-breeding stages would clarify the timescale over which preen oil changes. In addition, knowing at which specific period(s) (e.g. mate choice, incubation, chick rearing) seasonal changes occur will allow more specific, testable predictions about the function of changes in preen oil composition to be made.

Based on our findings that sex differences depend on season, and seasonal differences were nearly ubiquitous, these factors should be carefully considered in sampling design and analysis. If sex differences are of interest, sampling should be conducted during the breeding season, and the breeding stages (e.g. pair formation, egg laying, incubation, brood care) during which sampling occurs should be recorded. If sex differences are not of interest, it may be ideal to sample during the non-breeding season when sex differences may be less likely to be observed, as this may reduce potential confounds. The conditions, date(s), and duration of sampling should always be taken into consideration.

(2) Hypothesis testing

The olfactory crypsis and sex semiochemical hypotheses are not mutually exclusive. Studies that examine changes in preen oil over a finer timescale (e.g. comparing different periods within the breeding season, such as pre-breeding, incubation, and chick guarding; Reneerkens *et al.*, 2002), quantify hatching and fledging success (Whittaker *et al.*, 2013), and measure additional variables of interest (e.g. measures of quality; Whittaker *et al.*, 2019a) should provide important insights into the functions of avian preen oil in crypsis and social signalling.

The existing literature concerns mainly two bird orders (shorebirds and passerines). Most studies supporting the olfactory crypsis hypothesis have been conducted on shorebirds (but see Fluen, 2008), while studies supporting the sex semiochemical hypothesis have been conducted on predominantly passerines and shorebirds (Table S4). This could be due to a taxonomic bias, as evidence of olfactory crypsis was first collected in shorebirds (Reneerkens *et al.*, 2002), and evidence for sex semiochemicals is rapidly accumulating in passerines (Whittaker & Hagelin, 2020). Alternatively, this could be because seasonal and/or sexual variation in preen oil chemical composition has different functions in each taxon, or that such differences depend on the environment or ecology of a given species. These hypotheses could be tested by analysing variation in preen oil composition in ground-breeding passerines and shorebirds that co-occur in the same habitat, taking care to collect and analyse preen oil separately from the pre-breeding stage (mate choice, pairing, nest building) and during incubation and brood care.

(*a*) *Olfactory crypsis hypothesis*

Here, we outline specific predictions that should be tested to evaluate support for the olfactory crypsis hypothesis.

1. Predators should be less able to detect nests treated with the preen oil of incubating birds compared to preen oil of non-incubating birds. To test this, field experiments should be conducted, although we recognize that experiments on nest predation are difficult to implement in the field. For example, one could measure the predator detection rate of artificial nests where eggs are smeared with preen oil secreted during incubation *versus* outside of incubation, compared to nests with no preen oil treatment. Such an experiment should ideally be combined with chemical analyses to verify and quantify chemical differences among treatments.
2. During incubation, preen oil should become less volatile in the incubating sex compared to the non-incubating sex. This can be tested by taking repeated measurements of preen oil collected from incubating and non-incubating birds and performing chemical analyses to measure the volatility of preen oil at different time points.
3. Preen oil may be transferred from parents to eggs and/or chicks. To test for evidence of preen oil transfer, one could search for traces of preen oil on the eggs and/or chicks, or determine (e.g. using video recording; Martín-Vivaldi *et al.*, 2014) whether adults actively deposit preen oil onto the eggs and/or chicks. Currently, preen oil transfer has only been documented in a single species (Eurasian hoopoe, *Upupa epops*). In this species, preen oil becomes malodorous during breeding (Soler *et al.*, 2014), suggesting it does not provide olfactory crypsis but may instead repel predators and/or parasites.
4. Preen oil could increase olfactory crypsis at the nest *via* background matching (Soini *et al.*, 2007). To test this,

one could analyse the chemical composition of preen oil secreted by the incubating parent(s) compared to the chemical composition of the nest and surrounding environment (e.g. using headspace sampling; Díez-Fernández *et al.*, 2021) and assess whether preen oil is more chemically similar to the environment than would be expected by chance.

(*b*) *Sex semiochemical hypothesis*

Here, we outline specific predictions that should be tested to evaluate support for the sex semiochemical hypothesis.

(1) Birds should use preen oil odour cues to discriminate between the sexes and among individuals of varying quality. There is growing evidence for avian olfactory sex discrimination in the literature (e.g. Zhang *et al.*, 2010; Whittaker *et al.*, 2011a; Amo *et al.*, 2012; Grieves *et al.*, 2019a; Van Huynh & Rice, 2019). Additional experiments should be designed to test for evidence of olfactory discrimination based on indicators of genetic quality and compatibility such as genome-wide heterozygosity (Whittaker *et al.*, 2019a) and MHC genotype (Grieves *et al.*, 2019c).

(2) Preen oil should become more volatile and/or more abundant (e.g. to increase detectability) during breeding than non-breeding. This can be tested by taking repeated measurements of preen oil collected from breeding and non-breeding birds and performing chemical analyses to measure the volatility of preen oil and the abundance of compounds of interest (i.e. those that have been associated with sex or individual differences) at different time points and between the sexes.

(3) The preen oil preferences of birds tested in a laboratory should translate to mate choice in the wild. Such experiments are difficult to perform, not least because numerous factors affect mate choice in the field, but one could start by looking for evidence of non-random mating based on features birds have been shown to discriminate using odour cues, such as MHC genotype (Grieves *et al.*, 2019c) or the relative 'maleness' of preen oil composition (Whittaker *et al.*, 2011a).

