Olfactory kin recognition in a songbird

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The ability to recognize close relatives in order to
cooperate or to avoid inbreeding is widespread
across all taxa. One accepted mechanism for
kin recognition in birds is associative learning
of visual or acoustic cues. However, how could
individuals ever learn to recognize unfamiliar
kin? Here, we provide the first evidence for a
novel mechanism of kin recognition in birds.
Zebra finch (*Taeniopygia guttata*) fledglings are
able to distinguish between kin and non-kin
based on olfactory cues alone. Since olfactory
cues are likely to be genetically based, this find-
ing establishes a neglected mechanism of kin
recognition in birds, particularly in songbirds,
with potentially far-reaching consequences for
both kin selection and inbreeding avoidance.

1. INTRODUCTION

Distinguishing related from unrelated individuals is a
crucial ability for animals, facilitating cooperation
among relatives and avoidance of excessive kin compe-
tition or inbreeding [1–4]. Helping relatives and
avoiding mating with them are two very different
fitness-enhancing behaviours, which share an impor-
tant feature: relatives must be somehow identified
first. Recognition of kin can be based on (i) spatial dis-
tribution, (ii) familiarity or association, (iii) phenotype
matching, and/or (iv) recognition alleles; however,
these are not mutually exclusive mechanisms [4,5].
The first two mechanisms are relatively imprecise, as
the assessment is not directly based on genetic related-
ness, but is indirectly estimated from cues that are
likely to be correlated with kinship [5]. The latter
two mechanisms, in contrast, refer more directly to
the underlying genetic similarity of kin and are inde-
pendent of experience [5]. There is a large body of
evidence for kin recognition in birds and especially
songbirds [4,6–12]. Songbirds are primarily known
for their visual and acoustic discrimination abilities,
and most research on kin recognition cues has focused
on songs or calls [7,10,11]. Acoustic patterns learned
during the nestling phase from a parental template
facilitate kin recognition based on such learned cues
[11,13]. Associative learning can thereby explain how
individuals recognize parents and siblings of the same
brood. However, these mechanisms fail to explain

how other, unfamiliar kin, which are not present
during the association period, are recognized. For
example, Petrie *et al.* [8] have shown that peacock
(*Pavo cristatus*) males prefer to lek together with unfa-
miliar relatives and that this case of kin recognition is
not based on social and environmental cues. Further-
more, during associative learning recognition errors
can occur if traits from other non-related individuals
are learned, which would lead to the acceptance of
non-kin as kin.

The recognition of relatives, even if they are unfa-
miliar, in most other animals such as mammals,
amphibians, fish and insects, is based on olfactory cues
[14–16]. Most birds were long thought to be anosmic
[17]. Hence, olfactory-based kin recognition and the
possibility of olfactory phenotype matching have so far
been largely neglected. Zebra finches, however, have
been shown to possess large numbers of olfactory recep-
tor gene-like sequences in their genome [18,19], and the
actual use of olfaction for purposes of nest recognition
has been recently discovered [20]. Furthermore, zebra
finches are ideal model organisms to test for olfactory
kin recognition in birds, since they are altricial, highly
social and colonial songbirds [21]. Here, we asked
whether olfactory cues might provide a mechanism for
kin recognition [12]. In a foster experiment, we tested
whether zebra finches can distinguish the nest of kin
from that of non-kin, based on olfactory cues alone.

