How to Design Experiments in Animal Behaviour*

5. How Do Ants Estimate the Distance Walked?

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In this article, I will describe experiments designed to understand how ants estimate the distance they have walked. They rival in their simplicity, the experiments described in my previous article, designed to understand how bees estimate the distance flown. Although ants can also use optic flow to estimate distance, in the absence of optic flow cues and of pheromone/chemical trails, as may sometimes be the case in the desert ants, *Cataglyphis*, ants estimate the distance walked, not by the energy expended but, believe it or not, by 'counting' (or integrating) the number of steps they have taken. This was proved by showing that ants on stilts (elongated legs) overshot the required distance to return home while ants on stumps (shortened legs) undershot the required distance.

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How Do Ants Assess How Far They Have Travelled?

Ant workers walk rather than fly, and this has made them perhaps even more attractive model systems than flying insects for studying navigation. Being so-called central place foragers, foraging ants have to regularly return to their nests, and being social, large numbers of workers find and bring back food to the colony [1]. Many species of ants lay pheromone trails as they walk and use these to guide them on their return path. It is well-known that naïve ants will rely almost entirely on pheromone trails laid by themselves or their nestmates, but experienced ants can augment this with visual landmark cues. But what if the ants are incapable of laying chemical trails, as is true for many species, and there

*DOI: https://doi.org/10.1007/s12045-019-0850-0

Keywords
Animal behaviour, experimental design, dead reckoning, path integration, idiothetic cues, allothetic cues, pedometer hypothesis.

are no sufficiently conspicuous visual landmarks? Would the ants in such a situation be able to successfully navigate their way back to the nest? The answer is yes, and many species do so. That foraging ants in such situations successfully return back to the nest from long distances is astonishing enough. But even more astonishing is the fact that, while they may follow complicated, circuitous search routes on the way out, they return by a short straight path to the nest, at the end of their foraging effort. To accomplish this feat, they must somehow keep track of every turn they make and the distance they travel before making every new turn. Such a method of returning to the starting point by the shortest, straight path from the endpoint is called 'dead reckoning', a technique and term long used by humans in marine navigation.

Ants are hypothesized to use cues not derived from the environment, but those derived from the movement of their own bodies, legs in this case. Such cues are known as 'idiothetic' cues, in contrast to 'allothetic' cues derived from outside oneself.

When animals use the method of dead reckoning, it is called 'path integration'. To successfully perform path integration, the ants must continuously keep track both of their angular displacement (turns) as well as their linear displacement (distance travelled). It is well-known that ants track their angular displacement using a celestial compass. On the other hand, how they measure distance was not clear until recently. As in the case of the honey bees, the energy hypothesis, suggesting that the ants estimate distance travelled by the energy consumed in the process, was a favourite candidate. But as in the case of the honey bees again, there has been growing evidence against the energy hypothesis. For e.g., copper or lead weights attached to the ant's bodies have no effect on their distance estimation. The hypothesis that ants might simply estimate distance by the time elapsed, assuming that they walk at a constant speed throughout the entire round-trip journey, has also not found favour in empirical research. What remains is an audacious idea, first proposed by the French psychologist H Pieron that the ants might estimate distance travelled by counting the steps they take in the process. Of course, they need not literally count every time they take a step, the value of a continuous variable might be incremented by a fixed quantity. In other words, ants are hypothesized to use cues not derived from the environment, but those derived from the movement of their own bodies, legs in this case. Such cues are known as 'idiothetic' cues, in contrast to 'allothetic' cues derived from outside oneself [2].

In an attempt to test this 'pedometer' hypothesis (also known as 'stride integrator hypothesis'), Matthias Wittlinger and Harald Wolf from the University of Ulm in Germany, and Rüdiger Wehner from the University of Zürich in Switzerland (*Figure* 1), decided to study the Saharan desert ant *Cataglyphis fortis*, which is an efficient navigator without pheromone trails and without visual landmarks in its desert environment. Their experiment to test this hypothesis is as audacious as the hypothesis itself. They reasoned that ants walking on stilts (with elongated legs) should have increased stride lengths compared to normal ants and should, therefore, underestimate distance travelled. Conversely, ants walking on stumps (with shortened legs) should have decreased stride lengths compared to normal ants and should, therefore, overestimate distance travelled.

The 'pedometer' hypothesis (also known as 'stride integrator hypothesis'), has been tested by Matthias Wittlinger and Harald Wolf from the University of Ulm in Germany, and Rüdiger Wehner from the University of Zürich in Switzerland (*Figure* 1),on the Saharan desert ant *Cataglyphis fortis*, which is an efficient navigator without pheromone trails and without visual landmarks in its desert environment.

