

Article

Can house sparrows recognize familiar or kin-related individuals by scent?

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Abstract

In the last decades, higher attention has been paid to olfactory perception in birds. As a consequence, a handful of avian species have been discovered to use olfaction in different contexts. Nevertheless, we still have a very limited knowledge about the use of odor cues in avian social life, particularly, in the case of songbirds. Here, we investigate if female house sparrows *Passer domesticus* show any preference for the odor of kin and nonkin conspecifics and we also test a possible role of familiarity based on male scent in female choice. We performed the experiment with captive birds twice, during the nonbreeding and breeding seasons. Our results show that female house sparrows strongly avoided the odor of unrelated familiar (UF) males, both in the breeding and nonbreeding seasons. Our results suggest recognition for olfactory stimuli related to familiarity and kinship. We suggest that avoidance for UF males is associated with previous experience in this species. Also, we provided further evidence to the use of olfaction in passerine species by using a new experimental setup.

Key words: familiarity, house sparrow, kinship recognition, odor, olfaction, *Passer domesticus*.

In the past decades, an increasing number of investigations dealt with olfaction in avian species (Roper 1999; Caro et al. 2015). Up to now, all bird species studied have shown an anatomically functional olfactory system (Bang 1960; Bang and Wenzel 1986; Roper 1999; Hagelin 2007a).

Volatile chemicals in birds may derive from very different mechanisms or body parts but the main producer of avian odor is likely the uropygial (or preen) gland through the production of secretions composed by volatile and nonvolatile compounds (Jacob and Ziswiler 1982; Mardon et al. 2011) which can vary between sexes (Zhang et al. 2009, 2010) and seasons at least in some species (Soini et al. 2007; Amo et al. 2012a). Recent studies found evidence for individual smell characteristics (olfactory signature) in different species (Bonadonna et al. 2007; Soini et al. 2007; Mardon et al. 2011; Whittaker et al. 2011). Furthermore, behavioral studies

indicate that olfaction is used in many avian species and in a variety of contexts. For instance, odor cues play a role in orientation (Dell'Arciccia and Bonadonna 2013; Gagliardo 2013), foraging (Nevitt 2008; Amo et al. 2016), and predator detection (Roth et al. 2008; Amo et al. 2011; Zidar and Løvlie 2012; Stanbury and Briskie 2015). Birds produce and emit many chemical volatile compounds with a wide spectrum of different aromas (Hagelin and Jones 2007) that may be used in chemical communication with conspecifics (Roper 1999; Caro et al. 2015).

So far, increasing evidence suggests that birds use olfaction for recognition at species (Hagelin et al. 2003; Krause et al. 2014; Mihailova et al. 2014), sex (Whittaker et al. 2011; Mihailova et al. 2014), kin (Bonadonna and Sanz-Aguilar 2012; Krause et al. 2012; Caspers et al. 2013), and individual levels (Bonadonna and Nevitt 2004; Mardon and Bonadonna 2009; see Balthazart and Taziaux

2009 and Caro et al. 2015 for reviews). In crested auklets *Aethia cristatella*, odor may function as a social signal during the breeding season acting as a chemical ornament (i.e., sexually selected phenotypic trait; for a review, see Hagelin 2007b). Studies on Antarctic prions *Pachyptila desolata* and petrels evidenced self-odor avoidance and partner smell recognition (Bonadonna and Nevitt 2004; Jouventin et al. 2007; Mardon and Bonadonna 2009) whereas in mallards *Anas platyrhynchos*, it has been suggested that olfaction is involved in the control of sexual behavior (Balthazart and Schoffeniels 1979; Balthazart and Taziaux 2009). Other experimental studies also suggest that olfaction might play a role in mate choice (Bonadonna and Nevitt 2004; Amo et al. 2012a, 2012b; Whittaker et al. 2013).

