Biologically active substances of bird skin: a review

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ABSTRACT: Bird skin has a number of specific properties. The uropygial gland is a significant skin gland in many species. The secretion of this gland is particularly necessary for maintaining physical characteristics, including feather waterproofing. In some bird species this gland secretion has a repellent effect against potential mammalian predators; in other species it affects the final colour of feathers. In the investigated species of storks (genus *Ciconia*), secretions of the uropygial gland have been found to be mixtures of monoester waxes, diester waxes, triester waxes and triglycerides. Wax diesters were also found in the red knot *Calidris canutus* (order Charadriiformes). Lipid substances in the secretions of the rock dove (*Columba livia*) consist mainly of unsaturated fatty acids (59% secretion; mostly oleic acid – 37%, linoleic acid – 6% and arachidonic acid – 7%). Free fatty acids, which are decomposition products of epidermal lipids, can regulate microbial colonization of skin (e.g., by modification of pH); a shift of these values was detected in poultry in battery husbandry. Analysis of fatty acids from lipids shows the influence of age, diet, and also the relationship to feather pecking – the individual composition affects the smell and taste, and thus the attractiveness to other individuals. The antibacterial activity of skin secretions has been demonstrated. Secretions of the hoopoe (*Upupa epops*) have besides the function of maintenance of physical properties of feathers also a repellent effect on parasites and predators. Its active substance is a peptide bacteriocin, produced by strains of *Enterococcus faecalis*. This substance is active against a number of both G + and G-bacteria and helps to sustain the nest hygiene, it is also effective against *Bacillus licheniformis* that produces keratin-decompositing enzymes. A similar antimicrobial activity of uropygial secretion against bacteria which degrade feathers was demonstrated in the wild house finch *Carpodacus mexicanus*. Changes in skin microflora have been demonstrated in parrots kept for breeding in comparison with those living in the wild, which may have significance for husbandry practices and veterinary care. Passerines of the genus *Pitohui* and *Ifrita* living in New Guinea store in their skin and feather batrachotoxins, which they receive from food - beetles of the genus *Choresine*. These toxins are active against parasites (e.g. lice – Phthiraptera). In contrast, substances that act as potential attractants for hematophagous insects (e.g. mosquitoes of genus *Culex*) were found in the skin of chickens. Alcohols, ketones and diones were detected in these substances. The composition of uropygial gland secretions may be a guide in assessing the relatedness of bird species. Feather waxes can be analyzed also from old museum specimens. Lipid-enriched organelles, multigranular bodies in the epidermis mean that zebra finches (*Taeniopygia castanotis*) are facultatively waterproof, which appears to have a function in protecting the organism against dehydrating when water is unavailable.

Keywords: bird skin; uropygial gland; secretion; repellent effect; antibacterial; antiparasitic

Contents

1. Introduction

Bird skin is marked out by some specific morphological and physiological features. It does not have any sudorific glands, it is thin, elastic and its epidermis is both keratinized and lipogenic. Bird skin also acts as a sebaceous secretory organ. The uropygial gland is a large sebaceous gland (glandula uropygii) at the base of the tail, most developed in some waterfowl. Other sebaceous secretory glands are present in the ear canals (Stettenheim, 2000). The uropygial gland is in many bird species rudimentary or is only developed in the embryo and is missing in adulthood. However, the physiological role of this gland does not depend upon its mass; the percentage of secretion can be high also in a small gland: in the rock dove (*Columba livia*) secretion represented 32% of the gland's mass (Montalti et al., 2005). The common components of uropygial gland secretion are acidic mucins, neutral lipids, glycolipids and phospholipids. For example, in the gland of the Rock Pigeon (*Columba livia*), the extracted lipid mixture contained C14 to C20 fatty acids, mostly unsaturated. Uropygial gland secretion confers suppleness and water resistance to feathers (but no correlation between the size of the gland and aquatic and terrestrial bird species was found). Other physiological roles of gland secretion may be associated with pheromone production, plumage hygiene control, thermal insulation and defence against predators (Salibian and Montalti, 2009). Some earlier studies suggest an influence on the decrease of feather-degrading lice, e.g., in *Columba livia* (Moyer et al., 2003).

