A tangerine-scented social odour in a monogamous seabird

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Social odours, conspecific chemical signals, have been substantiated in every class of vertebrate except birds. The apparent absence is surprising, as every bird examined has a functional olfactory system and many produce odours. The crested auklet (*Aethia cristatella*), a monogamous seabird, exhibits a distinctive, tangerine-like scent closely associated with courtship. Using T-maze experiments, we tested whether auklets preferred conspecific odours or distinguished between different types of scents, two necessary pre-requisites of chemical communication. Crested auklets exhibited: (1) attraction to conspecific feather odour, (2) preference for two chemical components of feather scent (cis-4 decenal and octanal), which we identified as seasonally elevated, and (3) differential responses to odours, as indicated by a preference for auklet odour, aversion to mammalian musk, but no significant response to banana essence (amyl acetate). Our results suggest that crested auklets detect plumage odour and preferentially orient toward this stimulus. The striking and well-described courtship display that involves the scented neck region, the “ruff sniff,” provides a conspicuous behavioural mechanism for odour transmission and potential for scent assessment during sexual selection. Although the importance and full social function of chemical signals is just beginning to be realised in birds, including crested auklets, social odours promise to reveal a largely unexplored and possibly widespread means of avian communication.

Keywords: chemical communication; social signal; sexual selection; crested auklet; *Aethia cristatella*; Alcidae.

1. INTRODUCTION

Compared to other vertebrate groups, chemical signals in birds have been treated as a peculiarity, for which only rudimentary data are available (Roper 1999). Yet, every bird that has been tested exhibits a functional sense of smell (Bang & Wenzel 1985; Roper 1999). Readily detectable odours are also widespread in the class Aves (177 species, 80 genera, 19 orders; Weldon & Rappole 1992). Though human perception of an avian odour does not imply function, abundant evidence indicates that birds, like other vertebrates, recognise and respond to chemical cues in their immediate environment. Some avian species, for example, use odours during foraging (e.g. Stager 1964; Wenzel 1971; Verheyden & Jouventin 1994; Nevitt et al 1995), navigation (Waldvogel 1989; Wallraff 1990), and even in selection of nest materials (Clark & Mason 1985, Petit et al. 2002). Birds, therefore, not only detect and produce odours, they appear capable of recognising and adaptively employing them.

Data on the identity and use of social odours in birds are scarce (Roper 1999). Similarity between the avian oil gland and mammalian scent glands suggests an odour source, as do stomach oils or faeces (Jacob & Zisweiler 1982; Roper 1999). In behavioural studies, responses to avian scent are often implied, as in preferences for the odour of nests (e.g. Jones & Gentle 1985; Mínguez 1997), or up-wind navigation to locate burrows (e.g. Grubb 1974). Perhaps the most detailed experimental evidence for an avian social odour comes from differential responses of anosmic vs. sham-operated male mallards (*Anas platyrhynchos*; Balthazart & Schoffeniels 1979). Changes in male behaviour correlated with seasonal differences in the oil gland chemistry of females (Jacob et al. 1979).

Despite intriguing evidence, studies have never examined the response of birds to the chemical components of any self-produced, odour compound. Such information is fundamental to demonstrating chemical communication (Preti et al. 1977). Furthermore, previous investigations have focused on avian systems where the process of odour transmission is generally unclear, thereby making responses difficult to interpret.

Here we present chemical and behavioural experiments of a highly social seabird, the crested auklet (*Aethia cristatella*), which exhibits two key features of chemical communication: (1) scent production (Jones 1993a; Douglas et al. 2001), and (2) a behavioural means of scent reception. The plumage of both sexes of crested auklets exhibits a strong, tangerine-like scent (Jones 1993a). Courtship in this monogamous species also involves a frequently repeated 'ruff-sniff' display, in which individuals place their bills within the nape feathers of a display partner, a region of the body where tangerine-odour appears to be particularly strong (Jones 1993a; Jones

& Hunter 1993; Fig. 1a,b). Although the winter behaviour and odour of this species are unknown, several observations suggest that both are seasonal. In a captive colony, ‘ruff sniff’ behaviours are absent during non-breeding (S. Devereaux, pers. comm.). Also, a detailed description of both live and dead birds, collected following an unusual, mid-winter encounter at sea (Dick & Donaldson 1978), fails to mention scent, suggesting that odour may have been mild, or lacking altogether. Finally, to human observers, odours of both wild and captive crested auklets waned at the end of the breeding season, coincident with moult and the loss of other ornaments (crests, orange beak plates; Hagelin, unpubl. data).

