Factors reducing the expected deflection in initial orientation in clock-shifted homing pigeons

Anna Gagliardo*, Francesca Odetti and Paolo Ioalè

Dipartimento di Etologia, Ecologia ed Evoluzione, Via Volta 6, I-56126 Pisa, Italy

*Author for correspondence (e-mail: annag@discau.unipi.it)

Summary

To orient from familiar sites, homing pigeons can rely on both an olfactory map and visual familiar landmarks. The latter can in principle be used in two different ways: either within a topographical map exploited for piloting or in a so-called mosaic map associated with a compass bearing. One way to investigate the matter is to put the compass and the topographical information in conflict by releasing clock-shifted pigeons from familiar locations. Although the compass orientation is in general dominant over a piloting strategy, a stronger or weaker tendency to correct towards the home direction by clock-shifted pigeons released from very familiar sites has often been observed. To investigate which factors are involved in the reduction of the deviation due to clock-shift, we performed a series of releases with intact and anosmic pigeons from familiar sites in unshifted and clock-shifted conditions and a series of releases from the same sites with naive clock-shifted birds. Our data suggest that the following factors have a role in reducing deviation due to the clock-shift: familiarity with the release site, the lack of olfactory information and some unknown site-dependent features.

Key words: homing pigeon, sun compass, anosmia, visual landmark, orientation.

Introduction

Kramer's 'map and compass' concepts are useful tools to explain in general the mechanisms underlying navigation in birds (Kramer, 1953). After displacement, homing can take place if a bird is able to establish the new position with respect to home (map step), and if it can determine a direction in space following which it will reach the goal (compass step). Most of the experimental work investigating the homing process in detail has been done with homing pigeons and aimed at determining the nature of cues composing the navigational map. A large body of evidence, accumulated over 30 years of research, has shown that olfactory cues are critical for homing pigeon navigation (Papi, 1982, 1990; Wallraff, 1990, 2001), although this issue has stimulated a lively debate (Wallraff, 1996; Wiltschko, 1996). Indeed, anosmic pigeons are greatly impaired in homing, when released at unfamiliar sites located within unfamiliar areas (Benvenuti et al., 1992; Wallraff and Neumann, 1989; Wallraff et al., 1993). By contrast, at familiar locations olfactory cues become redundant, as the anosmic pigeons are able to home. The most intuitive explanation of this phenomenon is that homing pigeons can rely on familiar landmarks to find their way home. However, the role of the landscape in homing from familiar locations has also not found general agreement among the researchers of pigeon orientation. The observations causing some scepticism concerning the relevance of visual landmarks in the pigeon navigation system were essentially two: (1) pigeons wearing frosted lenses were still homeward oriented (Benvenuti and

Fiaschi, 1983; Schmidt-Koenig and Schlichte, 1972; Schmidt-Koenig and Walcott, 1978); (2) clock-shifted pigeons released from familiar locations displayed a marked deviation toward the expected direction, as predicted by the use of the sun compass rather than the use of a topographical map (Füller et al., 1983). More recently, two different kinds of experiments have provided convincing evidence that homing pigeons do use familiar landmarks during their homing process. In fact, viewing the landscape at familiar sites before take-off affects homing speed (Braithwaite and Guilford, 1991; Braithwaite and Newman, 1994; Gagliardo et al., 2001b) and orientation in a circular arena (Gagliardo et al., 2001b).

Several experiments in which the sun compass and the topographical information were put into conflict, by releasing pigeons from familiar sites after the manipulation of their internal clock, yielded variable results in terms of the extent of the deviation in their initial orientation (Bingman and Ioalè, 1989; Bonadonna et al., 2000; Füller et al., 1983; Gagliardo et al., 1999, 2001a; Holland et al., 2000; Luschi and Dall'Antonia, 1993). These inconsistencies might be explained by two different hypotheses. (1) The first hypothesis takes into account two distinct strategies that may be used by the birds for orienting at familiar locations. During previous homing flights from a certain location, a bird might memorise different information relative to both the site and the homing route. For example, the pigeons can learn the general feature of a location and its compass bearing with respect to home, but can also

learn several landmarks and their geometrical relationship, so as to build a topographical representation of the familiar site (and/or familiar area); or they can learn both. These concepts have been discussed in detail by several authors and different terminology is sometimes used (Chappell, 1997; Holland, 2003; Wallraff, 1991; Wallraff et al., 1999). In the first strategy, the landmarks have to be used in association with a compass bearing; this mechanism was named 'mosaic map' by Wallraff (1974) and also more recently by Holland (2003), and 'point map' by Wallraff (1991). In the second strategy, the landmarks are independent from the 'map and compass' system and are used for piloting (Holland, 2003). Wallraff (1991) referred to the latter mechanism as a 'pattern map'. Recent experiments on hippocampal ablated pigeons released at familiar sites support the existence of both strategies, as the hippocampal lesions disrupt the ability to refer to a topographical map, but leave intact the compass orientation (Gagliardo et al., 1999, 2002). Other studies, in which the homing routes of clock-shifted intact pigeons were tracked from familiar sites, suggest that the release site might also determine which strategy is used (Bonadonna et al., 2000). (2) The second hypothesis attributes the variable reduction of the deflection due to the clock-shift treatment to the conflicting information given by the sun and the magnetic compass (Chappell, 1997; Wiltschko and Wiltschko, 2001; Wiltschko et al., 1994).

In some of the papers cited above the orientation of clock-shifted anosmic pigeons has been reported, and a tendency of the anosmic birds to deviate less than the clock-shifted smelling controls can be observed. This suggests that the use of the olfactory navigational map might bias the choice of the pigeons towards the use of the 'mosaic map and compass' strategy. As a possible contribution in clarifying the role of the landscape and the sun compass for orientation over familiar areas, we trained a large number of pigeons from three familiar release sites located in different directions with respect to home. We compared the initial orientation of a group of anosmic birds and a group of intact (smelling) pigeons either in natural dark-light cycle or after a fast clock-shift manipulation. Moreover, we also recorded the orientation of the birds in the arena (Gagliardo et al., 2001b; Mazzotto et al., 1999) with the aim of assessing whether, even before take-off, they use the visual cues for piloting or within a 'map and compass strategy', as Holland (2003) also recently suggested.

Materials and methods
Series I

The experiments were carried out in 2002, with 110 adult experienced homing pigeons. The birds were housed in a loft in Arnino field station (about 10 km SW of Pisa, Italy) and were allowed to perform spontaneous flights. From the end of June, all the birds were subjected to eight training releases in one group from each of the three sites chosen for the experimental tests (see Table 1 for details of the release sites).

