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Uropygial Gland-Secreted Alkanols Contribute to Olfactory Sex Signals in Budgerigars

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Abstract

The possible role of uropygial gland-secreted compounds in olfactory discrimination of sex or sex attractants in the budgerigar, *Melopsittacus undulatus*, was investigated using behavioral 2-choice tests and gas chromatography–mass spectrometry analysis. Our data showed that female budgerigars were capable of distinguishing males from females in a Y maze via body odor, indicating its sexual dimorphism. When we conducted a chemical assay of the uropygial preen gland secretions, we found 4 times more volatile octadecanol, nonadecanol, and eicosanol in ratios in males than in females, making them putative male pheromone candidates. Female birds also showed overt preferences for the odor of male preen gland secretions or the 3-alkanol blend equivalent preened onto the plumage of a male over that of female counterparts. Removal of any one alkanol was associated with a loss of attractiveness to the female. In another test device (a test cage) with visible male bird stimulus, females chose the male with the 3-alkanol blend of males over the other male with female preen gland secretion, whereas did not differentiate their responses between the males with either this blend or male preen gland secretions. The behavioral data robustly suggested that the 3 alkanols synergistically created a female attractant odor or male pheromone in the budgerigar and that bird uropygial glands have broader implications in sexual behavior than previously known. This is the first investigation with bioassay of components of the gland in a bird species.

Key words: Aves, female attractant, male pheromone, *Melopsittacus undulatus*, olfactory sex recognition, uropygial gland

Introduction

Work on a number of bird species suggests that the capacity for chemical communication in birds is likely to be comparable with that of mammals with special reference to both olfactory reception such as food searching and predator assessment and odor production (Grubb 1972; Jones and Roper 1997; Weldon and Rappole 1997; Hagelin et al. 2003; Bonadonna and Nevitt 2004; Nevitt and Bonadonna 2005; Amo et al. 2008; Nevitt 2008; Steiger et al. 2008; Balthazart and Taziaux 2009). Sex recognition serves as the first step in breeding behavior of many animals and sex attractants or sex pheromones released by animals to attract opposite sex mates are therefore likely to exist in birds as in mammals and are worthy of exploration (Brennan and Zufall 2006; Hagelin and Jones 2007; Douglas et al. 2008). Some previous studies have shown that olfactory cues are involved in sexual behavior in birds. For example, sexual behavior was significantly inhibited in male domestic ducks (*Anas platyrhynchos*) with olfactory nerve section (Balthazart and Schoffeniels 1979); uropygial gland-removed females are less attractive to males in domestic chickens (*Gallus gallus domesticus*; Hirao et al. 2009). On the other hand, sexual differences in the chemical composition of the uropygial gland waxes and volatiles in some birds such as domestic ducks, dark-eyed Juncos (*Junco hyemalis*), and Bengalese finches (*Lonchura striata*) have been detected (Jacob et al. 1979; Soini et al. 2007; Zhang et al. 2009). However, it has not been directly demonstrated that birds can use body-emitted odor for sex discrimination (Bonadonna et al. 2009).

The budgerigar (*Melopsittacus undulatus*) is a small parrot native to Australia that lives in flocks, is the most popular caged bird worldwide, and is known to use vocal behavior, plumage coloration, and fluorescent and ultraviolet colors for sexual attractiveness (Dooling et al. 1987; Arnold et al. 2002, Eda-Fujiwara et al. 2003; Zampiga et al. 2004).

Because budgerigar males and females exhibit visible sex monomorphism except cere color and lack sexually dimorphic vocalization (Dooling et al. 1987), we hypothesized that sexual odor could contribute to sex discrimination in the budgerigar.

