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ORIGINAL PAPER

Effects of access to preen gland secretions on mallard plumage

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Abstract Preen glands exist in almost every bird species and several non-exclusive functions have been proposed for this gland and the oils that it produces. One function generally admitted is that the oily secretions of the preen gland would provide a waterproofing layer when spread over feathers. Alternatively, several authors have proposed that plumage waterproofness is mostly due to the spatial micro-structure of feathers. The purpose of this study was to examine, by manipulating the access to the preen gland, the effect of the preen oil on the plumage waterproofness and condition. To explore this question, we carried out two independent experiments where we temporarily blocked access to the preen gland secretions with a removable mechanism in one group of captive mallards (*Anas platyrhynchos*), whilst a second group of birds had access to gland secretions. In a long-term experiment (3 months of treatment) and a short-term experiment (10 days), we measured plumage water retention and condition. After 3 months without access to preen glands, we found a significant decrease of plumage condition and an associated increase in plumage water retention. Moreover, we found a significant correlation between plumage condition and water retention ability. In contrast, after 10 days of treatment, no significant effect was found on plumage condition and water retention. Our study shows that preen oil acts to maintain plumage condition and suggests that feather micro-structure is essential to maintain plumage waterproofness.

Keywords Preen oil · *Anas platyrhynchos* · Plumage condition · Water repellency

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Introduction

Nearly all birds have a preen gland on their rump (Moyer et al. 2003). This gland secretes a lipid-rich oil spread by birds over their plumage with the bill during frequent preening sequences (Jacob and Zisweiler 1982). Several non-exclusive functions have been proposed for preen oil (Jacob and Zisweiler 1982, Reneerkens 2007). First, preen oil may act against feather-degrading bacteria through the action of anti-bacterial properties of its compounds or the action of others bacteria hosted in the preen gland (Shawkey et al. 2003;

Ruiz-Rodriguez et al. 2009). In addition, it has been proposed that the oily secretions spread over the feathers may provide waterproofing layers due to their hydrophobic compounds (Jacob and Zisweiler 1982). However, it was suggested that the water repellency of feathers was mostly determined by a structural process resulting from the diameter and spacing of the barbs and barbules (Rijke 1970).

Previous experiments, where preen glands have been surgically removed from mallards, showed that the plumage of bird without gland contained twice as much water compared to control birds (Joseph 1891). More recently, Rutschke (1960) found that the buoyancy of ducks was not affected by the removal of fat components from the belly feathers. However, Van Rhijn (1977) criticized the protocol used since all the fat was apparently not removed. The latter author did not find convincing effect of a removal of preen wax with benzene and ethanol on water absorption of gull feathers. Van Rhijn (1977) thus suggested that feather microstructure is the most important mechanism implicated in plumage waterproofness. Although it is often claimed that the main function of preen oil is water repellence (Jacob and Zisweiler 1982), current evidence is based on dated experiments and appears to be contradictory (see Reneerkens 2007).

Our aim was to investigate the link between preen oil, plumage waterproofness, and condition with a non-invasive method. To explore this question, we temporarily blocked the access to the preen gland with a removable mechanism in one group of captive mallards (*Anas platyrhynchos*), whilst a second group of birds had access to the gland. To test whether access to the preen gland modifies plumage quality, we compared plumage water retention and its integrity between our two groups of birds. We replicated this test in two independent experiments. In a long-term experiment, we measured plumage water retention and condition 3 months after the start of the treatment. In a short-term experiment, we measured the same parameters to examine if 10 days of treatment led to a decrease of plumage waterproofness without the potential effects of long-term feather degradation.

Concerning the long-term experiment, we predicted a negative effect of our treatment on plumage condition. This effect and the absence of preen oil hydrophobic compounds on the feathers of experimental birds are expected to result in an increase of plumage water retention. For the short-term experiment, we predicted no difference of plumage condition between the two groups of birds but a lower plumage waterproofness of birds without preen gland access due to the absence of hydrophobic compounds on their feathers.

Material and methods

Experiments were carried out between February and April 2007 and in February 2009 on a group of mallards kept in semi-captivity. Individuals (3–5 years old) were held in a large open pen (4 ha of grassland) with free access to water and food. This study was conducted in agreement with French legislation and veterinary services.

Long-term experiment

We randomly assigned and fitted birds in a group with anti-preening mechanism (APM, N=16, equal sex ratio). APM was designed to prevent bill-preen gland contact and thus the spreading of the preen oil onto the feathers. As a control, a second group of birds (N=22, equal sex ratio) was fitted with control preening mechanism (CPM) which were identical to the APM, except that it did not prevent birds from spreading preen oil on their feathers (see electronic supplementary material for detailed methods).