Given the intriguing association between odour, breeding, and behaviour, our primary goal was to substantiate that crested auklet scent exhibited attributes of a social odour. That is, we hypothesized that the odour acted as a chemical stimulus that affected the behavioural state of conspecifics (Johnston 2000). We predicted birds would preferentially approach: (1) the natural tangerine scent of breeding plumage, and (2) specific volatile chemicals of feather odour that we identified as seasonally significant. Furthermore, for crested auklets to employ scent as a meaningful stimulus, they must not only recognise an odour, but also distinguish between different types of volatile stimuli. Therefore, we ran a series of tests to determine whether auklets responded differently to other kinds of scents, such as the skunk-like odour of mammalian musk or the novel, sweet scent of banana essence (amyl acetate).

2. METHODS

a) Odour Chemistry

Fresh plumage samples were collected from a total of 16 live, adult crested auklets (*n*= 8 males, *n*=8 females) during breeding (May-August) and non-breeding (November-January) seasons 2000-2001. Approximately ten feathers were clipped from the dorsal portion of the nape, placed into sealed, glass vials, and frozen at (–80C) upon return to the lab. Scented feathers (*n*=10), collected during the breeding season, came from wild (*n*=6) and captive birds (*n*=4). Field samples came from a breeding colony on Buldir Island, Aleutian Islands, Alaska (52°23'N 175°54'E). Captive samples were collected at the Aquarium of the Pacific, Long Beach, California. During winter months, wild auklets disperse to unknown regions of extremely treacherous arctic seas, making captives (*n*=6) the only feasible source of fresh feathers. For behavioural tests of plumage odour (below) we used feathers from adult parakeet auklets (*A. psittacula*) as a control. To chemically confirm that this species lacks specific compounds recognisable as citrus scent, as suggested by Jones et al. (2001), we analysed (*n*=3) parakeet auklet nape samples collected on Buldir Island.

Protocols for chemical analysis closely followed Rasmussen (2001). Briefly, we used solid phase microextraction (SPME) and gas chromatographic/mass spectrometric (GC/MS) procedures to process the volatile, chemical components of odour present in the head-space of plumage vials. Compound identity was confirmed from library spectral match (77-99%) and authentic standards. Concentration estimates were calculated relative to authentic standards. Any breeding-season concentration falling below 0.1μg/g feathers was not subject to statistical tests, as reliable quantitation at such levels is difficult (L.E.L. Rasmussen, pers. comm.).

Data Analysis

As many volatiles did not meet the requirements of MANOVA (normally distributed, equal variance), we analysed each separately via Kruskal-Wallis ANOVA to determine any differences based on feather type (captive breeding, wild breeding, captive winter). For each significant ANOVA ($p<0.05$), we assessed pairwise patterns in detail via Wilcoxon 2-sample tests. Given multiple comparisons, we also judged statistical significance via the sequential Bonferroni procedure (Rice 1989).

b) Behavioural Experiments

We tested the odour response of adult crested auklets on Buldir Island between 6 June-26 July 2001. Following capture in noose-carpets (Jones 1993b), each adult was used in only one 20-minute trail within a plexiglass T-maze. The maze, constructed in a shelter adjacent to the trapping site, provided a simultaneous choice between an odour and control. It consisted of a centrally located start box connected to a choice area (Fig 2). A solar-powered, 12V fan pulled in equal amounts of outside air through the two arms of the maze at approximately 0.5 m^3/s (Fig 2). Each bird acclimated for 10 minutes in the darkened start box and, when a screen wall was lifted, entered the 76 x 25 cm "choice area" (Fig. 2). We divided the choice area into three equal sectors, such that each outer sector (or arm) of the maze was adjacent to an experimental cue (Fig. 2).