Animal sebaceous glands, particularly in mammals, are important sources of secreted pheromones, in addition to other substances (e.g., oily wax esters) (Wyatt 2003; Brennan and Zufall 2006). In rodents, the sebum gland-secreted volatiles being either male specific or greater in relative concentrations in males than in females can be viewed as male pheromone candidates and are usually verified as pheromones by bioassay (Singer et al. 1997; Sun and Müller-Schwarze 1998; Zhang et al. 2007, 2008). The uropygial gland (also called preen, oil, or scent gland) is a large gland at the base of a bird's tail and is found in the large majority of birds that secrete oil used in preening. A bird typically transfers this oil to its feathers by rubbing its head against the oil and then around the rest of the body. Like the preputial gland in rodents, the preen gland serves as the largest exocrine gland and is the most likely pheromone source in most birds. For 2 particular examples, a synthetic analog of uropygial secretion of mother hens increase growth and decrease stress of chicks (Madec et al. 2006); the role of uropygial glands in mediating sexual behavior relies on olfaction domestic chickens (Hirao et al. 2009). Previous research has mainly focused on the glandular nonvolatile wax composition and functional association with light reflectance and plumage waterproofing in birds (Arnold et al. 2002; Zampiga et al. 2004; Soini et al. 2007; Jacob and Ziswiler 1982; Zhang et al. 2009). Emerging evidence shows that the avian uropygial gland produces low molecular weight volatiles that quantitatively vary with reproductive status, structurally resemble insect and mammalian pheromones, and can be preened into body plumage for likely airborne chemosignal transmission (Wyatt 2003; Burger et al. 2004; Zampiga et al. 2004; Madec et al. 2006; Bonadonna et al. 2007; Soini et al. 2007; Zhang et al. 2007, 2008; Douglas 2008), implying that the secretions might contribute to chemical sexual signaling in budgerigars. We thus hypothesized that if the gland secretion of males could attract females, it must include some volatiles exhibiting male-specific properties and/or be quantitatively heightened in males and affect behavior. Therefore, we characterized the constituents of the gland secretions to ascertain the putative male pheromone components via qualitative and quantitative comparison using gas chromatography–mass spectrometry (GC-MS) and then validated the activity of the pheromone candidates using behavioral 2-choice tests.

Materials and methods

Animals

Twenty-four male and 24 female budgerigars (yellow and green-based coloration) at 6–14 months of age were obtained from 3 large colonies maintained by respective pet owners. Eight males and 8 females were assigned randomly to each of 3 sex-mixed groups. The color of the cere of the subjects showed an overt difference between the sexes: blue in males and brown in females, indicative of sexual maturity. We held each group in a large cage composed of 2 connected wire cages ($47.5 \times 33 \times 32.5$ cm each) at 25 ± 2 °C and in the natural Beijing, China photoperiod that occurred April–August, 2008. The birds were tested after 4 weeks of acclimation. We provided mixed grain seeds, vegetables, eggs, and tap water ad libitum.

We exclusively used 1 of the 3 groups of birds as body odor, uropygial gland secretion and feather donors ($N = 8$). From the other 2 groups, all the females were used as detectors to validate the activity of pheromone analogs. When testing females' choice between living birds, the birds from these 2 groups were mutually used as either stimuli or the recipients ($N = 16$), where the stimulus birds were placed in the rear compartment partitioned by wire nettings in each arm of the Y maze as whole sensory stimuli or sealed in the glass jars beside the test cage as the visual and acoustic stimuli. In toto, 16 females from the 2 groups were tested at all stages of the experiment.

Odor collection and preparation

To collect gland secretions, we put on PE disposable plastic gloves, picked up a living bird, sanitized its gland openings with 75% alcohol swabs that we held with a pair of scissors, gently pressed on its paired glands to expel the secretions, and loaded 3–6 mg into a vial. We also used the scissors to sample ≈30 mg of wing feather.

Prior to extraction, we weighed the secretions or feathers and added dichloromethane into the vial at a proportion of 1-mg secretion or feathers in 20-μL dichloromethane (purity > 99.5%; Dima Technology, Inc.) and incubated the sample at 0 °C for 12 h. Then, we transferred the extract to vials and stored them at –20 °C until GC-MS assay.

Odor presentation and 2-choice test devices

To prepare the odor stimulus used in behavioral tests, we used dichloromethane to dissolve and dilute a mixture of octadecanol (18OH) (850 ppm), nonadecanol (19OH) (650 ppm), and eicosanol (20OH) (1250 ppm) (the 3 alkanols' purity > 98%, purchased from ACROS Organics made by Toshima, Kita-Ku) or glandular secretions (5% w/v) mixed in equal parts from either 8 females or 8 males and then evenly painted the solution onto the inside bottom of a petri dish (inner diameter = 6 cm). Prior to the petri dish presentation, we laid the prepared dish aside for 5 min to let the solvents evaporate. As the measured results described below, 40 μL and 10 μL of the mixed alkanol solution stand as an equivalent of one male and one female, respectively; 200 μL of the glandular secretion solutions (5% w/v) stand as an equivalent preened on one budgerigar plumage.

