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Evidence for counting in insects

Marie Dacke · Mandyam V. Srinivasan

Abstract Here we investigate the counting ability in honeybees by training them to receive a food reward after they have passed a specific number of landmarks. The distance to the food reward is varied frequently and randomly, whilst keeping the number of intervening landmarks constant. Thus, the bees cannot identify the food reward in terms of its distance from the hive. We find that bees can count up to four objects, when they are encountered sequentially during flight. Furthermore, bees trained in this way are able count novel objects, which they have never previously encountered, thus demonstrating that they are capable of object-independent counting. A further experiment reveals that the counting ability that the bees display in our experiments is primarily sequential in nature. It appears that bees can navigate to food sources by maintaining a running count of prominent landmarks that are passed en route, provided this number does not exceed four.

Keywords Counting · Honeybee · Cognition

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Introduction

One of the popular measures of the cognitive capacity of animals is the ability to count objects (Hauser 2000). Studies of numerical cognition have over the last decades extended from a focus on humans and non-human primates to include other mammals (Gallistel 1990; West and Young 2002; Killian et al. 2003), as well as birds (Pepperberg 2006; Roberts et al. 2002; Rugani et al. 2007), fish (Agrillo et al. 2007) and salamanders (Uller et al. 2003). Little is known about the numerical abilities of invertebrates. To date, only two studies suggest a counting ability in insects (Chittka and Geiger 1995; Karban et al. 2000), but a recent study questions the generality of these claims (Franks et al. 2006).

The process of counting, or estimation of numerosity, can be carried out in a number of different ways: 'Subitizing' refers to the ability to rapidly recognize the cardinality of a small set of objects at a single glance, without registering them sequentially. Subitizing appears to be possible with small numbers of objects, typically four or fewer (Wiese 2003, Gallistel and Gelman 2000), although the exact nature of the underlying process is unclear and controversial (Gallistel 1990). Sequential counting is the ability to count objects or events that are encountered one after another. This requires the ability to maintain a running tally of the number of events, incrementing the tally by one each time an event occurs (Dehaene 1999). A related process is that of summation, or integration, which involves accumulating, over time or space, some attribute of the objects that are to be counted. For example, if the task is to count the number of blue discs that are visible simultaneously on a display screen, this would mean estimating the total area of blue that is present in the screen; or if the discs are presented sequentially, it would involve accumulating

the total area of blue that is observed through the succession of presentations (Davis and Pérusse 1988; Franks et al. 2006). According to Gallistel and Gelman (2000), summation (or accumulation) could, under certain circumstances, constitute a form of counting, and perform a function that is equivalent to the counting of integers. However, if an animal that uses this technique is trained to count to a certain number by using objects of a fixed size, it could potentially overcount when it is subsequently tested with smaller objects, and undercount when tested with larger objects.

True counting involves assigning successive numbers to objects, irrespective of item size (Davis and Pérusse 1988). Apart from humans, this ability has been demonstrated, or been claimed to exist, in a relatively small number of animal species that includes chimpanzees, dolphins, raccoons, chicks, rats and parrots (Davis and Pérusse 1988; Dehaene 1999; Pepperberg 2006; Rugani et al. 2007). The existence of a counting ability has also been suggested in bees (Chittka and Geiger 1995) and cicadas (Karban et al. 2000).

Chittka and Geiger (1995) explored whether honeybees could learn to fly along a row of yellow tents in a field to find a food reward placed half way between the third and the fourth tent on the route. When the bees were subsequently tested for their ability to locate the feeder, a proportion of the bees did indeed land on a control feeder after they had flown over the three tents, whether or not the landmarks were closer together or further apart than during training. Thus, while these findings provide evidence in favour of a counting ability in honeybees, they do not prove it. This is because the study, using the same size and shape of the landmarks throughout, did not demonstrate a capacity of the bee to learn and use numbers in an object-independent way. In addition, the overwhelming majority of the bees landed on a control feeder that was located approximately at the same distance from the nest as during training. This is probably because the training paradigm, which used a constant separation between landmarks—and hence a constant distance to the food reward—was such that it encouraged the bees to attend primarily to the distance they had flown, rather than to the number of landmarks they had passed.