Hidden from subjects, an observer (JCH) recorded two types of data, following bird emergence from the start box: (1) relevant behaviours, including time spent in each sector of the maze during a 20-minute trial, and (2) the first outer sector visited. Odour location was not detectable to the observer. Odour and control samples were in identical, sealed jars or aluminium foil packets (plumage samples), each of which was opened *after* placing it in an arm of the maze. The fast-moving air and sealed maze kept odours from escaping. Each day, odour location of the first trial was determined at random. At the end of each trial, with the fan still running, vials were sealed and their positions swapped. At day's end, the observer shut off the fan, determined the location of the odour sample, and calculated the odour position of previous runs. Following each run, the maze was cleaned with "Odor Mute" (Ryder Products), an enzyme solution that breaks down organic compounds. Sex of each bird used in the maze was estimated at capture from bill characteristics. Sex was confirmed, post-test, from bill measurements (Jones 1993b). Our study was approved under University of Connecticut Animal Care and Welfare Permit A3124-01, and Alaska State and Federal permits MB09696-0 and 0-110, respectively.

Experiment 1: Fresh plumage odour

To test whether crested auklets preferred the tangerine scent of fresh feathers, we presented birds with a choice between the breeding plumage of conspecifics and plumage of the parakeet auklet, an unscented congener (Jones et al. 2001). Given that winter conspecific plumage was unavailable, parakeet auklets provided a "natural" alternative, as we confirmed this species lacks key seasonal components of crested auklet odour (see Results). We avoided "creating" a control (i.e. washing scented feathers), as we could not determine in the field whether soaps or solvents would: (a) chemically alter pre-existing compounds, (b) add other, unnatural fragrances, or (c) adequately remove key compounds tested in Experiment 2 (below). Reaction to fresh plumages also allowed us to explore the crested auklet's natural capacity for conspecific recognition.

Since crested auklet pairs (Fig. 1a) and groups (Fig. 1b) often engage in ruff-sniff displays at their breeding colony (Jones 1993a; Jones & Hunter 1993), we believed birds might respond more readily to the scent of multiple members of the opposite sex. Therefore, the odour cue consisted of the entire, scented plumage from two crested auklets that were the opposite sex of the bird run in the maze. The control consisted of identical samples from parakeet auklets. For ethical reasons we restricted feather collection to eight adults of each species (*n*=4 of each sex) collected over a period of two days. Freshly plucked plumage was put into tightly woven, black mesh bags that allowed airflow during a trial. Post-test, each was stored separately in an aluminium bag. Pairs of plumage samples were used an equal number of times. We ran trials over an eight-day period (2-10 July) only, as evaporation of volatiles caused feathers to lose scent.

Experiments 2-4: Synthetic components of auklet odour, mammalian musk, banana essence

To test whether synthetic, chemical components of feather odour were involved in auklet attraction, we presented birds with a mixture of cis-4-decenal and octanal, two compounds that we identified as seasonally significant (see Results: Chemical Analysis; Table 1). We placed 0.03mL of a 1:1 mixture of the tangerine-scented aldehydes on a cotton ball hidden in a black-coloured, open-topped, 4x4cm, 35ml glass jar. This treatment was more concentrated (approximately 39μg/g of cotton) than natural breeding plumage (Table 1). The control lacked the odour and consisted of an identical jar and cotton ball at the opposite end of the maze (Fig 2).

To test for odour aversion, we used a similar set-up of jars to present the experimental odour and control stimulus. Instead of aldehyde scent, the odour consisted of 10 mL of “Gusto” (Caven Lures), a blend of mammalian musks with a strong, skunk-like scent. For tests of a novel odour, banana essence, the experimental cue consisted of 25 mL of 40% amyl acetate. Data for all trials were collected in an identical fashion to Experiment 1. Odours were tested on different days throughout the study.