In passerines, olfactory perception may also be important for nest building, especially in those species that make use of aromatic plants, that is, nest protection hypothesis (Clark 1991; Petit et al. 2002; Mennerat 2008). Also, zebra finch fledglings *Taeniopygia guttata* have been shown to use odors for recognizing their own nest (Caspers and Krause 2011; Caspers et al. 2015a) and their genetic mother even after egg cross-fostering (Caspers et al. 2017). Moreover, odor seems to convey phenotypic information in house finches *Haemorhous mexicanus* as male finches of worse quality than the scent of the resident male avoid the male-scented area, whereas males of better quality move toward the other male odor (Amo et al. 2012b). However, despite major improvements in our understanding of olfaction in birds, very little is known about many aspects of avian chemical communication. In particular, our knowledge of socially relevant body scents is still scant and mainly based on nonpasserine birds. It is still unclear, for example, if birds use the odor of conspecifics during the assessment of mating partners or in social contexts (e.g., when choosing a site to roost). Studies offer contrasting results concerning olfaction with regards to mate choice. For example, when anosmic and control female zebra finches was given the possibility to mate with 2 unfamiliar males, an unrelated male and a brother, birds with an intact olfactory mucosa showed a reduced number of fledglings respect to the anosmic females (Caspers et al. 2015b). In contrast, in a nonpasserine bird, namely the Humboldt penguin *Spheniscus humboldti*, individuals distinguished between familiar and unfamiliar and between kin and non-kin odors (Coffin et al. 2011).

In this study, we focused on the role of olfactory cues in social and possibly sexual context, by examining female preferences in passerines. Therefore, we chose the house sparrow *Passer domesticus* as the study species for several reasons. First, this species was recently shown to perceive odor cues in an ecological context (Griggio et al. 2016). Second, house sparrows are essentially monogamous, highly gregarious, and live in discrete colonies establishing complex social relations with their flock mates (Cramp and Perrins 1992; Anderson 2006). Hence, odor recognition of familiar conspecifics and siblings might be important especially during the breeding season. In fact, recognizing a kin-related individual may help to avoid inbreeding while identifying familiar sparrows could confer social advantages (Kohn et al. 2015; Kohn 2017; Tóth et al. 2017). Thus, olfaction may play a role in a social context, mate assessment and inbreeding avoidance (Hagelin et al. 2003; Mardon and Bonadonna 2009). So far, house sparrows have mainly been investigated on visual cues for mate assessment during the breeding season (Møller 1990; Griffith et al. 1999). The aim of this study is to investigate if house sparrow females recognize and are attracted by kin-related and familiar males' body odors. We simultaneously offered the scent of a related familiar (RF) male (namely a brother) and of 2 unrelated males, 1

familiar and 1 unfamiliar to the female. By doing so, we investigated if familiarity plays a role in attracting conspecifics. Bird females are known to carefully evaluate nests in the wild as the latter are both under natural and sexual selection (Mainwaring et al. 2014). Hence, nest-boxes were used to present odor sources. We took into consideration possible behavioral differences between nonbreeding and breeding seasons repeating the experiment in both seasons.

Given the above-mentioned evidences, we hypothesized that house sparrow females 1) should prefer to spend more time in front of the scent of a conspecific respect to a control (both seasons); 2) should prefer familiar scents rather than unfamiliar ones (both seasons); 3) would spend less time in front of the odor of a RF (a brother), especially during the breeding season if mate assessment is involved. However, although in other studies individuals avoided the odor of related flock-mates possibly due to inbreeding avoidance (Krause et al. 2012; Caspers et al. 2013), in our setup the odor cues presented could be mainly used to evaluate a possible roosting site, hence not indicating mate choice.

Materials and Methods

Housing and study species

The experiment was conducted at the Konrad Lorenz Institute of Ethology (KLIVV, University of Veterinary Medicine) in Vienna, Austria (48°13' N, 16°17' E) from February to May. The house sparrows tested in this study are the third generation of a captive population (Griggio et al. 2011). All birds were less than 1 year old with no breeding experience. The house sparrows were kept in mixed-sexes outdoor aviaries (measuring 2 × 3.9 m and 2.6 m high). Every aviary was provided with wooden nest-boxes. House sparrows received food and water *ad libitum* (Griggio and Hoi 2010; Griggio et al. 2016). All experimental birds (i.e., males and females) were in the same housing conditions and received the same nutrition before and during the trials. Hence, it is unlikely that nutrition may have shaped body odors in different ways.

