

Chapter 13

Exploring the Use of Olfactory Cues in a Nonsocial Context in Zebra Finches (*Taeniopygia guttata*)

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13.1 Introduction

The use of chemical signals in bird communication received increasing attention over the last years (Roper 1999; Hagelin and Jones 2007; Caro and Balthazart 2010; Caspers and Krause 2013; Caro et al. 2015). Several avian taxa have been found to make use of olfactory cues in numerous contexts (Table 13.1). Even songbirds have been shown to be able to use their sense of smell, although they have been long thought to be anosmic due to their relative small olfactory bulbs (Bang and Cobb 1968) and the lack of obvious odor-guided behavior. However, several studies revealed that songbirds, including the zebra finch, possess numerous olfactory receptor genes (Steiger et al. 2008, 2009; Warren et al. 2010). The zebra finch is one of the most used avian laboratory model organisms (Zann 1996; Griffith and Buchanan 2010). As all songbirds, zebra finches are mainly visual and acoustically dominated (Zann 1996), but apart from these sensory modalities they make also use of olfactory cues in several social contexts. Fledglings as well as mothers can distinguish between their own and a conspecifics nest based on olfactory cues (Caspers and Krause 2011; Krause and Caspers 2012; Kohlwey et al. 2015; Fig. 13.1a).

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Table 13.1 Some examples when olfactory cues are used in nonsocial context by birds

Nonsocial context	Species	Reference
(a) Navigation	Pigeons	Papi et al. (1974), Gagliardo (2013)
	Antarctic prions	Nevitt and Bonadonna (2005)
	Cory's shearwaters	Gagliardo et al. (2013)
	Catbird	Holland et al. (2009)
(b) Foraging	Blue tit	Mennerat et al. (2005)
	Great tit	Amo et al. (2013)
	Several procellariiformes	Nevitt et al. (1995)
	Penguins	Wright et al. (2011)
(c) Response to predators cues	Blue tit	Amo et al (2008)
	House finch	Roth et al. (2008)
	Chicken	Zidar and Løvlie (2012)
(d) Nest material selection	Blue tit	Petit et al. (2002) Mennerat (2008) Mennerat et al. (2009)
	European starlings	Gwinner and Berger (2008) Gwinner (2013)

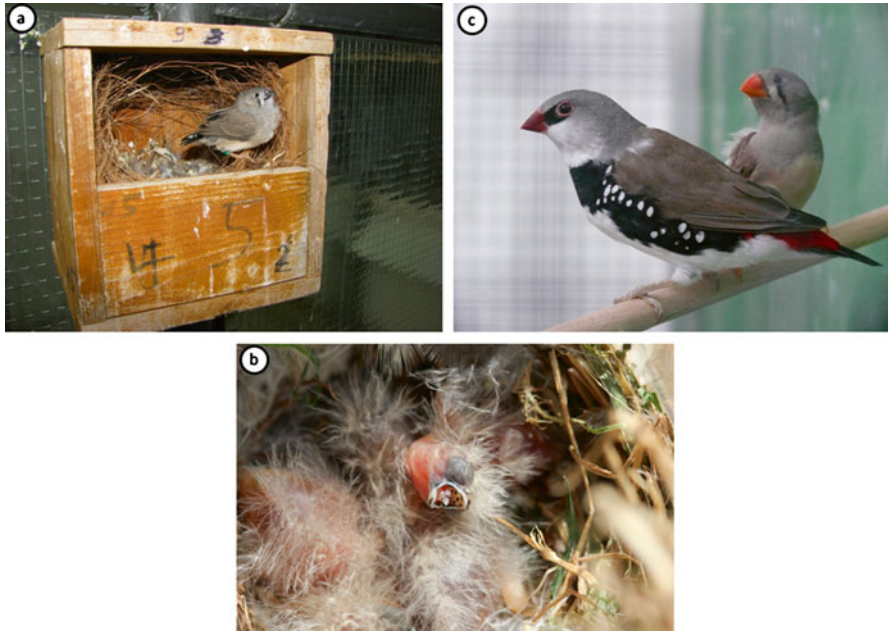


Fig. 13.1 (a) Fledgling zebra finch at the entrance of its natal nest, zebra finches fledge at an age of around 19 days post-hatching; (b) a zebra finch brood shortly after hatching, one of the chicks is raising the head for begging; and (c) interspecific interaction between a diamond firetail (front) and a zebra finch