Data Analysis

We calculated the proportion of time each bird spent in the experimental vs. the control arm of the maze. Differences in response were compared via paired t-tests, as each bird was given a simultaneous, dyadic choice. Data met the requirements for this statistical procedure (normally distributed, homogeneity of variance), and all tests were two-tailed. Chi-square tests determined whether birds preferred the experimental or control arm of the maze during their initial visit. In some cases, the first arm visited was accidentally omitted during data collection (n=2 mammal musk, n=8 odour chemicals treatment), therefore chi-square tests analyzed a slightly smaller subset of data.

To examine differential responses to odours, we compared three tests (tangerine aldehydes, banana scent, mammalian musk), as each involved an odour vs. a control that lacked volatiles. For each bird, we calculated a net olfactory response by subtracting the proportion of time spent in the outer sector next to the odour cue from the control. Using two-way ANOVA, we tested the effect of odour treatment, sex of bird run in the maze, and interaction.

3. RESULTS

a) Odour Chemistry

Twenty compounds from scented feathers were present in quantifiable amounts and tested via Kruskal-Wallis ANOVA. Nine exhibited significant variation between feather types ($5.98 \leq \chi^2 \leq 11.27$, $df = 2$, $0.003 \leq p \leq 0.05$; Table 1). Wilcoxon two-sample tests revealed that feather types differed *only* with respect to season (breeding vs. winter, Table 1). That is, scented, breeding-season feathers (from both wild and captive birds, n=10) differed from winter feathers ($n = 6$; Wilcoxon two-sample test: $21.0 \leq S \leq 71.0$, $0.001 \leq p \leq 0.03$). Scented feathers of wild and captive birds, however, did not exhibit striking differences ($p > 0.22$; Table 1). Evidence for seasonal variation persisted, even when we compared each type of scented feather (captive or wild) separately to winter specimens ($15.0 \leq S \leq 52.0$, $0.001 \leq p \leq 0.08$).

For Experiment 2 (synthetic components of feather odour), we chose two aldehydes that were both significantly elevated during the breeding season, cis-4-decenal and octanal. Of the compounds detected during the breeding season only, cis-4-decenal was the most concentrated (Table 1). Octanal, though detectable year-round, was the most concentrated of all elevated, breeding-season components (Table 1). Synthetic samples of both compounds also have notable, tangerine-like odours.

A general similarity in scent between captive and wild samples (described above; Table 1) enabled us to explore differences between males and females. For each compound, data for all breeding-season samples were pooled (n=5 males, 5 females; equal sex ratio in wild and captive samples) and each compound was tested via a Wilcoxon 2-sample test. We found no striking differences relative to sex (p>0.40). All ANOVA and 2-sample tests we conducted were capable of detecting 'large' differences between groups (i.e. effect size>0.8; Cohen 1988), while retaining 80% statistical power. Assessing patterns of lesser magnitude requires more sampling. Nape feathers of parakeet auklets lacked detectable amounts of all seasonally-elevated components found in crested auklets (Table 1). Instead, principle volatiles of parakeet auklets included a series of C_{12}-C_{16} alcohols and hydrocarbons dominated by pentadecane.

Behavioural Experiments

Of 174 birds run in four T-maze experiments (Fig 3), 154 emerged from the start box and spent an average of 65% of the trial in the outer sectors, or arms, of the maze (mean ± s.d., 13.1 ± 5.9 min). Crested auklets spent more time near the scent of fresh feathers (t_{33} =2.9, p=0.007) and the mix of tangerine-scented aldehydes (cis-4-decenal and octanal; t_{48}=3.8, p=0.0004) than by controls (Fig. 3). During the initial visit to the choice area, birds preferred the sector nearest the tangerine aldehydes (30 vs.11 visits, χ^2=9.04, p=0.002). Fresh feathers did not elicit a similar, initial attraction (p=0.8). Auklets avoided mammalian musk (Fig. 3; t_{35}=2.4, p=0.02) and preferred to visit the unscented arm of the maze first (24 vs.10 visits, χ^2=4.89, p=0.02). For banana odour, we found no significant response (p=0.7; Fig. 3) or pattern of initial visits (p=0.9).