A Y maze was used initially to validate the activity of body odor and the presence of putative male pheromones. The Y maze was made of a galvanized iron sheet that had two 50-cm long symmetrical choice arms, each placed at an angle of 90°, and a 25-cm starting arm equipped with a sliding wire mesh door that regulated access to the choice arms. Each choice arm contained another wire mesh door that separated a rear compartment used to hold a living bird for stimulus bird presentation. Each arm also was connected by a silicone tube (100-cm long; inner diameter = 3 cm) with outside transparent glass vacuum desiccators as above mentioned, into which the test odor materials, including living birds, glandular secretions, and the blends of 3 alkanols, could be sealed for odor presentation. A pump-forced airstream flowed through the 2 jars and into the respective choice arms at 60 mL/min. "Investigating time" was recorded when the test birds entered each choice arm (Figure 1).

A test cage was used to confirm the identity of pheromones. In this case, transparent glass jars were used to prevent the body odor emission of stimulus birds and allow examination of the significance of chemical communication in comparison with visual and acoustical communication in budgerigars. The test cage consisted of a "start" wire cage (20 × 13 × 12 cm) that had a sliding wire door to a "choice" wire cage (26 × 18 × 12 cm), where 2 opposite ends of 6-cm long for each were designated as the "choice areas," and the middle 14 cm was the neutral area. We placed 2 transparent glass desiccators (as described above) beside the test cage and sealed (to prevent odor emission) one living male or female bird inside each; each of the 8 paired stimulus budgerigars used had a similar appearance except the cere color between males and females. The airflow in these jars was redirected outside the room in order to screen demonstrator odor from the test birds. To present odor stimuli, we added the blends of 3 alkanols (i.e., 18OH, 19OH, and 20OH) at the doses preened onto the whole plumage of a male or a female. Each petri dish held a different dose of the pheromonal analogs, and the dishes were placed under the cage close to the jars. Investigating time was recorded when the test bird's head was oriented toward the jar within 6 cm (Supplementary Figure S1).

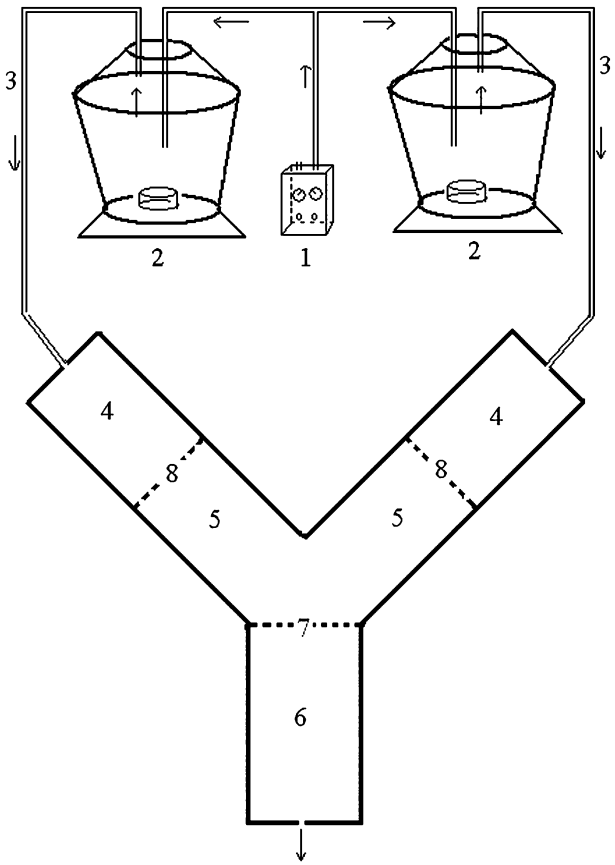

Figure 1 A sketch map of the Y maze linked with glass desiccators with pump-forced airstream for odor presentation. 1, air pump; 2, glass desiccators for odor stimulus presentation; 3, connecting silicone tube; 4, the rear compartments of the choice arms of Y maze for living bird stimulus presentation; 5, the choice arms of Y maze; 6, the starting arm of Y maze; 7, wire mesh gate; 8, wire mesh partitions.

A focal bird was placed in the start arm of the Y maze or the start wire cage of the test cage and acclimated for 3 min, and the bird then was released by opening the sliding door for a 3-min habituation to the entire Y maze or test cage. We then restricted the focal bird to the start area for another 3-min acclimation and set up the stimuli. Finally, we released the test birds for a 3-min investigation and videotaped the investigating time.

Prior to trial, each sex-mixed group was separated into male or female subgroup for 12 h. Each bird was used only once every other day. All tests were performed in the daytime.