Experimental apparatus

All birds were tested in metal cages (cage model: Montana Terenzo; 1 × 0.5 m and 0.5 m high) held in a room (6.60 × 3.30 m and 2.25 m high) individually. Inside the experimental cages conditions were standardized. In order to do so, every cage was equipped with 4 identical feeders (9 × 8 × 5 cm), 4 wooden perches (50 cm long), and 4 plastic nest-boxes (23 × 15 × 12 cm; entrance hole diameter: 5 cm). Overall, 3 rooms, 3 cages, and 24 plastic nest-boxes were used (12 at a time).

A stick (17 cm long) was placed underneath the entrance hole of each nest-box to allow females to perch in front of the odorous stimuli for inspection. Sticks were crafted from leafless branches of the same local tree. Every experimental room was visually isolated from the other rooms and the outside and held at a temperature of about 18–20 °C. Inside the rooms, artificial light reproduced the natural light/dark cycle for the date. To simulate sunset, we reduced the light intensity over a period of 60 min before complete darkness. The start of the trials was synchronized to the natural sunset time. In the cages, we provided water and commercial food for granivorous passerines *ad libitum*.

Experimental procedures

We tested 12 females in 2 different seasons. Focal females were caught in the morning (11:30 AM–12:30 AM). Immediately after capture,

sparrows were held in an acclimatization cage for about 4 h. Subsequently, we moved the house sparrow females to their experimental cages. The females' behavior was video recorded during the whole time spent in the cage (75 min) by using iSpy, v. 6.3.0.0, an open source software (www.ispyconnect.com). All videos were scored by 1 experimenter who was blind to bird identity and position of the odorous stimuli. A visit to an odorous stimulus was considered when a bird perched on the stick located in front of the entry hole of a nest-box or entered inside the nest-box itself (choice area). This is a similar method to that used in other studies (Amo et al. 2011; Caspers and Krause 2011; Krause and Caspers 2012; Golüke et al. 2016; Griggio et al. 2016). Choice time was defined as the total time spent by each bird in the choice area (i.e., on the nest-box sticks or inside the nest-boxes) as similarly done by Krause and Caspers (2012) and by Griggio et al. (2016). Focal females were considered to be motivated to explore the nest-boxes and possibly smell their odor when they were inside the choice area. Time spent in the choice area was calculated in seconds with the help of a digital timer superimposed on video recordings (an iSpy feature). We determined preference time as the proportion of time in front of each odor over the total time in the choice area.

All trials were carried out before sunset when birds search for a safe place to rest during the night. Hence, we considered the experimental conditions to be sufficiently reliable to observe eventual preferences if any. In these conditions, females were stimulated to explore the scented nest-boxes and to spend more time in front of the preferred one. Birds were introduced in the experimental cages 75 min before light was off (i.e., 15 min before the start of artificial sunset). Hence, females could explore the experimental cage in full artificial light for 15 min prior to sunset (75 min recorded and analyzed).

Four different odors were presented to the females, namely the scent of a RF, unrelated familiar (UF), unrelated unfamiliar (UU) male, and a control (C). In all tests, the RF male was a brother of the focal female. Each chemical stimulus was placed inside a nest-box and covered with cotton wool (see Odor stimuli preparation). During the year before the experiment took place, all familiar males were housed in the same aviary with the focal females whereas unfamiliar sparrows were completely unknown to the tested females. We used male scent because it is usually preferred by both males and females (Whittaker et al. 2011; Amo et al. 2012a, 2012b). Moreover, females are usually the choosy sex as supported by the results on mate choice based on the Major Histocompatibility Complex (MHC) in house sparrows (Cramp and Perrins 1992; Bonneaud et al. 2006; Griggio et al. 2011). We used different males for each tested female to avoid pseudoreplication. In total, we used 36 house sparrow males and 2 tree sparrow *Passer montanus* males (control feathers, see Odor stimuli preparation). In the breeding season, each female was tested with the same group of males of the nonbreeding season.