ANOVA analysis of net olfactory response for tests of tangerine aldehydes, mammalian musk, and banana essence was significant ($F_{5, 114}$=3.54, p=0.005; Fig. 3). Differences in response were related to the different odour treatments (F=8.05, p=0.0005; Fig. 3), rather than to sex (p=0.6) or interaction (p=0.4). Unlike other tests, we noticed that fresh feathers lost their tangerine scent over the eight-day plumage experiment. Using a median test, we compared the net olfactory response of birds on the first day (*n*=8) versus the last day (*n*=7), as non-normality of the data made linear regression unsuitable. Birds exhibited a marked decrease in response (*Z*=-227, *p*=0.02) over the eight-day experiment. That is, they spent more time (23.5% or 3.2 min) near fresh feathers than the control on the first day, but dropped to 3.6% or 0.5 min by the eighth day. Subsequent chemical analyses of several individual feathers from scented plumage were consistent with this decrease in response. Tested daily over an eight-day period, feathers lost, on average, 30-40% of their volatile components, including cis-4-decanal and octanal.

4. DISCUSSION

In birds, the basic assumption of most behavioural studies is that vision and hearing primarily govern the social cues to which individuals respond. After identifying and testing key chemical components that comprise a seasonally-elevated avian odour, we provide experimental evidence that brings this assumption into question. Specifically, we have shown that crested auklets preferentially orient toward chemical scents that occur on feathers during the breeding season.

Four aspects of crested auklet behaviour, three of which we tested experimentally, are consistent with tangerine scent acting as a relevant social stimulus. First, birds preferred natural plumage odour, which our chemical analyses indicate is elevated during the breeding season (Table 1). Second, birds were attracted to two key compounds that make-up the tangerine feather scent (Table 1; Fig. 3). Third, auklets not only recognised feather odour and two chemical components, they also distinguished between different kinds of odours (Fig. 3), both of which are pre-requisites for chemical communication (Bradbury & Veherencamp 1998). Fourth, the primary courtship display, the ‘ruff-sniff,’ involves a strongly-scented body region and implicates odour in a specific social context (Jones 1993a). The seasonal display provides an unequivocal, behavioural means for odour transmission; it is a self-evident mechanism for obtaining olfactory information. Several authors have already associated ‘ruff-sniff’ displays with mutual sexual selection (Jones 1993a; Hunter & Jones 1999; Douglas et al. 2001), as birds simultaneously rub their bills in the scented nape of a display partner

(Fig. 1). Thus, tangerine odour could function as an olfactory ornament. Mutual sexual selection for a visually conspicuous plumage trait (the crest) is already well-documented in this monogamous species (Jones & Hunter 1993; Hunter & Jones 1999). Clearly, future testing is required to uncover the full social context of auklet odour and whether it represents a compelling case for an avian pheromone (Karlson & Luscher 1959; Meredith 2001). At present, the facts strongly indicate that the tangerine odour of crested auklets is the most convincing example of a social odour for any bird.

Several other aspects of our tests are also germane to the role of odours in avian behaviour. First, auklet responses were suggestive of odour concentration, as birds were strongly attracted to the concentrated mix of tangerine aldehydes during initial visits and, overall, preferred this treatment somewhat more so than natural plumage (Fig. 3). During the eight-day plumage experiment, a decreased attraction to natural feather scent was also consistent with odour loss from fresh, scented feathers tested in the laboratory. Second, auklet avoidance of mammalian musk (Fig. 3) has two possible explanations. Either birds simply found the scent unpleasant, or possibly indicative of a predator (Fluck et al. 1996). Third, a lack of preference for the novel, sweet odour of banana scent (amyl acetate), suggests that this unusual odour simply did not evoke a meaningful response (Fig. 3). It is also possible that auklets may not be able to detect amyl acetate; however, a variety of avian species respond to this compound (e.g. ducks: Balthazart & Schoffeniels 1979; pigeons: Walker et al. 1986).