The paired odorants were put in left and right at random. No significant differences between the time spent by birds in left and right choice areas were found across all behavioral trials.

GC-MS analysis

We performed analytical GC-MS (Agilent Technologies, Inc.) using an Agilent Technologies Network 6890N GC system in combination with a 5973 mass selective detector and the MS Library (National Institute of Standards and Technology 2002; Agilent Technologies 2002; Windows 2000). The GC had an HP5-MS column (30 m × 0.25 mm internal diameter × 0.25 μm film thickness; Agilent Technologies, Inc.), carrier gas helium at 1.0 mL/min, injector, and was set at 280 °C. We programmed the oven at 5 °C/min from 70 to 280 °C. MS was in the electron impact mode (70 eV), and the transfer line was set at 280 °C. In total, 1 μL of the gland secretion extract or 5 μL of the feather extract was injected in the splitless mode.

We made tentative identifications using the MS library and diagnostic ions. We further confirmed 3 fatty acids, 5 linear alkanols and squalene by matching their retention times, and mass spectra with authentic analogs (all purity > 98%;

purchased from ACROS Organics) following separation with both a nonpolar column (HP5-MS) and a polar column (DBWAX, 30-m long, 0.25 mm internal diameter × 0.25 μm film thickness; Agilent Technologies, Inc.). To measure the relative abundances of the compounds, we converted the peak area (obtained by HP5-MS column) of a particular compound into a percentage of the summed peak area using the 23 detected volatile GC peaks from budgerigar uropygial glands. To measure the amounts of 18OH, 19OH, and 20OH in the samples, we compared their GC areas in the samples with the established standard curve of GC areas versus concentrations.

Statistical analysis

We used a Kolmogorov–Smirnov test in SPSS (version 13.0) for Windows to examine the distribution of raw data. Subsequently, we analyzed the amounts (or ratios) of the volatiles with either an independent 2-tailed *t*-test (if the data were normally distributed) or a Mann–Whitney *U* test (if the data were not normally distributed). Likewise, we used a paired *t*-test and a Wilcoxon signed-rank test for normal and nonnormal behavioral data, respectively. For all tests, $P = 0.05$ was set as the level of significance.

Ethical notes

The procedures of animal care and use in this study fully complied with the legal requirements of China and were approved by the Animal Use Committee of the Institute of Zoology, Chinese Academy of Sciences, where the experiments were conducted.

Results

Behavioral responses to whole birds and bird odor

By using a Y maze, we found that female budgerigars exhibited significant preferences for living male birds over female birds ($Z = 2.701$, $N = 16$, $P = 0.007$) and male body odor over female body odor ($Z = 2.298$, $N = 16$, $P = 0.025$) (Figure 2).

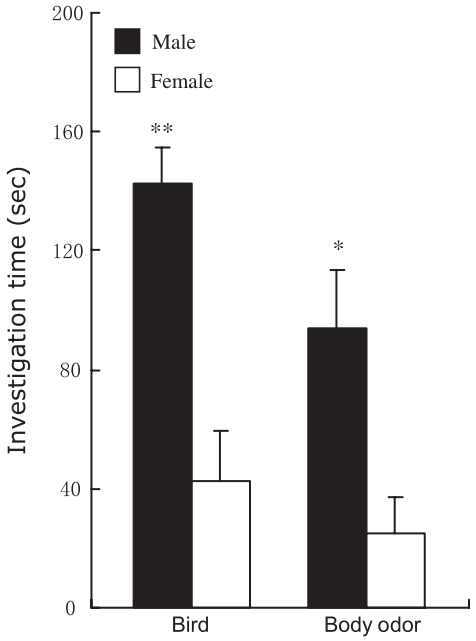

Figure 2 Binary choices by female budgerigars, *Melopsittacus undulatus*, ($N = 16$ birds) between birds themselves ($N = 16$) or between body odor of male (M) and female (F) birds ($N = 8$ tests for each sex) in a Y maze (values = mean ± standard error of the mean; Wilcoxon test, $*P < 0.05$; $**P < 0.01$).

Sexually dimorphic compounds from uropygial glands

GC-MS results revealed the early (before 40 min) peaks eluting from the capillary GC as hexadecanoic acid, heptadecanol, 18OH, 19OH, 20OH, heneicosanol, and 15 pentanoates with linear alkanol or alkenol chains (C_{16}–C_{20}) and the late peaks (after 40 min) as ester waxes with long-chain fatty acids (C_{16}–C_{18}) (Figure 3 and Supplementary Figure S2).