After 3 months, plumage condition and water retention were measured. Plumage wettability was obtained by weighing the bird's dry and wet weights. The difference between the two weights indicated the amount of water retained by the plumage and provided a measure of plumage wetness (see electronic supplementary material for detailed methods).

In order to measure plumage condition, we used a similar method as Moyer et al. (2003). Plumage quality was scored (N=32) from 1 to 4: 1=poor condition, 2=fair, 3=good, 4=very good. Five body parts (belly, back, head, upper wing, and under wing) were considered for each bird by three independent observers blind to the treatment identity (see electronic supplementary material for detailed methods).

Short-term experiment

Twenty birds were assigned to the CPM and 19 to the APM groups (equal sex ratio). We measured, in the same way as described above, plumage condition after 10 days and water retention at the start of the experiment and 10 days after.

Statistical analyses

As plumage scores were significantly repeatable between body parts (R=0.78, P<0.0001) and observers (R=0.6, P<0.001, Lessells and Boag 1987), analyses of plumage condition were performed using mean values of all the scores for each body part and observers per bird. We then performed a two-way analysis of variance (ANOVA) for ranked data. Finally, we computed a correction factor to take into account the ties (Sokal and Rohlf 1995).

Analyses of plumage waterproofness changes between the end and the beginning of the short-term experiment were performed by using an ANOVA. For the long-term experiment, ANOVA were performed on plumage water retention measured at the end of the experiment because only these data

Table 1 Short and long-term effects of access to preen gland on plumage condition and water retention in mallards (*Anas platyrhynchos*)

	Short-term experiment			Long-term experiment		
Plumage condition						
Effect	Df	F	P	Df	F	P
Sex	1	0.0019	0.99	1	4.9	0.001
Treatment	1	1.79	0.82	1	9.4	$\mathbf{10^{-5}}$
Sex×treatment	1	2.32	0.79	1	0.0002	0.98
Plumage water retention						
Effect	Df	F	P	Df	F	P
Sex	1	0.25	0.62	1	2.3	0.14
Treatment	1	1.99	0.17	1	36.11	$\mathbf{10^{-6}}$
Sex×treatment	1	0.38	0.54	1	0.03	0.85

were available. Mass and tarsus length factors were excluded from the models since they always showed non significant effects (All $P>0.2$).

We did not have the same sample sizes for all the measurements since birds were held in semi-captive condition in a large area where it was difficult to catch all of them since they could hide in vegetation. Statistical analyses were conducted with the software STATISTICA 6.0 and R.

Results

Long-term experiment

After the 3 months that the experiment lasted, the plumage of APM birds had a lower condition with a loss of the normal fluffy appearance than CPM birds (Table 1 and Fig. 1). Sex also influenced plumage condition; females had plumage in poorer condition than males at the end of the experiment. No effect of sex×treatment interaction on plumage condition was found. Birds' plumage returned to good condition once the experimental device had been removed and after a normal moult (Giraudeau, personal observation).

Treatment had a highly significant negative effect on plumage waterproofness. Water retention was greatest for APM than for control (CPM) birds (Table 1 and Fig. 2). Sex did not significantly affect plumage water retention although females had more degraded feathers than males (Table 1). Finally, we found a negative correlation between plumage scores and water retention ($F_{1,36}=9.6$; $P=0.004$; $y=-14.17x+96.48$; Fig. 3).

Short-term experiment

We found no significant difference of plumage condition between birds with or without access to the preen gland after 10 days. There was also no significant effect of sex and sex× treatment interaction on plumage condition (Table 1 and Fig. 1).

There was no significant difference in plumage water retention between the treatments (t test: $t=-0.67$; $P=0.5$) at the start of the experiment.

Treatment did not significantly affect plumage water retention after 10 days (Table 1 and Fig. 2). In contrast to the long-term experiment, we did not find a significant correlation between plumage score and water retention ($F_{1,37}=2.26$; $P=0.14$).