Hence, they elongated the legs of the ants by attaching pig bristles to the tips of their legs with superglue and shortened their legs by clipping off their tips. The effective change in leg length was about 2 mm in either case (*Figures* 2 and 3). Remarkably, the operated ants not only survived but also resumed their foraging duties and successful navigation [3]. See for yourself how well the ant on stilts walks [http://bit.ly/2YVlAJp] or go the Supporting Material in [3].

The Experiments

Ants were trained to walk from their nest to a feeder kept 10 m away. The walking was performed inside a channel 7 cm wide with walls 7 cm high. The open-top allowed the ants a view of the sky, to facilitate their use of the celestial compass. Even though it was thought at that time that optic flow, which we encountered in the honey bee experiment in the previous article [4], plays a rather small role in these ants in this environment (the ants can estimate distances accurately even in featureless environments and even in total darkness), great care was taken to further minimise

Figure 1. Photos of the authors and the apparatus. Top: Matthias Wittlinger with the experimental apparatus (photo: Harald Wolf), middle: Harald Wolf (left) and Rüdiger Wehner (right) (photo: Sibylle Wehner) and bottom: Rüdiger Wehner (photo: Sibylle Wehner), at the study site in Mahrès in Tunisia.

optical flow cues. The floor of the channel was coated with fine grey sand to provide traction for walking, but the sand particle size was carefully chosen to be below the ants' optical resolution. The walls of the channel were painted with matt grey varnish to

Figure 2. Photo of *Cataglyphis fortis* ants used in the current experiment, right, with normal legs, middle, on stilts and left, on stumps (left) (Photo: Matthias Wittlinger).

provide a featureless environment. It must be noted however that the same ants can estimate distance travelled entirely by means of optic flow under the right conditions. Hence it was all the more important to deprive them of optic flow cues in the present experiment. After a day of training during which the ants walked up and down this channel, they were put to a test. Ants reaching the feeder were transferred to a different channel placed a little

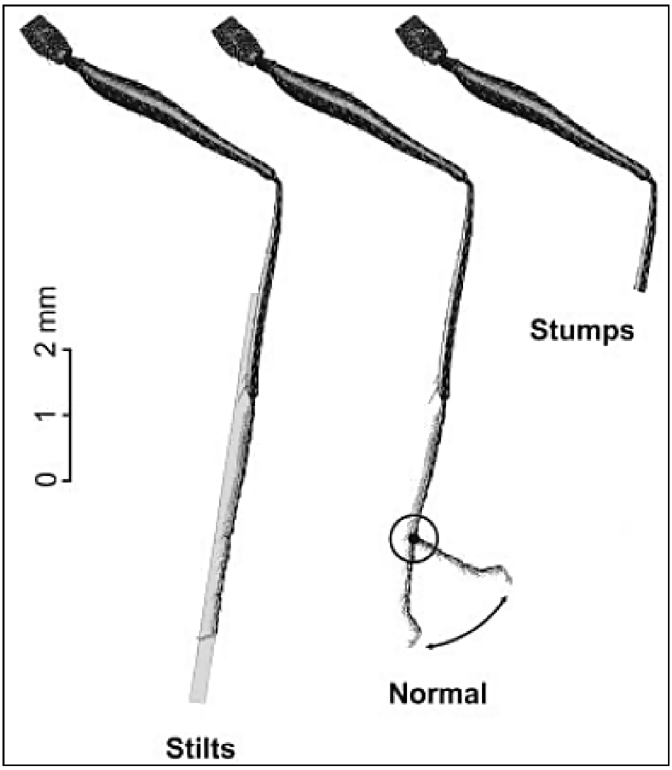

Figure 3. Images of the normal and manipulated ant legs. Left, elongated legs (stilts) due to the attached pig bristles, middle; normal unmodified legs, with approximate range of tarsus movement indicated; right, shortened legs (stumps). The right hind leg is shown from anterior, in all cases. Reproduced with permission from An Ant Odometer: Stepping on Stilts and Stumps by Matthias Wittlinger, *et al.*, *Science*, 312, pp.1965, 2006, American Association for the Advancement of Science.