Zebra finch chicks learn around hatching (Fig. 13.1b) the scent of their family (Caspers et al. 2013) and can use odors to discriminate kin from non-kin (Krause et al. 2012). Odor also seems to play a role at an interspecific level as zebra finches have different olfactory fingerprints than individuals from a sympatric close-related species and can use those to distinguish between the scent of conspecifics and heterospecifics (Krause et al. 2014; Fig. 13.1c). Our studies so far focused on the use of chemical cues for social communication. Here we explore the use of olfactory cues in zebra finches (*Taeniopygia guttata*) in a nonsocial context without any semi-chemical cues.

Here we put our emphasis on the use of olfaction for foraging. Zebra finches are granivores that usually forage on the ground (Zann 1996). In experimental contexts, food can be used as a good motivational stimulus to engage birds in learning and/or exploration tasks (Bischof et al. 2006; Boogert et al. 2008; Krause and Naguib 2011; Brust et al. 2014). A previous study explored the role of olfactory cues in a foraging-related context. Kelly and Marples (2004) suggested that neophobia to a familiar food was not triggered when additionally a new artificial odor was presented. Neophobia was induced when the food was dyed in a new color or when the new color of the food was accompanied by the new artificial odor. We wanted to investigate whether zebra finches can use the scent of their known food alone to locate the food patch.

13.2 Methods

We used 23 adult zebra finch females from the domesticated lab stock at Bielefeld University (Forstmeier et al. 2007; Hoffman et al. 2014). The birds were kept in single-sex groups of 3–4 birds in cages (30×40×83 cm). The birds had *ad libitum* access to standard seed food and water and received a mixture of germinated seeds and egg food (CéDé Premium Eggfood) once a week. The floor of each cage was cleaned twice a week. All birds were housed in the experimental room with a light–dark cycle of 14:10 h. No other birds were housed in this room.

13.2.1 Olfactory Foraging Experiment

The test arena consisted of four identical choice arms and one start box (Fig. 13.2a). It was built out of gray plastic (PVC) and was covered during the test by transparent Plexiglas, to avoid birds from escaping. In each of the four choice arms, a wooden divider was located (Fig. 13.2b). The wooden dividers were varnished with white paint. Behind this divider the odor samples or controls were located, in a way that the birds needed to hop around the divider to see the sample behind. Each divider had small holes (Fig. 13.2c) with a slope of 45°. This way we guaranteed that an air

