

HOMING BEHAVIOUR OF PIGEONS SUBJECTED TO UNILATERAL ZINC SULPHATE TREATMENT OF THEIR OLFACTORY MUCOSA

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Summary

Pigeons were made anosmic by unilateral treatment of their olfactory mucosa with a zinc sulphate solution and by plugging the contralateral nostril. In a series of releases at unfamiliar sites, 55–79 km from the home loft, the experimental birds' homing behaviour was compared with that of two control groups: unmanipulated control birds, and birds subjected to unilateral zinc sulphate treatment and equipped with an ipsilateral nasal plug. The experimental pigeons exhibited homing behaviour – in terms of both homeward initial orientation and homing performance – significantly poorer than that of both

unmanipulated and treated control pigeons. In addition, the homing behaviour of the treated controls turned out to be only slightly, and not significantly, poorer than that of the unmanipulated birds. The results show that the impaired homing capabilities of the zinc-sulphate-treated birds are due to the lack of navigational information and not to non-specific brain damage caused by the experimental treatment.

Key words: pigeon, zinc sulphate, anosmia, olfactory navigation, homing, *Columba livia*.

Introduction

During the last two decades, intense investigation of the navigational mechanisms of pigeons has been focused on the olfactory hypothesis developed by Papi's research group in Pisa (references in Papi, 1991, 1995; Wallraff, 1990; Benvenuti *et al.* 1992*b*; for debates on controversial aspects of olfactory navigation, see Schmidt-Koenig, 1987; Wiltschko *et al.* 1987; Waldvogel, 1989; Able, 1996; Wallraff, 1996; Wiltschko, 1996). Some of the many experiments aimed at verifying the role of olfactory information in pigeon navigation were carried out by Schlund and Schmid (Schlund and Schmid, 1991; Schlund, 1992; Schmid and Schlund, 1993), who ran tests with German pigeons made anosmic by bilateral treatment of their olfactory mucosa with zinc sulphate (see Benvenuti *et al.* 1992*a*, for references on zinc-sulphate-induced anosmia). These authors admit that their results are 'suggestive' and 'seem to support the hypothesis of olfactory navigation', but they then conclude that their results may have been produced by a non-specific zinc-sulphate-induced disturbance of the birds' behaviour, with possible effects on learning, motivation, circadian rhythms or magnetic perception. Thus, the German authors reject the method they had chosen on the basis of the results they obtained. They propose, against the parsimony principle, new explanations for a phenomenon – impairment of navigation following anosmia – which had been recorded by

several authors with birds made anosmic using different methods (for references, see Papi, 1990). In any case, the implications of the conclusions of Schlund and Schmid are serious for two reasons. First, they suggest that the zinc sulphate method – which is frequently used on vertebrates – is not a sound one; second, they leave unsettled the problem that they wanted to solve, i.e. whether pigeons in their area use olfactory cues to home.

We therefore ran a series of tests aimed at verifying whether the loss of navigational ability in zinc-sulphate-treated birds is actually related to non-specific effects. For this experimental series, we developed a new technique to achieve zinc-sulphate-induced anosmia. We used unilaterally treated control and experimental birds; the former and the latter were then equipped with ipsilateral and contralateral, respectively, nasal plugs. This experimental plan, like that used in previous tests using different methods of olfactory deprivation (Papi *et al.* 1972; Baldaccini *et al.* 1975; Benvenuti, 1979), allows one to subject both bird groups (controls and experimentals) to identical experimental manipulations and to a similar exposure to the risk of a non-specific disturbance. The only crucial difference is that the controls, unlike the experimentals, are able to smell through the non-treated side of their olfactory apparatus.