Odor stimuli preparation

In order to collect males' body odor, we used a procedure similar to that used in Antarctic prions (Bonadonna and Nevitt 2004), zebra finches and diamond firetails *Stagonopleura guttata* (Krause et al. 2014). We caught male sparrows in the outdoor aviaries and held them individually in cotton bags (~20 cm²) for 1 h. During their stay in the bag, males could move and swipe the uropygial gland and the preened feathers against the fabric. Inside the bags, we placed a sheet of blotting paper (~13 cm²). Bags were handled in the same way to ensure homogeneous handling effect among individuals and trials. Then, each scented bag was turned inside out and put at the bottom of a randomly assigned plastic nest-box as odor source. We covered

each bag with 2 g of cotton wool and made sure that in all instances the odorous nest-boxes were visually indistinguishable. Mihailova et al. (2014) showed that feathers of crimson rosella *Platycercus elegans* are sufficient for odor recognition in this species. Hence, we increased the intensity of the odorous stimuli by cutting a couple of feathers from the belly of our scent donor males. One of the feathers and the paper sheet (previously stored with the male in the cotton bag) were fixed at the entrance of each nest-box with a small piece of masking tape. The second feather was put inside the nest-box. We think that a clearly visible feather in each nest-box may stimulate house sparrows to explore the nest-boxes.

The control treatment was prepared using a cleaned bag and covered with 2 g of cotton wool. Then, a cleaned 13 cm² piece of paper was placed at the entrance of the control nest-box together with a tree sparrow feather. All feathers were identical in color and size both within and between species (i.e., house sparrows and tree sparrows). Feathers were cut from the belly of 2 tree sparrow males (as a single individual could not provide the amount of feathers required without distress) at the start of the experimental session and stored altogether in a different ziplock[®] storage bag for each individual. Since tree sparrow feathers belonged to 2 different individuals, they were randomly assigned to the experimental trials. Control nest-boxes were visually identical to the other experimental odorous stimuli. Hence, in control nest-boxes the only avian odor source comes from the tree sparrow' feather. At the end of each day, all bags were washed at 95 °C in a washing machine. To avoid odor contaminations during the cleaning we did not use any cleanser.

Every day, we cleaned and ventilated the rooms for at least 60 min after the removal of odorous stimuli. All cages and nest-boxes were rinsed with hot water (~70 °C) and dried with unscented kitchen roll before being cleaned with 70% ethanol. Brown wrapping paper covered the bottoms of each experimental cage. The nest-boxes, sticks, and perches were removed and cleaned daily. Every 2 days, we washed the cages and removed the wrapping paper. We wore latex examination gloves for cleaning and nest-box preparation. A random number generator was used to randomize the position of the different males' odors, nest-box identity and experimental room number at every trial (Haahr 1998). Nest-boxes were provided with a couple of hay stems partially protruding from the entrance hole to stimulate exploration and improve the naturalness of the experimental setup.

Statistical analyses

All data were analyzed using R version 3.2.1 (The R Foundation for Statistical Computing, Vienna, Austria, <http://www.r-project.org>). Analyses met the assumptions of parametric statistics. We checked for the presence of outliers and residual error distribution visually. All tests are two-tailed. All females visited all 4 choice areas in every test. Results from all female odor preference tests were analyzed with General Linear Mixed Models (GLMMs) using the package lme4 (1.0.5) for R version 3.2.1. We used both seconds spent in front of each nest-box (gamma distribution, log link) and proportion of time spent in front of each nest-box (logistic regression for proportion, logit link) as dependent variables in 2 separate sets of models. In both sets of models we fitted odor stimulus, season, and their interaction as the categorical fixed effects, whose estimates and significance were obtained using the "car" package. All models used a Kenward–Roger correction to approximate the degrees of freedom. In order to differentiate between 4 or more groups we performed Tukey post hoc tests, implemented within the package "lsmeans" (Lenth 2016). We entered female individual identity as random term

in the analysis in order to control for repeated measures on the same individuals. Repeatability was tested using package “rptR” (Stoffel et al. 2017). Marginal R -squared (R_m) and conditional R -squared (R_c) variances explained by the model were calculated with the method recently detailed by Nakagawa et al. (2017). Results are presented as mean \pm SE.

Results

During the nonbreeding season, the average proportion of time females spent in the choice area was $42.2\% \pm 9.7\%$, whereas in breeding season the average time spent in the choice area was $47.6\% \pm 10.5\%$. Within-individual, between-season repeatability of odor stimulus choice was $R = 0.15 \pm 0.091$ for time measured in seconds and $R = 0.08 \pm 0.042$ for the proportion of time spent in the choice area.