In addition, we observed similar GC profiles between dichloromethane extracts of the glandular secretions and feathers (Figure 3 and Supplementary Figures S2, S3, and S4).

We did not find compounds unique to males. However, further comparison of percent GC peak areas of volatile compounds revealed significantly higher relative abundances of glandular hexadecanoic acid and alkanols in males than in females ($N = 8$, $P < 0.05$ in Table 1). In particular, 18OH, 19OH, and 20OH were 4 times greater in relative abundance in males than in females ($N = 8$, $P < 0.01$ in Table 1) and constituted 73% of the male volatiles (Table 1).

The available amounts of 18OH, 19OH, and 20OH for signaling

The contents of 18OH, 19OH, and 20OH determined by GC-MS were 3.58 ± 3.06 μg, 2.78 ± 2.67 μg, and 5.32 ± 3.10 μg per mg of secretion in males ($N = 8$, mean ± standard deviation), respectively.

Using dichloromethane extraction, we determined the amount of 18OH preened into budgerigar body plumage to be 8.38 ± 4.47 ng ($N = 8$) per mg of feather (Supplementary Figure S4). We could not directly measure the amounts of 19OH and 20OH with GC-MS because they coeluted with other feather-derived compounds in GC chromatograms (Supplementary Figure S4). Instead, we calculated their averages with the ratio of their amount versus the amount of 18OH (w/w) in the secretions to be 6.51 ng and 12.45 ng per mg of feather, respectively. Thus, each male bird had approximately 34 μg 18OH, 26 μg 19OH, and 50 μg 20OH on whole plumage (≈4-g total plumage), which collectively would be equivalent to the quantities in 10-mg uropygial gland secretion spread over all body plumage. As a result, we blended the synthetic analogs of 18OH, 19OH, and 20OH at these quantities (34 μg 18OH, 26 μg 19OH, and 50 μg 20OH) into one unit of male secretion to be used in the binary choice tests as described below. Meanwhile, to mimic the lower quantities found in females,