2. MATERIAL AND METHODS

We fostered single zebra finch hatchlings between days 1 and 4 of life
(on average, day 2 of life $\pm$ 1 day s.d.) into unrelated broods of a
similar age (average brood size prior to fostering 2.5 ± 1.6 s.d.
chicks; for details of the fostering protocol see the electronic sup-
plementary material). We tested the foster chick and a randomly
chosen unrelated nest-mate shortly after fledging (days 20–23 of
life). In a simultaneous choice situation (see the electronic sup-
plementary material), we presented the familiar nest odour in
which both test individuals were raised, and the nest odour of the
nest from which the foster chick came and which it had not experi-
enced for 19–22 days. Individuals were tested for 10 min in total.
To control for side preferences, we switched odour samples after
5 min [20]. The two stimuli differed in their genetic source and
time of exposure to the two test individuals. For each pair of
chicks, we used nest material from the nest they were raised in,
and nest material from the nest the foster chick hatched in, as
odour stimuli. Hence, fostered chicks had a choice between the
odour of the nest used by unrelated, but familiar, individuals and
the odour of the nest used by closely related individuals, which
they had not experienced for the last 20 days. In contrast, non-fos-
tered chicks had a choice between the odour of the nest used by
closely related and familiar individuals, and the unfamiliar odour
of a nest used by unrelated and unfamiliar individuals. Owing to
our unidirectional fostering, the genetic purity of odour stimuli dif-
fered. The nest from which the fostered chick originated consisted
of one genetic source only, whereas the other consisted of two genetic
sources (non-fostered and fostered chicks). We analysed the time the
individual spent in each preference zone as the proportion of time
spent in the respective zone relative to the time the individual
spent in both preference zones (see the electronic supplementary
material). The preference strength was calculated as the net differ-
ence between the times the individual spent in each preference zone.

3. RESULTS

In total, we performed experiments with 33 individ-
uals, 17 fostered chicks and one of the unrelated
nest-mates (non-fostered chicks) each. Since the
non-fostered chicks of one brood died, we used
16 non-fostered chicks. Foster chicks significantly pre-
ferred the odour of the nest in which they hatched,
over the nest odour in which they were raised

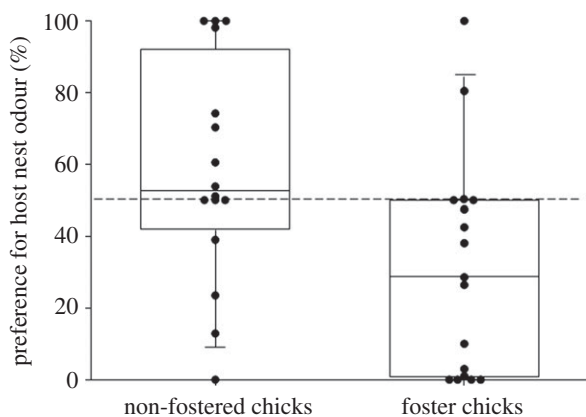Figure 1. Evidence for olfactory kin recognition in a songbird. Proportion of time that foster ($n = 17$) and non-fostered chicks ($n = 16$) spent in the preference zone of the odour of the host nest odour. Foster chicks showed a significant preference for the odour of the nest they originated from, i.e. their genetic nest odour and the preferences from foster and non-fostered chicks were significantly different (central line in the boxes, median; spread of the box, 25 and 75% quartile; whiskers, entire data range; dots, individual data points).

(Wilcoxon; $n_{foster\ chicks} = 17$, $Z = -2.22$, $p = 0.026$; mean preference strength: $121\ s \pm 141$ s.d.; figure 1). Furthermore, foster chicks and their unrelated nest-mates differed significantly in their preference for the nest odour of the nest in which they were raised (χ^2-test; $\chi^2 = 6.68$; $p = 0.009$; figure 1).

In contrast to the foster chicks, non-fostered chicks did not show a clear preference for the home nest odour which provided a familiar and genetically related stimulus (Wilcoxon; $n_{non\text{-}fostered\ chicks} = 16$; $Z = -1.05$, $p = 0.29$, mean preference strength: $148\ s \pm 188$ s.d.; figure 1). The preference of non-fostered chicks for the home nest odour was positively correlated with the number of full siblings in the nest ($n_{non\text{-}fostered\ chicks} = 16$, $r_S = 0.55$, $p = 0.029$; figure 2). The number of full siblings is an indirect measure of relative nest odour purity, as the more full siblings are present in the nest, the lower the relative contamination from the non-related fostered chick, and thus the higher is the expected preference for the home nest odour.