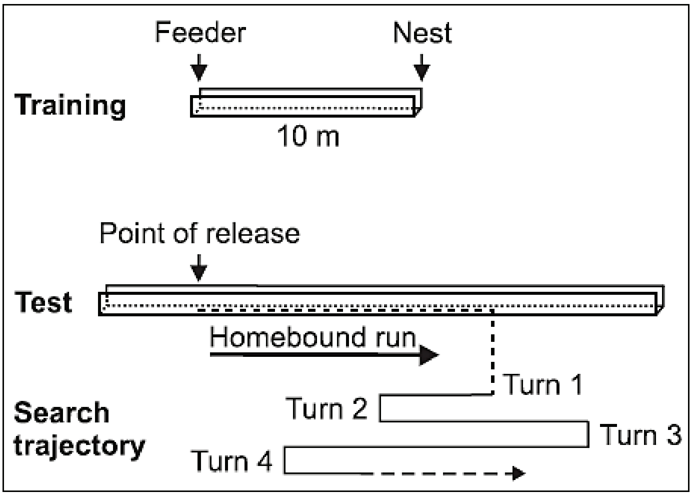

Figure 4. Schematic representation of the training and testing layout Reproduced with permission from An Ant Odometer: Stepping on Stilts and Stumps by Matthias Wittlinger, *et al*., *Science*, 312, pp.1965, 2006, American Association for the Advancement of Science.

away from the original channel. These ants were given a piece of biscuit to increase their motivation to return home, and they promptly began walking in the new channel in the homeward direction.

After walking a certain distance, presumably their estimate of where the nest ought to be, and not finding it, they abandoned their straight and steady homeward run and began to search for the missing nest (goal) (*Figure* 4). The point of the experiment was to see how far the ants will walk in the homeward direction before beginning to search for the missing nest. This will tell us what the ants had estimated as the distance they had walked from the nest to the feeder and hence the distance they needed to walk back to reach the nest. In such an experiment, normal (unoperated) ants walked up to 10.2 m before abandoning the straight path and beginning to search, as might be expected because they had walked a distance of 10 m to reach the nest in the first place.

In the first experiment with operated ants (which the authors call test 1), ants reaching the feeding station were collected and their legs were elongated or shortened as described above. After the operation, the ants were briefly rested in a separate chamber and offered biscuits. When they picked up a piece of biscuit, it was assumed that they were ready to go home and were transferred to the

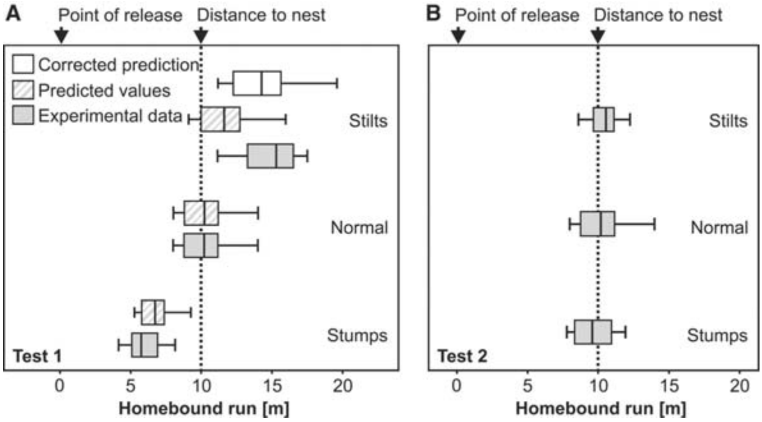

Figure 5. Comparison of homing distances of normal ants and of those whose leg lengths had been modified at the feeding site. (Panel A) Leg lengths were normal during the outbound journey but manipulated during the homebound run, resulting in different homing distances. (Panel B) Ants tested after re-emerging from the nest after manipulation so that the leg lengths were the same during outbound and homebound runs, for each kind of ant. Box and whisker plots show median, inter-quartile margins, and 5th and 95th percentiles. of the homing distances recorded for 25 ants per experiment. The hatched box plots in (A) illustrate the homing distance predicted from the high-speed video analyses of stride lengths in normal and manipulated animals. The open box represents the prediction further corrected for slow walking speed of ants on stilts. Reproduced with permission from An Ant Odometer: Stepping on Stilts and Stumps by Matthias Wittlinger, *et al.*, *Science*, 312, pp.1965, 2006, American Association for the Advancement of Science.

second channel. These ants now began their straight homeward run. As expected from the pedometer hypothesis, ants walking on stilts (elongated legs) overshot the distance and walked up to 15.3 m before beginning to search for the missing nest. On the other hand, ants walking on stumps (shortened legs) undershot and began to search after walking 5.75 m. The values of 10.2 m, 15.3 m and 5.75 m mentioned above, are just the median values (*Figure* 5). To see whether the differences between normal ants and those on stumps and stilts are statistically significant, the experimenters needed to measure the variation around these median values. This they did by dividing the running channel into 10 cm bins and noting how often ants that had left the straight homebound path and begun searching for the missing nest were seen in each bin.

