Age versus clade congruence

Three measures were used to assess age versus clade congruence (Fig. 2): the stratigraphic consistency index, SCI (ref. 24), the relative completeness index, RCI (ref. 25) and the gap excess ratio, GER (ref. 21). The SCI is the ratio of consistent to inconsistent nodes in a cladogram, and it can range from 0 to 1.0 in a fully pectinate (unbalanced) tree, but the minimum value lies between 0 and 0.5 in balanced trees[21,22]. The RCI and GER depend on numerical age estimates of the branching points on a cladogram, and the calculation of 'ghost ranges'. The ghost range[26] is the difference in age, or number of stratigraphic intervals, between the oldest known fossils of two sister taxa. The RCI is assessed as the ratio between the sum of ghost ranges to the sum of recorded fossil ranges in any cladogram. The GER focuses solely on the estimated dates of origin of groups, and compares the sum of actual ghost ranges in a cladogram with the theoretical minimum and maximum ghost ranges if the various branches in the cladogram are rearranged. The metrics were calculated using the software 'Ghosts 2.4', developed by M.A.W., which assesses all three metrics (SCI, RCI, and GER) for individual cladograms, or for large batches of cladograms (available from http:// palaeo.gly.bris.ac.uk/cladestrat/cladestrat.html).

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Ontogeny of orientation flight in the honeybee revealed by harmonic radar

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Cognitive ethology focuses on the study of animals under natural conditions to reveal ecologically adapted modes of learning. But biologists can more easily study what an animal learns than how it learns. For example, honeybees take repeated 'orientation' flights before becoming foragers at about three weeks of age[1]. These flights are a prerequisite for successful homing.[2] Little is known[2,3] about these flights because orienting bees rapidly fly out of the range of human observation. Using harmonic radar, we show for the first time a striking ontogeny to honeybee orientation flights. With increased experience, bees hold trip duration constant but fly faster, so later trips cover a larger area than earlier trips. In addition, each flight is typically restricted to a narrow sector around the hive. Orientation flights provide honeybees with repeated opportunities to view the hive and landscape features from different viewpoints, suggesting that bees learn the local landscape in a progressive fashion. We also show that these changes in orientation flight are related to the number of previous flights taken instead of chronological age, suggesting a learning process adapted to changes in weather conditions, flower availability and the needs of bee colonies.

Figure 1 Bee wearing transponder used for harmonic radar tracking of flight. The transponder weighs less than an average load of nectar or pollen.

We began our investigations without harmonic radar, by introducing one-day-old adult worker bees tagged with numbered disks (n = 125) into a colony of about 20,000 workers and a queen, and simply watching them as they exited and re-entered the hive. All flight activities of the tagged bees were recorded. We found that no bee became a forager (defined as a bee that returns to the hive with nectar or pollen) without taking at least one orientation flight and that almost all bees took multiple orientation flights before they began to forage. The number of orientation flights taken by bees before foraging was highly variable, ranging from 1 to 18 (mean ± s.e., 5.6 ± 0.29 flights). Equally variable was the age at which these flights began, ranging from 3 to 14 days (mean ± s.e., 6.2 ± 0.18 days). In this study the mean age at onset of pollen foraging was 14 ± 2 days.

We examined whether, given this variability, individual orientation flights differ after bees leave the vicinity of the hive. We used harmonic radar[4,5] to extend the range of our observations in the next study. Tagged one-day-old bees were introduced into a standard hive, and all of their subsequent flights were visually observed and recorded. We were thus able to select bees of known age and flight history for radar tracking. Transponders were attached to bees as they departed from the hive (Fig. 1) and orientation flight tracks were recorded by the radar ($n = 53$). Each bee was tracked only once. We analysed 29 complete (out-and-back) orientation flights taken by bees of different ages (3–27, median = 6 days), with differing degrees of experience (1–17 orientation flights). For comparison, we also tracked flights (n = 15) taken by experienced foragers of unknown age from the same hive. All flights were naturally occurring; that is, all tracked bees had moved spontaneously from inside the hive to the hive entrance before attachment of the transponder.

Most bees fitted with transponders began their orientation flights with a brief period of hovering facing the colony entrance before departure (see Methods), just as those without transponders did and as has been previously reported[3]. The duration of the orientation flights of bees with and without transponders was not significantly different (with transponders: mean ± s.e., 331.6 ± 59.2 s (n = 29) versus without transponders: 340.1 ± 26.4 s (n = 219), Wilcoxon test, $P > 0.05$, n.s.). We therefore concluded that the attachment of transponders did not significantly alter flight behaviour.