Time spent in the choice area (seconds)

The interaction between odorous stimulus and season was not significant in the model using seconds spent in front of each nest-box as the dependent variable ($\chi^2 = 4.460$, $P = 0.216$). Thus it was removed from this model. The effect of the odorous stimuli was, however, highly significant (GLMM, $R^2_M = 0.10$, $R^2_C = 0.21$, $\chi^2 = 13.618$, $P = 0.003$) whereas we did not find a significant effect of season ($\chi^2 = 0.845$, $P = 0.358$). The UF odor stimulus was the least visited in both nonbreeding and breeding season with females spending significantly less time at this odor stimulus than at any other odor stimulus (Table 1). We then limited the analysis to only 1 season at the time in order to see if the rejection of the UF odorous stimulus could still be detectable. Only the difference between time spent in front of the UF and UU odorous stimuli (Z-ratio = -3.035 , $P = 0.013$) remained significant during nonbreeding season (all other $P > 0.19$). However, during the breeding season, differences between time spent at the UF stimulus and at all the other stimuli were still significant (Table 2).

Time spent in the choice area (proportion)

Repeating the analysis using proportion of time spent at each stimulus yielded very similar results to those obtained using time in seconds. Results using proportion of time are given henceforth. The odorous stimuli had a significant effect (GLMM, $R^2_M = 0.12$, $R^2_C = 0.30$, $\chi^2 = 15.907$, $P = 0.001$) whereas season did not ($\chi^2 = 0.122$, $P = 0.727$). The UF stimulus was the least visited in both nonbreeding and breeding season in terms of proportion of time spent in the choice area (Figure 1), with females spending significantly less time at the UF odor stimulus than at any other odor stimulus (Figure 1; see Table 3 for the statistical analysis). Similarly to the models with time in seconds as the dependent variable, the rejection of the UF odor stimulus was still significant when limiting the analysis to the breeding season (see Appendix Table A1) while it was weaker but still detectable during nonbreeding season (see Appendix Table A2).

Discussion

In line with some evidence for kin (Bonadonna and Sanz-Aguilar 2012; Krause et al. 2012; Caspers et al. 2013) and partner (Bonadonna and Nevitt 2004; Jouventin et al. 2007; Mardon and Bonadonna 2009) recognition in avian species, our results seem to suggest that female house sparrows can recognize odor stimuli and avoid UF males (Figure 1). Even if this difference was slightly more

Table 1. GLMM post hoc test (Tukey honestly significant difference test) showing the comparison between the time in seconds spent by females in front of odors of males with a different relationship with respect to the females

Fixed effect	Variance	<i>P</i>
Season <i>df</i> = 1, 71	2.849	0.130
Male body odor <i>df</i> = 3, 72	10.340	0.003
UF versus		
UU	−2.990	0.015
Familiar brother	3.352	0.004
Control	−3.187	0.008
UU versus		
Familiar brother	0.483	0.963
Control	−0.329	0.988
Familiar brother versus		
Control	0.170	0.998

Variance is shown as *F*-statistic for fixed effects and Z-ratio for contrasts. Only time in the choice area was considered. Both nonbreeding and breeding seasons are included.

P-values lower than 0.05 are in bold.

Table 2. GLMM post hoc test (Tukey honestly significant difference test) showing the comparison between the time in seconds spent by females in front of the odors of males with a different relationship with respect to the females

Fixed effect	Variance	<i>P</i>
Male body odor <i>df</i> = 3, 42	16.174	0.002
UF versus		
UU	−2.666	0.039
Familiar brother	2.851	0.023
Control	−3.638	0.002
UU versus		
Familiar brother	0.485	0.963
Control	−1.581	0.390
Familiar brother versus		
Control	−1.054	0.717

Variance is shown as *F*-statistic for fixed effects and Z-ratio for contrasts. Only time in the choice area was considered. Only the breeding season is analyzed.