The pigeons were also trained to exit from a circular arena, used for the experimental tests (see later in this section for details on the apparatus). The pigeons were subjected to one pre-training session, during which they were put in the arena in groups of three at a time, and to two individual training sessions, during which they were put singly in the arena.

During the training releases seven pigeons got lost. The remaining 103 birds were divided into two groups. 3 days before the first test they were subjected to the following treatments: (Group 1) FA-pigeons (N=52), birds familiar with the release sites, were made anosmic by washing their olfactory mucosa with a 4% $ZnSO_4.7H_2O$ solution, according to the procedure described in Guilford et al. (1998). (Group 2) FC-pigeons (N=51), control birds, familiar with the release sites, whose olfactory mucosa was washed with Ringer solution.

A first set of three releases, one for each site, was performed (No-Shift condition). In each test the pigeons' orientation while exiting from the arena and at vanishing were recorded. After homing, the same birds were subjected to a fast clock-shift treatment, keeping them in a light-tight room with the light:dark cycle 6 h fast with respect to the natural one for a period ranging from August 15th to September 5th, except for a few hours in which the birds took part in the release tests. The phase-shifted pigeons were then tested in a second series of releases from the same sites (Clock-Shift condition). Thus we adopted an experimental protocol allowing intra-individual comparisons. Although we could not record the initial orientation of the unshifted and shifted birds in the same day by this method, we consider this protocol valid due to the extreme stability of the initial orientation of both anosmic and intact unshifted pigeons familiar with Calambrone, La Costanza and Arnaccio (see the data reported in Diekamp et al., 2002; Gagliardo et al., 2001b). In this case each release took 3 days to be performed (see Table 1 for details) since the clock-shifted pigeons had to be released before the beginning of their subjective night. Once homed from each release site, the birds were immediately caught and placed again in the light-tight room until the next test release. Due to their familiarity with the release sites, all the birds homed within the same day of the release and most of them homed before the end of their subjective day. Therefore they were unlikely to re-adjust their internal clock to the natural light:dark cycle.

All the experimental releases took place in sunny conditions, with no or light wind.

The circular arena used to record the orientation before take-off is described in Gagliardo et al. (2001b) and Mazzotto et al. (1999). In brief, the arena (1.8 m in diameter) was made of non-magnetic material and placed on a 1.2 m high pedestal. The ceiling consisted of a net which allowed both a full view of the sky and free circulation of the air. The birds were able to escape by pushing through aluminium bars hanging down around the edge of the arena. The bars are familiar to the pigeons since they are the same as those used at the entrance to their loft. At the centre of the arena there is a remotely

Table 1. *Series I: orientation in the arena*

Site	Treatment	Experimental group	N	n	α (degrees)	r	hc	H' (degrees)	h'c	U²	at (s)
La Costanza	No-Shift,	C	51	51	239	0.54***	+0.32**				12
(185°,	12/08/02	A	52	52	237	0.59***	+0.36***			NS	9
18.1 km)	Clock-Shift,	C	44	42	111	0.08	+0.02	071	+0.06		13
	02-04-05/09/02	A	46	46	115	0.08	+0.03	068	+0.05	NS	38
Arnaccio	No-Shift,	C	51	51	303	0.46***	+0.39***				8
(270°,	13/08/02	A	52	52	290	0.35**	+0.32**			NS	8
12.7 km)	Clock-Shift,	C	49	49	107	0.17	−0.17	155	+0.11		10
	21-22-23/08/02	A	50	49	059	0.10	−0.01	153	−0.01	NS	19
Calambrone	No-Shift,	C	49	48	009	0.18	+0.18				5
(356°,	14/08/02	A	52	52	341	0.43***	+0.41***			NS	8
7.4 km)	Clock-Shift,	C	44	44	088	0.34**	−0.01	235	+0.28		8
	26-27-28/08/02	A	45	45	045	0.24	+0.16	236	−0.24	NS	15

Site, name of the release site, home direction and distance are indicated; C, control pigeons; A, anosmic pigeons; N, number of birds released; n, number of birds for which the orientation was recorded; α, mean vector direction; r, mean vector length; hc, homeward component, hc=$\mathbf{r}$cos (α–H), where H is the home direction; H', shifted home direction; h'c, shifted homeward component, h'c=$\mathbf{r}$cos(α–H'); U^2, results of the Watson U^2 test; at, median time spent in the arena before take-off; the asterisks in the r and hc columns indicate the results of the Rayleigh and V test, respectively; *$P<0.05$, **$P<0.01$, ***$P<0.001$; NS, not significant, that is $P>0.05$.

See text for an explanation of the clock-shifted treatment.

operated release box made of a net, where the pigeons were kept for 2 min before the beginning of the test.

Each bird was placed in the release box, which was opened by the experimenter pulling a rope while sitting under the arena out of the view of the bird. The escape bearing was recorded using a compass referring to the mid-point between the bars lifted by the pigeon. For each bird, the time spent in the arena before taking off was also recorded. If a bird spent more than 20 min before exiting from the arena, we tried to catch it in order to release it later. If the bird escaped as the experimenter approached, the exit direction was not recorded.

After take-off the pigeon's flight was observed using 10×40 binoculars and the azimuth of the vanishing bearing was recorded. The vanishing time was also recorded.

For each release, two bearing distributions were obtained: one referring to the pigeons' directional choices while exiting from the arena and the second referring to the birds' vanishing directions. For each distribution a mean vector and homeward component were calculated, the latter ranging from –1.0 to +1.0. The 95% and 99% confidence limits of the mean vectors were also calculated. The circular distributions were tested for randomness by means of both the Rayleigh and V-test, the latter taking into account an expected direction (Batschelet, 1981). For the non-shifted group distributions the expected direction was the home direction, while for the clock-shifted distributions the V-test was also performed considering the shifted home direction (see below). Comparisons between two circular distributions were achieved by the Watson U^2 test.

Second order mean vectors were calculated for each pigeon, for both the orientation in the arena and at vanishing. The vectors were obtained by pooling the orientation data obtained from each single pigeon in the three tests and setting the home direction to 360°. For most of the birds each vector was

calculated from three bearings, but the individual mean vectors of a few pigeons were calculated from the data of two sites. If only one bearing was recorded the datum was excluded from the second order statistics.

The one-sample Hotelling test (Batschelet, 1981) was applied to test for randomness the individual mean vector distributions relative to the orientation in cage and at vanishing. The two-sample Hotelling test (Batschelet, 1981) was applied to compare the mean vector distributions of C-pigeons and A-pigeons. The Hotelling test for paired samples of angles (Zar, 1984) was used in order to compare, for each experimental group of birds, the orientation in the arena in the no-shift and clock-shift conditions.