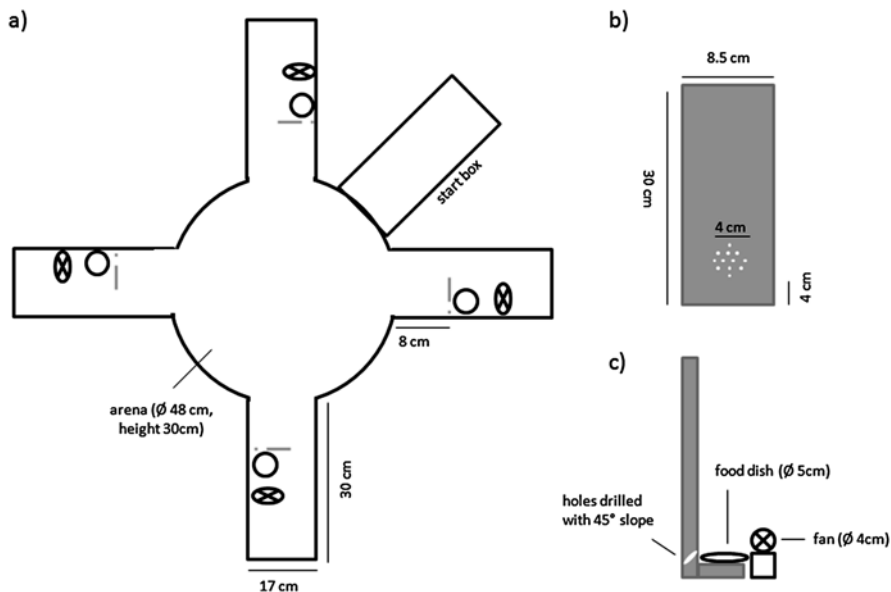


Fig. 13.2 Experimental test arena. (a) Test arena from a top view perspective. The arena consisted of four arms where food could potentially be hidden behind dividers. The birds are released to the test arena from a start box. (b) Each divider has small holes that allowed an airstream to pass through, created by a fan. (c) Holes in the dividers had a slope of about 45° to prevent birds from looking through the holes

stream could pass through the barrier, whereas birds cannot look through. Behind the dividers a white plastic food dish for odor samples or controls and a small fan were placed (Fig. 13.2c). In the experiments, in three of the four arms, the food dish remained empty (control) and in only one arm food was placed in the respective dish to provide a food-related odor. The fans ran in all four arms. A mixture of germinated seeds and protein-rich egg food was used as the food sample. This food represents high-quality nutrition to zebra finches (e.g., Krause and Naguib 2011). We used the odor of the bird's familiar food only, as we wanted to know whether in their daily life, food-related cues might be potentially relevant. Despite this, germinated seeds are a natural food source (Zann 1996) and zebra finches in natural population also occasionally take in insects (Zann and Straw 1984). After each trial the entire test arena was uncovered from the Plexiglas to allow an air exchange with fresh air. After the habituation period and after each experimental trial, the arena and the plastic food dishes were cleaned with 70 % ethanol and water. All experiments were observed using four wireless cameras (dnt, QuattSecure Profiset).

Individuals were habituated to the arena before the tests began. Therefore, a group of 3–4 birds (i.e., all birds from their home cage) was housed inside the

arena for 48 h. During this habituation period, water was provided in the central area of the test arena and food was offered in each of the four arms visible to the birds beside the dividers. The start box was not accessible to birds during the habituation phase. Birds were deprived for food in their home cages for about 3 h (mean, 213 min \pm 45 min S.D.) prior to the experiment, to ensure that they participate in the behavioral experiments (Krause and Naguib 2011). Each bird was tested individually at four subsequent days and on four trials per day. At the beginning of each trial, birds were placed in the start box and were allowed to habituate there for three minutes. Thereafter, the start box was opened and the birds were individually released to the test arena. During the experiments a fan in each arm created an air stream directed to the center of the arena. Only one of the four air streams transported the smell of food. The location of the food rewarded arm was randomly altered in each trial. Each trial lasted up to 15 min. If a bird located the food in a trial, it was allowed to feed for 10 s before the trial ended. The four trials each day were conducted in a row.

We recorded in each trial and measured on a daily average basis: (1) the latency to leave the start box, (2) the first choice (whether it was correct (=1) or not (=0), the chance level was 0.25), (3) the time to enter the rewarded arm, (4) the time to find the food (i.e., looking behind the barrier in the rewarded arm), and (5) the number of errors (i.e., entering wrong arms). The first choice has been shown to be a valid measure in olfactory choice experiments (e.g., Bonadonna et al. 2006; Amo et al. 2012; Krause et al. 2014).

13.2.2 Statistical Analysis

All measures were analyzed as a mean for each respective experimental day (1–4). We analyzed the average first choices with one-sample t-tests, testing whether the bird's performance differed significantly from the expected probability that the choice was correct (1/4, i.e., 0.25) by chance. Performance significantly better than chance level would indicate that they have used olfactory cues to find the food. We further tested whether the bird's performance differed between days using a Friedman test. With this test we examined whether their performance improved over time, i.e., whether the bird, for example, learned to use olfactory cues to search the food in the tests. The average first choices were analyzed for correlation across the four experimental days using Spearman rank correlations; however due to multiple comparisons the p-values of all correlation have been Bonferroni corrected. The average time to leave the start boxes, to enter the correct arm, and to find the food and the number of errors were analyzed using a Friedman test, to check whether the performance of the birds improved over time or not. All tests were calculated using SPSS 22.