In some bird species, uropygial and other skin secretions can also influence feather coloration; this cosmetic function is an important factor in intraspecific relations in these species (Delhey et al., 2007). From this point of view, two main types of uropygial secretions exist. The first one occurs mainly in passerines, the other in non-passerines. Both of them reduce relative UV reflectance of a white background. Although this probably does not have a major role, it can be a factor influencing on visual signalling through plumage reflectance (Delhey et al., 2008).

Besides the above mentioned functions, other important biological features of the substances included in bird skin or secreted on its surface have been demonstrated.

2. Composition and characteristics of uropygial gland secretions

The uropygial gland is a gland of the holocrinne type. The composition of its secretion is specific to bird taxonomic groups; it consists of waxes, fatty acids and alcohols. Mixtures of monoester waxes, diester waxes, triester waxes and triglycerides were found in the secretions of the uropygial gland of birds of the order Ciconiiformes (five species of the genus *Ciconia*, family Ciconiidae were tested). The components of monoester waxes are unbranched fatty acids and alcohols, whereas diester waxes are derived from both 2- and 3-hydroxy fatty acids esterified with unbranched alcohols and fatty acids. Triester waxes included in these secretions are derived either from 2-hydroxy alkylmalonic acids or from erythro-2,3-dihydroxy fatty acids. These latter compounds have been found only in the above mentioned vertebrates (Jacob and Raab, 1996).

From many species of the order Charadriiformes, the red knot *Calidris canutus* (arctic bird of the order Charadriiformes, wintering mainly on the coast of all contiguous continents) was investigated. In the secretion of the uropygial gland of the red knot intact C-32 to C-48 diester wax esters, comprising predominantly C-12 to C-16 alkane-1,2-diols esterified with octanoic, decanoic, and dodecanoic acid at one position, and with predominantly even-numbered carbon fatty acids at the other position were found (Damste et al., 2000). More detailed studies by the methods of gas chromatography/mass spectrometry showed, e.g., that the presented C-21 to C-32 wax esters of this bird species are composed of complex mixtures of hundreds of individual isomers. The odd carbon-numbered wax esters are predominantly composed of even carbon-numbered n-alcohols (C-14, C-16, and C-18) esterified predominantly with odd carbon-numbered 2-methyl fatty acids (C-7, C-9, C-11, and C-13) (Dekker et al., 2000). Reneerkens et al. (2008) conclude that preen waxes of the red knot protect feathers by forming a physical barrier to microbes rather than through any chemical properties of these substances.

Typical birds with a small gland are e.g., birds of the order Columbiformes. Lipids extracted from the gland secretion of the rock dove (*Columba livia*) were investigated during the nonbreeding season. These lipid substances consisted of C14 to C20 fatty acids, predominantly unsaturated fatty acids. The total unsaturated fatty acids comprise 59% of the secretion; oleic acid predominated (37%), while linoleic acid (6%) and arachidonic acid (7%) were also present. No gender differences in the observed parameters were found, probably because the birds were investigated during the nonbreeding season (Montalti et al., 2005).

An important feature of the skin surface is pH and it influences, e.g., microbial colonization. Bartels et al. (1991) studied the surface-pH of the bird skin in 36 bird species (wild and domesticated). The results of measurements on three different apteria showed relatively constant pH values between 4.93 and 6.03, thus mildly acidic. Free fatty acids, products of decomposition of epidermal lipids, can have a specific role in the regulation of microorganism colonization (not only by pH value modification) of the skin surface.

An important finding for animal husbandry and veterinary medicine is the fact that significantly higher pH values could be obtained in poultry from intensive floor husbandry and battery husbandry. This could cause an unnatural microbial colonization of skin with a potential impact on animal health.

Sandilands et al. (2004) examined the gland secretion in laying hens. Nevertheless, the lipids present in feather are a combination of secretion from the preen gland and of sebaceous skin secretion. Analysis of fatty acids of lipids by the method of gas chromatography demonstrated the influence of age, diet (lipid source) and also impact of particular behaviour (the case of individuals whose feathers were pecked by others). Age influences the composition of secretion by changes in circulating hormone concentrations. The individual composition of secretion influences the plumage odour and thus its taste, which causes feathers to become attractive for pecking by other birds. However, Karlsson et al. (2010) reported that in the red jungle fowl (*Gallus gallus*, biologically the same species) individual body odours are present (probably mostly based on differences in the relative abundance of aliphatic carboxylic acids), but they observed no differences in body odours between pecked and non-pecked birds.