For the three releases after the Clock-Shift treatment, individual expected directions in arena and at vanishing were also calculated as follows. On the basis of the sun azimuth at the time of release of each single bird, we calculated the expected deviation after clock-shift. The latter was added to the home direction to calculate the individual, and then the mean, shifted home direction (used in the V-test and in the shifted homeward component for the clock-shifted birds). The expected deviation was also added to the mean direction of both C and A groups in the No-Shift condition to calculate the individual expected directions. We used the mean direction of the groups released in the No-Shift condition, to keep possible release site bias out of the analysis. The individual expected directions were pooled, setting the home direction to 360°, in order to obtain the expected individual mean vectors, which were used to calculate the second order expected mean vectors and their confidence ellipses (Batschelet, 1981).

The times spent in the arena and the vanishing times of FC- and FA-pigeons were compared using the Mann–Whitney U test (Siegel, 1956).

Series II

A second series of test releases was carried out in order to observe the initial orientation of pigeons unfamiliar with the three release sites used for Series I and therefore to get a baseline for the pigeon orientation without any influence of the landscape. An optimal experimental plan would have provided the simultaneous release of the birds familiar and unfamiliar with the release sites. Unfortunately this was not possible due to the large number of pigeons needed and because the releases would have taken too long. The circular arena was not used and only vanishing bearings were recorded.

Ninety-seven adult pigeons unfamiliar with the release sites were used. They were randomly assigned to two experimental groups: UUC, unshifted unfamiliar controls, and SUC, clock-shifted unfamiliar controls, subjected to a 6 h fast shift of their internal clock. Each bird took part in only one experimental release. Each release took place in the same month of the following year as the clock-shift test of Series I (see Table 4 for details). For other details of the methods, see Series I.

In order to compare the vanishing bearing distributions of the pigeons familiar with the release sites, either intact (FC) and anosmic (FA), and the vanishing distributions of pigeons unfamiliar with the release sites (UUC and SUC), we applied the Watson–Williams test (Watson and Williams, 1956).

Results

Series I

Orientation in arena

The directional choices displayed in the single releases by the C-pigeons and A-pigeons when exiting the arena are reported in Table 1 and Figs 1 and 2 (inner diagrams).

For releases performed in the No-Shift condition (see Fig. 1), at La Costanza and Arnaccio, both control and anosmic birds displayed directional choice distributions in the arena significantly different from random, according to both the Rayleigh and the *V*-test (see Table 1 for significance levels). The homeward component of the distributions' mean vectors for both C- and A-pigeons was positive at both sites and the mean vector directions were close to the home direction. At Calambrone, the unshifted
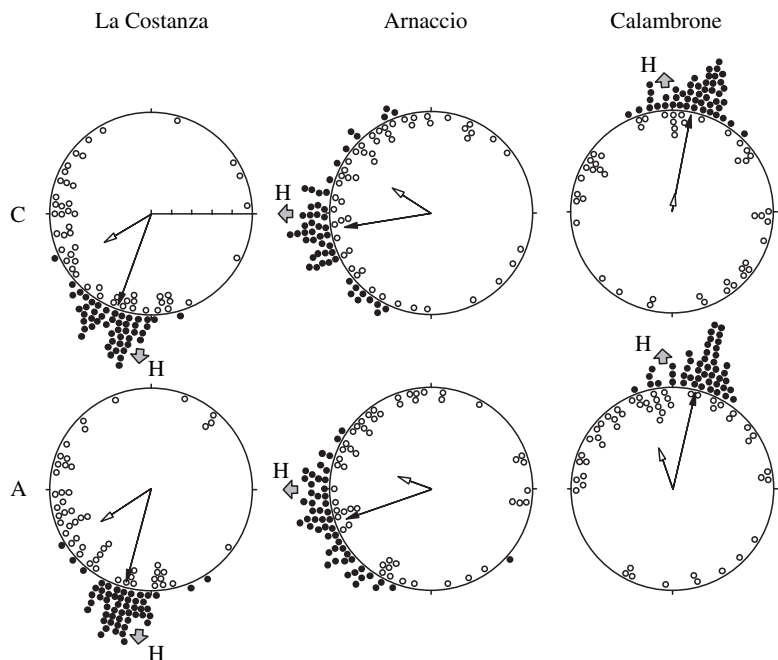
Fig. 1. Orientation distributions of pigeons released in the unshifted condition at the three experimental sites. C, control (intact) pigeons; A, anosmic pigeons. The inner diagrams (open circles) indicate the orientation of the pigeons while exiting from the circular arena; the outer diagrams (black circles) show the orientation at vanishing. Each symbol within a diagram represents a single pigeon. Inner arrows represent the distributions' mean vectors; white and black arrows are relative to arena and vanishing distributions respectively. The vector length can be read using the scale in the first diagram. The outer grey arrows indicate the home directions (H). Other details are reported in Tables 1 and 2.

anosmic birds were significantly oriented while exiting the arena, according to both the Rayleigh and the *V*-test, while the control (intact) pigeons' distribution turned out to be not significantly different from uniform according to the Rayleigh test. However, the C-birds' mean vector direction was oriented close to the home direction (see also the positive results of the *V*-test and other details in Table 1). On the whole, in the No-Shift condition, both control and anosmic birds were homeward oriented even before take-off.

In the Clock-Shift condition (Fig. 2), neither C- nor A-pigeons displayed distributions significantly different from random, except for the control birds at Calambrone, which were significantly oriented (Rayleigh test, *P*<0.01) in a direction different from the home direction (see also the negative homeward component in Table 1).

Within each single release test, in both the No-Shift and the Clock-Shift conditions, the Watson U^2 test never revealed a significant difference between the distributions in the arena of C- and A-pigeons.

The median time spent in the arena by the C- and A-pigeons before take-off, which is reported in Table 1, was not significantly different in the three releases (Mann–Whitney U test, *P*>0.05).

The second order statistics results are reported in Table 2 and Fig. 3, which show the individual mean vectors distributions. In the No-Shift condition, the second order distributions in the arena were significantly different from random for both anosmic and control (intact) pigeons (one-sample Hotelling test, *P*<0.001 for both A- and C-birds) and the second order mean vector directions were close to the home direction. By contrast, in the Clock-Shift condition, both second order distributions were not significantly different from random (one-sample Hotelling test, *P*>0.05 for both A- and C-birds). The comparison between the A and the C vector

La Costanza   Arnaccio   Calambrone

C

A

distributions did not reveal any statistical difference in either No-Shift or Clock-Shift conditions (two-sample Hotelling test on vectors, *P*>0.05 in both cases).