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Materials and methods

The pigeons and experimental treatment

The birds used in our experiments were born in the spring of 1995 in our field station in Arnino (Pisa, Italy) and housed in a loft there. Their pre-test experience was very limited, consisting of spontaneous daily flights and 2–3 training tosses at a maximum distance of about 30 km from the home loft, in different directions. The day before each single test, the birds were subjected to unilateral washing of the olfactory mucosa through one nostril with an aqueous solution of $ZnSO_4 \cdot 7H_2O$ (4%). In order to avoid possible contamination of the other nostril by gravity or capillarity during the unilateral treatment with zinc sulphate solution, the other nostril was washed with a Ringer's solution. This contemporaneous washing of the nostrils with two different solutions was achieved by using two paired syringes, one of which contained 4–5 ml of zinc sulphate and the other an equal volume of Ringer's solution. The needles of the syringes had been replaced with thin Teflon tubes inserted into the pigeon's nostrils. Gentle pressure on the syringes allowed the solutions to wash the olfactory apparatus from the nostrils to the choanae. In order to avoid the ingestion of the solutions, another operator removed them from the mouth – while they were flowing from the choanae – by aspiration with a suction pump. The treated birds were then subdivided into two groups, control and experimental birds, which were subjected to ipsilateral (controls) and contralateral (experimentals) plugging of one nostril (ipsilateral and contralateral with respect to the nostril that had received the zinc sulphate solution). The plugs were made with an adequate amount of paste (Xantoprene®) which turns into a solid, rubbery plug after insertion into the nostril. The pigeons were then allotted to treatment groups (controls and experimentals) in such a way as to have an equal number of right-side- and left-side-treated birds in the control and experimental groups. A third group of birds was transported unmanipulated to the release sites. The nasal plugs were removed as soon as the pigeons had homed (no birds lost the plugs, either during the passive displacement or during the homing flight).

Release tests and statistical methods

The experimental series started when pigeons were 3–4

months old. Each pigeon took part in only one test release. The tests were run on sunny days with no wind or only light winds. The three treatment groups, untreated controls (UC), treated controls (TC) and anosmic experimentals (E), were transported to the unfamiliar release sites in the same well-ventilated van. The birds were released singly, alternating different treatments. Vanishing times and bearings and homing performances were recorded according to standard procedures used in pigeon homing studies.

Each set of vanishing bearings was tested for randomness using the Rayleigh and *V*-tests; the Watson U^2 -test was then used to compare different bearing distributions (Batschelet, 1981). Homing performances of UC, TC and E birds were tested using the Kruskal–Wallis analysis of variance by rank (Siegel, 1956) and Dunn's multiple comparison test (Zar, 1984). The homing performances of left-side- and right-side-treated controls (TC) were compared using the Mann–Whitney *U*-test (Siegel, 1956).

Results

Initial orientation

Table 1 reports the results of the single release tests. In all the tests, both treated (TC) and untreated (UC) controls exhibited better initial homeward orientation than anosmic experimentals (E) in all the four release experiments, as revealed by the higher values of the homeward component (Hc) in the former with respect to the latter. According to the Watson U^2 -test, the difference in initial orientation between the experimentals (E) and each of the two control groups (UC and TC) is significant in all experiments but the fourth, i.e. in six out of eight possible comparisons. Conversely, significant differences between treated (TC) and untreated (UC) controls never occurred, although the homeward orientation of the UC birds was higher in three out of four experiments, as shown by the values of the homeward component (see Table 1). When the vanishing bearings recorded in the complete experimental series were pooled with the home direction set to 0° (Fig. 1), the bearings of both UC and TC birds turned out to be different from random (Rayleigh and *V*-tests, $P < 0.001$ in both cases) and not significantly different from each other (Watson U^2 -test, $P > 0.1$). The confidence interval of the distributions of both

Fig. 1. The vanishing distributions of pigeons subjected to the three treatments (untreated controls, treated controls and experimentals). Each symbol represents the vanishing bearing of a single pigeon; the inner arrows are mean vectors whose length is proportional to the scatter of the related bearing distributions and can be read using the scale in the left-hand diagram. Results from four release experiments are pooled with the home direction (H) set to 0° . Other explanations in text.