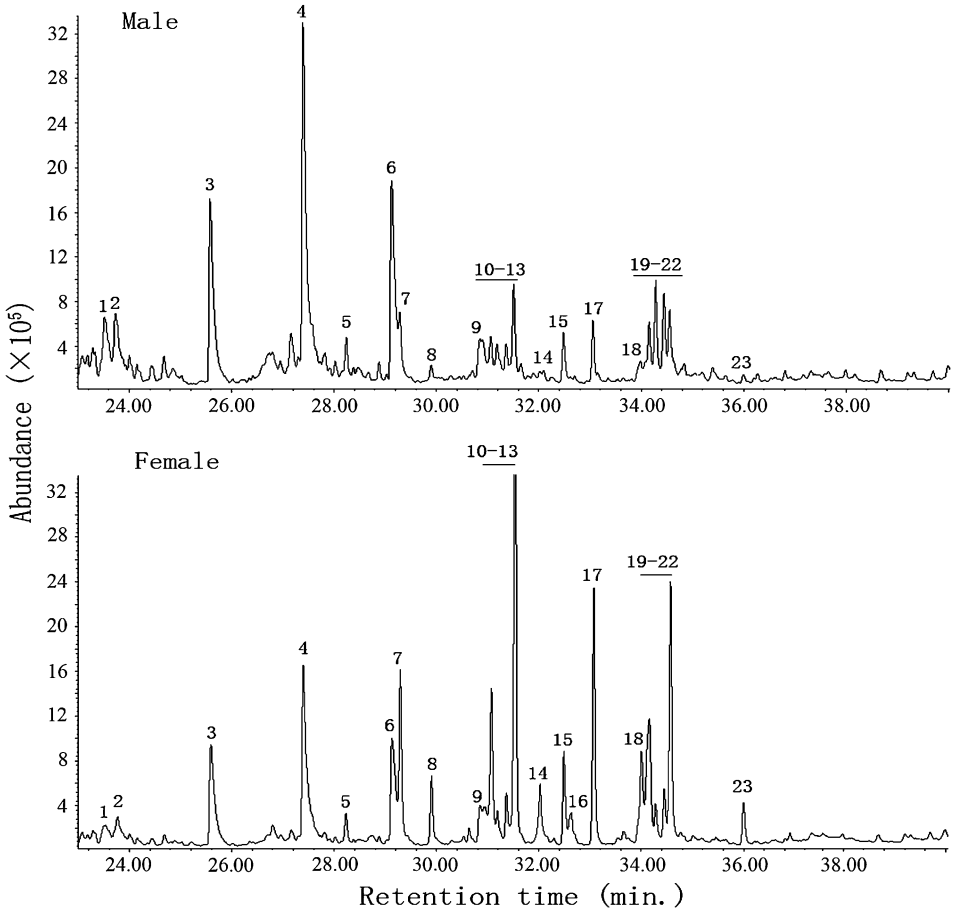

Figure 3 Compound fractions (23–40 min of retention time) on representative GC chromatograms of uropygial gland secretion of males (brown line) and females (green line). GC Peaks 1, 2, 3, 4, 6, and 9 refer to hexadecanoic acid, heptadecanol, octadecanol, nonadecanol, eicosanol, and heneicosanol, respectively. Peaks 5, 7, 8, and 10–23 refer to pentanoates identified by characteristic ions at m/z 85 and 103 and with different linear alkanol or alkenol chains (C_{16}–C_{20}). Note that females have more abundant pentanoates than males, resulting in higher ratios of the acid and alkanols in males.

Table 1 Sexual differences in GC peak areas (using nonpolar GC column, HP5-MS) and percent GC areas of hexadecanoic acid and 5 alkanols extracted from the uropygial gland secretion of budgerigars, *Melopsittacus undulatus*

		GC area (×10⁶)		Significance test		Percent GC area (%)		Significance test	
		Male	Female	*Z*	*P*	Male	Female	*t*	*P*
1	23.51	3.15 ± 0.50	2.88 ± 1.11	0.235	0.819	2.16 ± 1.52	0.42 ± 0.70	2.484	0.031
2	23.69	4.87 ± 4.50	4.37 ± 5.16	0.840	0.401	2.82 ± 2.25	0.59 ± 1.11	2.506	0.025
3*	25.57	23.40 ± 24.64	39.30 ± 39.53	1.050	0.294	12.77 ± 5.88	3.97 ± 5.25	3.159	0.007
4*	27.38	55.70 ± 46.64	63.01 ± 50.46	0.315	0.753	30.38 ± 5.72	7.55 ± 8.61	6.247	0.000
6*	29.12	65.03 ± 53.84	87.45 ± 84.70	0.315	0.753	32.01 ± 9.00	7.34 ± 9.35	4.723	0.000
9	30.67	21.30 ± 16.89	28.12 ± 0.72	1.050	0.294	8.17 ± 4.07	1.23 ± 0.66	4.762	0.000

Mean ± standard deviation, $N = 8$. Notes: 1) we calculated percent GC peak area for each volatile of each bird as a percentage of the sum of the 23 measured volatile peak areas, which presumably reflects the relative concentration of each volatile compound; 2) peaks 1, 2, 3, 4, 6, and 9 refer to hexadecanoic acid, heptadecanol, octadecanol, nonadecanol, eicosanol, and heneicosanol, respectively; the 3 asterisked alkanols were blended for behavioral tests; 3) independent 2-tailed *t*-test and the Mann–Whitney *U* test used for percent GC peak areas and GC peak areas, respectively. Significance set at $\alpha < 0.05$; 4) on GC polar column, the retention time for 3 major alkanols (compounds 3,4, and 6) is respective 29.16, 30.75, and 32.29 min.

we created a 4-fold reduced-dose alkanol blend of 8 μg 18OH, 6.5 μg 19OH, and 12.5 μg 20OH).

Behavioral responses to glandular secretions and synthetic pheromone mixtures

We applied 10-mg glandular secretion or the aforementioned alkanol blends in 40-μL dichloromethane to a petri dish and placed the dishes inside Y maze stimulus jars. Test results showed female budgerigars preferred either the 10-mg glandular secretion ($Z = 2.639$, $N = 16$, $P = 0.008$) or the high-dose alkanol blend ($t = 2.236$, $N = 16$, $P = 0.041$) of males over the female counterparts (Figure 4). Upon removal of any one alkanol, the male blend no longer caused female attraction (Figure 5).

Behavioral response of females to visual stimuli and the chemical stimulus of the putative pheromone

By using a test cage, we found that focal females chose the sealed jar with a male bird significantly more than the jar with a female bird ($Z = 2.501$, $N = 16$, $P = 0.012$) (Figure 6).

Furthermore, we sealed a male in each of 2 stimulus jars and added the high-dose alkanol blend under one jar and an equivalent amount (10 mg) of female uropygial gland secretion to the other. Focal females showed a preference for the male with the high-dose blend over the other with the female secretion ($Z = 2.058$, $N = 16$, $P = 0.040$), although responded equally between males presented together with male glandular secretion and the high-dose alkanol blend (Figure 6).