Sample flight tracks are shown in Fig. 2, and characteristics of the orientation tracks are summarized in Table 1. We found significant positive correlations between flight number and speed relative to the ground (averaged over the journey), round trip journey distance, maximum range from the hive and the area covered by the convex polygon circumscribing each flight (Table 1, Fig. 3). There was no significant positive correlation between flight number and

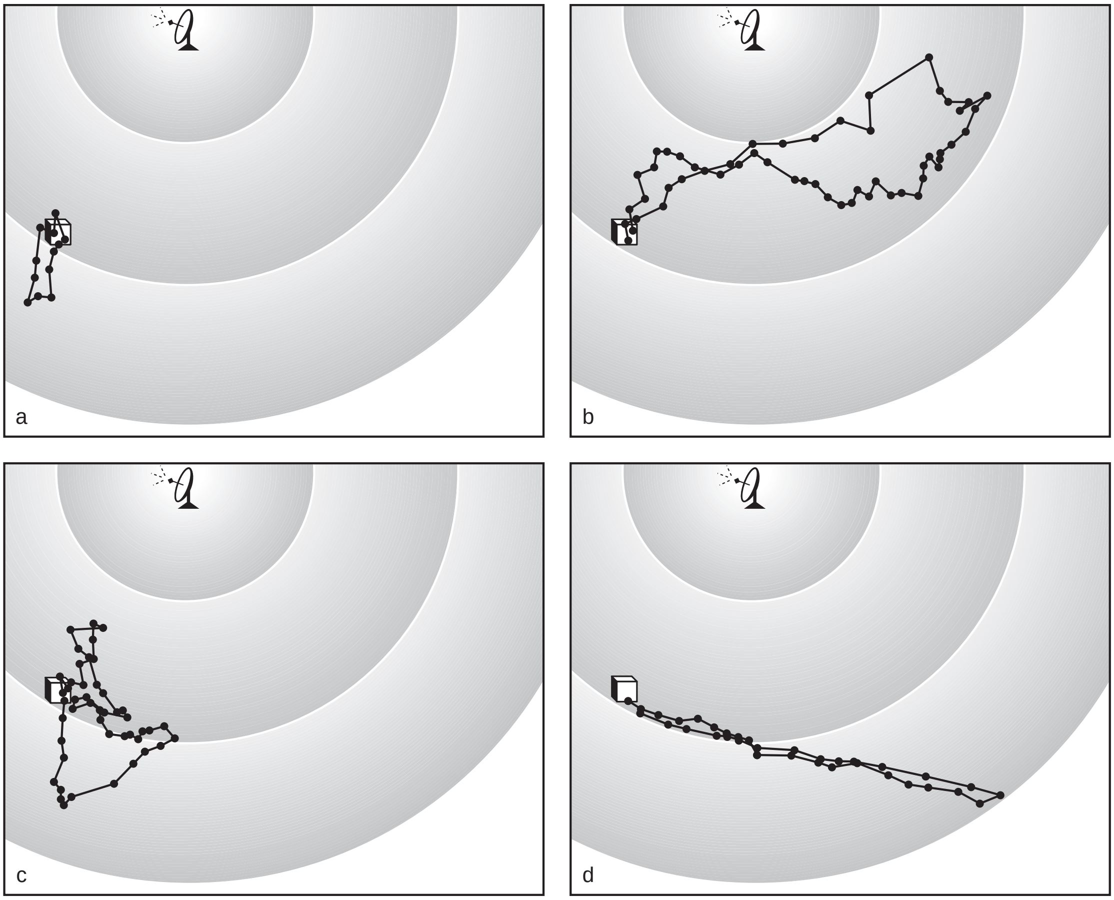

Figure 2 Representative radar tracks from four bees. Orienting bees (**a**–**c**) were of known age and flight experience. The foraging bee (**d**) was definitively identified as a forager upon her return to the hive with a pollen load. The position of the hive and the harmonic radar are indicated. Shaded area indicates extent of radar coverage. Distance between concentric rings is 116 m. **a**, Orientation flight number 1, age 4 days, duration 228 s. **b**, Orientation flight number 2, age 6 days, duration 1,011 s. **c,** Orientation flight number 14, age 6 days, duration 486 s. **d,** Foraging flight, unknown age forager. Unlike the tracks shown for orienting bees, this track is not complete, as the bee left the region of radar tracking, re-entering on its homeward journey. Duration, 1,215 s. Comparisons between orientation and foraging flights suggest that both ground speed and flight distance continue to increase as bees mature into foragers. Foraging bees had a significantly faster average ground speed than orienting bees (5.6 ± 1.0 m s^{-1} versus 3.6 ± 1.4 m s^{-1}) and also flew further and straighter, in agreement with previous reports that honeybees forage over distances of hundreds and even thousands of metres[10,15].

flight duration; as the bees gained experience they apparently travelled further by moving faster rather than by staying out longer. Partial correlation analysis indicated that the observed change in ground speed is sufficient to explain the significant correlation between flight number and area (Pearson partial ρ= 0.14 for flight number and area