This information can help explain one of the problems in poultry farming practice.

The results of the study by Haribal et al. (2009) suggest that the volatility of the secreted components is correlated to the environmental temperature; saturated and unsaturated components in the secretion of tropical bird species have higher molecular weights. The secretions of birds of the family Thamnophilidae consist of long chain acids, alcohols, esters, unsaturated hydrocarbons and isoprenoids. Birds of the family Formicaridae secrete exclusively squalene and its derivatives; in the secretions of birds of the family Pipridae complex long chain esters were found. In birds of the family Dendrocolaptidae long chain esters of both saturated and monounsaturated acids combined with mono-alcohols were identified, in the case of birds of the family Tyrannidae long chain esters of saturated, mono- and tri-unsaturated acids with mono- and di-ols were found.

3. Antibacterial characteristics of uropygial gland secretions

The composition of the uropygial gland secretion of the above mentioned hoopoe (*Upupa epops*) and some other bird species is influenced by both seasonal and sexual factors. It is darker and more malodorous in females and their nestlings during the nesting phase and is spread throughout the plumage. Besides the function of keeping feathers flexible and waterproof, it also has an important role in defending the bird against predators and parasites. However, the active substances are not produced by the bird organism immediately, but by a specific bacterial strain. The finding of a monospecific culture of bacterium determined as *Enterococcus faecalis* in the uropygial secretion of nestlings is very significant. This bacterial strain produces the peptide bacteriocin within the group known as enterocins; it is active against all gram-positive bacteria assayed and also against some gram-negative strains. The production of these broad-spectrum antibacterial substances by an enterococcal strain living in the uropygial gland is an important factor in the nest microecology. It is an example of a biological method of nest hygiene maintenance and thus influences the health of eggs and chicks (Martin-Platero et al., 2006).

Effectiveness against the ubiquitous bacteria *Bacillus licheniformis* is very important as it is one of the bacterias which produce enzymes that digest keratin. Ruiz-Rodriguez et al. (2009) investigated how the keratinolytic effects of *B. licheniformis* were reduced by the *E. faecalis* MRR10-3 strain, isolated from hoopoes, and their bacteriocins. A significant decrease of feather degradation in the presence of the symbiotic *Enterococcus faecalis* or its bacteriocin was found. This chemical defence against feather degradation is applied by hoopoes during feather preening.

The volatile substances in secretions of the European hoopoe (*Upupa epops*) and also of the green woodhoopoe (*Phoeniculus purpureus*) have shown strong antibacterial activity. The role of symbiotic bacteria in the production of substances of this gland was experimentally demonstrated using antibiotics. Thus, the presence of these symbiotic bacteria in the uropygial gland of hoopoes is essential for the protecting the nest from microbes and for general hygiene (Martin-Vivaldi et al., 2010). Further studies also confirmed seasonal changes and intersexual differences in the properties of the above mentioned hoopoe species' uropygial glands and secretions, and the natural occurrence of bacteria within the secretions has been demonstrated. Male glands exhibited no seasonal changes; their secretions were similar to the female products outside the breeding season. However, secretion properties (colour, odour) of female glands showed very expressive changes depending on the breeding season phase. Nestling glands produced a secretion similar to that of the breeding females. A high abundance of symbiotic bacteria was found in these glands during intensive secretion (Martin-Vivaldi et al., 2009).

Seasonal changes in volatile components of the secretions were also analyzed in *Junco hyemalis* (Order Passeriformes, Family Emberizidae), living in North America. Changes in the secretion of linear alcohols (C-10 to C-18), the major volatile compounds, were demonstrated; their levels were higher in both sexes when birds were kept in long-day conditions, similar results were found in methyketones levels. Linear but not branched carboxylic acids (C-12, C-14, and C-16) showed some differences between the breeding and nonbreeding season. However, there was a large individual variation. More sulfur-containing compounds were found in males than in females during the breeding season (Soini et al., 2007).