(4) Mate choice based on preen oil odour cues should also be linked to measures of mate quality and fitness. For example, in lance-tailed manakins, male reproductive success is correlated with microsatellite heterozygosity – chicks of more heterozygous males are more likely to fledge, and heterozygosity is correlated with lower proportions of certain preen oil chemicals, but whether female manakins use preen oil odour during male mate choice is still unknown (Whittaker *et al.*, 2019a). Odour preferences can be tested using a two-choice maze, and preferences can subsequently be linked back to field data on metrics such as heterozygosity, quality, and fitness.

VI CONCLUSIONS

(1) Determining the functions of preen oil chemical differences in birds has the potential to shift our understanding of avian behaviour.

(2) We conducted an extensive literature review to evaluate the evidence for seasonal and sex differences in the chemical composition of avian preen oil (a proxy of avian body odour). Seasonal differences were nearly ubiquitous, while sex differences were found in almost half of the species studied.

(3) We conducted a comparative analysis to test two hypotheses that may explain seasonal and sex differences in preen oil: the olfactory crypsis and the sex semiochemical hypotheses.

(4) Our comparative analyses on both seasonal and sex differences supports the olfactory crypsis hypothesis. However, direct evidence of a role for preen oil differences in olfactory crypsis is still lacking, notably from experimental studies with natural predators and from studies on species other than shorebirds.

(5) Our comparative analysis supports the sex semiochemical hypothesis. Evidence for the sex semiochemical hypothesis is growing, but more research is needed to connect preen oil differences to odour preferences, measures of quality, and mating success in the wild.

(6) We suggest numerous predictions that can be tested to allow researchers to disentangle the olfactory crypsis and sex semiochemical hypotheses. Doing so will enable us to gain deeper insights into the role of chemical masking and chemical signalling in birds.

VII ACKNOWLEDGEMENTS

We thank the Wissenschaftskolleg Berlin for the time to formulate ideas (B.A.C., I.C.C., T.S.), two anonymous reviewers for comments that improved this manuscript, J. Winternitz for advice on statistical analysis, D. Whittaker for helpful discussion, and F. Bonadonna and J. Reneerkens for sharing information. This study was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) through a Discovery Grant to E.A.M.-S. and a Vanier Canada Graduate Scholarship to L.A.G. B.A.C. was funded through a Freigeist-Fellowship from the Volkswagen Foundation and M.G. was funded by the German Science Foundation (421568765). Illustrations in Figs 2 and 3 were created by M.G.

VIII DATA ACCESSIBILITY

The data and code used for the literature review and the comparative analyses are available at the repository PUB –

Publications at Bielefeld University (https://pub.uni-bielefeld.de/record/2956821, doi: https://doi.org/10.4119/unibi/2956821).

IX. REFERENCES

Abalain, J. H., Amet, Y., Daniel, J. Y. & Floch, H. H. (1984). Androgen regulation of secretions in the sebaceous-like uropygial gland of the male Japanese quail. *Journal of Endocrinology* **103**, 147–153.

Amat, J. A., Garrido, A., Portavia, F., Rendón-Martos, M., Pérez-Gálvez, A., Garrido-Fernández, J., Gómez, J., Béchet, A. & Rendón, M. A. (2018). Dynamic signalling using cosmetics may explain the reversed sexual dichromatism in the monogamous greater flamingo. *Behavioral Ecology and Sociobiology* **72**, 1–10.

Amo, L., Avilés, J. M., Parejo, D., Peña, A., Rodríguez, J. & Tomás, G. (2012). Sex recognition by odour and variation in the uropygial gland secretion in starlings. *Journal of Animal Ecology* **81**, 605–613.

Angelstam, P. (1986). Predation on ground-nesting birds' nests in relation to predator densities and habitat edge. *Oikos* **47**, 365.

Apandi, M. & Edwards, H. M. Jr. (1964). Studies on the composition of the secretions of the uropygial gland of some avian species. *Poultry Science* **43**, 1445–1462.

Azzani, L., Rasmussen, J. L., Gieseg, S. P. & Briskie, J. V. (2016). An experimental test of the effect of diet on preen wax composition in New Zealand silvereyes (*Zosterops lateralis*). In *Chemical Signals in Vertebrates* (ed. C. D. Buesching), pp. 511–525. New York, NY: Springer.

Bairlein, F. & Gwinner, E. (1994). Nutritional mechanisms and temporal control of migratory energy accumulation in birds. *Annual Review of Nutrition* **14**, 187–215.

Balthazart, J. & Taziaux, M. (2009). The underestimated role of olfaction in avian reproduction? *Behavioural Brain Research* **200**, 248–259.

Bang, B. G. & Cobb, S. (1968). The size of the olfactory bulb in 108 species of birds. *The Auk* **85**, 55–61.

Barros, Á., Romero, R., Munilla, I., Pérez, C. & Velando, A. (2016). Behavioural plasticity in nest-site selection of a colonial seabird in response to an invasive carnivore. *Biological Invasions* **18**, 3149–3161.

Bhattacharyya, S. P. & Chowdhury, S. R. (1995). Seasonal variation in the secretory lipids of the uropygial gland of a sub-tropical wild passerine bird, *Pycnonotus cafer* (L) in relation to the testicular cycle. *Biological Rhythm Research* **26**, 79–87.

Bisson, I.-A., Marra, P. P., Burtt, E. H. Jr., Sikaroodi, M. & Gillevet, P. M. (2009). Variation in plumage microbiota depends on season and migration. *Microbial Ecology* **58**, 212–220.