Here we re-examine the ability of bees to count by using an experimental design that encourages them to find a food source by counting landmarks, rather than by relying on their odometer.

Methods

Experimental set-up

Bees (*Apis mellifera* L.) were trained to forage from a tunnel placed outdoors under a homogenous veranda roof. The

tunnel was 4 m long, 20 cm wide and 20 cm high, and was placed with its entrance 7 m from the hive. The walls and floor of the tunnel were lined with light grey paper, and the tunnel was covered with clear Perspex sheets. The tunnel carried a series of prominent landmarks, described below. For each experiment, up to 30 individually marked bees were trained to enter the tunnel and receive a food reward at one of the landmarks. The food was provided by five inconspicuous small cylindrical containers, each 1 cm dia and 0.5 cm tall, placed in the base of the rewarded landmark. The reward-bearing landmark was thus identical in appearance to the other landmarks, which carried no reward.

Procedure

In the first series of experiments, each landmark consisted if a yellow strip of paper, 3.5 cm wide and 60 cm long, decorating the walls and floor of the tunnel as shown in Fig. 1a. Depending upon the particular experiment, the reward was placed at the first, second, third, fourth or fifth landmark. A separate group of bees was trained for each experiment. The landmarks were always spaced at regular intervals. During training, the separation between the landmarks— and consequently the position of the rewarded landmark in the tunnel—was varied every 5 min. This was done to eliminate any tendency of the bees to otherwise learn to identify the correct landmark in terms of its distance from the tunnel entrance, rather than in terms of its numerical position in the landmark sequence. During training, the position of the landmarks varied randomly over a set of eight different separations, carefully chosen to ensure that the average position of the rewarded landmark did not coincide with the expected position of search in the subsequent test situation (see below). Each of the eight separations was an integer multiple of the smallest separation. This ensured that the position of the rewarded landmark was uniformly distributed over a large range of distances during the training. The position of the rewarded landmark from the tunnel entrance varied between 120 and 320 cm, to ensure that the bees were trained within the same section of the tunnel in all of the experiments. The bees were trained for a minimum of 3 and a maximum of 5 days. The training was considered to be complete when no improvement in the bees' performance could be recorded during the tests.

After training, the bees were tested individually in a tunnel that carried no reward. Prior to each test, the last position of the rewarded landmark was recorded to cheque for any possible influence of this position on the performance in the test.

In the first experimental series, the tests carried the same landmarks as in the training (Fig. 1a). The separation between landmarks was 70 cm for all tests, except for two tests in which the bees were initially trained to landmark 3,
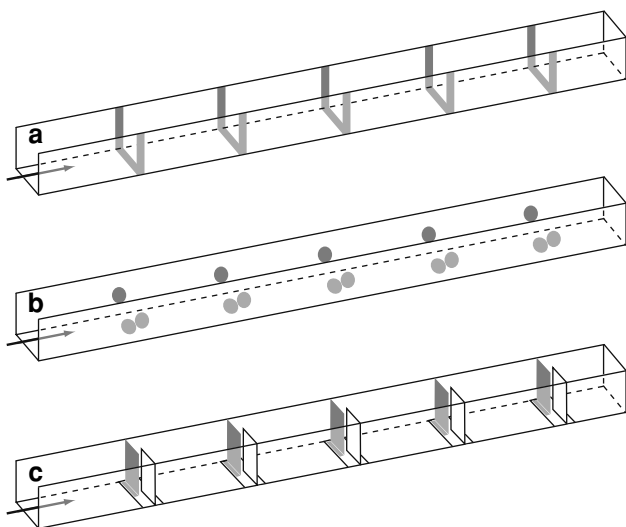
Fig. 1 Illustration of the experimental tunnels with landmarks consisting of *stripes* (**a**), *circles* (**b**) and *baffles* (**c**) spaced at regular intervals

and then tested with the landmarks spaced regularly at 40 cm (Fig. 3a) or irregularly (Fig. 3b).