The antimicrobial activity of uropygial secretion (oil) against feather-degrading bacteria of the wild house finch *Carpodacus mexicanus* was also tested. The results of these experiments suggest that these birds may suppress the growth of some feather-degrading bacteria by their own uropygial oil. The experiments included: identification of 13 bacterial isolates from the plumage, measuring of bacterial keratinase production as an index of feather-degrading activity and use of the disc-diffusion method to test bacterial responses to uropygial secretion. The same tests were performed using a strain of *Bacillus licheniformis*, known as a feather-degrading bacterium. The growth of three strongly feather-degrading isolates (including *Bacillus licheniformis*), one weakly feather-degrading isolate and one non-feather-degrading isolate was inhibited. Growth of most other isolates was unaffected; certain weak growth stimulation appeared in one case of a weakly feather-degrading isolate (Shawkey et al., 2003).

In *Carpodacus mexicanus* (Passeriformes, Fringillidae) antibacterial activity is not the only function of the uropygial secretions. Besides this function, a role for preen waxes as cosmetics in influencing the coloration intensity of plumage was proven, which is important in visual communication and in intraspecific relationships (Lopez-Rull et al., 2010). The results of the study on the Burn Swallow (*Hirundo rustica*, Passeriformes, Hirundinidae) showed that the abundance of feather-degrading bacteria decreased with the increasing size of the uropygial gland and was influenced by sex (females had more feather-degrading bacteria than males). It was also shown that swallows living in larger colonies harboured more of these bacteria than less social conspecifics (Moller et al., 2009).

The microbial circumstances of bird skin can be interesting also from another point of view. Recently, Briscoe et al. (2009) have found different species of *Staphylococcus* in commensal cutaneous microflora of psittacine birds in households and pet stores compared to those living in natural conditions. The finding that the microflora of bird skin in captive birds can be different from the microflora of the same species living in natural conditions is very important for husbandry practice and veterinary care as there may be a shift towards potentially pathogenic microorganisms to the detriment of the birds.

Antibacterially active substances have been described in many bird tissues. In the last ten years, over 20 novel proteins with antibacterial activity were isolated from avian tissues, most of them domestic chickens. Moreover, it can be assumed that this represents a small part of avian antimicrobial proteins (Wellman-Labadie et al., 2007).

The discovery of defensins represents significant milestone in the field. Beta-defensins are cationic, cysteine-rich peptides that play a major role in the innate immune response against bacterial and viral pathogens. The duck beta-defensin-2 homologue, *Anas platyrhynchos* avian beta-defensin 2 was one of the identificated and described defensins (Soman et al., 2009). Three types of avian beta-defensins (AvBD1, AvBD2, AvBD7) were obtained from chicken bone marrow extract (Derache et al., 2009). The peptide avian beta-defensin (AvBD) was isolated from the duck pancreas. A recombinant peptide exhibited strong antibacterial activity against *Bacillus cereus, Staphylococcus aureus* and *Pasteurella multocida*, and milder effects on other bacteria. Expression of the appropriate gene was demonstrated in many tissues, but only low levels were shown in the skin (Ma et al., 2009).

Other peptides with broad antimicrobial activity are the cathelicidins, which have been described in mammals. Another defensin is the chicken myeloid antimicrobial peptide 27 (CMAP27) that was found in chicken bone marrow cells (van Dijk et al., 2005).

These are only some informative examples from a wide spectrum of antimicrobial active substances isolated from avian tissues (other than skin). They have been subjected to intensive research, but probably do not participate in antimicrobial activity on the skin surface. Their detailed characterization is beyond the thematic sphere of this article.

An interesting but also logical finding is that the function of the uropygial gland can influence predation by bird predators: antimicrobial properties of the secretion reduce the feather degrading bacteria and thus would enhance the flight capabilities of birds. This fact was confirmed by a study on 56 bird species of prey of the goshawk (*Accipiter gentilis*) (Moller et al., 2010b).