Bohnet, S., Rogers, L., Sasaki, G. & Kolattukudy, P. E. (1991). Estradiol induces proliferation of peroxisome-like microbodies and the production of 3-hydroxy fatty acid diesters, the female pheromones, in the uropygial glands of male and female mallards. *Journal of Biological Chemistry* **266**, 9795–9804.

Bonadonna, F., Caro, S. P. & de Brooke, M. L. (2009). Olfactory sex recognition investigated in Antarctic prions. *PLoS One* **4**, e4148.

Bonadonna, F., Miguel, E., Grosbois, V., Jouventin, P. & Bessiere, J.-M. (2007). Individual odor recognition in birds: an endogenous olfactory signature on petrels' feathers? *Journal of Chemical Ecology* **33**, 1819–1829.

Bonadonna, F. & Nevitt, G. A. (2004). Partner-specific odor recognition in an Antarctic seabird. *Science* **306**, 835–835.

Bonadonna, F. & Sanz-Aguilar, A. (2012). Kin recognition and inbreeding avoidance in wild birds: the first evidence for individual kin-related odour recognition. *Animal Behaviour* **84**, 509–513.

Buitron, D. & Nuechterlein, G. L. (1985). Experiments on olfactory detection of food caches by black-billed magpies. *The Condor* **87**, 92–95.

Burger, B. V., Reiter, B., Borzyk, O. & Du Plessis, M. A. (2004). Avian exocrine secretions. I. Chemical characterization of the volatile fraction of the uropygial secretion of the green woodhoopoe, *Phoeniculus purpureus*. *Journal of Chemical Ecology* **30**, 1603–1611.

Campagna, S., Mardon, J., Celerier, A. & Bonadonna, F. (2012). Potential semiochemical molecules from birds: a practical and comprehensive compilation of the last 20 years studies. *Chemical Senses* **37**, 3–25.

Caro, S. P. & Balthazart, J. (2010). Pheromones in birds: myth or reality? *Journal of Comparative Physiology A* **196**, 751–766.

Caro, S. P., Balthazart, J. & Bonadonna, F. (2015). The perfume of reproduction in birds: chemosignaling in avian social life. *Hormones and Behavior* **68**, 25–42.

Caspers, B. A., Gagliardo, A. & Krause, E. T. (2015). Impact of kin odour on reproduction in zebra finches. *Behavioural Ecology and Sociobiology* **69**, 1827–1833.

Caspers, B. A., Hagelin, J. C., Paul, M., Bock, S., Willeke, S. & Krause, E. T. (2017). Zebra Finch chicks recognise parental scent, and retain chemosensory knowledge of their genetic mother, even after egg cross-fostering. *Scientific Reports* **7**, 12859.

Catry, I., Catry, T., Alho, M., Franco, A. M. A. & Moreira, F. (2016). Sexual and parent-offspring dietary segregation in a colonial raptor as revealed by stable isotopes. *Journal of Zoology* **299**, 58–67.

Catry, T., Alves, J. A., Gill, J. A., Gunnarsson, T. G. & Granadeiro, J. P. (2012). Sex promotes spatial and dietary segregation in a migratory shorebird during the non-breeding season. *PLoS One* **7**, e33811.

Coffin, H. R., Watters, J. V. & Mateo, J. M. (2011). Odor-based recognition of familiar and related conspecifics: a first test conducted on captive Humboldt penguins (*Spheniscus humboldti*). *PLoS One* **6**, e25002.

Cox, W. A., Thompson, F. R. & Reidy, J. L. (2013). The effects of temperature on nest predation by mammals, birds, and snakes. *The Auk* **130**, 784–790.

Damsté, J. S., Dekker, M. H. A. & Piersma, T. (2000). Molecular analysis of intact preen waxes of *Calidris canutus* (Aves: Scolopacidae) by GC/MS and GC/MS/MS. *Lipids* **35**, 533–542.

Dawson, S. J., Adams, P. J., Huston, R. M. & Fleming, P. A. (2014). Environmental factors influence nest excavation by foxes: excavation of artificial turtle nests. *Journal of Zoology* **294**, 104–113.

DeGregorio, B. A., Chiavacci, S. J., Weatherhead, P. J., Willson, J. D., Benson, T. J. & Sperry, J. H. (2014). Snake predation on North American bird nests: culprits, patterns and future directions. *Journal of Avian Biology* **45**, 325–333.

del Hoyo, J., Elliott, A. & Christie, D. A. (eds) (2009). *Handbook of the Birds of the World*. Lynx Edicions, Barcelona.

Díez-Fernández, A., Martínez-de la Puente, J., Gangoso, L., Ferraguti, M., Soriguer, R. & Figuerola, J. (2020). House sparrow uropygial gland secretions do not attract ornithophilic nor mammophilic mosquitoes. *Medical and Veterinary Entomology* **34**(2), 225–228.

Díez-Fernández, A., Martínez-de la Puente, J., Martín, J., Gangoso, L., López, P., Soriguer, R. & Figuerola, J. (2021). Sex and age, but not blood parasite infection nor habitat, affect the composition of the uropygial gland secretions in European blackbirds. *Journal of Avian Biology* **2021**, e02630.

Douglas, H. D. (2006). Measurement of chemical emissions in crested auklets (*Aethia cristatella*). *Journal of Chemical Ecology* **32**, 2559–2567.

Fischer, I., Haliński, L. P., Meissner, W., Stepnowski, P. & Knitter, M. (2017). Seasonal changes in the preen wax composition of the Herring gull *Larus argentatus*. *Chemoecology* **27**, 127–139.