In a second experiment, the bees were trained as before on stripe landmarks, with the reward offered at landmark 3. The trained bees were then tested on a novel set of landmarks, each consisting of three yellow discs, 7 cm in diameter, one placed on the floor and one on each wall (Fig. 1b). In the tests, the landmarks were separated by 70 cm.

In a third experiment, the landmarks were designed to ensure that the bees saw only one landmark at a time as they flew through the tunnel (Fig. 1c). Here each landmark consisted of a baffle, with slightly overlapping left and right partitions, separated by 4 cm. Each partition was 20 cm tall and 10.5 cm wide. When viewed from the front, the left partition was yellow and the right partition was grey; when viewed from behind, both partitions were grey. The overlap between the partitions ensured that a bee could not see beyond one landmark, at any location in the tunnel.

Analysis of counting performance

The test data were analysed by subdividing the tunnel into 40 units, each 10 cm long. In their search for food, the bees typically flew back and forth along the tunnel, making a number of U-turns as they searched for the reward. This searching behaviour was quantified by recording visually the positions in the tunnel in which the bee made the first six turns. By measuring the number of times the bee entered each unit during these six turns, we could estimate the spatial distribution of each search. To ascertain the maximum number of landmarks that could be counted, we used a 'Counting Performance Index' (CPI) defined as the ratio of the frequency of search in the three units surround-

ing the position of the correct landmark, to the mean of the equivalent frequencies of search at the other landmarks. The larger this ratio, the better is the discrimination of the correct landmark from the others. A CPI of 1 represents uniform searching at all landmarks, i.e. a breakdown of the counting process.

Results

We began by asking whether bees can learn to 'count' the number of landmarks that they encounter on the way to a food source. Individually marked bees were trained to receive a reward of sugar solution after they had flown past a specific number of regularly spaced yellow stripes during their flight through a narrow tunnel. Depending upon the experiment, this number was 1, 2, 3, 4 or 5. After training, the bees were individually tested by removing the food reward, and observing their searching behaviour in the tunnel to determine which landmark they had associated most strongly with the reward during the training.

We see from Fig. 2a that bees trained to landmark 1 show a strong preference to search in the vicinity of landmark 1. Bees trained to landmark 2 similarly prefer to search near landmark 2 (Fig. 2b); and so on (Fig. 2c–e). Even when trained on landmark 5, bees spend the greatest time searching in the vicinity of this landmark, although clearly discernible peaks now appear at all landmarks. One interpretation of this data is that the bees were learning to count the number of landmarks passed en route to the feeder, and using this learned number to guide their search in the tests.

A hallmark of the ability to truly 'count' landmarks is the capacity to count accurately, irrespective of how these landmarks are arranged spatially. To investigate this, we conducted two further tests in which bees were trained to receive a reward at landmark 3. The trained bees were then tested with a configuration in which the landmarks were separated by 40 cm rather than 70 cm (Fig. 3a), and with another configuration in which the landmarks were spaced irregularly (Fig. 3b). The bees always showed a clear preference for searching in the vicinity of the third landmark, regardless of the position of this particular landmark in the tunnel, and irrespective of the layout of the other landmarks. A similar ability has been demonstrated recently in young chicks (Rugani et al. 2007).