13.3 Results

13.3.1 First Choice

The mean first choice at all days of the birds was not significantly different from chance level (0.25) at any of the 4 days (all one-sample t-tests, $t > -0.42$, $p > 0.68$; Fig. 13.3a). Birds did not show any improvement over the subsequent 4 days (Friedman test, $df=3$, $X^2=4.05$, $p=0.26$; Fig. 13.3a). However, the mean first choice ratio between day 3 and day 4 correlated significantly (Fig. 13.3b; Table 13.2).

13.3.2 Latency for Leaving the Start Box

Birds in general left the start box relatively fast, but latency for leaving the start box reduced significantly over days (medians: day 1, 3.3 s; day 2, 2.5 s; day 3, 1.3 s; day 4, 1.5 s; Friedman test; $N=23$, $df=3$, $X^2=21.24$, $p=0.00009$).

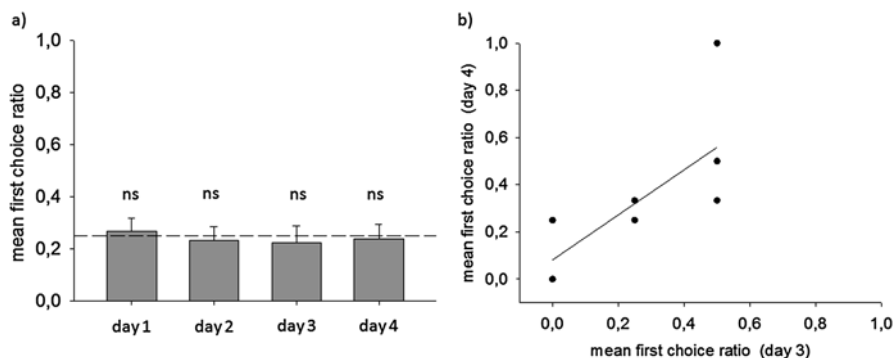


Fig. 13.3 (a) Birds' first choice in the test arena shown as means (\pm SE) for all four test days. The dashed line indicates the chance level, i.e., here 0.25. (b) Significant correlation between the mean first choices at days 3 and 4 of testing in the arena. Some data point overlap

Table 13.2 The Spearman correlations of the average first choices of all four testing days are shown. The p-values in all cases have been Bonferroni corrected as indicated by $p\#$ -values

	Mean first choice ratio			
	Day 1	Day 2	Day 3	Day 4
Day 1	–	$r=-0.18$ $p\#=1$	$r=0.01$ $p\#=1$	$r=-0.35$ $p\#=1$
Day 2		–	$r=0.12$ $p\#=1$	$r=0.09$ $p\#=1$
Day 3			–	$r=0.87$ $p\#=0.0001$

13.3.3 Time to Enter Rewarded Arm

The time to enter the correct arm did not differ significantly between days, i.e., birds did not become faster (medians: day 1, 620 s; day 2, 552 s; day 3, 687 s; day 4, 631 s; Friedman test $N=23$, $df=3$, $X^2=1.57$, $p=0.67$).

13.3.4 Time to Find the Food

The time to find the food did not differ between days and the birds did not become faster in finding the food over the 4 days (Friedman test, $N=23$, $df=3$, $X^2=1.35$, $p=0.14$; Fig. 13.4).

13.3.5 Number of Errors

The mean number of errors did not differ between days (Friedman test, $N=23$, $df=3$, $X^2=3.93$, $p=0.27$; Fig. 13.5). The error rate, i.e., number of errors per time in the test, did not differ across test days (median errors per min, day 1, 0.20; day 2, 0.29; day 3, 0.05; day 4, 0.11; Friedman $N=23$, $df=3$, $X^2=1.09$, $p=0.78$).