Fischer, I., Meissner, W., Haliński, Ł. P. & Stepnowski, P. (2020). Preen oil chemical composition in herring gull *Larus argentatus*, common gull *Larus canus* and black-headed gull *Chroicocephalus ridibundus* confirms their status as two separate genera. *Biochemical Systematics and Ecology* **89**, 103987.

Fluen, T. (2008). A comparative analysis of evolutionary changes in island birds. MSc Thesis, University of Canterbury, Christchurch.

Ford, N. B. & Burghardt, G. M. (1993). Perceptual mechanisms and the behavioral ecology of snakes. In *Snakes: Ecology and Behavior* (eds R. A. Seigel and J. T. Collins), pp. 117–164. McGraw-Hill, New York.

Forrette, L.M. (2018). A description and analysis of preen oil volatiles in wild white-throated sparrows (*Zonotrichia albicollis*). PhD Thesis, Indiana State University, Terre Haute, Indiana.

Fracasso, G., Tuliozi, B., Hoi, H. & Griggio, M. (2018). Can house sparrows recognise familiar or kin-related individuals by scent? *Current Zoology* **65**, 53–59.

Gabirot, M., Buatois, B., Müller, C. T. & Bonadonna, F. (2018). Odour of King Penguin feathers analysed using direct thermal desorption discriminates between individuals but not sexes. *Ibis* **160**, 379–389.

Gabirot, M., Raux, L., Dell'Ariccia, G., Bried, J., Ramos, R., González-Solís, J., Buatois, B., Crochet, P.-A. & Bonadonna, F. (2016). Chemical labels differ between two closely related shearwater taxa. *Journal of Avian Biology* **47**, 540–551.

Gelman, A. & Rubin, D. B. (1992). Inference from iterative simulation using multiple sequences. *Statistical Science* **7**, 457–472.

Goodenough, A. E., Stallwood, B., Dandy, S., Nicholson, T. E., Stubbs, H. & Coker, D. G. (2017). Like mother like nest: similarity in microbial communities of adult female Pied Flycatchers and their nests. *Journal of Ornithology* **158**, 233–244.

Grieves, L. A., Bernards, M. A. & MacDougall-Shackleton, E. A. (2019a). Behavioural responses of songbirds to preen oil odour cues of sex and species. *Animal Behaviour* **156**, 57–65.

Grieves, L. A., Bernards, M. A. & MacDougall-Shackleton, E. A. (2019b). Wax ester composition of songbird preen oil varies seasonally and differs between sexes, ages, and populations. *Journal of Chemical Ecology* **45**, 37–45.

Grieves, L. A., Bottini, C. L. J., Branfireun, B. A., Bernards, M. A., MacDougall-Shackleton, S. A. & MacDougall-Shackleton, E. A. (2020). Food stress, but not experimental exposure to mercury, affects songbird preen oil composition. *Ecotoxicology* **29**, 275–285.

Grieves, L. A., Gloor, G. B., Bernards, M. A. & MacDougall-Shackleton, E. A. (2019c). Songbirds show odour-based discrimination of similarity and diversity at the major histocompatibility complex. *Animal Behaviour* **158**, 131–138.

Grieves, L. A., Gloor, G. B., Kelly, T. R., Bernards, M. A. & MacDougall-Shackleton, E. A. (2021). Preen gland microbiota of songbirds differ across populations but not sexes. *Journal of Animal Ecology* **90**, 2202–2212.

Grieves, L. A., Kelly, T. R., Bernards, M. A. & MacDougall-Shackleton, E. A. (2018). Malarial infection alters wax ester composition of preen oil in songbirds: results of an experimental study. *The Auk* **135**, 767–776.

Grond, K., Sandercock, B. K., Jumpponen, A. & Zeglin, L. H. (2018). The avian gut microbiota: community, physiology and function in wild birds. *Journal of Avian Biology* **49**, e01788.

Hackett, S. J., Kimball, R. T., Reddy, S., Bowie, R. C. K., Braun, E. L., Braun, M. J., Chojnowski, J. L., Cox, W. A., Han, K.-L., Harshman, J., Huddleston, C. J., Marks, B. D., Miglia, K. J., Moore, W. S., Sheldon, F. H., et al. (2008). A phylogenomic study of birds reveals their evolutionary history. *Science* **320**, 1763–1768.

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of Statistical Software* **33**, 1–22.

Hagelin, J. C. & Jones, I. L. (2007). Bird odors and other chemical substances: a defense mechanism or overlooked mode of intraspecific communication? *The Auk* **124**, 741–761.

Hagelin, J. C., Jones, I. L. & Rasmussen, L. E. L. (2003). A tangerine-scented social odour in a monogamous seabird. *Proceedings of the Royal Society B: Biological Sciences* **270**, 1323–1329.

Haribal, M., Dhondt, A. A., Rosane, D. & Rodriguez, E. (2005). Chemistry of preen gland secretions of passerines: different pathways to same goal? why? *Chemoecology* **15**, 251–260.

Haribal, M., Proctor, H., Dhondt, A. A. & Rodriguez, E. (2011). Biology of House Finch feather mites, *Proctophyllodes pinnatus* (Acari: Proctophyllodidae), parallels variation in preen gland secretions. *International Journal of Acarology* **37**, 75–90.

Hirao, A., Aoyama, M. & Sugita, S. (2009). The role of uropygial gland on sexual behavior in domestic chicken, *Gallus gallus domesticus*. *Behavioural Processes* **80**, 115–120.