The individual mean orientation in the arena in the two phase conditions (No-Shift *versus* Clock-Shift) was compared by means of the Hotelling test on paired vectors. According to this test, the mean orientation in the arena of both control and anosmic pigeons was significantly different in the No-Shift *versus* the Clock-Shift condition (C-pigeons: $F_{(2,42)}$=9.804, *P*<0.001; A-pigeons: $F_{(2,43)}$=17.37, *P*<0.001). Therefore, before the manipulation of their internal clock the birds belonging to both experimental groups (A and C) were already homeward oriented before take-off, while the clock-shift treatment caused a general scattering of both groups in the arena.

Orientation at vanishing

The orientation at vanishing of the C- and A-pigeons in the single releases is reported in Table 3 and Figs 1 and 2 (outer diagrams).

In the No-Shift condition, the two experimental groups (C and A) were significantly oriented both according to the Rayleigh and *V*-test at the three release sites (see Table 3 for significance levels). In all releases, the homeward component relative to C- and A-pigeons' distributions was positive and the mean vector direction was close to the home direction. As expected, the vanishing distributions of both groups were on the whole gathered around the home direction.

In the Clock-Shift condition (Fig. 2, outer diagrams), the vanishing distributions of both C- and A-pigeons were significantly different from random according to the Rayleigh test in all releases, and according to the *V*-test (performed by considering the home direction as expected direction) at La Costanza and Calambrone (see details in Table 3). In fact, only at Arnaccio did both experimental groups display a negative homeward component as a consequence of the clock-shift treatment (see also in Table 3, columns h′c, the statistically
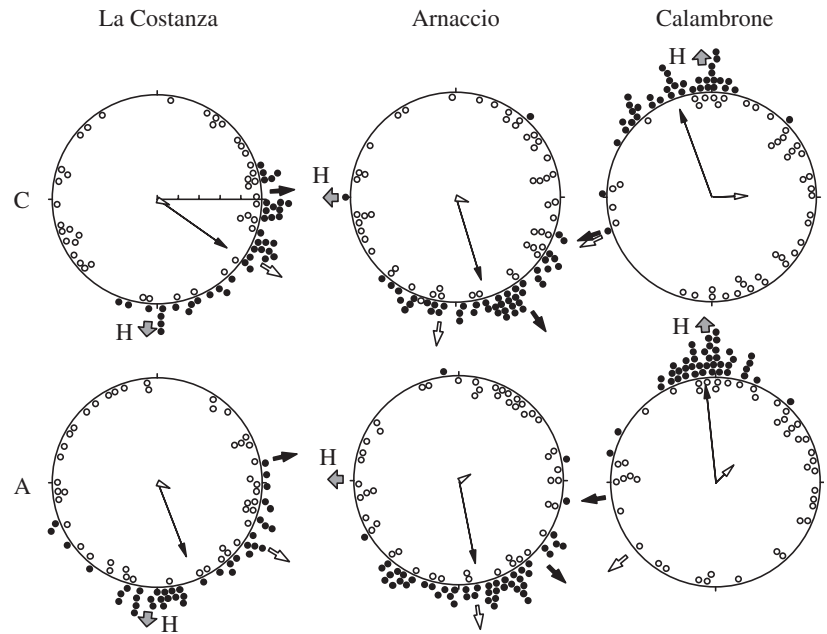

Fig. 2. Orientation distributions of pigeons released after 6 h fast clock-shift. White and black outer arrows represent the expected mean directions after the clock-shift treatment, for arena and vanishing distributions, respectively. Other explanations are given in Fig. 1.

significant results given by the *V*-test performed by considering the shifted home direction as expected direction). Therefore, the clock-shift manipulation did not, at least at La Costanza and Calambrone, produce the expected deviation for C- and A-pigeons (see also Fig. 2, in which the expected mean direction for each release is reported). The Friedmann-repeated-measures ANOVA on ranks applied to the deviations from the mean direction recorded in the No-shift release revealed a significant effect of the release site on the extent of the deviation for both groups of pigeons (C-pigeons, *N*=38, *P*<0.0001; A-pigeons, *N*=36, *P*<0.0001; *post hoc* analysis: Student–Newman–Keuls method, *P*<0.05 in all comparisons for both C- and A-pigeons).

Within each single release, in the No-Shift condition, the Watson U^2 test never revealed a significant difference between

Table 2. *Series I: second order statistics*

	Treatment	Experimental group	*n*	α (degrees)	**r**	Hotelling
Arena	No-shift	C	51	039	0.39***	NS
		A	52	026	0.34***	
	Clock-Shift	C	44	119	0.09	NS
		A	45	046	0.06	
Vanishing	No-shift	C	51	008	0.91***	NS
		A	52	003	0.91***	
	Clock-Shift	C	41	297	0.67***	**
		A	44	321	0.62***	

n, number of birds for which the individual mean orientation vector was calculated; α, second order mean vector direction; **r**, second order mean vector length; Hotelling, results of the two-sample Hotelling test for vector distributions; **P<0.01; NS, not significant, that is *P*>0.05.
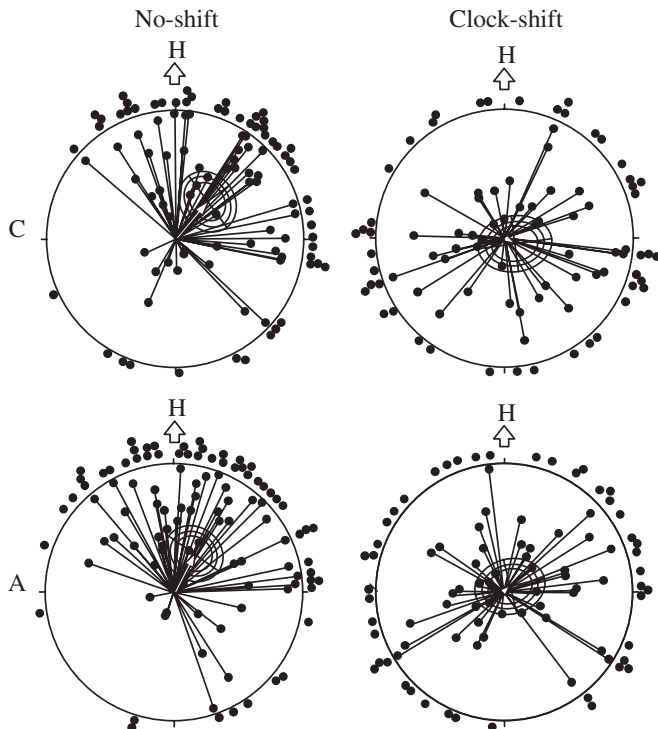
Fig. 3. Individual mean vector distributions relative to the orientation in the arena, in the No-Shift and Clock-Shift conditions. C, control (intact) pigeons; A, anosmic pigeons. All directions are calculated with respect to the home direction, which is set at 360°. Each inner line-and-circle represents the orientation mean vector with respect to home, calculated for a single pigeon. The outer circles indicate the mean vector directions. The inner white arrows represent the second order mean vectors. Confidence ellipses at 95%, 99% and 99.9%, according to the Hotelling one-sample test for vector distributions, are also reported.
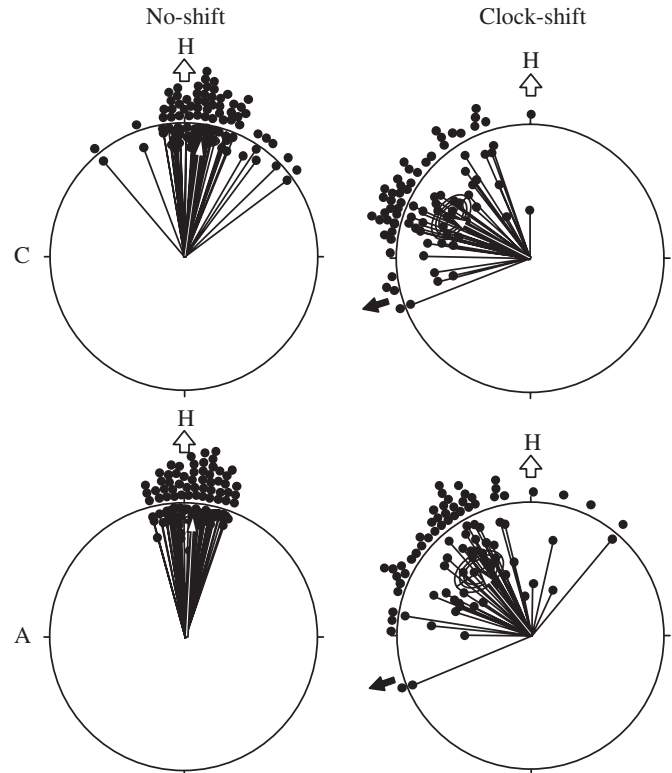