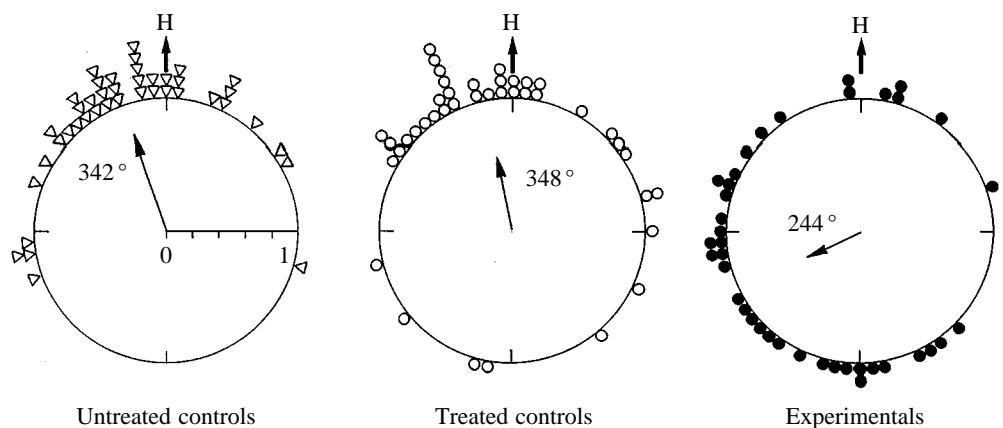


Table 1. Results of the experimental series

Experiment	Tr	n (N)	α (degrees)	r	Hc	U ²	Hp
1							
27 June	UC	11 (11)	161	0.67**	+0.66**	NS	8.2.1
152°	TC	11 (11)	212	0.74***	+0.37*	***	2.4.5
57km	E	11 (11)	324	0.63**	-0.62	**	0.3.8
2							
25 July	UC	10 (11)	283	0.94***	+0.92***	NS	5.5.1
293°	TC	10 (11)	262	0.78***	+0.67**	***	5.4.2
79km	E	11 (11)	181	0.43	-0.16	**	2.1.8
3							
27 July	UC	11 (11)	296	0.78***	+0.60**	NS	9.2.0
336°	TC	11 (11)	296	0.70**	+0.53**	**	7.3.1
55km	E	11 (11)	231	0.73**	-0.19	***	7.1.3
4							
20 October	UC	10 (10)	215	0.85***	+0.75**	NS	7.1.2
243°	TC	9 (11)	220	0.83***	+0.76**	NS	8.1.2
60km	E	6 (11)	217	0.80*	+0.71**	NS	2.2.7

For each of the four experiments, the date of release, the home direction and the distance from home are given.

Tr, bird treatment (see text); n, number of vanishing bearings recorded; N, number of birds released; α and r, direction and length of the mean vector; Hc, homeward component; U², level of statistical significance of the Watson U²-test; Hp, homing performance: the three values separated by stops indicate the number of birds that homed on the day of release, the number of birds that homed later and the number of birds lost, respectively.

Levels of statistical significance of the Rayleigh (r column), V-test (Hc column) and U²-tests are given as asterisks: *P<0.05, **P<0.01, ***P<0.001; NS, not significant.

In the U² column, the first (top) level of significance refers to the comparison between UC and TC, the second (middle) to that between UC and E, the third (bottom) to that between TC and E.

UC, untreated controls; TC, treated controls; E, anosmic experimentals.

groups included the home direction (confidence coefficient 0.99 for UC and 0.95 for TC; Hc=+0.73 for TC and +0.57 for UC). Conversely, the bearing distribution of the experimentals, though significantly different from random (Rayleigh test, P<0.01), gives a negative value for the homeward component (Hc=-0.16) and is significantly different from each of the two control groups' distributions (Watson U²-test, P<0.001, in both comparisons).

As reported in the Materials and methods section, treated controls were equipped with an ipsilateral plug inserted into one of the nostrils. As half of the birds were treated with the zinc sulphate solution on the right side and half on the left side, we were able to compare the initial orientation of right-side-treated controls (RT) with that of left-side-treated controls (LT). Fig. 2, in which pooled data are reported, shows that both the RT and LT bearing distributions were different from random (Rayleigh test, P<0.01 and P<0.05, respectively) and homeward-oriented (the home direction is included in the 95 % confidence interval in both cases; Hc=+0.68 for RT and +0.40 for LT). Despite the apparent difference in the lengths of the mean vectors, the Watson U²-test does not reveal any significant difference between the two bearing distributions (P>0.05).