Jacob, J., Balthazart, J. & Schoffeniels, E. (1979). Sex differences in the chemical composition of uropygial gland waxes in domestic ducks. *Biochemical Systematics and Ecology* **7**, 149–153.

Jacob, S., Immer, A., Leclaire, S., Parthuisot, N., Ducamp, C., Espinasse, G. & Heeb, P. (2014). Uropygial gland size and composition varies according to experimentally modified microbiome in great tits. *BMC Evolutionary Biology* **14**, 134.

Jennings, S. L. & Ebeler, S. E. (2020). Individual chemical profiles in the Leach's Storm-Petrel. *Journal of Chemical Ecology* **46**, 845–864.

Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K. & Mooers, A. O. (2012). The global diversity of birds in space and time. *Nature* **491**, 444–448.

Johansson, B. G. & Jones, T. M. (2007). The role of chemical communication in mate choice. *Biological Reviews* **82**, 265–289.

Johnson, D. H., Sargeant, A. B. & Greenwood, R. J. (1989). Importance of individual species of predators on nesting success of ducks in the Canadian Prairie Pothole Region. *Canadian Journal of Zoology* **67**, 291–297.

Johnston, D. W. (1988). *Morphological Atlas of the Avian Uropygial Gland*. London, UK: British Museum (Natural History).

Jones, I. L., Hagelin, J. C., Major, H. L. & Rasmussen, L. E. L. (2004). An experimental field study of the function of Crested Auklet feather odor. *The Condor* **106**, 71–78.

Kanakri, K., Muhlhausler, B., Carragher, J., Gibson, R., Barekatain, R., Dekoning, C., Drake, K. & Hughes, R. (2016). Relationship between the fatty acid composition of uropygial gland secretion and blood of meat chickens receiving different dietary fats. *Animal Production Science* **58**, 828–833.

Kolattukudy, P. E., Bohnet, S. & Rogers, L. (1985). Disappearance of short chain acids from the preen gland wax of male mallard ducks during eclipse. *Journal of Lipid Research* **26**, 989–994.

Kolattukudy, P. E., Bohnet, S. & Rogers, L. (1987). Diesters of 3-hydroxy fatty acids produced by the uropygial glands of female mallards uniquely during the mating season. *Journal of Lipid Research* **28**, 582–588.

Krause, E. T., Brummel, C., Kohlwey, S., Baier, M. C., Müller, C., Bonadonna, F. & Caspers, B. A. (2014). Differences in olfactory species recognition in the females of two Australian songbird species. *Behavioral Ecology and Sociobiology* **68**, 1819–1827.

Krause, E. T., Kruger, O., Kohlmeier, P. & Caspers, B. A. (2012). Olfactory kin recognition in a songbird. *Biology Letters* **8**, 327–329.

Leclaire, S., Chatelain, M., Pessato, A., Buatois, B., Frantz, A. & Gasparini, J. (2019). Pigeon odor varies with experimental exposure to trace metal pollution. *Ecotoxicology* **28**, 76–85.

Leclaire, S., Merkling, T., Raynaud, C., Giacinti, G., Bessière, J.-M., Hatch, S. A. & Danchin, É. (2011). An individual and a sex odor signature in kittiwakes? Study of the semiochemical composition of preen secretion and preen down feathers. *Naturwissenschaften* **98**, 615–624.

Leclaire, S., Strandh, M., Mardon, J., Westerdahl, H. & Bonadonna, F. (2017). Odour-based discrimination of similarity at the major histocompatibility complex in birds. *Proceedings of the Royal Society B: Biological Sciences* **284**, 20162466.

Leclaire, S., van Dongen, W. F. D., Voccia, S., Merkling, T., Ducamp, C., Hatch, S. A., Blanchard, P., Danchin, E. & Wagner, R. H. (2014). Preen secretions encode information on MHC similarity in certain sex-dyads in a monogamous seabird. *Scientific Reports* **4**, 1–6.

Lenhard, W. & Lenhard, A. (2016). *Calculation of Effect Sizes*. Psychometrica, Dettelbach. https://www.psychometrica.de/effect_size.html.

Livezey, B. C., Jacob, J. & Humphrey, P. S. (1986). Biochemical composition of secretions from uropygial glands of steamer-ducks. *Biochemical Systematics and Ecology* **14**, 445–450.

Loiselle, B. A. & Hoppes, W. G. (1983). Nest predation in insular and mainland lowland rainforest in Panama. *The Condor* **85**, 93–95.

López-Perea, J. J. & Mateo, R. (2019). Wax esters of uropygial gland secretion as biomarkers of endocrine disruption in birds exposed to treated sewage water. *Environmental Pollution* **250**, 323–330.

Macdonald, M. A. & Bolton, M. (2008). Predation on wader nests in Europe. *Ibis* **150**, 54–73.

Mallord, J. W., Orsman, C. J., Cristinacce, A., Butcher, N., Stowe, T. J. & Charman, E. C. (2012). Mortality of wood warbler *Phylloscopus sibilatrix* nests in Welsh oakwoods: predation rates and the identification of nest predators using miniature nest cameras. *Bird Study* **59**, 286–295.

Mardon, J., Saunders, S. M., Anderson, M. J., Couchoux, C. & Bonadonna, F. (2010). Species, gender, and identity: cracking petrels' sociochemical code. *Chemical Senses* **35**, 309–321.

Mardon, J., Saunders, S. M. & Bonadonna, F. (2011). From preen secretions to plumage: the chemical trajectory of blue petrels' *Halobaena caerulea* social scent. *Journal of Avian Biology* **42**, 29–38.