with speed as the partial variable; P = 0.48). Partial correlation analysis also confirmed that the observed relationship between flight number and ground speed was not caused by changes in wind speed (Pearson partial ρ = 0.53 for flight number and ground speed with wind speed as the partial variable; P = 0.004). Surprisingly, changes in orientation flights that were correlated with flight number were not also correlated with age (Table 1, Fig. 3), indicating that the rate at which bees acquired orientation experience was variable. The observed experience-related increase in average ground speed during flight is a finding that has not been considered before in studies of orientation behaviour.

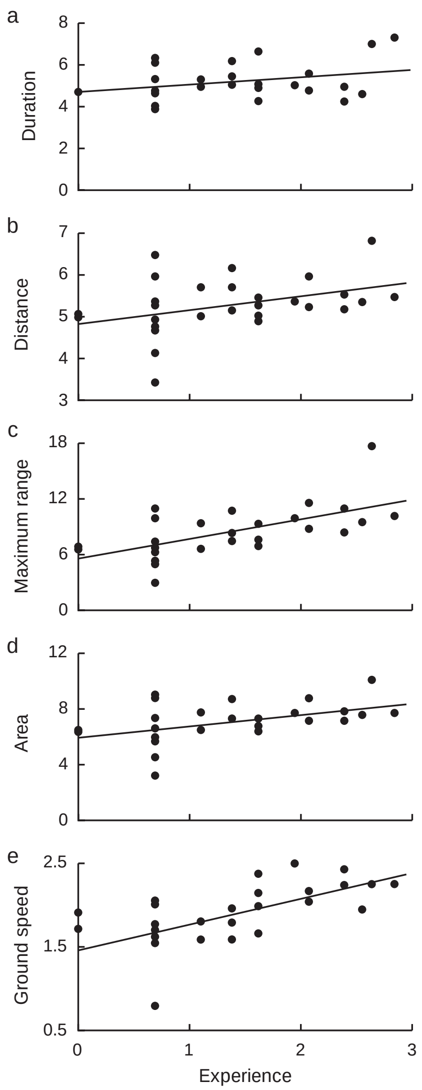

Figure 3 Plots illustrating the correlations between flight experience with the flight attributes. **a–e**, Experience (number of flights), duration (s), distance (m), and area (m^2) were natural log transformed. Ground speed (m s^{-1}) and maximum range (m) were square root transformed. Only the correlation shown in **a** (between duration and flight experience) is not significant (ρ = 0.334, P=0.07). Nonparametric correlation analyses (Spearman rank order test) on untransformed data matched the conclusions of the parametric correlation analyses.

Table 1 Attributes of honeybee orientation flights tracked with harmonic radar and correlation of flight attributes with age and experience

	Flight Attributes		Age		Experience*	
	Median	Range	ρ†	P	ρ‡	P
Duration (s)	135	48–1,485	*0.191*	0.32	*0.334*	0.08
Round trip distance# (m)	189	30–900	*–0.234*	0.22	*0.386*	0.04
Maximum range (m)	58	9–314	*0.032*	0.87	*0.619*	0.0003
Area covered (m^2)	1418	27–23,997	*–0.146*	0.45	*0.474*	0.009
Average ground speed (m s^{-1})	3.6	0.6–6.2	*0.211*	0.27	*0.599*	0.0006

* N = 29 flights, one per bee; explanations of measures in Methods.
† Number of orientation flights taken
‡ Pearson correlation coefficient from transformed data, see Fig. 3 for details
§ Even the long duration flights began with hovering, an indicator of orientation.
# See Methods for details