In addition, when we offered females one jar with a sealed male bird but no alkanol blend under it and an empty jar with the high-dose blend under it, the females exhibited a clear preference for the jar with the bird to the empty jar treated with the high-dose blend ($Z = 2.111$, $N = 16$, $P = 0.035$) (Figure 6).

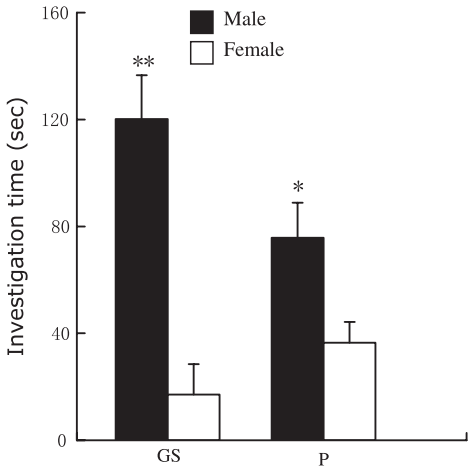

Figure 4 Binary choices by female budgerigars, *Melopsittacus undulatus*, ($N = 16$ birds) between male and female uropygial gland secretions (GS) or blends of octadecanol, nonadecanol, and eicosanol (P) (see Materials and methods for quantities) in a Y maze. (GS, uropygial gland secretion in males or females; P, the high-dose alkanol blends in males or the low-dose alkanol blends in females; values = mean ± standard error of the mean; Wilcoxon test, $*P < 0.05$; $**P < 0.01$.)

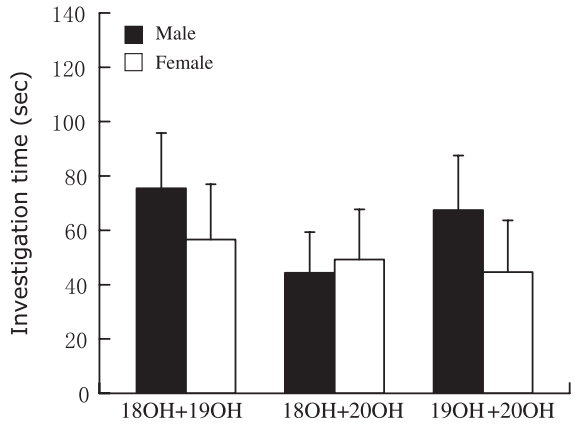

Figure 5 Binary choices by female budgerigars, *Melopsittacus undulatus*, between male and female blends of any 2 of octadecanol (18OH), nonadecanol (19OH), and eicosanol (20OH) in a Y maze, showing the inactivation of female attraction. (Black column, the high-dose alkanols in males; white column, the low-dose alkanols in females; values = mean ± standard error of the mean; $N = 16$ birds; Wilcoxon tests revealed no significant differences, $P < 0.05$, $N = 16$ female birds.)

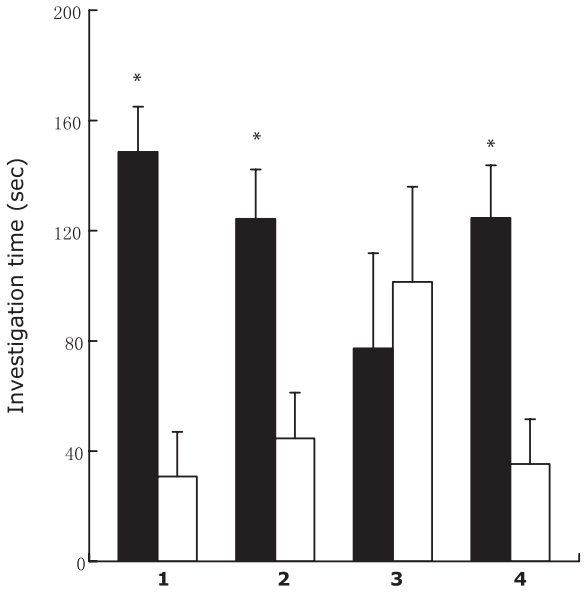

Figure 6 Binary choices by female budgerigars, *Melopsittacus undulatus*, in a test cage. Two jars beside the cage have a stimulus male bird in each ($N = 8$ tests, $N = 16$ birds), and the petri dishes under the bottom of the cage, by each jar, are scented with a male blend of octadecanol, nonadecanol, and eicosanol or 10-mg female uropygial gland secretion (in *x* axis: 1, MB vs. FB; 2, MB + MP vs. MB + FGS; 3, MB + MP vs. MB + MGS; 4, MB vs. MP, where MB, male living birds; FB, female living birds; MP, the high-dose alkanol blends in males; MGS, male uropygial gland secretion; FGS, female uropygial gland secretion). (Values = mean ± standard error of the mean; Wilcoxon or *t*-test, $*P < 0.05$; $**P < 0.01$.)