Martin, T. E. (1993). Nest predation among vegetation layers and habitat types: revising the dogmas. *The American Naturalist* **141**, 897–913.

Martin, T. E. (1995). Avian life history evolution in relation to nest sites, nest predation, and food. *Ecological Monographs* **65**, 101–127.

Martínez-de la Puente, J., Díez-Fernández, A., Soriguer, R. C., Rambozzi, L., Peano, A., Meneguz, P. G. & Figuerola, J. (2020). Are malaria-infected birds more attractive to mosquito vectors? *Ardeola* **68**, 205–218. BioOne.

Martín-Vivaldi, M., Pena, A., Peralta-Sánchez, J. M., Sánchez, L., Ananou, S., Ruiz-Rodríguez, M. & Soler, J. J. (2010). Antimicrobial chemicals in hoopoe preen secretions are produced by symbiotic bacteria. *Proceedings of the Royal Society B: Biological Sciences* **277**, 123–130.

Martín-Vivaldi, M., Ruiz-Rodríguez, M., José Soler, J., Manuel Peralta-Sánchez, J., Méndez, M., Valdivia, E., Manuel Martín-Platero, A. & Martínez-Bueno, M. (2009). Seasonal, sexual and developmental differences in hoopoe *Upupa epops* preen gland morphology and secretions: evidence for a role of bacteria. *Journal of Avian Biology* **40**, 191–205.

Martín-Vivaldi, M., Soler, J. J., Peralta-Sánchez, J. M., Arco, L., Martín-Platero, A. M., Martínez-Bueno, M., Ruiz-Rodríguez, M. & Valdivia, E. (2014). Special structures of hoopoe eggshells enhance the adhesion of symbiont-carrying uropygial secretion that increase hatching success. *Journal of Animal Ecology* **83**, 1289–1301.

Mihailova, M. (2014). Olfactory signaling in an avian species complex: the crimson rosella. PhD Thesis, Deakin University, Geelong.

Molina-Morales, M., Castro, J., Albaladejo, G. & Parejo, D. (2020). Precise cache detection by olfaction in a scatter-hoarder bird. *Animal Behaviour* **167**, 185–191.

Montalti, D., Gutiérrez, A. M., Reboredo, G. & Salibián, A. (2005). The chemical composition of the uropygial gland secretion of rock dove *Columba livia*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **140**, 275–279.

Moreno-Rueda, G. (2017). Preen oil and bird fitness: a critical review of the evidence. *Biological Reviews* **92**, 2131–2143.

Müller, C., Caspers, B. A., Gadau, J. & Kaiser, S. (2020). The power of infochemicals in mediating individualized niches. *Trends in Ecology & Evolution* **35**, 981–989.

Mullin, S. J. & Cooper, R. J. (1998). The foraging ecology of the gray rat snake (*Elaphe obsoleta spiloides*)—visual stimuli facilitate location of arboreal prey. *The American Midland Naturalist* **140**, 397–401.

Pan, P. R., Dilworth, B. C., Day, E. J. & Chen, T. C. (1979). Effect of season of the year, sex, and dietary fats on broiler performance, abdominal fat, and preen gland secretion. *Poultry Science* **58**, 1564–1574.

Pearce, D. S., Hoover, B. A., Jennings, S., Nevitt, G. A. & Docherty, K. M. (2017). Morphological and genetic factors shape the microbiome of a seabird species (*Oceanodroma leucorhoa*) more than environmental and social factors. *Microbiome* **5**, 146.

Phillips, R. A., McGill, R. A. R., Dawson, D. A. & Bearhop, S. (2011). Sexual segregation in distribution, diet and trophic level of seabirds: insights from stable isotope analysis. *Marine Biology* **158**, 2199–2208.

Piersma, T., Dekker, M. & Sinninghe Damsté, J. S. (1999). An avian equivalent of make-up? *Ecology Letters* **2**, 201–203.

Pietz, P. J. & Granfors, D. A. (2000). Identifying predators and fates of grassland passerine nests using miniature video cameras. *The Journal of Wildlife Management* **64**, 71–87.

Potier, S., Besnard, M. M., Schikorski, D., Buatois, B., Duriez, O., Gabirot, M., Leclaire, S. & Bonadonna, F. (2018). Preen oil chemical composition encodes individuality, seasonal variation and kinship in black kites *Milvus migrans*. *Journal of Avian Biology* **49**, e01728.

R Development Core Team (2017). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.

Rasmussen, J.L. (2013). Investigations of evolutionary arms races and host diversity in avian brood parasite systems. PhD Thesis, University of Canterbury, Christchurch.

Reidy, J. L. & Thompson, F. R. III (2012). Predatory identity can explain nest predation patterns. In *Video Surveillance of Nesting Birds. Studies in Avian Biology* (eds C. A. Ribic, F. R. Thompson III and P. J. Pietz), pp. 135–148. University of California Press, Berkeley.

Reneerkens, J., Almeida, J. B., Lank, D. B., Jukema, J., Lanctot, R. B., Morrison, R. G., Rijpstra, W. I. C., Schamel, D., Schekkerman, H. & Sinninghe Damsté, J. S. (2007a). Parental role division predicts avian preen wax cycles. *Ibis* **149**, 721–729.

Reneerkens, J., Piersma, T. & Damsté, J. S. S. (2002). Sandpipers (Scolopacidae) switch from monoester to diester preen waxes during courtship and incubation, but why? *Proceedings of the Royal Society B: Biological Sciences* **269**, 2135–2139.