4. Antiparasitic substances

The skin and feather of some bird species from the genus *Pitohui* and *Ifrita*, living in the New Guinean primavean forest contain poisons from the batrachotoxin group, described primarily in amphibians. *Pitohui* belong to the order Passerines (Passeriformes), family Pachycephalidae. The genus *Pitohui* has seven species (*P. cristatus*, *P. dichrous*, *P. ferrugineus*, *P. incertus*, *P. kirhocephalus*, *P. nigrescens*, *P. tenebrosus*), from which primarily *P. dichrous*, *P. kirhocephalus* and *P. ferrugineus* are known as poisonous. Another known poisonous bird, *Ifrita kowaldi*, also belongs to the order Passerines, family Timaliidae. In feathers and skin the alkaloids batrachotoxinin-A *cis*-crotonate (1), an allylically rearranged 16-acetate (2), which can form from (1) by sigmatropic rearrangement under basic conditions, batrachotoxinin-A and its isomer (3 and 3a, respectively), batrachotoxin (4), batrachotoxinin-A 3'-hydroxypentanoate (5), homobatrachotoxin (6), and mono- and dihydroxylated derivatives of homobatrachotoxin have been identified. The highest levels of these toxins were generally detected in the contour feathers of belly, breast; smaller amounts were found in the head, back, tail, and wing feathers. Batrachotoxin and homobatrachotoxin were found only in feathers and not in skin. The levels of batrachotoxins varied widely among different populations of *Pitohui* and *Ifrita*, which suggests that these birds sequester the toxins from a dietary source (Dumbacher et al., 2000). The analysis of various tissues and organs of *Pitohui dichrous* using radioligand binding assays confirmed that the highest concentration of toxins is in the skin and feathers, and significant levels are present in some internal tissues and organs: skeletal muscle, heart, liver. The presence of toxins in these tissues indicates that pitohui is probably insensitive to these toxins. Investigation by scanning and transmission electron microscopy of skin histology of this bird species showed that it has a typical, common dermal and epidermal morphology. Thus, its unique physiological role in transport and storage of very specific substances, batrachotoxins, is very interesting (Dumbacher et al., 2009). All vertebrate groups, in which batrachotoxins were found, do not produce these toxins *de novo*, but likely sequester them from dietary sources instead. This source is apparently beetles of the genus *Choresine* (family Melyridae), found in the stomach of these birds (a beetle of the family Melyridae occurring in rain forests of South America could be a source of batrachotoxins found in the highly toxic arrow frogs) (Dumbacher et al., 2004). The accumulation of batrachotoxins in Pitohui skin and feathers is regarded as chemical protection against predators and parasites. The phenomenon of tissue toxicity in birds is probably not restricted to this New Guinean species (Bartram and Boland, 2001). Eighty genera and 10 families representing 17 orders, including birds of the order Passeriformes, include malodorous or uniquely odorous birds. Thirty genera and three families representing 13 orders, primarily Passeriformes, were reported as unpalatable. Biologically active compounds may arise for a variety of reasons, e.g., as dietary by-products

(Weldon and Rappole, 1997). Homobatrachotoxin repels and kills chewing lice (order Phthiraptera) and may thus protect birds against lice infestation, but some results suggest that the Pitohui's own lice can have some degree of insensitivity to this toxin (Dumbacher, 1999).

Jonsson et al. (2008) reported that Pitohuis are polyphyletic species, representing several lineages among the corvoid families of passerines. Thus, many related bird species have the ability to ingest the toxin through their insect diet and excrete it through the uropygial gland. The secret of this gland is applied to the skin and feathers as in other birds. The main function of these toxins is regarded as an active substance against ectoparasites and bacterial infection rather than defence against predators.