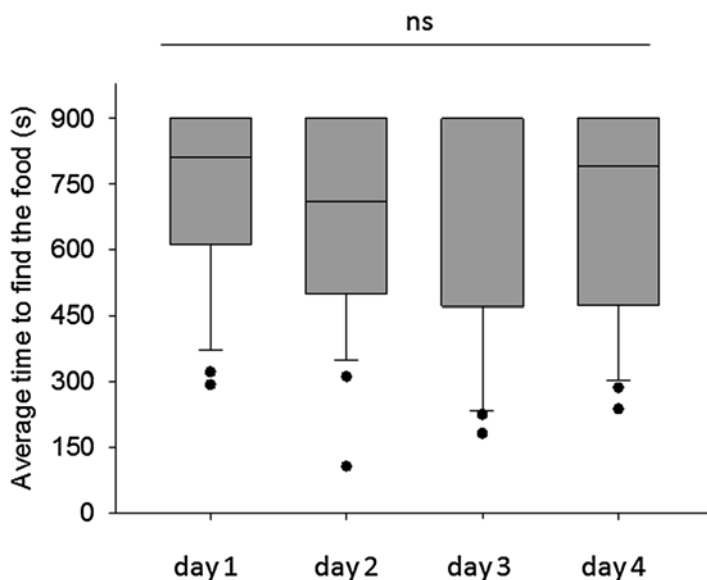


Fig. 13.4 The median time to find the food did not significantly improve over days. Birds showed no training effect, with respect to this parameter. Shown are box plots with median and the quartiles. The dots represent outliers, i.e., data points that lie outside the 10th and 90th percentiles

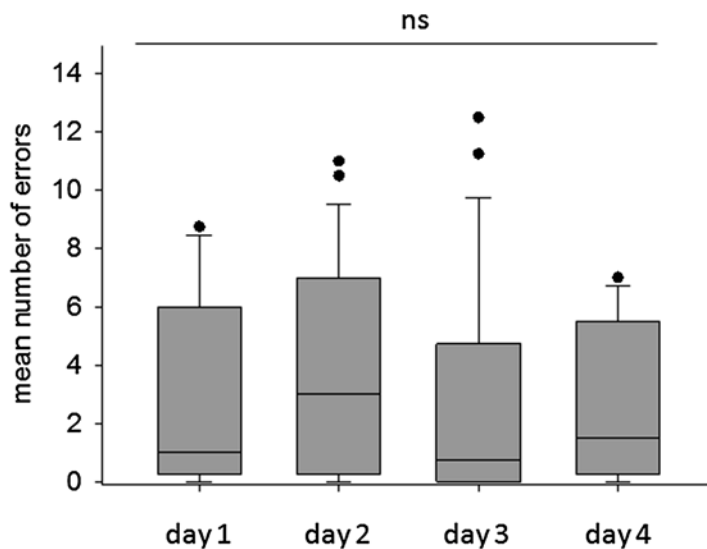


Fig. 13.5 The median number of errors did not significantly decrease over days, indicating that no training effect occurs. Shown are box plots with median and the quartiles. The dots represent outliers, i.e., data points that lie outside the 10th and 90th percentiles

13.4 Discussion

Although zebra finches are known to be able to use olfactory cues for social communication (Krause et al. 2012, 2014; Caspers et al. 2013), our present data shows that they do not seem to use olfactory cues in every context. Zebra finches found the food at none of the four experimental days at a higher probability than at chance level. In addition, the time to find the food and the number of errors until ending up at the food did not improve over time. Zebra finches become faster over the different test days in leaving the start box which might indicate that they become more familiar with the experimental setup and the testing procedures.