From this data, they calculated a search density distribution for 25 ants of each type and plotted them as box-and-whisker plots indicating the medians, interquartile range (boxes) and the 5th and 95th percentile values (whiskers). They found that the lengths of the straight homeward runs of the normal ants, ants walking on stilts and those walking on stumps were all statistically significantly different from each other. These results clearly support the pedometer hypothesis. Thus, ants must estimate distance travelled by 'counting' the number of steps needed to cover the distance.

The Clinching Experiment

Even though ants with elongated and shortened legs behaved in opposite ways as expected, could the modified legs have influenced the behaviour of the ants in some other way?

Abundant caution never hurts. As I have emphasized before, we must always worry about potential confounding factors. Even though ants with elongated and shortened legs behaved in opposite ways as expected, could the modified legs have influenced the behaviour of the ants in some other way? To rule out this possibility, the authors performed a second, clinching experiment (which they call test 2). They reasoned that in test 1 the ants on stilts and stumps overshot and undershot respectively because they had estimated the distance from the nest to the feeder with normal legs, but had to travel back from the feeder to the nest with altered legs. Thus, if the ants also walk from the nest to the feeder with altered legs then they should not make the same error of over- or undershooting. So, they let the ants on stilts and stumps rest in the nest and then walk back from the nest to feeder in the first channel, and finally transferred them to the second channel. Now all the ants, normal as well as those on stilts and stumps walked the same distance before searching; ants on stilts now walked 10.55 m (instead of the previous 15.3 m), and those on stumps walked 10.25 m (instead of their previous 5.75 m). These new distances travelled by ants on stilts and stumps are not significantly different from each other nor are they significantly different from the 10.2 m of the unmanipulated ants. This experiment strongly reinforces support for the pedometer hypothesis because it eliminated the possible confounding factors associated with the operation of the ants.

Can One Be Even More Cautious?

What might constitute an even better proof of the pedometer hypothesis? One must always think along these lines, rather than worry that another experiment might just rock the boat and spoil the party. The goal should be to try one's best to disprove a hypothesis, and only failing to do so should one accept the hypothesis and always only for the time being. In the present case, it would be even more convincing if we can count the number of steps made by the ants and show that in test 1, the number of steps taken during the return homeward run are the same, whether the ants are normal, on stilts or on stumps, hence providing the basis for the error in distance estimation. In test 2 however, the number of steps while walking from the nest to the feeder should be different for each kind of ant – normal, those on stilts, and those on stumps; and on the return journey, each kind of ant should take the same number of steps that it made in the outbound journey – hence the absence of overshooting and undershooting. Despite recording high-speed videos of the ants, we are told that it was technically impossible to actually count the number of steps the ants took during these experiments (test 1 and test 2) across the whole distance of 10 m.

The next best thing they could do was to check if the exact extent of undershoot and overshoot was what one might predict due to the exact increase or decrease in the length of the legs. To predict the extent of overshoot and undershoot, we need to know the changes in the stride lengths due to the operation. Hence, they measured the stride lengths for normal as well as manipulated ants, in a separate set of experiments. This creates its own problem because stride length is expected to be influenced by the body size in addition to the leg length. But this problem is solvable – the researchers corrected for differences in body size by normalizing the stride lengths obtained for variations in body size. This means that that they divided the stride lengths of each ant by a measure of the body size of that ant in all cases. After such normalization, they found that normal ants had stride lengths of 13.0 ± 1.98 mm, ants on stilts had stride lengths of 14.8 ± 2.5 mm and ants on stumps had stride lengths of 8.6 ± 1.73 mm. These three values are significantly different from each other. Now, one could make predictions about how far the ants should walk on the way to the nest. From the altered stride lengths, they made predictions about how far the ants of stilts and stumps should have travelled homeward in test 1, before beginning to search for their missing nests, if they were guided by their pedometers i.e., they took the same number of steps as they had taken on the outbound journey with normal lengths .

These predictions further upheld the pedometer hypothesis because the predicted homeward travel distances of the modified ants were in general agreement with the observed values. In other words, the ants behaved as if they were counting the number of steps in the outbound journey and taking a similar number of steps in their homebound journey. But the authors were not satisfied with a 'general agreement'. Instead, they focussed on tiny differences between the observed and predicted values, comparing them statistically. They found that while the differences between the observed and predicted values were not significant in the case of ants on stumps, ants on stilts overshot significantly more than predicted. Now, why should this be so? If a satisfactory explanation cannot be found for this subtle quantitative discrepancy between the observed and predicted overshoots, the entire interpretation may be suspect, or so the authors fearlessly reasoned.