Fig. 4. Individual mean vector distributions relative to the orientation at vanishing, in the No-Shift and Clock-Shift conditions. The outer black arrows represent the expected second order mean directions after the clock shift treatment. Other explanations are given in Fig. 3.

the vanishing distributions of C- and A-pigeons, while in the Clock-Shift condition C- and A-birds turned out to have different vanishing orientations at Calambrone (see Table 3 for significance levels).

The median vanishing times are reported in Table 3. The Mann–Whitney U test revealed a statistical difference between A- and C-pigeons in the time taken to vanish from the observer's view in only one release test (Clock-Shift condition, La Costanza $P<0.01$).

The results of the second order statistics are reported in Table 2 and Fig. 4. The second order vanishing distributions were significantly different from random for the anosmic and control (intact) pigeons in both the No-Shift and Clock-Shift condition (one-sample Hotelling test, $P<0.001$ in all cases). In the No-Shift condition the second order mean vector direction was close to the home direction for both C- and A-pigeons and the two vector distributions were not statistically different (two-sample Hotelling test, $P>0.05$). In the Clock-Shift condition the mean vector direction relative to both pigeon groups deviated counterclockwise from the home direction. Although both A- and C-pigeons displayed a deviation smaller than expected (see expected mean direction in Fig. 4) the

anosmic birds deviated significantly less than the controls (two-sample Hotelling test, $P<0.01$).

According to the Hotelling test for paired data applied to the individual mean vector distributions, the orientation of both control and anosmic pigeons was significantly different in the No-Shift *versus* the Clock-Shift condition ($P<0.001$ for both groups).

Series II

Orientation at vanishing

The initial orientation of the unshifted (UUC) and clock-shifted (SUC) pigeons unfamiliar with the release sites is reported in Table 4 and Fig. 5.

At the three release sites the UUC-pigeons were significantly oriented at vanishing, according to both the Rayleigh and V-test (see Table 4 for significance levels). In all releases, the homeward component of the mean vector was positive and its direction was close to the home direction.

The SUC-pigeons displayed significantly oriented vanishing distributions at the three release sites, according to the Rayleigh test. As a consequence of the clock-shift treatment, the V-test, when performed taking into account the home direction, never turned out to be significant, and in two releases the homeward component of the mean vector was negative. By contrast, when the V-test was performed taking into account

Table 3. *Series I: orientation at vanishing*

Site	Treatment	Experimental group	N	n	α (degrees)	r	hc	H′ (degrees)	h′c	U^2	vt (s)
La Costanza	No-shift	C	51	49	200	0.97***	+0.93***				139
(185°,	12/08/02	A	52	52	195	0.97***	+0.95***			NS	158
18.1 km)	Clock-shift	C	44	41	125	0.81***	+0.40***	071	+0.48***		184
	02-04-05/09/02	A	46	41	159	0.75***	+0.68***	068	–0.01	NS	230
Arnaccio	No-Shift	C	51	51	261	0.86***	+0.85***				173
(270°,	13/08/02	A	52	52	251	0.89***	+0.84***			NS	181
12.7 km)	Clock-Shift	C	49	48	163	0.82***	–0.24	155	+0.81***		172
	21-22-23/08/02	A	50	49	169	0.80***	–0.15	153	+0.77***	NS	180
Calambrone	No-Shift	C	49	48	011	0.97***	+0.94***				152
(356°,	14/08/02	A	52	47	013	0.98***	+0.94***			NS	151
7.4 km)	Clock-Shift	C	44	38	340	0.89***	+0.85***	235	–0.23		201
	26-27-28/08/02	A	45	41	354	0.94***	+0.94***	237	–0.43	*	212

vt: median vanishing time.

See Table 1 for other explanations.

Table 4. *Series II. Orientation at vanishing*

Site	Experimantal group	N	n	α (degrees)	r	hc	H′ (degrees)	h′c	U^2	vt (s)
La Costanza	UUC	16	15	218	0.83***	+0.69***				245
04/09/03	SUC	16	15	091	0.75***	–0.06	066	+0.68***	***	259
Arnaccio	UUC	16	16	244	0.74***	+0.66***				178
21/08/03	SUC	16	15	128	0.64***	–0.51	152	+0.58***	***	268
Calambrone	UUC	16	15	336	0.70***	+0.65***				236
27/08/03	SUC	17	15	268	0.53*	+0.16	234	+0.44**	**	229

Site, name of the release site and date of the experiment; for home direction and distance see Tables 1 and 3; UUC, unshifted unfamiliar controls; SUC, shifted unfamilir controls.

For other explanations, see Tables 1 and 3.

the shifted home direction, it gave statistically significant results (see Table 4 for details).