This suggests that zebra finches do not primarily rely on olfactory cues while foraging and that the use of olfaction is context dependent in this species. However, their first choices were correlated at the last two days of testing probably giving at least a hint that some initiated an association between the odor of the food and the respective location. In this nonsocial foraging context, investigated here, zebra finches might not primarily rely on chemical cues, which may highlight the context specificity of chemical communication in birds and songbirds in particular. Although other birds, including passerines (e.g., Mennerat et al. 2005) and non-passerines (e.g., Nevitt et al. 1995, Wright et al. 2011), have been shown to use chemical cues for foraging, it always needs to be considered that different species are faced with different natural environments and different selective pressures. Zebra finches forage

mainly on seeds, which can probably be easily located by vision. The context specificity has also been found to be important in social contexts, where adult zebra finch females react differently to their own nest odor depending on age of their chicks and the stimulus odor that is presented simultaneously (Krause and Caspers 2012).

It might also be possible that zebra finches do not learn this task. Kelly and Marples (2004) found a similar pattern although they aimed to initiate aversive reactions. However, in other context, such as spatial learning, it is possible to train zebra finches to learn the association between location and food (e.g., Krause and Naguib 2011; Mayer et al. 2010). An alternative explanation might be that the food-related odor used in our apparatus was not strong enough to be located by the zebra finches or that turbulences in the air streams inhibit the birds to find the food in our arena. It would be interesting to test whether zebra finches can find food when the stimuli are reinforced by artificial odors (e.g., Würdinger 1990). As we wanted to investigate whether food-related odors may play a role in the normal housing conditions, we used a food source our zebra finches are quite familiar with.

It could also be possible that zebra finches do not use food-related odors directly, but odors that are linked to the foraging sites as it is known from Procellariiformes and great tits. Procellariiformes, for example, use dimethyl sulfide (DMS) as a foraging cue (Nevitt et al. 1995). DMS is produced by phytoplankton and is an indicator for productive areas. Great tits use chemical cues of infested plants to locate potential insect prey items (Amo et al. 2013). Although this is a fascinating idea, it has so far only been demonstrated in non-herbivorous species, in which the prey is feeding on plants and the plants release olfactory cues. It seems rather unlikely that a similar mechanism may be present in herbivore species, but we cannot rule out this possibility and it might be interesting to explore this idea in future studies.

Whether zebra finches have not perceived or just did not react to the food-related odors cannot fully be answered here. However, it seems extremely unlikely that they cannot perceive the odors as they have been shown to be able to smell in several other experimental studies, but in other mainly social context (Caspers and Krause 2011; Krause et al. 2012; Krause and Caspers 2012; Caspers et al. 2013; Krause et al. 2014). The fact that the outcomes of the third and fourth trial are highly correlated might lead to the conclusion that longer training and testing procedures are needed to train zebra finches to locate food on the basis of smell. This needs to be tested in future experiments. At the moment we can summarize that olfactory cues do not seem to be of major importance for foraging in zebra finches. Taken together, we have shown that the use of chemical cues may be context dependent in zebra finches. In a nonsocial context, such as individual foraging, olfactory cues do seem not to play a primarily role. However, based on previous work, it is well documented that in social context olfactory cues provide an important source of information to these birds (Caspers et al. 2013; Krause et al. 2012, 2014).