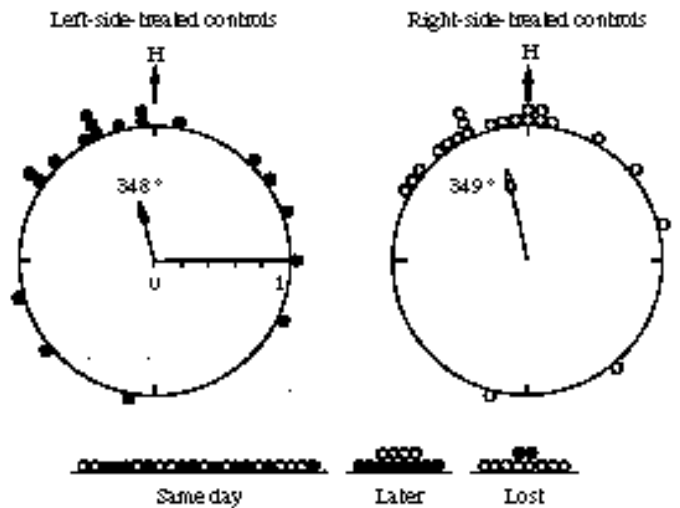


Fig. 2. The initial orientation of right-side- and left-side-treated control pigeons. The homing performance of the two treatment groups is given below. For birds that homed on the same day as they were released, symbols are listed according to the homing speed. Later, birds homing later than the first day; Lost, birds lost. Further explanation is given in Fig. 1 and in the text.

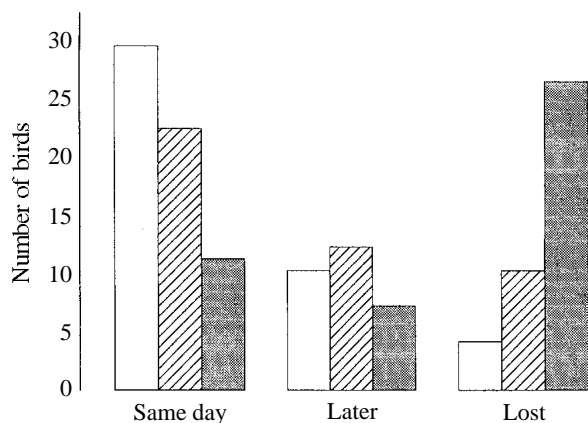


Fig. 3. Homing performance of the three treatment groups; open, hatched and filled bars denote untreated controls, treated controls and anosmic experimental pigeons, respectively. Further explanation is as in Fig. 2 and in the text.

Homing performance

The results for the initial orientation are in agreement with those for the homing performance. The Kruskal–Wallis analysis of variance revealed a statistically significant difference among the groups in three out of four releases ($P < 0.02$ in experiments 2 and 4; $P < 0.001$ in experiment 1). The comparisons TC *versus* E and UC *versus* E revealed a statistically significant difference in two (experiment 2, $P < 0.01$; experiment 4, $P < 0.025$; Dunn's test) and three (experiment 1, $P < 0.0005$; experiments 2 and 4, $P < 0.025$; Dunn's test) releases respectively. A significant difference between treated (TC) and untreated (UC) controls was reported only in the first experiment ($P < 0.02$, Dunn's test). A clear picture emerges from Fig. 3, which shows the pooled homing performance. The analysis of the pooled data showed a significant difference among groups ($P < 0.0001$, Kruskal–Wallis analysis of variance). On the whole, the performance of the treated controls is not significantly different from that of the untreated controls (Dunn's test, $P > 0.25$), whereas the homing ability of anosmic experimentals (E) is significantly different from that of both control groups, UC and TC (Dunn's test, $P < 0.0005$ and $P < 0.0025$ respectively). No significant differences were reported between the homing performance of the right-side- and left-side-treated controls ($P > 0.2$, Mann–Whitney *U*-test; see Fig. 2).

Discussion

Our homing experiments have shown that birds made anosmic by washing the olfactory mucosa on one side and by placing a plug in the contralateral nostril performed very poorly in both initial orientation and homing when compared with both treated and untreated controls. It is worth emphasising that the treated controls were subjected to the same manipulation as the anosmic pigeons, but the plugged nostril was ipsilateral to the zinc-sulphate-treated one. Only in the fourth test did the initial orientation of the experimentals

not differ from that of the two control groups. This result was attributable to the southwest bias of our pigeons (Ioalè, 1995), which 'simulated' the homeward orientation, and not to an unimpaired navigational ability. In fact, the experimentals' homing performance was extremely poor compared with both their own high level of homeward orientation and the homing performance of the treated and untreated controls.