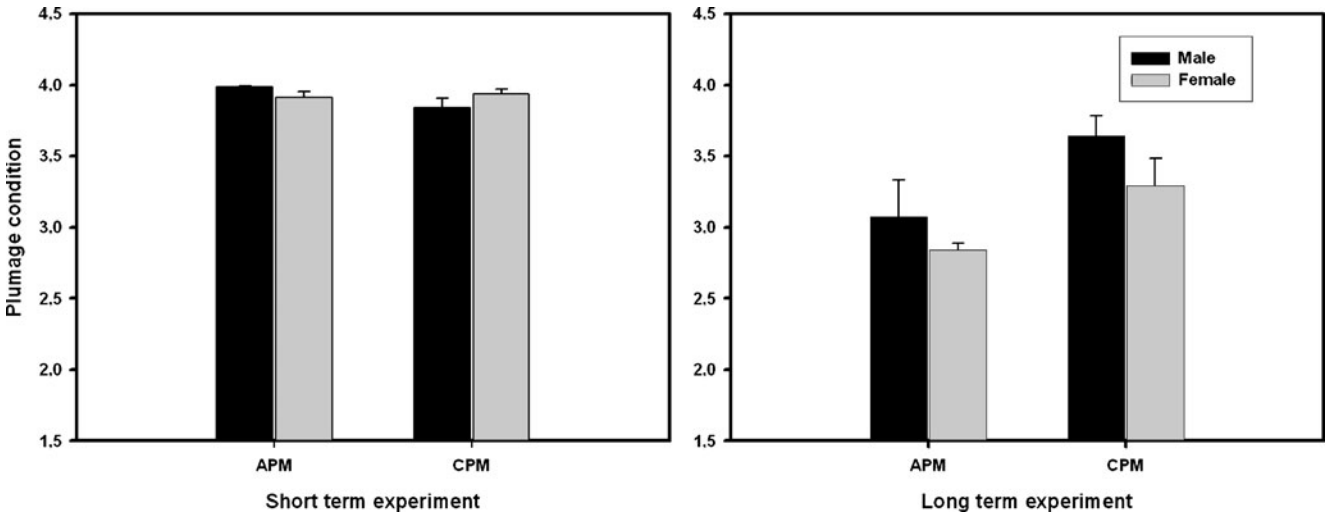

Fig. 1 Mean (+SE) plumage condition of mallards (*Anas platyrhynchos*) without (APM) and with preen gland access (CPM) after 10 days (short-term experiment) and 3 months of treatment (long-term experiment). *Black bars* represent males and *grey* represent females

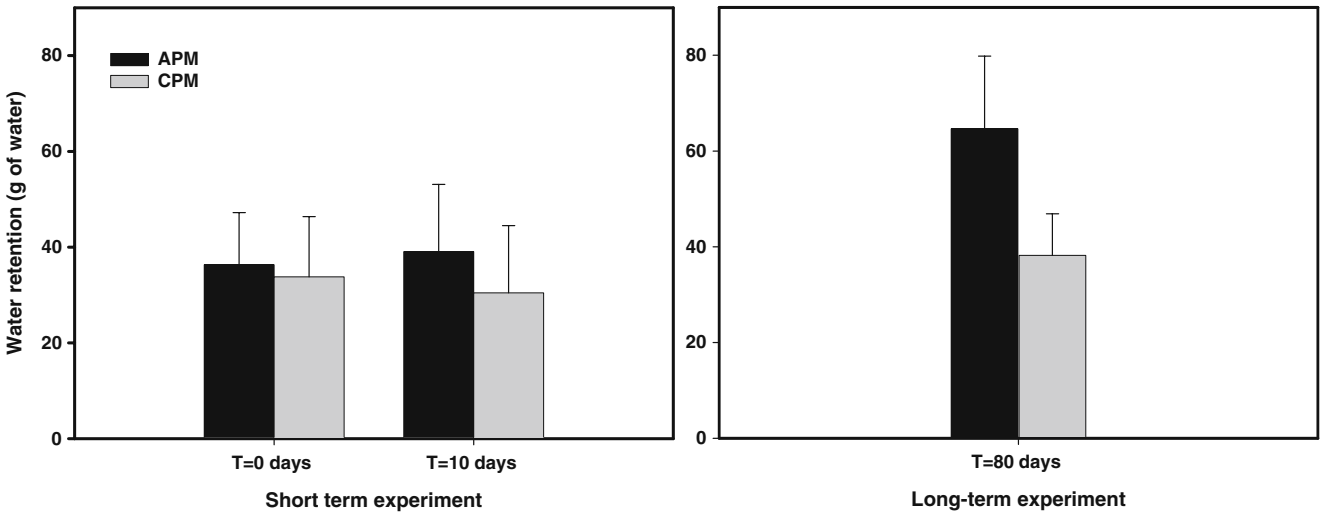

Fig. 2 Mean (+ SD) water retention (grams of water retained in plumage) of mallards (*Anas platyrhynchos*) with access (CPM, *grey bars*) or no access (APM, *black bars*) to their preen gland before and after 10 days (short-term experiment) and after 3 months of treatment (long-term experiment)

Discussion

During the long-term experiment, birds without access to the gland showed a plumage in poorer condition with a lower water repellency efficiency compared to control birds. In addition, plumage condition and water retention were negatively correlated at the end of this long-term experiment. Our results are consistent with previous studies which showed a decrease of plumage condition after surgical removal of preen glands in waterfowl (Hou 1928, Elder 1954) and Rock doves (*Columbia livia*, Moyer et al. 2003). Absence of preen oil on plumage led to plumage degradation either through feather deterioration or structural organisation of barbules. Either of these mechanisms could be associated with the decline of plumage waterproof efficiency observed in our experiment (Rijke 1970).