The positive correlation between uropygial gland size (and thus its secretion activity) and the resistance to chewing lice was investigated and confirmed also in the house sparrow (*Passer domesticus*, Passeriformes, Passeridae). The consequence was a positive correlation between uropygial gland size and body condition, immunocompetence and thus the birds' health. This relationship was not proved in birds living in captivity for longer than one year, which was perhaps due to their access to feeding resources. Similarly to the above mentioned species *Carpodacus mexicanus*, the positive effect of uropygial gland secretions on the colouring mark of plumage was also found in the house sparrow. In the house sparrow, it is the size of the wingbar acting as a sexual signal, which indicates the resistance of a potential mate to ectoparasites (Moreno-Rueda, 2010). Antiparasitic substances were also isolated from the feather of the crested auklet (*Aethia cristatella*). Compounds isolated from the feathers of this species include *n*-hexanal, *n*-octanal, *n*-decanal, Z-4-decenal and a 12-carbon unsaturated aldehyde. Octanal and hexanal are ectoparasite repellents and a signal of mate quality at the same time (Douglas et al., 2001). Uropygial gland secretions seem to operate differently in different taxa of ectoparasites. Moller et al. (2010a) analyzed the variation of uropygial gland size in 212 bird species. Bird species with larger uropygial glands had more genera of chewing lice of the sub-order Amblycera, but not of the sub-order Ischnocera, and more feather mites. These authors report also higher hatching success in the birds with larger uropygial glands.

Bird skin may contain substances that act as potential attractants for hematophagous insects. A remarkable case is the finding of substances with differential attractiveness for ornithophilis mosquitos (*Culex* spp.) in hexane and ether extracts from skin, feet and feathers of chickens (*Gallus gallus domesticus*). The active attractive extracts were hexane extracts, in which alcohols, ketones and diones were found, while in inactive ether extracts, there were aldehydes (nevertheless also contained in hexane extracts) (Bernier et al., 2008).

5. Similarity of the composition of secretions as an indicator of phylogenetic affinity

The characteristics of uropygial gland secretion composition can make a certain contribution to the evaluation of bird taxon affinity and thus can affirm the results of other domains of research (DNA-study, comparative morphological studies etc.). Gebauer et al. (2004) found considerable similarities in the fatty acid composition of uropygial gland secretions in the Hume's ground jay (*Pseudopodoces humilis*) and tits (birds of the family Paridae), but differences in the birds of the family Corvidae. This result corresponds to other independent characteristics (comparative osteology, nuclear and mitochondrial DNA sequences). It shows a nearer affinity of *Pseudopodoces humilis* to birds of the family Paridae than to the family Corvidae (in which it has been classified so far).

Feather waxes are omnipresent in the feathers of passerine birds (the order with the highest number of birds), however its characteristics are not well known. An important component of feather waxes – saturated fatty acids – is highly stable. Therefore it is possible to use samples from museum specimens that are up to a hundred years old. Analyses of these specimens provide comparable results to analyses of extracts from fresh material. Although feather-wax composition is not critical for the reconstruction of phylogenetic relationships, elucidating its diversity can make a small contribution to comparative phylogenetic studies. A study of saturated fatty acid diversity in feather waxes in 91 passerine species was performed using gas chromatography/mass spectrometry. In all investigated species, unbranched fatty acids were found, mainly those from the group of saturated fatty-acids; the occurence of other classes of fatty acids was more variable. The specific findings were the following: 2-methyl fatty acids were found in a number of passerine families, often in high proportions, however they were absent or low in all investigated fringillid species except for the Dickcissel (*Spiza americana*); 3-methyl fatty acids were conversely found only in the birds of the family Fringillidae and in birds of the genus *Zoothera* from the family Turdidae. 4-methyl compounds were rare and were only isolated from birds of families Paridae, Polioptilinae and Troglodytidae (Sweeney et al., 2004).

6. The unique function of specific lipid-enriched organelles in bird skin

Sebokeranocytes of the bird epidermis process and secrete sebum-like lipids and they also process, but rarely secrete, specific lipid-enriched organelles – multigranular bodies. These organelles form a cutaneous barrier reserve in bird epidermis. Under normal conditions, multigranular bodies are not secreted and the organism is able to cool itself, i.e., to thermoregulate, by evaporation. Under conditions of water deficit, multigranular body secretion allows for facultative waterproofing, as described in zebra finches (*Taeniopygia guttata*), often used as experimental birds. A high degree of lipid secretion has been described in naturally glabrous skin regions in some bird species; lipid-rich material is also found in some types of specialized feathers (Menon and Menon, 2000). The epidermal structure of zebra finches consuming ample water and water-deprived zebra finches and also the neck epidermis in the ostrich (*Struthio camelus*) has been studied previously (Menon et al., 1996). Facultative waterproofing is apparently a mechanism of protecting the organism against water loss in the case that water is unavailable.

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