We examined the distribution of the flight tracks around the hive to gain insight into the orienting bee's view of the landscape. Previous studies have shown that bees in some landscapes are able to home after displacement after a single flight in a landscape[2,6]. Based on these findings, we expected that the tracked bees would explore all quadrants around the hive during a single flight. This rarely occurred. Only 6 out of 29 orientation flights visited all 4 quadrants. In 28 out of 29 tracks, at least 50% of the flight was confined to a single quadrant; thus bees did not typically investigate the entire area surrounding the hive during a single orientation flight. There was a bias towards occupancy of the two quadrants in front of the hive (frequency analysis of radar position fixes per quadrant in each flight: χ^2 = 109.5, degrees of freedom (d.f.) = 3, $P < 0.05$). It has previously been reported that honeybee flight direction is biased toward the position of the sun's azimuth and toward directions in which forage is available[3]. We studied bees from just one colony, with its entrance continuously facing due east, so we did not test for ecological correlates of this slight directional bias.

Our findings suggest that bees take multiple orientation flights before becoming foragers in order to visit different, and larger, portions of the landscape around the hive. These flights provide them with repeated opportunities to view the hive and its surroundings from different positions, suggesting that bees learn the local landscape in a progressive fashion. Bees navigate using a combination of cues, including the position of the sun and the location of salient landscape features[7–9], but it is not known how or whether information about these cues, obtained during sequential flights, is integrated.

It is an extraordinary feat for an animal the size of a honeybee to be able to find a small nest from distances as great as 10 km (ref. 10). Our finding of a progressive process of orientation behaviour begins to demystify this feat for honeybees and may have relevance for other species that face analogous navigational tasks. As these results are incorporated into future models of animal navigation, they will enhance the value of the bee in the fields of cognitive ethology and, eventually, cognitive neuroethology. ☐

Methods

Bees

Bees were typical of North American and United Kingdom populations of *Apis mellifera*, and were probably a mix of the European subspecies *mellifera* and *ligustica*.

Entrance observations

One-day-old bees were obtained by collecting adults that emerged from incubated frames of capped brood. Numbered, coloured plastic tags were applied to the thorax. For hive entrance observations conducted in Illinois, flight activities of marked bees were recorded from 12:00 to 21:00 until all observed bees had initiated pollen and/or nectar foraging (mean ± s.e., 14 ± 0.18 days). (Once a bee becomes a forager, she takes orientation flights only when displaced to a new environment or when the visual scene around a familiar location has changed[11].) Simulated rain at the hive exit prevented flight activities during the morning hours. Complete records were obtained for 125 bees. In the radar-tracking study at Rothamsted, flight activities of marked bees were recorded from 08:00 to 20:00. Over the five-week course of the study, 625 marked one-day-old bees were introduced into a colony of approximately 10,000 bees. Flights were again prevented by simulated rain when no observer was present. Flight activities were recorded for all bees until any bee in the cohort was identified as a pollen forager. This procedure ensured that tracked bees were taking orientation flights and were not foragers. Bees for radar tracking were selected on the basis of their flight experience and age. They were captured as they departed from the hive. A queen-marking cage was used to restrain the bee as the transponder was attached to the number tag using double-sided foam tape. The bee was then released from the cage at the height of the hive entrance. When the bee returned to the hive-front, the transponder was removed before she was permitted to re-enter the hive.

Radar tracking

The radar study site was a flat region of farmland (700 m × 900 m) at the Institute of Arable Crops Research, Rothamsted (Harpenden, Hertfordshire, UK). It was planted with cereal crops and was surrounded by flowering crops, hedges, wooded areas and buildings[5]. The radar and transponders have been previously described[4,5]. The transponder worn by the bees consisted of a 16 mm vertical dipole aerial and a Schottky diode. Two sizes were used, 0.8 mg and 12.0 mg. The larger version weighs less than an average pollen or nectar load[1]. Glass or plastic tubing was attached to the bottom of the transponder so that it could be inserted into a small hole drilled into the number tag. This created a base for the transponder, facilitating its attachment to the bee. Tracks were obtained during June and July 1997, on days when the sun or blue sky was visible. Five anemometry stations were set up around the study site, and these recorded wind speed and direction once every 10 s. To obtain a single measure of wind magnitude and direction for each bee flight, the mean wind vector at the midpoint between the beginning and end of the flight path was obtained by interpolation of the vectors recorded at the anemometry stations, averaged over the flight period.