Discussion

It has been previously shown in birds that olfaction and uropygial glands have an impact on sexual behavior by behavioral tests (Balthazart and Schoffeniels 1979; Hirao et al. 2009) and that sexual differences in the chemical composition of the uropygial gland secretions by chemical analysis (Jacob et al. 1979; Soini et al. 2007; Zhang et al. 2009). However, no study to date has successfully chemically analyzed uropygial gland secretions and demonstrated in bioassays that birds are attracted to opposite sex odors and synthetic mixtures mimicking them (Bonadonna et al. 2009).

Here, we first found from Y maze tests that female budgerigars are capable of distinguishing male body odor from female body odor. As the uropygial gland serves as the major exocrine sebaceous gland of the budgerigar, it was thus considered as most possible odor source and put to further chemical investigation.

The preputial gland of rodents secretes some volatiles, at higher ratios or relative concentrations in males, to attract female conspecifics (Zhang et al. 2008). As such, we revealed that 18OH, 19OH, and 20OH of the uropygial glands of the budgerigars had significantly higher ratios in males than in females, indicative of male pheromone candidates by using GC-MS analysis. Like in other birds, the similarity of the compounds between uropygial gland secretion and plumage in budgerigars indicated that the glandular secretions are preened onto the whole plumage in order to transmit chemical information (Burger et al. 2004; Soini et al. 2007; Zhang et al. 2009). In addition, the 3 alkanols composed 75% of all male glandular volatiles. Therefore, we focused our subsequent efforts on behavioral tests of the 3 alkanols.

Our behavioral results also showed that females exhibited an olfactory preference for the glandular secretion of males over that of females in the quantities on the plumage of one bird as measured, suggesting the presence of sex attracting compounds in the secretion.

The further behavioral tests revealed that female budgerigars exhibited olfactory preferences for the 3-alkanol blend at the dose equivalent to total body plumage of a male over the 4-fold reduced-dose alkanol blend or female uropygial gland secretion, and in particular, that removing any 1 of the 3 alkanols disabled attractiveness of the male blend for female budgerigars and the high-dose alkanol blend and male uropygial gland secretion had similar attractiveness to females. Because the pheromone has been termed as chemical substances secreted externally by some animals that influences the physiology or behavior of other animals of the same species (Karlson and Lüscher 1959), our behavioral and chemical results robustly suggested the 3 alkanols synergistically created a female attractant scent or male pheromone in the budgerigar. Whether the 3 minor male-biased compounds (hexadecanoic acid, heptadecanol, and heneicosanol) of uropygial glands as shown in Table 1 also contributed to olfactory sex attractiveness or not remains to be done in the future.

In addition, the cage tests showed that female budgerigars were capable of distinguishing male and female birds using visual cues in the absence of strong acoustic signals and body odor, as previously reported (Dooling et al. 1987; Arnold et al. 2002; Eda-Fujiwara et al. 2003; Zampiga et al. 2004) and that visual signals might evoke stronger female responsiveness than chemical signals alone. Namely, female birds might prefer the visible male to the male scent/no visible male. Thus, we conclude that olfactory cues alone may not be sufficient to dictate preference in the presence of living birds rather the combination of olfactory, visual, and auditory cues is important. This may have important implications for the design of bioassays investigating chemical communication in other bird species.

In conclusion, by combining GC-MS analysis and bioassay of the uropygial gland secretion of budgerigars, we first showed that birds could use some volatiles in the uropygial gland as sex attractant odor or sex pheromones, which are preened into the whole plumage to transmit olfactory sex information. The uropygial glands and preening behavior may have much broader implications than the previously believed light reflectance and plumage waterproofing in birds (Arnold et al. 2002; Zampiga et al. 2004; Jacob and Ziswiler 1982). Some birds may integrate olfactory cues derived from uropygial glands with visual and auditory cues to increase the precision of sex discrimination and sex selection especially in inaudible (e.g., noisy) and poor visibility (e.g., dark) surroundings (Bonadonna et al. 2004).

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Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/

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