The Kruskall–Wallis test applied on the deviations displayed by the clock-shifted birds (SUC) from the mean direction of unshifted pigeons (UUC) at the three release sites, did not reveal a significant effect of the release site on the extent of the deviation (SUC-pigeons, N=15, P=0.059).

The Watson U^2 test revealed a significant difference between the unshifted and the shifted pigeons' distributions in all releases (see Table 4 for significance levels).

The median vanishing times are reported in Table 4. The Mann–Whitney U test never revealed a statistical difference between UUC- and SUC-pigeons in the time taken to vanish from the observer's view.

A comparison between the vanishing distributions of the experimental groups of Series I and Series II, for each release site, is reported in Fig. 6. In particular, Fig. 6N-S shows the mean vectors and their 95% confidence limits of unshifted birds (FC, FA, UUC) and Fig. 6C-S refers to the shifted birds (FC, FA, SUC). Consistent with what can be observed in Fig. 6, the Watson–Williams test applied to compare the three experimental groups revealed a significant difference between

the distributions in all cases, except at Arnaccio in the unshifted condition (Unshifted condition: Calambrone and La Costanza, P<0.001; Arnaccio, P>0.05; Clock-shifted condition: Calambrone and La Costanza, P<0.001; Arnaccio, P<0.01). Although these results must be considered with caution because the experiments of the Series I and II were performed in different years, they are indicative of an effect of familiarity with the release site on the initial orientation in both unshifted and shifted conditions. In particular, in the shifted condition the birds unfamiliar with the release site displayed a deflection greater than both familiar groups (see Fig. 6C-S). Moreover, the anosmic birds familiar with the sites (FA) tended to deviate less than the familiar controls (FC).

Discussion

The aim of our work was to investigate which factors affect compass orientation in homing pigeons, by inducing a reduction of the deflection in initial orientation of clock-shifted pigeons. During the releases we collected data on orientation in a circular arena both before take-off and at vanishing. As regards the orientation at vanishing, on the whole our data are
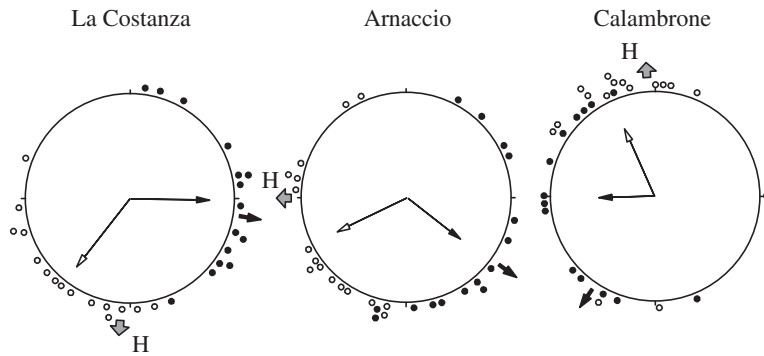
Fig. 5. Orientation at vanishing of pigeons unfamiliar with the release sites. Open and black circles represent the vanishing bearings of pigeons released without or after phase-shift manipulation respectively. The outer black arrows indicate the expected mean direction of the clock-shifted pigeons. Other explanations as in Fig. 1.

consistent with previous findings, which reported on the one hand a general dominance of the sun compass mechanism over the pilotage strategy and on the other a relevant role of familiarity with the release site in reducing the deflection induced by the clock-shift treatment (Bingman and Ioalè, 1989; Bonadonna et al., 2000; Füller et al., 1983; Gagliardo et al., 1999; Holland, 2003; Wallraff et al., 1999). Essentially three relevant issues on the relationship between the familiarity with a site and the use of the sun compass mechanism emerged: (1) familiarity with a release site reduces the extent of the deviation consequent to the clock-shift treatment; (2) anosmia enhances this reduction; (3) the extent of the deviation at familiar locations is site-dependent.

The combined effect of familiarity with the release site and the general dominance of the sun compass mechanism over the pilotage strategy determines an orientation towards an intermediate direction between the true and the erroneous home direction indicated by the topographical map and the sun compass, respectively. Some authors have reported a certain degree of reduction in the deviation of clock-shifted pigeons, regardless of the level of familiarity with the release site (Wiltschko et al., 1994) and they have explained this phenomenon with a contemporary use of the sun and magnetic compass information, the latter not being influenced by the clock-shift (Wiltschko and Wiltschko, 2001). However, we can reasonably interpret the reduction of the deviation observed in our pigeons released at familiar locations, as mainly due to the use of topographical information. In fact, different from what was observed for the pigeons familiar with the release sites, the clock-shifted birds unfamiliar with the release sites showed a deviation consistent with expectations (see also Fig. 5). If

the magnetic compass information were responsible for a correction mechanism, we would have observed the same extent of reduction in deviation in pigeons both familiar and unfamiliar with the release sites.

In principle, the observed reduced deflection showed by the birds familiar with the release site, might also be interpreted as a consequence of a recalibration of the sun compass due to consecutive releases in the phase-shifted condition (Wiltschko et al., 1976, 1984). However Foà and Albonetti (1980) produced clear evidence against the occurrence of the recalibration of the sun compass due to flight experience during the clock-shift period. In fact they observed that releasing the birds several times at the same familiar site reduced the deflection, while the homing experience during the clock-shift *per se* (tested by releasing the birds at unfamiliar locations) did not. Moreover, according to the recalibration hypothesis, we should expect in our data a gradual reduction of the deflection from the first to the third release, which is actually not observed. In fact, although the deviation in the first release (Arnaccio) was the largest, it was larger in the third release than in the second. Finally, the evidence that the anosmic clock-shifted pigeons familiar with the release site deviated significantly less than their intact (smelling) companions does not support the recalibration hypothesis, which should in principle occur in both groups to the same degree.