The experiments so far do not indicate whether the bees were truly 'counting' the landmarks, or whether they were simply performing a cumulative integration of the areas of the landmarks as they went by. For example, could the bees have been summing the area of yellow that they had flown past, stopping when the appropriate area had been accumulated? To test for this possibility, we again trained bees to
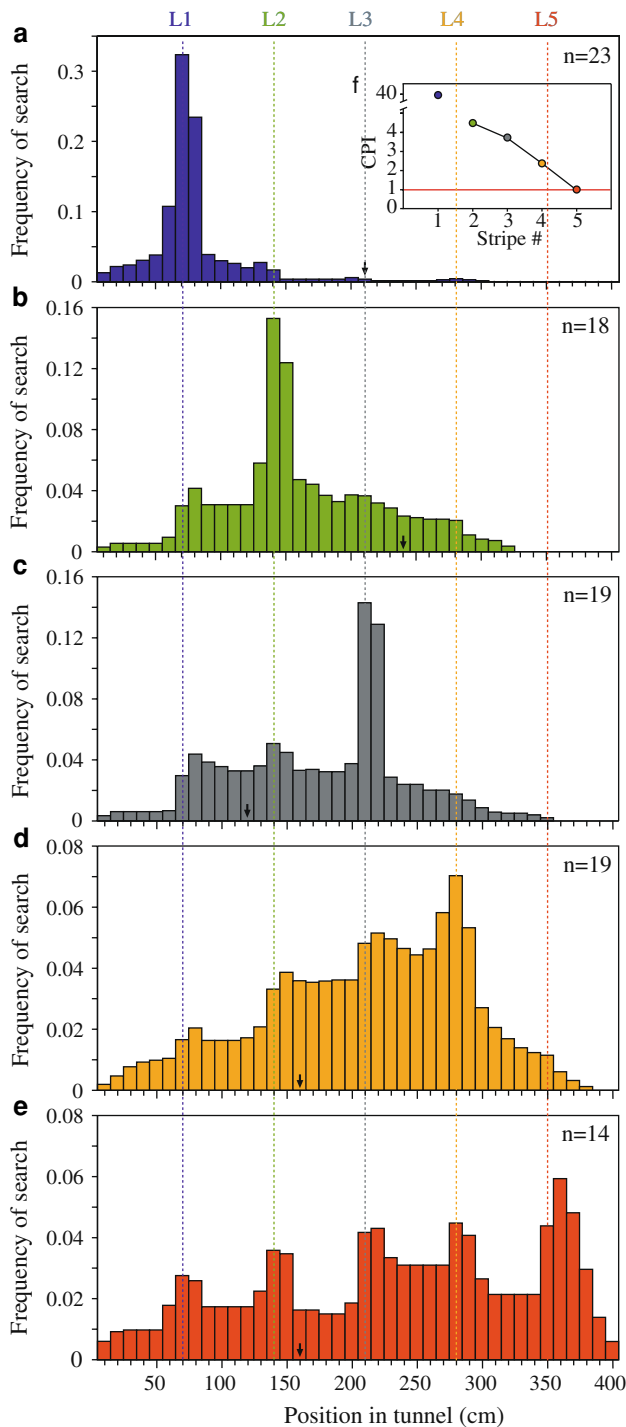
Fig. 2 Pinpointing the correct landmark in a series of landmarks. Search distributions of bees that are tested after being trained to receive a reward at landmark 1 (**a**), landmark 2 (**b**), landmark 3 (**c**), landmark 4 (**d**) and landmark 5 (**e**). Bees trained to landmark 1 show a strong preference to search in the vicinity of landmark 1 (**a**). Bees trained to landmark 2 similarly prefer to search near landmark 2 (**b**); and so on (**c–e**). The *arrows* mark the position of the rewarded landmark in the training, just prior to each test. **f** Variation of CPI with the number of the rewarded landmark. The CPI is greater than 1 when the bees are rewarded at landmark 1, 2, 3 or 4 and is equal to 1 at landmark 5. This indicates that bees can count up to four landmarks, but no more
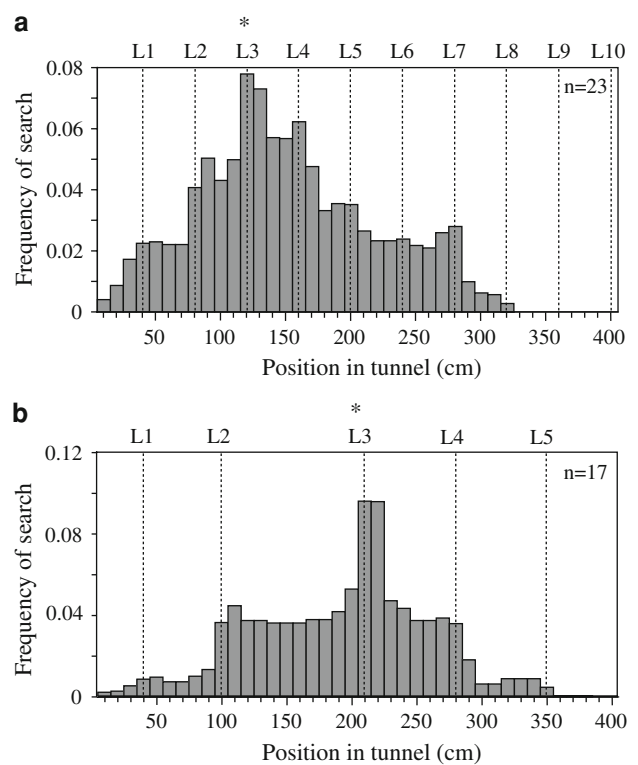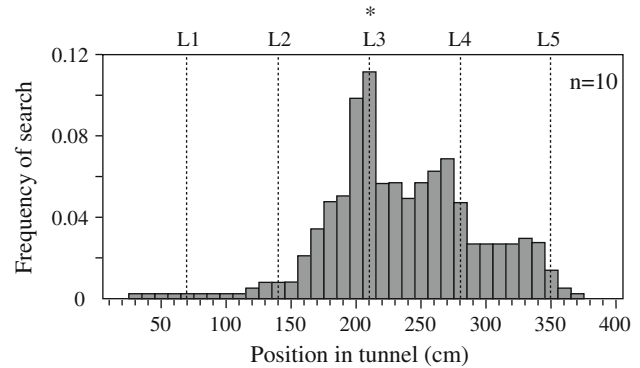