4. DISCUSSION

Our results show that zebra finch fledglings are able to distinguish between kin and non-kin by using olfactory cues alone. Foster fledglings prefer the nest odour of close relatives, even though they have been fostered into a host nest shortly after hatching.

The preference of non-fostered chicks for their home nest odours is positively linked to the proportion of genetic relatives in the nest, i.e. the stimulus quality, indicating the ability to perceive kin labels from olfactory nest cues.

Since olfactory cues are likely to be genetically based [14–16,22], olfactory kin recognition bears the potential to be a novel mechanism for kin recognition in songbirds and possibly birds in general. A potential olfactory kin label has very recently been documented in petrels (*Halobaena caerulea*) [23], in which mice were used to demonstrate the similarity of odours from closely related
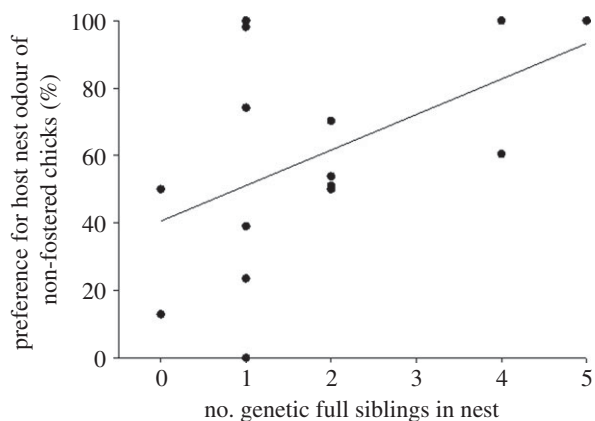
Figure 2. Genetic purity (number of genetic full siblings in the nest) affects odour preference from non-fostered chicks. Preference strength of the non-fostered chicks for the home nest odour, i.e. their familiar and genetically related nest odour is significantly correlated with the number of full-siblings in the nest (some dots overlap).

petrel individuals, but no ecologically relevant evidence of actual usage and perception of these cues has been provided. One potential reason for the use of olfaction in zebra finches might be to detect extra-pair paternity or conspecific brood parasitism, but this selection pressure is likely to be negligible, as extra-pair paternity is relatively rare, as is the frequency of egg dumping [24,25]. The ability to distinguish closely related from non-related individuals is crucial, not only for parent–offspring recognition but also for the evolution of cooperative breeding and inbreeding avoidance [1,3]. The use of olfaction in mate recognition has been demonstrated in Antarctic prions (*Pachyptila desolata*) [26], and zebra finches, which are not explicitly cooperative breeders, might also use olfactory cues during mate choice decisions in terms of inbreeding avoidance [12]. Furthermore, if olfactory cues indeed facilitate kin recognition, this might well explain results that have so far escaped a plausible mechanistic explanation in other birds, as for example the finding that peacocks within a lek are more related than expected by chance [8].

The ability of zebra finches to recognize the nest odour of closely related individuals may result from learning the genetic nest odour within the first few hours after hatching as a type of olfactory familial imprinting [2], or it may be innate and distinguishing kin from non-kin may be based on self-referent phenotype matching [15]. If learning is the underlying mechanism, the acquisition of the nest odour might occur pre-hatching [27] or shortly after hatching [20,28], as is known from other bird species. Nevertheless, if the ability of zebra finches to distinguish the two nest odours results from olfactory imprinting, the sensitive phase during which the olfactory template is acquired is much earlier in life than for visual [21,29] and acoustic cues [13,21]. Thus, olfactory kin recognition decreases the possibility of recognition errors owing to learning kin cues from other than kin.

The finding that even songbirds, and possibly birds in general [18,30], might be able to rely on olfactory-based labels for kin recognition leads to the conclusion that the general mechanism of kin

recognition might be based on phenotype matching, for example, by genetically based markers, such as the major histocompatibility complex [2].

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