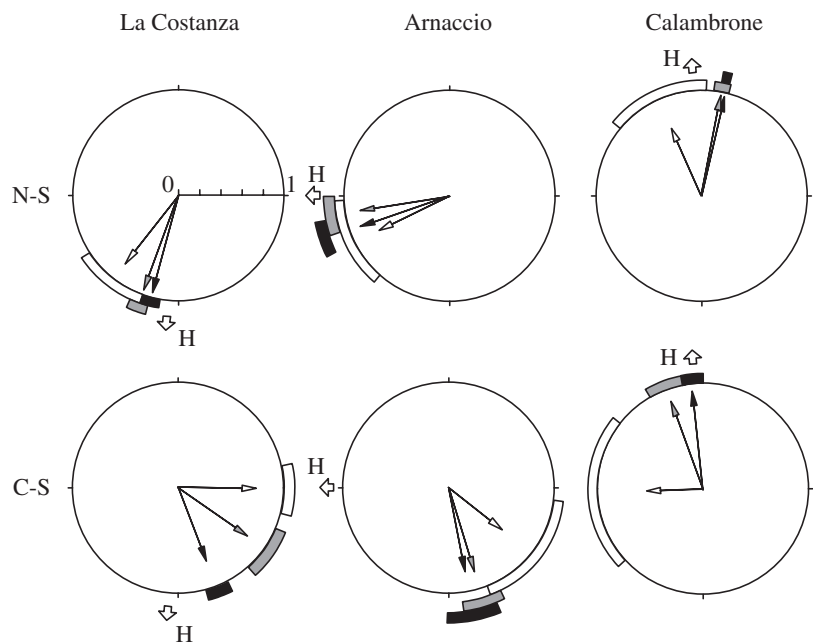It has been shown that at familiar sites pigeons have access

Fig. 6. Comparison between the mean vectors and their 95% confidence limits of pigeons with different experience and treatment. The diagrams of the upper row (N-S, No-Shift) and the lower row (C-S, Clock-Shift) refer to the orientation of pigeons released without or after clock-shift treatment, respectively. White sectors correspond to pigeons unfamiliar with the release site; grey and black sectors correspond to pigeons familiar with the release site, intact and anosmic, respectively.

to two maps, based on olfactory and visual information (Gagliardo et al., 2001b): the olfactory map can only be used in association with a compass, while the map based on visual cues can be used either coupled with a compass or for piloting within an array of landmarks.

As already mentioned, we observed that, at familiar locations, anosmic pigeons tend to deviate less than intact birds after clock-shifting. Therefore, it seems that the lack of an olfactory navigational map, whose functionality in homing is strictly dependent on a compass mechanism, leads the pigeons to use topographical information more in a pilotage-like strategy rather than in a 'map and compass' one.

A study reporting homing routes of clock-shifted pigeons released from two familiar sites, showed a marked effect of the release site on the homing strategy (Bonadonna et al., 2000). In fact, from one site most of the pigeons followed straight routes towards home, while from the other location most of them made a long detour, as was expected after the internal clock manipulation. Therefore, at one site piloting was the preferred strategy while at the other site the 'map and compass strategy' prevailed. Our data are consistent with the observation that different homing strategies can have different importance depending on the site. In fact, our clock-shifted pigeons familiar with the sites tended to orient straight home at Calambrone and to deviate to differing degrees at the other two locations.

Although it is not clear which local factors generally influence the choice of the homing strategy after a clock-shift we can speculate that the homeward orientation observed at Calambrone, for both anosmic and intact birds, might have been due to their short distance from home. In fact we can hypothesise that a pilotage strategy is easier to use when fewer landmarks need to be remembered. Nevertheless, at least for the anosmic birds, a difference in the extent of deviation can also be observed between La Costanza and Arnaccio, which are located further from home. It is worth noting that La Costanza is much closer (about 5 km) to the coast than Arnaccio (about 14 km) and therefore the sea could represent a useful orienting cue. The latter explanation has also been suggested by other authors, who observed a similar reduction of the deflection in initial orientation of clock-shifted birds released from La Costanza (Bonadonna et al., 2000). At Arnaccio the anosmic birds, in compromising between the use of topographical information in a 'piloting-like' and in a 'map and compass' strategy, seemed to rely more on the latter.

Since, as already mentioned in the Introduction, different authors have reported a different degree of deflection in the initial orientation consequent to the clock-shift at familiar locations, it is worth comparing our data with those collected by other authors performing similar experiments. Similar to our observations, Bingman and Ioalè (1989) and Wallraff et al. (1994) also reported a strong effect of combined familiarity and anosmia in reducing the deviation of initial orientation after clock-shift. However, different from our observations in the present work and in Bingman and Ioalè (1989), Wallraff et al. (1994) and Füller et al. (1983) reported an almost full deflection in the initial orientation of clock-shifted smelling pigeons at familiar sites. It is worth noting that all these studies are not homogeneous with respect to the training procedure used for acquisition of familiarity with the release sites. For example, Wallraff et al. (1994), in contrast to all the other papers on the subject, also trained the birds from several locations around the experimental release site. Although the aim of the latter training procedure was to prevent directional bias and, at the same time, induce a wider topographical map learning, actually it might have made the use of a pilotage-like strategy more difficult, due to the higher number of landmarks and their spatial relationship to be remembered. On the other hand, the extreme directional training (more than 50 times from the same site) as reported in Füller et al. (1983) is likely to have induced the birds to display a stereotyped directional response that disregarded the topographical information.

As regards the orientation of our birds while exiting from the circular arena, our data confirm previous findings that pigeons in the unshifted condition are already homeward-oriented before take-off (Chelazzi and Pardi, 1972; Diekamp et al., 2002; Gagliardo et al., 2001b; Mazzotto et al., 1999). Contrary to our expectation, the clock-shift manipulation increased the scattering so much that all the distributions of clock-shifted pigeons turned out to be no different from uniform. Therefore, our data relative to the orientation in the arena of clock-shifted birds are inconclusive, so that we could not verify which strategy, piloting or 'map and compass', the clock-shifted pigeons used referring to the visual cues before take-off. It is difficult to speculate about the causes of the observed scattering as a consequence of the clock-shift; however, we can suggest that the observed random distributions might result from the combination of two treatments (test in the arena and clock-shift), each of which usually produces some scattering.

References

Batschelet, E. (1981). *Circular Statistics in Biology*. London: Academic Press.

Benvenuti, S., Ioalè, P., Gagliardo, A. and Bonadonna, F. (1992). Effects of zinc-sulphate induced-anosmia on homing behaviour of pigeons. *Comp. Biochem. Physiol.* **103A**, 519-626.

Benvenuti, S. and Fiaschi, V. (1983). Pigeon homing: combined effect of olfactory deprivation and visual impairment. *Comp. Biochem. Physiol.* **76A**, 719-723.

Bingman, V. P. and Ioalè, P. (1989). Initial orientation of anosmic homing pigeons based on information gathered at familiar release sites remains homeward directed following clock-shift. *Behaviour* **110**, 1-4.

Bonadonna, F., Holland, R., Dall'Antonia, L., Guilford, T. and Benvenuti, S. (2000). Tracking clock-shifted homing pigeons from familiar release sites. *J. Exp. Biol.* **203**, 207-212.

Braithwaite, V. A. and Guilford, T. (1991). Previewing familiar landscapes affect pigeon homing. *Proc. R. Soc. Lond. B* **245**, 183-186.

Braithwaite, V. A. and Newman, J. A. (1994). Exposure to familiar landmarks allows a pigeon to home faster. *Anim. Behav.* **48**, 1482-1484.