Fig. 3 Pinpointing the correct landmark irrespective of spatial arrangement along the tunnel. Searching distributions of bees trained to landmark 3 as in Fig. 1c, and then tested on their ability to count landmarks that are spaced regularly at 40 cm (**a**), or irregularly (**b**). In each case, the bees show a clear preference for searching in the vicinity of the third landmark (*). The CPI is 3.1 in **a** and 3.4 in **b**

find a reward on a given stripe, exactly as in the first series of experiments. In the test configuration each stripe was replaced by a yellow disc, with an area of only 55% of the area of the stripe in the training situation. The results are shown in Fig. 4. The bees showed a clear preference for searching at the correct landmark in four out of the five experiments. Thus, bees trained on landmark 1 searched preferentially at landmark 1 in the test even though the landmark was now of a different size and shape (Fig. 4a). The same was true when the bees were tested after training on landmark 2, 3 or 4 (Fig. 4b–d). Thus, the bees were not locating the goal by summing target areas: they were truly 'counting', in the sense that they were assigning a number to each individual landmark, regardless of its shape or size.

This conclusion of course relies on the assumption that the bees were able to distinguish between the two shapes. We confirmed the validity of this assumption by examining whether bees could be trained to distinguish between the disc and the stripe in a Y-maze (*for set-up and method*, see Srinivasan and Lehrer 1988). After training by reward on the disc, bees displayed a choice frequency of 72% for the disc. After training by reward on the stripe, bees displayed
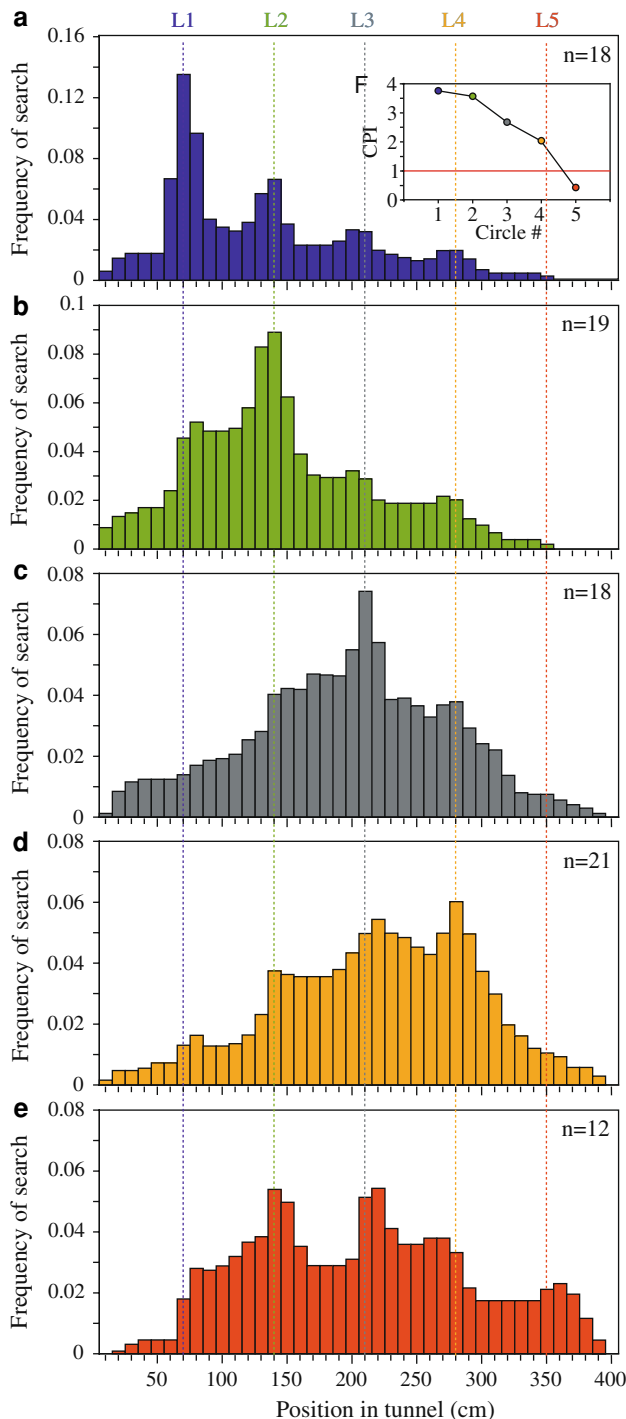
Fig. 4 Pinpointing landmarks of novel shape. Searching distribution of bees trained to count stripes, and then tested on their ability to count landmarks of a different size and shape (*circles*). Bees trained on landmark 1 (*stripe*) search preferentially at landmark 1 (*L1*) (*circle*) in the test (**a**). Bees also pinpoint the correct landmark when tested after training on landmark 2 (*L2*), 3 (*L3*) and 4 (*L4*) (**b**–**d**). After training on landmark 5 (*L5*), the bees no longer search preferentially at this landmark in the test (**e**), but rather show a strong preference to search in the vicinity of landmarks 2 and 3. **f** Variation of CPI with the number of the rewarded landmark. The CPI is greater than 1 when the bees are rewarded at landmark 1, 2, 3 or 4, and is less than 1 at landmark 5. This indicates that bees can count up to four landmarks, but no more