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References

- Amo L, Galván I, Tomás G, Sanz JJ (2008) Predator odour recognition and avoidance in a songbird. *Funct Ecol* 22:289–293
- Amo L, Avilés JM, Parejo D, Peña A, Rodríguez J, Tomás G (2012) Sex recognition by odour and variation in the uropygial gland secretion in starlings. *J Anim Ecol* 81:605–613
- Amo L, Jansen JJ, van Dam NM, Dicke M, Visser ME (2013) Birds exploit herbivore-induced plant volatiles to locate herbivorous prey. *Ecol Lett* 16:1348–1355
- Bang BG, Cobb S (1968) Size of olfactory bulb in 108 species of birds. *Auk* 85:55–61
- Bischof HJ, Lieshoff C, Watanabe S (2006) Spatial memory and hippocampal function in a non-foodstoring songbird, the zebra finch (*Taeniopygia guttata*). *Rev Neurosci* 17:43–52
- Bonadonna F, Caro S, Jouventin P, Nevitt GA (2006) Evidence that blue petrel, *Halobaena caerulea*, fledglings can detect and orient to dimethyl sulphide. *J Exp Biol* 209:2165–2169
- Boogert NJ, Giraldeau LA, Lefebvre L (2008) Song complexity correlates with learning ability in zebra finch males. *Anim Behav* 76:1735–1741
- Brust V, Krüger O, Naguib M, Krause ET (2014) Lifelong consequences of early nutritional conditions on learning performance in zebra finches (*Taeniopygia guttata*). *Behav Process* 103:320–326
- Caspers BA, Krause ET (2011) Odour-based natal nest recognition in the zebra finch (*Taeniopygia guttata*), a colony-breeding songbird. *Biol Lett* 7:184–186
- Caspers BA, Krause ET (2013) Intraspecific olfactory communication in zebra finches (*Taeniopygia guttata*) – Potential information apart from visual and acoustic cues. In: East ML, Dehnhard M (eds) *Chemical signals in vertebrates 12*. Springer Verlag, Berlin, pp 341–351
- Caspers BA, Hoffman JI, Kohlmeier P, Krüger O, Krause ET (2013) Olfactory imprinting as a mechanism for nest odour recognition in zebra finches. *Anim Behav* 86:85–90
- Caro SP, Balthazart J (2010) Pheromones in birds: myth or reality? *J Comp Physiol A* 196:751–766
- Caro SP, Balthazart J, Bonadonna F (2015) The perfume of reproduction in birds: Chemosignaling in avian social life. *Horm Behav* 68:25–42
- Forstmeier W, Segelbacher G, Mueller JC, Kempenaers B (2007) Genetic variation and differentiation in captive and wild zebra finches (*Taeniopygia guttata*). *Mol Ecol* 16:4039–4050
- Gagliardo A (2013) Forty years of olfactory navigation in birds. *J Exp Biol* 216:2165–2171
- Gagliardo A, Bried J, Lambardi P, Luschi P, Wikelski M, Bonadonna F (2013) Oceanic navigation in Cory's shearwaters: evidence for a crucial role of olfactory cues for homing after displacement. *J Exp Biol* 216:2798–2805
- Griffith SC, Buchanan KL (2010) The zebra finch: the ultimate Australian supermodel. *Emu* 110:v–xii
- Gwinner H, Berger S (2008) Starling males select green nest material by olfaction using experience-independent and experience-dependent cues. *Anim Behav* 75:971–976
- Gwinner H (2013) Male European starlings use odorous herbs as nest material to attract females and benefit nestlings. In: East ML, Dehnhard M (eds) *Chemical Signals in Vertebrates 12*. Springer Verlag, Berlin, pp 353–362
- Hagelin JC, Jones IL (2007) Bird odors and other chemical substances: a defense mechanism or overlooked mode of intraspecific communication? *Auk* 124:741–761
- Hoffman JI, Krause ET, Lehmann K, Krüger O (2014) MC1R genotype and plumage colouration in the zebra finch (*Taeniopygia guttata*): Population structure generates artefactual associations. *PLoS One* 9, e86519
- Holland RA, Thorup K, Gagliardo A, Bisson IA, Knecht E, Mizrahi D, Wikelski M (2009) Testing the role of sensory systems in the migratory heading of a songbird. *J Exp Biol* 212:4065–4071
- Kelly DJ, Marples NM (2004) The effects of novel odour and colour cues on food acceptance by the zebra finch, *Taeniopygia guttata*. *Anim Behav* 68:1049–1054