After 3 months of experiment, the plumage of females was in significantly poorer condition than those of males. This difference could be explained by the fact that plumage condition was estimated during the reproductive period, when females invest more in reproduction (nest building and egg laying) than males (Tamisier & Dehorter 1999). In support of this hypothesis, it was found in Starlings that a greater investment in reproduction was associated with greater plumage bacterial loads (Lucas et al. 2005). Another explanation could be that males invest more time in plumage maintenance because of the related sexual dimorphism in coloring and thus greater investment may be required by males to maintain the plumage in order to attract mates. However, this hypothesis must be considered cautiously as bill color seems to be a more important trait than plumage color for female mate choice decisions (Omland 1996). Finally, an alternative explanation may be that females were more parasited by feather-degrading bacteria than males as it was recently shown in the barn swallow (hirundo rustica, Moller et al. 2009).

In the short-term experiment, we observed that without access to preen glands, plumage degradation requires more than 10 days since there was no significant effect of treatment on plumage condition. Moreover, as expected if plumage water repellency efficiency is associated with feather structure, we showed that no access to the preen gland during 10 days did not affect plumage water retention. Our results suggest that preen oil may not act directly on the waterproof efficiency of plumage by the action of its hydrophobic compounds since after several days, preen waxes may be oxidized and thus have lost their

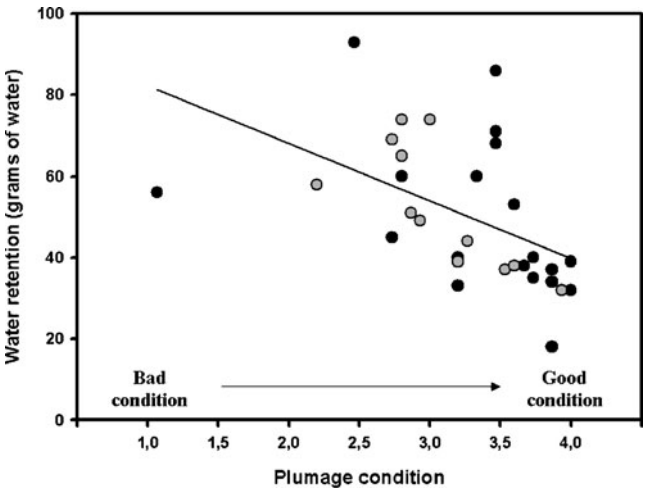

Fig. 3 Relationship between plumage condition and plumage water retention in mallards (*Anas platyrhynchos*) after 3 months of experiment. *Black points* represent males and *grey* represent females

waterproofing ability (Borchelt et al. 1973). However, an examination of preen wax oxidation kinetics is now needed to determine precisely the time after which preen wax are oxidized on feathers. Our study is in accordance with previous in vitro results which did not show convincing effect of preen oil removal on feather water absorption (Van Rhijn 1977). The importance of preening for plumage water repellency efficiency could be studied in greater detail with the use of others in vivo designs. For example, a study during which plumage water retention would be measured after an experimental removal of preen waxes on the plumage could confirm our results.

Two mechanisms could explain how preen oil protects feathers from degradation and indirectly maintain plumage water repellency efficiency. First, plumage can be damaged by breakage, wear of barbs, or photomechanical processes (Moyer et al. 2003). It has been proposed that the plumage of birds with preen gland access is more resilient to abrasion from preening (Moyer et al. 2003). Preen oil may help feathers to keep their strength and flexibility (Jacob and Zisweiler 1982). However, this property of preen oils has never been demonstrated and future studies should examine if preen oil acts to maintain barbule flexibility.

A second mechanism responsible for feather damage in the absence of preen oil could be plumage infection with parasites and particularly bacteria. Birds carry keratinolytic bacteria on their feathers which are able to degrade feathers (Burtt and Ichida 1999). Shawkey et al. (2003) demonstrated in vitro anti-microbial activity of preen oil of house finches (*Carpodacus mexicanus*) on some keratinolytic bacteria isolates. Moreover, antibacterial chemical compounds were recently found in preen glands of different bird species (Haribal et al. 2009; Martín-Vivaldi et al. 2010). These results suggest that birds may defend themselves against feather-degrading bacteria using preen oil and could explain why birds without access to the preen gland had plumage in poorer condition. Further experiments must be done to examine the effect of preen oil on keratinolytic bacteria. For example, a comparison of keratinolytic bacteria loads between birds equipped with anti-preening mechanisms or able to use their preen glands would constitute an interesting way forward.

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