Chappell, J. (1997). An analysis of clock-shift experiments: is scatter increased and deflection reduced in clock-shifted homing pigeons? *J. Exp. Biol.* **200**, 2269-2277.

Chelazzi, G. and Pardi, L. (1972). Experiments on the homing behaviour of caged pigeons. *Monit. Zool. Ital. (N.S.)* **6**, 63-73.

Diekamp, B., Prior, H., Ioalè, P., Odetti, F., Güntürkün, O. and Gagliardo, A. (2002). Effects of monocular viewing on orientation in an arena at the release site and homing performance in pigeons. *Behav. Brain Res.* **136**, 103-111.

Foà, A. and Albonetti, E. (1980). Does familiarity with the release site influence the initial orientation of homing pigeons? Experiments with clock-shifted birds. *Z. Tierpsychol.* **54**, 327-338.

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.

Gagliardo, A., Ioalè, P. and Bingman, V. P. (1999). Homing in pigeons: the role of the hippocampal formation in the representation of landmarks used for navigation. *J. Neurosci.* **19**, 311-315.

Gagliardo, A., Ioalè, P., Odetti, F., Bingman, V. P., Siegel, J. J. and Vallortigara, G. (2001a). Hippocampus and homing in pigeons: left and right hemispheric differences in navigational map learning. *Eur. J. Neurosci.* **13**, 1617-1624.

Gagliardo, A., Odetti, F. and Ioalè, P. (2001b). Relevance of visual cues for orientation at familiar sites by homing pigeons: an experiment in a circular arena. *Proc. R. Soc. Lond. B* **268**, 2065-2070.

Gagliardo, A., Odetti, F., Ioalè, P., Bingman, V. P., Tuttle, S. and Vallortigara, G. (2002). Bilateral participation of the hippocampus in familiar landmark navigation by homing pigeons. *Behav. Brain Res.* **136**, 201-209.

Guilford, T., Gagliardo, A., Chappell, J., Bonadonna, F., Burt, d. P. and Holland, R. (1998). Homing pigeons use olfactory cues for navigation in England. *J. Exp. Biol.* **201**, 895-900.

Holland, R., Bonadonna, F., Dall'Antonia, L., Benvenuti, S., Burt, d. P. and Guilford, T. (2000). Short distance phase shifts revisited: tracking clock-shifted homing pigeons (Rock Dove *Columba livia*) close to the loft. *Ibis* **142**, 111-118.

Holland, R. A. (2003). The role of visual landmarks in the avian familiar area map. *J. Exp. Biol.* **206**, 1773-1778.

Kramer, G. (1953). Wird die Sonnenhöhe bei der Heimfindeorientierung verwertet? *J. Ornithol.* **94**, 201-219.

Luschi, P. and Dall'Antonia, P. (1993). Anosmic pigeons orient from familiar sites by relying on the map-and-compass mechanism. *Anim. Behav.* **46**, 1195-1203.

Mazzotto, M., Nacci, L. and Gagliardo, A. (1999). Homeward orientation of pigeons confined in a circular arena. *Behav. Proc.* **46**, 217-225.

Papi, F. (1982). Olfaction and homing in pigeons: ten years of experiments. In *Avian Navigation* (ed. F. Papi and H. G. Wallraff), pp. 149-159. Berlin: Springer Verlag.

Papi, F. (1990). Olfactory navigation in birds. *Experientia* **46**, 352-363.

Schmidt-Koenig, K. and Schlichte, H. J. (1972). Homing in pigeon with impaired vision. *Proc. Natl. Acad. Sci. USA* **69**, 2446-2447.

Schmidt-Koenig, K. and Walcott, C. (1978). Tracks of pigeons homing with frosted contact lenses. *Anim. Behav.* **26**, 480-486.

Siegel, S. (1956). *Nonparametric Statistics for the Behavioral Sciences.* New York: McGraw-Hill.

Wallraff, H. G. (1974). *Das Navigationssystem der Vögel.* München: R. Oldenbourg Verlag GmbH.

Wallraff, H. G. (1990). Navigation by homing pigeons. *Ethol. Ecol. Evol.* **2**, 81-115.

Wallraff, H. G. (1991). Conceptual approaches to avian navigation systems. In *Orientation in Birds* (ed. P. Berthold), pp. 128-165. Basel: Birkhäuser Verlag.

Wallraff, H. G. (1996). Seven theses on pigeon homing deduced from empirical findings. *J. Exp. Biol.* **199**, 105-111.

Wallraff, H. G. (2001). Navigation by homing pigeons: updated perspective. *Ethol. Ecol. Evol.* **13**, 1-48.

Wallraff, H. G., Chappell, J. and Guilford, T. (1999). The roles of the sun and the landscape in pigeon homing. *J. Exp. Biol.* **202**, 2121-2126.

Wallraff, H. G., Kiepenheuer, J. and Streng, A. (1993). Further experiments on olfactory navigation and non olfactory pilotage by homing pigeons. *Behav. Ecol. Sociobiol.* **32**, 387-390.

Wallraff, H. G., Kiepenheuer, J. and Streng, A. (1994). The role of visual familiarity with the landscape in pigeon homing. *Ethology* **97**, 1-25.

Wallraff, H. G. and Neumann, M. F. (1989). Contribution of olfactory navigation and non-olfactory pilotage to pigeon homing. *Behav. Ecol. Sociobiol.* **25**, 293-302.

Watson, G. S. and Williams, E. J. (1956). On the construction of significance tests on the circle and the sphere. *Biometrika* **43**, 344-352.

Wiltschko, R. (1996). The function of olfactory input in pigeon orientation: does it provide navigational information or play another role. *J. Exp. Biol.* **199**, 113-199.

Wiltschko, R., Kumpfmuller, R., Muth, R. and Wiltschko, W. (1994). Pigeon homing: the effect of a clock-shift is often smaller than predicted. *Behav. Ecol. Sociobiol.* **35**, 63-73.

Wiltschko, R. and Wiltschko, W. (2001). Clock-shift experiments with homing pigeons: a compromise between solar and magnetic information? *Behav. Ecol. Sociobiol.* **49**, 393-400.

Wiltschko, W., Wiltschko, R. and Keeton, W. T. (1976). Effects of a 'permanent' clock-shift on the orientation of young homing pigeons. *Behav. Ecol. Sociobiol.* **1**, 229-243.

Wiltschko, W., Wiltschko, R. and Keeton, W. T. (1984). The effects of a 'permanent clock-shift' on the orientation of experienced homing pigeons. *Behav. Ecol. Sociobiol.* **15**, 263-272.

Zar, J. H. (1984). *Biostatistical Analysis* Englewood Cliffs: Prentice-Hall International.