P-values lower than 0.05 are in bold.

marked during the breeding season these results were mostly independent from the time of the year, as experiments were carried out during both nonbreeding (February–March) and breeding season (from mid-April to May). We suggest that the avoidance behavior shown by females is thus associated with the social environment of house sparrow flocks. This species is highly gregarious and it is known to establish dominance hierarchies (Watson 1970; Møller 1987; Solberg and Ringsby 1997). Hence, entering the nest-box of a known conspecific (familiar but unrelated, thus born in a different nest-box) may lead to aggression. In this scenario, females might have considered the nest-boxes containing the odor of RF males as friendly environments resembling their natal nest-box (all birds in the experiment were less than 1 year old). Correspondingly, the scent of UU males and the control nest-box might have been perceived as empty places or occupied by unknown conspecifics deserving further inspection. On the contrary, the scent of UF males might be associated with the cost of past aggressive interactions and hence should be avoided. The slightly more marked avoidance of the scent of UF males during the breeding season can be explained with an

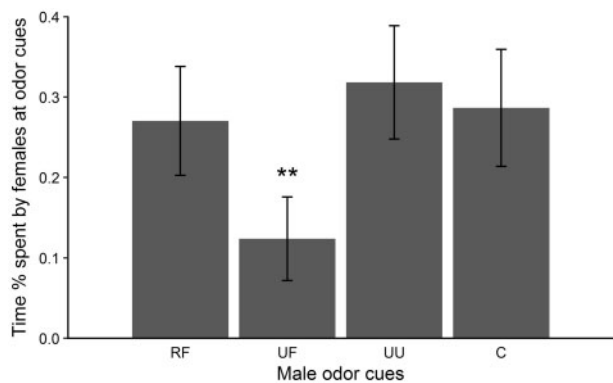


Figure 1. Proportion of time females ($n=12$) spent in front of the body odor of a RF, UF, UU male and of a control. Both breeding and nonbreeding season are included (see Table 3 for the statistical analysis). Only time in the choice area was considered. Females showed a significant rejection for the odor cue of UF males. ****** $P < 0.01$. Error bars indicate \pm SE.

Table 3. GLMM post hoc test (Tukey honestly significant difference test) showing the comparison between the percentage of time spent by females in front of the odors of males with a different relationship with respect to the females, including both nonbreeding and breeding seasons

Fixed effect	Variance	P
Season $df = 1, 71$	0.005	0.726
Male body odor $df = 3, 72$	24.315	0.002
UF versus		
UU	-3.153	0.009
Familiar brother	3.382	0.004
Control	-3.435	0.003
UU versus		
Familiar brother	0.180	0.998
Control	-0.250	0.995
Familiar brother versus		
Control	-0.072	0.999

Variance shown as F -statistic for fixed effects and Z -ratio for contrasts. Only time in the choice area was considered.

P -values lower than 0.05 are in bold.

increase in aggressive interactions during this period (Wingfield et al. 1987). Moreover, the uropygial gland increases in size (Pap et al. 2010), possibly increasing the clarity of the odorous signal. As the group composition did not change throughout the entire experiment and the life of the experimental birds, repeated aggressive interactions near the nest-boxes of UF individuals could have been a constant feature of the females' social environment.

Some hypotheses might explain why female sparrows did not spend less time in front of the odor of a RF. First, the use of chemical cues might be context dependent in many bird species, including the house sparrow. Focal females may have used the odor sources we provided only to inspect and choose the safest nest-box where to roost, that is, for reasons different than mate choice. The lack of conspecifics in the experimental setup and the time of the day during which the experiment was carried out (i.e., late afternoon) may have prevented the olfactory information from eliciting the assessment of potential reproductive partners during the breeding season. Our findings also raise the possibility that smell alone is not sufficient to evaluate potential partners in this species, or at least not in this setup.

This might also be due to the fact that females could need a multi-modal source of information to assess potential reproductive partners. Similarly, zebra finches do not seem to use odor cues when it comes to foraging despite using smell in social contexts (Caspers et al. 2013; Krause et al. 2016). Further studies should investigate if olfactory information acquires a different relevance or significance when coupled with visual information of conspecifics in social and sexual contexts.