The experimental birds' homing success was very poor in three out of four tests (first, second and fourth). In these three tests, only four out of 33 pigeons (12.1%) were able to home on the same day as the release; in the third test, the anosmic birds' homing success was only slightly – and non-significantly – lower than that of the control birds (seven out of 11 anosmic pigeons, 63.6%, homed on the same day as the release). In order to explain the homing ability of some of the anosmic birds, it is important to take into consideration that the home distance in the tests is not so great as to exclude the possibility that the birds would enter areas flown over during training tosses. As regards the unusually good homing success of the anosmic birds from release site 3, it may not be pure chance that this test site is closer than the others (22 km) to the site where most of the birds had been trained and was also in a similar direction with respect to home (336° *versus* 320°). In our opinion, the unusually good homing performance of the anosmic birds in the third experiment must be attributed to the above-mentioned cause rather than to a possible inefficiency of the zinc sulphate method (see Benvenuti *et al.* 1992a).

Our results, in agreement with a host of previous findings (see Introduction), confirm the fundamental role of olfactory information in homing when pigeons are released at unfamiliar sites.

Considering the lack of effect of zinc sulphate on the navigational abilities of the treated controls, our results cannot be attributed to a non-specific disturbance of the zinc sulphate treatment as suggested by Schlund (1992) and Schmid and Schlund (1993). Their deduction of a non-specific disturbance was based on apparently unrelated facts. Ablation of mouse olfactory bulbs has an effect on circadian rhythms (Possidente *et al.* 1990) and, hence, Schlund (1992) proposed that washing the olfactory mucosa of pigeons with zinc sulphate would affect their time-compensated sun compass. This connection, however, is not based on any experimental evidence. In addition, Schlund (1992) argued that treatment with zinc sulphate might interfere with the magnetic orientation of pigeons by impairing the ophthalmic nerve which, in the bobolink, was found to respond to magnetic variations (Semm and Beason, 1990). However, it is known that pigeons released under sunny conditions, such as those in Schlund's experiments, rely on their sun compass (Wallcott and Green, 1974; Visalberghi and Alleva, 1979; Füller *et al.* 1983).

It is worth observing that the homing behaviour of treated controls (see pooled results), though significantly better than that of the experimentals, is slightly (and non-significantly) poorer than that reported for untreated controls. This slight difference between untreated and treated controls can be put down to the stress experienced by the latter or to other minor

non-specific effects of the treatment. We have, however, to consider that the treated controls, unlike the unmanipulated birds, could only rely on half of their olfactory apparatus, and this limitation is a plausible obstacle to optimal perception of olfactory information.

A phenomenon of olfactory lateralization was recently observed in chicks (Vallortigara and Andrew, 1994a,b). The authors observed that only when the olfactory input is through the right olfactory pathway do the chicks learn the odour properties of objects in their rearing environment and use olfactory stimuli in choosing the objects on which they had been imprinted (Vallortigara and Andrew, 1994a,b). Conversely, our data show that olfactory stimuli received through the right nostril are as effective as those that reach the brain through the left nostril in eliciting an unimpaired navigational response. This result suggests a lack of lateralization of the pigeons' olfactory map.

Our results give further support to the olfactory navigation hypothesis and exclude the possibility that the marked impairment of the homing ability of pigeons made anosmic by washing their olfactory mucosa with zinc sulphate can be due simply to a systemic effect. It is therefore reasonable to conclude that our results, in agreement with those found in past years, show that the impaired homing abilities of pigeons, including the German birds used by Schlund (1992), were due to the specific deprivation of olfactory information.