Data analysis

53 tracks were obtained from orienting bees, and 29 of them met the following criteria for analysis: all signals fell within the 700 m radar range and the tracked path started and ended at the hive front. An orientation flight was defined as a flight that began with hovering in front of the hive entrance (23 out of 29); of the remaining 6 tracked bees, most were seen again on subsequent flights preceded by hovering. At the end of her tracked flight, one bee lost her identification tag and could not be studied further. Hovering is a widely accepted indicator of orientation[3]. Flight speed is equivalent to the mean ground speed for the flight, and was calculated by averaging the point-to-point speed of the moving bee based on 3-s radar sampling intervals. Track segments $\geqslant 9$ s in duration were used for this calculation to match flight-speed sampling intervals to wind-speed information. In cases in which a radar track was divided into segments by a missing signal, the flight-speed measure ignores the time the bee spent in these gaps unless the plotted data indicated that the flight path continued on the same course and speed. Proportion of time spent in gaps was not correlated with age or experience (data not shown), and probably represents short landings on vegetation. The area defined by a tracked flight was calculated using the minimum convex polygon method of home range analysis[12]. The x and y coordinates of each signal were imported into the Antelope spatial analysis software package (FTP://www-biolog.ucsd.edu.research/vehrenbury/programs.html). SAS Institute software was used for all other analyses[13]. All correlation analyses used the Pearson product moment coefficient[14]. The data were transformed to satisfy the requirements of this parametric test. Non-parametric tests (Spearman correlation) conducted on the untransformed data yielded identical results (data not shown). Quadrants around the hive were defined by the intersection of two perpendicular lines centred at the hive.

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Myoglobin-like aerotaxis transducers in Archaea and Bacteria

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Haem-containing proteins such as haemoglobin and myoglobin play an essential role in oxygen transport and storage. Comparison of the amino-acid sequences of globins from Bacteria and Eukarya suggests that they share an early common ancestor, even though the proteins perform different functions in these two kingdoms[1–6]. Until now, no members of the globin family have been found in the third kingdom, Archaea. Recent studies of biological signalling in the Bacteria and Eukarya have revealed a new class of haem-containing proteins that serve as sensors[7]. Until now, no haem-based sensor has been described in the Archaea. Here we report the first myoglobin-like, haem-containing protein in the Archaea, and the first haem-based aerotactic transducer in the Bacteria (termed HemAT-*Hs* for the archaeon *Halobacterium salinarum*, and HemAT-*Bs* for *Bacillus subtilis*). These proteins exhibit spectral properties similar to those of myoglobin and trigger aerotactic responses.

When the *hemAT-Hs* gene was originally cloned, its product was predicted to be a soluble signal transducer[8] (HemAT-*Hs* was originally named HtB)[8]. HemAT-*Bs* was identified in the *B. subtilis* genome-sequencing project as the product of an open-reading frame encoding a protein with marked similarities to methyl-accepting chemotaxis proteins (MCPs) (HemAT-*Bs* was previously named YhfV)[9]. The predicted translation products of the *hemAT-Hs* and *hemAT-Bs* genes, comprising 489 and 432 residues, respectively, exhibit two striking features: first, their amino termini (residues 1–184 in HemAT-*Hs* and 1–175 in HemAT-*Bs*) display limited homology to myoglobin (Fig. 1a); and, second, residues 222–489 of HemAT-*Hs* and 198–432 of HemAT-*Bs* are 30% identical to the cytoplasmic signalling domain of Tsr (ref. 10), a MCP from *Escherichia coli* (Fig. 1b).

The residues absolutely conserved among all globins are the proximal histidine in the F helix (F8) and phenylalanine in the CD region (CD1)[11,12]. Highly conserved residues include the distal His in the E helix (E7), Phe in the CD4 region and proline at the beginning of the C helix (C2). Three of these residues (Pro in C2, Phe in CD1, His in F8) are conserved in both HemAT-*Hs* and HemAT-*Bs* (asterisks in Fig. 1a). These features suggested to us that HemATs may be haem-containing proteins that generate signals in response to binding of oxygen.

To identify the prosthetic groups in HemAT-*Hs* and HemAT-*Bs*,