Fig. 5 Pinpointing the correct landmark in a series of sequentially presented landmarks. Searching distribution of bees trained in an experiment in which they are forced to count landmarks sequentially. Bees trained to feed from landmark 3 (*L3*), show a clear preference for searching in the vicinity of the third landmark (*) in subsequent tests. The CPI is 4.8

a choice frequency of 70% for the stripe. A binominal test (Zar 1999) revealed a significant difference from the random choice level of 50%, ($P < 0.001$, $n = 100$) for both results, indicating that the bees must have generalized their capacity from counting stripes to counting discs.

Can bees continue to count items if they are forced to encounter them in a truly sequential fashion? To examine this question, bees were trained in a tunnel in which each landmark now consisted of a baffle, with slightly overlapping left and right partitions. The overlap between the partitions ensured that a bee could not see beyond one landmark at any time. Thus, in order to identify the correct landmark, the bee would have to count the landmarks in a sequential fashion. In this experiment, bees were trained to find a food reward at the base of landmark 3. Again, the landmarks were regularly spaced, but their separation was varied randomly during training. The trained bees were then tested with the same set of landmarks, spaced regularly at 70 cm. The results of this test (Fig. 5) show a clear preference for landmark 3, revealing that bees are indeed capable of counting landmarks when they are presented sequentially.

Discussion

Our findings provide evidence that bees are capable of counting objects that are encountered on the way to a food source. In all probability, this counting is performed sequentially, and requires the ability to maintain a running tally of the number of events, incrementing the tally by one each time an event occurs. The number of landmarks that can be counted in this way appears to be four. When trained to landmark 4 the CPI is still greater than 2, indicating that the bees still spend more than twice the time searching in the vicinity of that landmark, compared with the other

landmarks (Figs. 1f, 2f). When rewarded at landmark 5 the CPI falls below 1, and the bees no longer show a clear preference to search in the vicinity of the correct landmark.