Reneerkens, J., Piersma, T. & Damsté, J. S. S. (2005). Switch to diester preen waxes may reduce avian nest predation by mammalian predators using olfactory cues. *Journal of Experimental Biology* **208**, 4199–4202.

Reneerkens, J., Piersma, T. & Damsté, J. S. S. (2006). Discerning adaptive value of seasonal variation in preen waxes: comparative and experimental approaches. *Acta Zoologica Sinca* **52**, 272–275.

Reneerkens, J., Piersma, T. & Damsté, J. S. S. (2007b). Expression of annual cycles in preen wax composition in red knots: constraints on the changing phenotype. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* **307A**, 127–139.

Rodríguez-Ruano, S., Martín-Vivaldi, M., Peralta-Sánchez, J., García-Martín, A., Martínez-García, Á., Soler, J., Valdivia, E. & Martínez-Bueno, M. (2018). Seasonal and sexual differences in the microbiota of the hoopoe uropygial secretion. *Genes* **9**, 407.

Ruxton, G. D. (2009). Non-visual crypsis: a review of the empirical evidence for camouflage to senses other than vision. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**, 549–557.

Saag, P., Mänd, R., Tilgar, V., Kilgas, P., Mägi, M. & Rasmann, E. (2011). Plumage bacterial load is related to species, sex, biometrics and fledging success in co-occurring cavity-breeding passerines. *Acta Ornithologica* **46**, 191–201.

Salibian, A. & Montalti, D. (2009). Physiological and biochemical aspects of the avian uropygial gland. *Brazilian Journal of Biology* **69**, 437–446.

Sardell, R. J., Kempenaers, B. & DuVal, E. H. (2014). Female mating preferences and offspring survival: testing hypotheses on the genetic basis of mate choice in a wild lekking bird. *Molecular Ecology* **23**, 933–946.

Schaefer, T. (2004). Video monitoring of shrub-nests reveals nest predators. *Bird Study* **51**, 170–177.

Shaw, C. L., Rutter, J. E., Austin, A. L., Garvin, M. C. & Whelan, R. J. (2011). Volatile and semivolatile compounds in gray catbird uropygial secretions vary with age and between breeding and wintering grounds. *Journal of Chemical Ecology* **37**, 329–339.

Shutler, D. (2019). Some important overlooked aspects of odors in avian nesting ecology. *Journal of Avian Biology* **50**, e02003.

Slade, J. W. G., Watson, M. J., Kelly, T. R., Gloor, G. B., Bernards, M. A. & MacDougall-Shackleton, E. A. (2016). Chemical composition of preen wax reflects major histocompatibility complex similarity in songbirds. *Proceedings of the Royal Society B: Biological Sciences* **283**, 20161966.

Söderström, B., Pärt, T. & Rydén, J. (1998). Different nest predator faunas and nest predation risk on ground and shrub nests at forest ecotones: an experiment and a review. *Oecologia* **117**, 108–118.

Soini, H. A., Schrock, S. E., Bruce, K. E., Wiesler, D., Ketterson, E. D. & Novotny, M. V. (2007). Seasonal variation in volatile compound profiles of preen gland secretions of the dark-eyed junco (*Junco hyemalis*). *Journal of Chemical Ecology* **33**, 183–198.

Soler, J. J., Pérez-Contreras, T., De Neve, L., Macías-Sánchez, E., Møller, A. P. & Soler, M. (2014). Recognizing odd smells and ejection of brood parasitic eggs. An experimental test in magpies of a novel defensive trait against brood parasitism. *Journal of Evolutionary Biology* **27**, 1265–1270.

Stager, K. E. (1967). Avian olfaction. *American Zoologist* **7**, 415–420.

Stake, M. M., Thompson, F. R., Faaborg, J. & Burhans, D. E. (2005). Patterns of snake predation at songbird nests in Missouri and Texas. *Journal of Herpetology* **39**, 215–222.

Stevens, M. & Merilaita, S. (2009). Animal camouflage: current issues and new perspectives. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**, 423–427.

Thomas, R. H., Price, E. R., Seewagen, C. L., Mackenzie, S. A., Bernards, M. & Guglielmo, C. G. (2010). Use of TLC-FID and GC-MS/FID to examine the effects of migratory state, diet and captivity on preen wax composition in White-throated Sparrows *Zonotrichia albicollis*. *Ibis* **152**, 782–792.

Thompson, F. R. (2007). Factors affecting nest predation on forest songbirds in North America: nest predation on forest songbirds in North America. *Ibis* **149**, 98–109.

Tuttle, E. M., Sebastian, P. J., Posto, A. L., Soini, H. A., Novotny, M. V. & Gonser, R. A. (2014). Variation in preen oil composition pertaining to season, sex, and genotype in the polymorphic white-throated sparrow. *Journal of Chemical Ecology* **40**, 1025–1038.

van Huynh, A. & Rice, A. M. (2019). Conspecific olfactory preferences and interspecific divergence in odor cues in a chickadee hybrid zone. *Ecology and Evolution* **9**, 9671–9683.

van Veelen, H. P. J., Salles, J. F. & Tieleman, B. I. (2017). Multi-level comparisons of cloacal, skin, feather and nest-associated microbiota suggest considerable influence of horizontal acquisition on the microbiota assembly of sympatric woodlarks and skylarks. *Microbiome* **5**, 156.