Our tests of natural plumage odour cannot exclude the possibility that birds may have found parakeet auklet feathers aversive. Such an explanation, however, would seem inconsistent with crested auklet attraction to specific, chemical components of scent, which we found lacking in parakeet auklet plumage. Future chemical and behavioural testing promise to reveal: (1) possible reactions of birds to heterospecific odour, (2) more subtle, conspecific differences between males and females, and (3) variation between wild and captive populations. With regard to captive birds, Douglas et al. (2001) anecdotally reported that at least one colony (different from that used in this study) might lack odour year-round. Interestingly, the breeding-season plumage we collected from captives exhibited a chemical profile that was generally similar to wild birds (Table 1).

The response of birds to environmentally-generated, chemical signals have already altered our views of avian foraging and navigation (e.g. Nevitt 1999). So, too, may avian odours alter interpretations of social behaviour. Our study and others (Shallenberger 1975; Thibault & Holyoak 1978; Balthazart & Schoffeniels 1979) relate seasonally significant scents or mate odours to breeding. Additional evidence implicates avian odours in alarm behaviour (Mason 1975) and conspecific recognition (Würdinger 1982). The widespread incidence of odours in birds (Weldon & Rappole 1992; Roper 1999), suggests that avian chemical signals may occur more often than we can readily detect. This idea is akin to the realisation that some birds respond to ultraviolet signals that humans cannot see (Bennett et al.1995), or the startling discovery that toxic secretions may serve as an avian chemical defense (Dumbacher et al. 2001). Though most birds do not appear to scent-mark or overtly assess odours, chemical signals could be transferred via more common or subtle behaviours, such as mutual preening (Roper 1999).

In summary, our data, combined with other studies of avian odour, indicate: (1) Birds can produce, and preferentially orient toward seasonally relevant, chemical components of conspecific odour. (2) Avian odours are linked to a variety of behavioural situations that are subject to natural or sexual selection. (3) Though chemical communication is common in every other class of vertebrate (Wingfield et al. 1994), its significance in birds stands as a promising, new area of avian behavioural ecology (Roper 1999).

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Table 1 Volatiles of crested auklet feathers that change in a seasonally significant manner. Bold type, compound tested in odour maze. ND, not detected (<0.0001 μg/g feathers).

Compound†	Concentration: μg/g feathers‡ Median (25–75%)		
	Breeding seasonù [median of wild birds, captives]	Winterψ	RT§
Cis-4-decenal* **	1.10 (0.80–1.60)*** [1.20, 1.10]	ND	27.1
Z-2-Decenal***	0.30 (0.10–0.89)*** [0.29, 0.35]	ND	29.3
Octanol**	0.18 (0.10–0.20)** [0.20, 0.13]	ND	24.2
Octanal* **	2.98 (2.02–4.40)** [2.98, 2.85]	0.25 (0.10–0.40)	21.8
Hexanoic acid*	0.84 (0.67–0.90)*** [0.88, 0.74]	0.36 (0.30–0.52)	20.7
Octanoic acid*	0.65 (0.58–2.88)** [1.68, 0.60]	0.15 (0.00–0.33)	26.5
Undecanal**	0.35 (0.30–1.35)*** [0.83, 0.35]	0.03 (0.02–0.20)	30.5

Tridecanal***	0.30 (0.10–1.34)**** [0.77, 0.24]	0.03 (0.02–0.05)	35.8
Heptanal*	0.15 (0.10–0.20) ** [0.10, 0.20]	0.35 (0.20–0.60)	18.0

****p***≤0.05, *****p***≤0.01, ******p***≤0.005, *******p***≤0.001

[†] Statistical significance from Kruskal-Wallis ANOVA of three feather types (see Results). Results for each compound was at least ***p***≤0.05. All compounds also met the criteria of the sequential Bonferroni procedure (Rice 1989) for ***n***=20 compounds tested, except for hexanoic acid (***p***=0.03) and heptanal (***p***=0.05).