To our knowledge, few studies have investigated avian odor recognition of familiar and unfamiliar conspecifics. A study conducted on blue tits suggests the possibility that nestlings of this species could discriminate between the odor of familiar and unfamiliar conspecifics adjusting their begging behavior accordingly (Rossi et al. 2017). In Humboldt penguins, birds spent more time next to familiar than unfamiliar odors (Coffin et al. 2011). When considering studies examining the choice between related and unrelated conspecifics, European storm petrels *Hydrobates pelagicus* showed avoidance for the odor of unrelated individuals (Bonadonna and Sanz-Aguilar 2012). On the contrary, adult zebra finches seem to avoid the odor of related individuals (Krause et al. 2012; Caspers et al. 2013) whereas chicks were shown to recognize the scent of their genetic mother (Caspers et al. 2017). In our study, sparrow females did not show any avoidance for the scent of brothers. Hence, our findings suggest the existence of a wide variation in the use of olfactory cues even between closely related species.

A number of experimental observations suggest that the odor source was sufficiently strong and reliable. First, the same sparrow population, during the nonbreeding season and with a similar experimental setup revealed positive results regarding the olfactory capabilities of house sparrows (Griggio et al. 2016). In this case, house sparrow males spent a significantly greater amount of time in front of hay odor than mouse *Mus musculus domesticus* urine. Second, cotton bags were already successfully used as odor sources in different species (Bonadonna and Nevitt 2004; Krause et al. 2014). Hence, we think that the experimental procedure can be considered sufficiently reliable. Many studies on olfaction mainly used a Y-maze or T-maze apparatus (Bonadonna et al. 2003; Hagelin et al. 2003; Bonadonna and Nevitt 2004; Amo et al. 2011) but we decided to use a different experimental setup where this species might experience a situation closer to the one faced in a natural environment. Finally, there could have been some variation in the quantity and concentration of body odors among different donor males as birds could leave a variable amount of odor molecules in the bag. Nevertheless, our result would appear to indicate that the setup made it at least possible to discern scents clearly enough to consistently avoid the UF odor. In future experiments, a gas chromatography-mass spectrometry analysis of the odor sources could help to detect possible differences between the stimuli presented.

In conclusion, our results indicate that female house sparrows do not show any preference or avoidance for the scent of kin-related individuals. On the contrary, female house sparrows seem to remember the scent of UF males avoiding their odor. We suggest that this behavior may be explained by house sparrow sociality. Also, when our findings are compared with the behavior of closely related species they suggest that there are considerable differences in the use of olfactory cues across different situations, with the rejection or the attraction towards other specific flock-mates being strongly context-dependent. Given the lack of knowledge on the role of olfactory cues in social contexts and in the light of our findings we believe that this topic deserves further and more accurate studies.

Ethical note

The experiments reported in this article comply with current laws on animal experimentation in Austria and the European Union. Housing conditions as well as the capture and bird handling were in accordance with the Austrian laws and were approved by the government of Vienna (MA22-231/2011). The experiments in this study were performed after the approval by the Ethical Commission of the University of Veterinary Medicine, Vienna (Austria), in accordance with Good Scientific Practice guidelines and national legislation (68.205/121-WF/V/3 b/2016). The condition and health of all captive birds were checked daily.

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APPENDIX

Table A1. GLMM post hoc test (Tukey honestly significant difference test) showing the comparison between the percentage of time spent by females in front of the odor stimuli of males with a different relationship with respect to the females, during nonbreeding season

Male body odor	Z-ratio	P
UF versus		
UU	-3.612	0.002
Familiar brother	2.147	0.139
Control	-1.390	0.505
UU versus		
Familiar brother	-1.593	0.382
Control	2.355	0.086
Familiar brother versus		
Control	0.787	0.860

Only time in the choice area was considered.

Table A2. GLMM post hoc test (Tukey honestly significant difference test) showing the comparison between the percentage of time spent by females in front of the odor stimuli of males with a different relationship with respect to the females, during breeding season

Male body odor	Z-ratio	P
UF versus		
UU	-2.563	0.051
Familiar brother	3.593	0.002
Control	-4.605	<0.0001
UU versus		
Familiar brother	1.177	0.641
Control	-2.386	0.080
Familiar brother versus		
Control	-1.234	0.605

Only time in the choice area was considered.

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