Whelan, R. J., Levin, T. C., Owen, J. C. & Garvin, M. C. (2010). Short-chain carboxylic acids from gray catbird (*Dumetella carolinensis*) uropygial secretions vary with testosterone levels and photoperiod. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **156**, 183–188.

Whittaker, D. J., Gerlach, N. M., Slowinski, S. P., Corcoran, K. P., Winters, A. D., Soini, H. A., Novotny, M. V., Ketterson, E. D. & Theis, K. R. (2016). Social environment has a primary influence on the microbial and odor profiles of a chemically signaling songbird. *Frontiers in Ecology and Evolution* **4**, 1–15.

Whittaker, D. J., Gerlach, N. M., Soini, H. A., Novotny, M. V. & Ketterson, E. D. (2013). Bird odour predicts reproductive success. *Animal Behaviour* **86**, 697–703.

Whittaker, D. J. & Hagelin, J. C. (2020). Female-based patterns and social function in avian chemical communication. *Journal of Chemical Ecology* **47**, 53–62.

Whittaker, D. J., Kuzel, M., Burrell, M. J. E., Soini, H. A., Novotny, M. V. & DuVal, E. H. (2019a). Chemical profiles reflect heterozygosity and seasonality in a tropical lekking passerine bird. *Animal Behaviour* **151**, 67–75.

Whittaker, D. J., Richmond, K. M., Miller, A. K., Kiley, R., Bergeon Burns, C., Atwell, J. W. & Ketterson, E. D. (2011a). Intraspecific preen oil odor preferences in dark-eyed juncos (*Junco hyemalis*). *Behavioral Ecology* **22**, 1256–1263.

Whittaker, D. J., Rosvall, K. A., Slowinski, S. P., Soini, H. A., Novotny, M. V. & Ketterson, E. D. (2018). Songbird chemical signals reflect uropygial gland androgen sensitivity and predict aggression: implications for the role of the periphery in chemosignaling. *Journal of Comparative Physiology A* **204**, 5–15.

Whittaker, D. J., Slowinski, S. P., Greenberg, J. M., Alian, O., Winters, A. D., Ahmad, M. M., Burrell, M. J. E., Soini, H. A., Novotny, M. V., Ketterson, E. D. & Theis, K. R. (2019b). Experimental evidence that symbiotic bacteria produce chemical cues in a songbird. *Journal of Experimental Biology* **222**, jeb.202978.

Whittaker, D. J., Soini, H. A., Atwell, J. W., Hollars, C., Novotny, M. V. & Ketterson, E. D. (2010). Songbird chemosignals: volatile compounds in preen gland secretions vary among individuals, sexes, and populations. *Behavioral Ecology* **21**, 608–614.

Whittaker, D. J., Soini, H. A., Gerlach, N. M., Posto, A. L., Novotny, M. V. & Ketterson, E. D. (2011b). Role of testosterone in stimulating seasonal changes in a potential avian chemosignal. *Journal of Chemical Ecology* **37**, 1349–1357.

Wilcove, D. S. (1985). Nest predation in forest tracts and the decline of migratory songbirds. *Ecology* **66**, 1211–1214.

Zhang, J.-X., Sun, L. & Zuo, M. X. (2009). Uropygial gland volatiles may code for olfactory information about sex, individual, and species in Bengalese finches *Lonchura striata*. *Current Zoology* **55**, 357–365.

Zhang, J.-X., Wei, W., Zhang, J.-H. & Yang, W.-H. (2010). Uropygial gland-secreted alkanols contribute to olfactory sex signals in budgerigars. *Chemical Senses* **35**, 375–382.

Zhang, Y.-H., Du, Y.-F. & Zhang, J.-X. (2013). Uropygial gland volatiles facilitate species recognition between two sympatric sibling bird species. *Behavioral Ecology* **24**, 1271–1278.

Zuria, I., Gates, J. E. & Castellanos, I. (2007). Artificial nest predation in hedgerows and scrub forest in a human-dominated landscape of central Mexico. *Acta Oecologica* **31**, 158–167.

X. Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Studies included in the literature review of seasonal and sex differences in preen oil chemical composition.

Table S2. Occurrences (i.e. sex in each species or season in each species) included in the analyses of seasonal and sex differences in preen oil chemical composition.

Table S3. Definitions used to categorize studies on preen oil according to their main focus.

Table S4. Species and studies included in comparative analysis testing whether sex-specific seasonal changes in preen oil chemical composition are related to incubation and nest ecology.

Table S5. Species and studies included in a comparative analysis testing whether sex-specific changes in preen oil chemical composition are related to season (breeding/non-breeding) and incubation type (uniparental/biparental).

Table S6. Sample sizes for the analyses testing for factors affecting the likelihood of detecting seasonal and sex differences in avian preen oil chemical composition.

Table S7. Effect of the inclusion of the random effects (species, phylogeny) on the fit of the Markov chain Monte Carlo generalized linear mixed effects (MCMCglmm) models on seasonal differences and sex differences in preen oil composition.

Table S8. Mean proportion of the total variance explained by the random effects (phylogeny, species, and residual variance) in the two models fitted to explain seasonal and sex differences in preen oil chemical composition across species.

Appendix S1. Supplementary methods.

Appendix S2. References for studies included in Tables S4 and S5.

Fig. S1. PRISMA flowchart for the systematic review and comparative analysis on seasonal and sex differences in preen oil chemical composition.

Appendix S3. R code for the comparative analysis on seasonal differences in preen oil composition.

Appendix S4. R code for the comparative analysis on sex differences in preen oil composition.

(*Received 27 August 2021; revised 19 January 2022; accepted 24 January 2022*)