[‡] Statistical significance from Wilcoxon 2-sample test of scented vs. unscented feathers. All tests met the criteria of the sequential Bonferroni procedure.

[ù] Nape feathers of ten adults (6 wild, 4 captive; 5 male, 5 female) collected May-August.

[ψ] Nape feathers of six captive adults only (4 male, 2 female), collected November-January.

[§] Retention time (min) during gas chromatographic analysis

A.

B.

Fig. 1 Stereo-typed "ruff-sniff" courtship. Individuals place their bills within the nape feathers of a display partner, a region of the body that is strongly scented.

A. Display between a courting pair.

B. Group display.

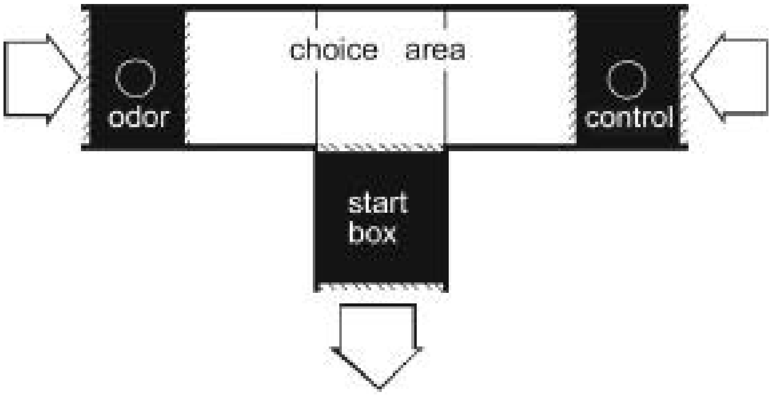

Fig. 2 T-maze used to test the response of crested auklets to odours. Odour samples and controls were visually hidden in darkened arms of the maze, which were constructed of black plexi-glas. Orthogonally arranged, fine mesh screens (dashed lines) provided an additional visual barrier. Screens were permeable to air flow (indicated by arrows); maze was otherwise air-tight. See methods for more detail.

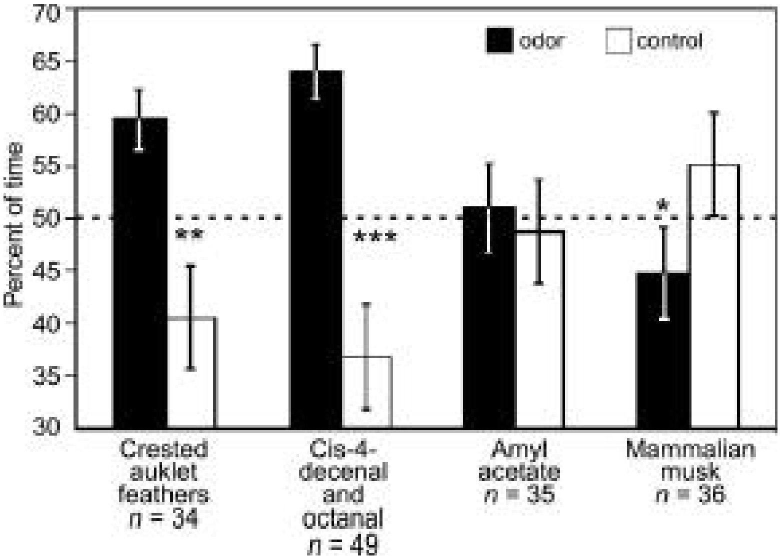

Fig. 3 Percent of time (mean ± s.e.) that crested auklets spent next to odour cues versus controls in the two outer sectors of the T-maze. Dashed line indicates the null hypothesis of equal preference. Paired ***t***-tests compared odour vs. control for each treatment.

****p***=0.02, *****p***=0.006, ******p***=0.0004