Could the bees' ability to count be mediated by a visually driven 'odometer', of the kind proposed by Esch and Burns (1996) and Srinivasan et al. (2000) for estimating distances to food sources? It has been shown that, when landmarks are close together and appear in rapid succession along the journey the odometer integrates the perceived optic flow (image motion) and measures the total angular motion of the image of the environment over the journey, irrespective of the number of landmarks passed (Si et al. 2003). However, when the landmarks are few and far between—as they are in some of the present experiments—one could consider whether the odometer plays a 'counting' role. If the odometer is to keep track of the number of sparse landmarks encountered during a journey, it must operate in a different mode in which the visual pathway produces a signal (e.g. a pulse) of a constant magnitude every time a landmark is passed, irrespective of the size or shape of the landmark. These pulses must then be accumulated at a subsequent stage of processing. While it is conceivable that the odometer could operate in this way under these conditions, it is unlikely because, in the initial phases of the training in our experiments, the bees tend to learn the (most recent) distance of the feeder from the entrance, rather than the number of landmarks passed. This suggests that the odometer operates in its normal, distance-measuring mode even when the landmarks are sparse. Indeed, our informal observations of the bees' performance during the course of the training suggest that bees have to be trained to disregard information on the distance actually traveled and to learn to attend to a different signal, namely, one that represents the number of landmarks passed. In other words, it is necessary to 'de-emphasize' the importance of the odometric signal before the counting ability can be revealed. This was the basis of our training paradigm, in which the distance to the goal was varied frequently and randomly, whilst keeping the number of intervening landmarks constant. The training protocol was further arranged such that, just prior to each test, the rewarded landmark was always at a fixed distance from the tunnel entrance, as indicated by the arrow in each panel of Fig. 1. In none of the tests is there a peak in the searching distribution at the position of the arrow. Thus, the bees could not have pinpointed the correct landmark in the tests by memorizing the most recent distance that they had flown to reach the reward.

Could the bees' ability to count be mediated by summing the amount of yellow they had flown past? To address this question, we conducted an experiment in which the bees were trained to count stripes, and then tested in a tunnel in which each stripe was replaced by a yellow disc, with an area of only 55% of the area of the stripe in the training situation. During the training the inter-stripe distance was varied frequently, as before. If the bees had simply been summing target areas to locate the correct landmark, we would expect bees trained to the second stripe to search at the fourth disc in the tests, and bees trained to the third stripe to search for the sixth disc in the tests—that is, they should have searched at the end of the tunnel. Clearly, this did not occur. Furthermore, this finding reveals that the bees were treating the novel landmarks (discs) in the tests in exactly the same way as the familiar landmarks (stripes) in the training, thus demonstrating a capacity to learn and use numbers in an abstract and object-independent way. The data from this second set of experiments suggest that bees are able to acquire a representation of a number, learnt from counting landmarks of one kind, and then to apply it to count a sequence of novel, unfamiliar objects. It is unlikely that this ability is mediated by any simple process of accumulation, because it functions even when the novel landmarks are of a different size and shape.

The third experiment in this study, where the overlap between the partitions ensured that a bee could not see beyond one landmark at any time, clearly shows that the counting performance in bees is not limited to situations in which the objects to be counted are viewed simultaneously. Subitizing has been studied extensively in human infants, and it would be of interest to enquire whether similar processes also underlie counting performance in bees.

The ability to count landmarks sequentially on the way to a food source, as we have shown here, should benefit an animal that visits a food source repeatedly. A running count of the number of prominent and recognizable landmarks encountered en route, e.g. trees, bushes or buildings, can be used to monitor progress and to indicate when the navigator is approaching the vicinity of its destination. This information could be supplemented by odometry, which serves to pinpoint the final destination. Indeed, landmarks can interact with odometric information to enhance the accuracy of navigation (Chittka et al. 1995; Srinivasan et al. 1997; Vladusich et al. 2005).

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