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References

- ABLE, K. P. (1996). The debate over olfactory navigation by homing pigeons. *J. exp. Biol.* **199**, 121–124.
- BALDACCINI, N. E., BENVENUTI, S., FIASCHI, V. AND PAPI, F. (1975). New data on the influence of olfactory deprivation on the homing behavior of pigeons. In *Olfaction and Taste V* (ed. D. Denton and J. P. Cogland), pp. 351–353. New York, London: Academic Press.
- BATSCHLET, E. (1981). *Circular Statistics in Biology*. New York: Academic Press.
- BENVENUTI, S. (1979). Impaired homing ability in anosmic pigeons. *Z. Tierpsychol.* **51**, 406–414.
- BENVENUTI, S., IOALÈ, P., GAGLIARDO, A. AND BONADONNA, F. (1992a). Effects of zinc sulphate-induced anosmia on homing behaviour of pigeons. *Comp. Biochem. Physiol.* **103A**, 519–526.
- BENVENUTI, S., IOALÈ, P. AND PAPI, F. (1992b). The olfactory map of homing pigeons. In *Chemical Signals in Vertebrates VI* (ed. R. L. Doty and D. Müller-Schwarze), pp. 429–434. New York: Plenum Press.
- FÜLLER, E., KOWALSKI, U. AND WILTSCHKO, R. (1983). Orientation of homing pigeons: compass orientation vs piloting by familiar landmarks. *J. comp. Physiol.* **153**, 55–58.
- IOALÈ, P. (1995). Preferred compass directions of homing pigeons in Italy. *Boll. Zool.* **62**, 13–21.
- PAPI, F. (1990). Olfactory navigation in birds. *Experientia* **46**, 352–362.
- PAPI, F. (1991). Olfactory navigation. In *Orientation in Birds* (ed. P. Berthold), pp. 52–85. Basel: Birkhauser Verlag.
- PAPI, F. (1995). Recent experiments on pigeon navigation. In *Behavioural Brain Research in Naturalistic and Semi-Naturalistic Settings* (ed. E. Alleva, A. Fasolo, H.-P. Lipp, L. Nadel and L. Ricceri), pp. 225–238. Dordrecht: Kluwer Academic Publishers.
- PAPI, F., FIORE, L., FIASCHI, V. AND BENVENUTI, S. (1972). Olfaction and homing in pigeons. *Monitore zool. ital. (N.S.)* **6**, 85–95.
- POSSIDENTE, B., LUMIA, A. R., MCGINNIS, M. Y., TEICHER, M. H., DELEMOS, E., STERNER, L. AND DEROS, L. (1990). Olfactory bulb control of circadian activity rhythm in mice. *Brain Res.* **513**, 325–328.
- SCHLUND, W. (1992). Intra-nasal zinc sulphate irrigation in pigeons: effects on olfactory capabilities and homing. *J. exp. Biol.* **164**, 171–187.
- SCHLUND, W. AND SCHMID, J. (1991). Auswirkungen von ZnSO₄ auf die olfaktorische Wahrnehmung, die Anfangsorientierung und den Heimkehrerfolg von Brieftauben (*Columba livia*). *Verh. dt. zool. Ges.* **84**, 360.
- SCHMID, J. AND SCHLUND, W. (1993). Anosmia in ZnSO₄-treated pigeons: loss of olfactory information during ontogeny and the role of site familiarity in homing experiments. *J. exp. Biol.* **185**, 33–49.
- SCHMIDT-KOENIG, K. (1987). Bird navigation: has olfactory orientation solved the problem? *Q. Rev. Biol.* **62**, 31–47.
- SEMM, P. AND BEASON, R. C. (1990). Responses to small magnetic variation by the trigeminal system of the bobolink. *Brain Res. Bull.* **25**, 735–740.
- SIEGEL, S. (1956). *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill Book Co.
- VALLORTIGARA, G. AND ANDREW, R. J. (1994a). Olfactory lateralization in the chick. *Neuropsychologia* **32**, 417–423.
- VALLORTIGARA, G. AND ANDREW, R. J. (1994b). Differential involvement of right and left hemisphere in individual recognition in the domestic chick. *Behav. Process.* **33**, 41–58.
- VISALBERGHI, E. AND ALLEVA, E. (1979). Magnetic influences on pigeon homing. *Biol. Bull. mar. biol. Lab., Woods Hole* **125**, 246–256.
- WALCOTT, C. AND GREEN, R. P. (1974). Orientation of homing pigeons altered by a change in the direction of an applied magnetic field. *Science* **184**, 180–182.
- WALDVOGEL, J. A. (1989). Olfactory orientation in birds. *Curr. Orn.* **6**, 269–321.
- WALLRAFF, H. G. (1990). Navigation by homing pigeons. *Ethol. Ecol. Evol.* **2**, 81–115.
- WALLRAFF, H. G. (1996). Seven theses on pigeon homing deduced from empirical findings. *J. exp. Biol.* **199**, 105–111.
- WILTSCHKO, R. (1996). The function of olfactory input in pigeon orientation: does it provide navigational information or play another role? *J. exp. Biol.* **199**, 113–119.
- WILTSCHKO, W., WILTSCHKO, R. AND WALCOTT, C. (1987). Pigeon homing: different effects of olfactory deprivation in different countries. *Behav. Ecol. Sociobiol.* **21**, 333–342.
- ZAR, J. H. (1984). *Biostatistical Analysis*. Englewood Cliffs: Prentice-Hall, Inc.