- Kohlwey S, Krause ET, Baier MC, Müller C, Caspers BA (2015) Chemical analysis reveal family specific nest odour profiles in Zebra finches (*Taeniopygia guttata*): A pilot study. In: Schulte BA, Ferkin MH, Goodwin TE (eds) Chemical Signals in Vertebrates 13. Springer Verlag, Berlin, pp
- Krause ET, Naguib M (2011) Compensatory growth affects exploratory behaviour in zebra finches, *Taeniopygia guttata*. *Anim Behav* 81:1295–1300
- Krause ET, Krüger O, Kohlmeier P, Caspers BA (2012) Olfactory kin recognition in a songbird. *Biol Lett* 8:327–329
- Krause ET, Caspers BA (2012) Are olfactory cues involved in nest recognition in two social species of estrildid finches? *PLoS One* 7, e36615
- Krause ET, Brummel C, Kohlwey S, Baier MC, Müller C, Bonadonna F, Caspers BA (2014) Differences in olfactory species recognition in the females of two Australian songbird species. *Behav Ecol Sociobiol* 68:1819–1827
- Mayer U, Watanabe S, Bischof HJ (2010) Hippocampal activation of immediate early genes *ZenK* and *c-Fos* in zebra finches (*Taeniopygia guttata*) during learning and recall of a spatial memory task. *Neurobiol Learn Mem* 93:322–329
- Mennerat A, Bonadonna F, Perret P, Lambrechts MM (2005) Olfactory conditioning experiments in a food-searching passerine bird in semi-natural conditions. *Behav Processes* 70:264–270
- Mennerat A (2008) Blue tits (*Cyanistes caeruleus*) respond to an experimental change in the aromatic plant odour composition of their nest. *Behav Process* 79:189–191
- Mennerat A, Perret P, Bourgault P, Blondel J, Gimenez O, Thomas DW, Heeb P, Lambrechts MM (2009) Aromatic plants in nests of blue tits: positive effects on nestlings. *Anim Behav* 77: 569–574
- Nevitt GA, Veit RR, Kareiva P (1995) Dimethyl sulphide as a foraging cue for Antarctic procelariiform seabirds. *Nature* 376:680–682
- Nevitt GA, Bonadonna F (2005) Sensitivity to dimethyl sulphide suggests a mechanism for olfactory navigation by seabirds. *Biol Lett* 1:303–305
- Papi F, Ioalé P, Fiaschi V, Benvenuti S, Baldaccini NE (1974) Olfactory navigation of pigeons: the effect of treatment with odorous air currents. *J Comp Physiol* 94:187–193
- Petit C, Hossaert-McKey M, Perret P, Blondel J, Lambrechts MM (2002) Blue tits use selected plants and olfaction to maintain an aromatic environment for nestlings. *Ecol Lett* 5:585–589
- Roth TC II, Cox JG, Lima SL (2008) Can foraging birds assess predation risk by scent? *Anim Behav* 76:2021–2027
- Roper TJ (1999) Olfaction in birds. *Adv Stud Behav* 28:247–332
- Steiger SS, Fidler AE, Valcu M, Kempenaers B (2008) Avian olfactory receptor gene repertoires: evidence for a well-developed sense of smell in birds? *Proc R Soc Lond B* 275:2309–2317
- Steiger SS, Kuryshev VY, Stensmyr MC, Kempenaers B, Mueller JC (2009) A comparison of reptilian and avian olfactory receptor gene repertoires: Species-specific expansion of group gamma genes in birds. *BMC Genomics* 10:446
- Warren WC, Clayton DF, Ellegren H, Arnold AP, Hillier LW, Künstner A, Searle S, White S, Vilella AJ, Fairley S et al (2010) The genome of a songbird. *Nature* 464:757–762
- Wright KL, Pichegru L, Ryan PG (2011) Penguins are attracted to dimethyl sulphide at sea. *J Exp Biol* 214:2509–2511
- Würdinger I (1990) Die Reaktionen von Zebrafinken (*Taeniopygia guttata*) auf Düfte—eine Pilotstudie. *Vogelwarte* 35:359–367
- Zidar J, Løvlie H (2012) Scent of the enemy: behavioural responses to predator faecal odour in the fowl. *Anim Behav* 84:547–554
- Zann RA, Straw B (1984) Feeding ecology and breeding of zebra finches in farmland in northern Victoria. *Aust Wildl Res* 11:533–552
- Zann RA (1996) *The Zebra Finch—a synthesis of field and laboratory studies*. Oxford University Press, Oxford