To fathom this discrepancy, they first reasoned that the operation performed on the ants could not be held responsible because, rather than be somehow incapacitated, the ants on stilts walked longer than predicted. Besides, they observed that the operated ants (on stilts and stumps), were making successful foraging trips several times a day for several days – not a sign of incapacitation. The other possibility is that altered walking speeds may account for the discrepancy. Stride length is expected to be dependent on both leg length as well as walking speed. To test this possibility that altered walking speeds might be responsible for the discrepency, they determined the walking speeds of normal and both kinds of operated ants. They did this both by recording the time required to walk a distance of 3 m, using a stopwatch as well as using a high-speed video camera. Normal ants walked at a speed of 0.31 m/s. As expected from their shorter legs and smaller strides, ants on stumps walked at a slower speed of 0.14 m/s. However, ants on stilts, rather than walking at higher speeds than normal ants, on account of their longer legs and greater strides, walked at a speed of 0.29 m/s, slower than normal ants. This was probably on account of the extra weight of the pig bristles attached to their legs. Now, the prediction about how far the ants on stilts should walk in a straight homebound direction before beginning their search was corrected for this altered walking speed and the corresponding changes in stride length. They did this using a standard graph relating the stride length to walking speed obtained for each kind of ant [5]. Thus, they finally obtained a value not significantly different from the observed value. Only at this point, did the authors put their weight behind the pedometer hypothesis and claim that *Cataglyphis* ants estimate distance travelled by 'counting' the steps. As the authors point out, these simple experiments open up exciting opportunities to understand how the ant brain, tiny as it is, counts steps and directs the legs to take the desired number of steps to go back home. And let us not forget, the brain has to perform path integration in between, to arrive at the number of steps required to trace a straight path back to the nest after having made a meandering outward journey. It is perhaps worth reiterating that the ants need not literally count their steps, the value of a continuous variable might be incremented by a fixed quantity every time they take a step, based for instance on the movements of the leg muscles.

Today we know that these desert ants can also use optic flow to estimate the distance. In another remarkable experiment, ants which did not walk but were carried by their nestmates were also shown to have correct information about the distance between their current position and their nest. The clinching experiment here was to show that when the carried ants were blindfolded, and thus could not gather optic flow information, they were quite lost. Ants can, therefore, estimate distance both with the help of their stride integrator and optic flow; they can use either, but anyone will do [6]. There are many more intriguing suggestions from similar simple behavioural experiments. E.g., distance information gathered from optic flow during the outbound journey (by carried ants that are not blindfolded) cannot be used to work out the number of steps to walk back (by walking ants that are blindfolded). And yet distance estimated by both methods can be integrated to arrive at the best possible estimate of the distance

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to walk back. I will not go into these details here, but readers will surely enjoy reading about them [6, 7]. I also recommend a delightful account of 50 years of research by Rüdiger Wehner's group on *Cataglyphis* ants in the Tunisian deserts near Mahrès [8].

Reflections

As the reader would be familiar by now, the broad message of this series is that cutting-edge science can be done with little or no access to large sums of money and sophisticated laboratory facilities. The aim of spreading this message is to make the production of scientific knowledge and not merely its consumption, as democratic and widespread an activity as possible. This will not just be good for large numbers of people in less endowed circumstances, but the participation of a large and diverse set of actors with many different perspectives would be good for science as a whole. As might be expected, I am choosing examples of experiments in animal behaviour that are especially appropriate to illustrate this message. Hence the lessons to be drawn from each experiment are much the same. Nevertheless, the experiments with ants on stilts and stumps does have something unique to ponder.

Try telling an intelligent, educated person with no knowledge or interest in insects that you think that ants might measure distance travelled by counting steps, and you will likely get a look suggesting that you must be mad to think so.

First, I would like to draw attention to the audacity, both of the hypothesis being tested and the experiment used to test it. Try telling an intelligent, educated person with no knowledge or interest in insects that you think that ants might measure distance travelled by counting steps, and you will likely get a look suggesting that you must be mad to think so. Try telling a scientist, even someone who works on insects that you plan to cut the legs of an insect or add pig bristles to its legs and study its behaviour, and you will most likely get a similar look. And yet, as JBS Haldane put it brilliantly, "The Universe is not only queerer than we suppose, but queerer than we can suppose." And that is why the Medawars included "courage in framing expectations" in their description of ethology, as you will recall from the first article in this series [8].

Second, I would like to draw attention to the simplicity of the experiments and the fact that very little was needed by way of instruments, money, or other facilities – pig bristles, super glue, some simple channels and some sand. It is true, however, that they used a high-speed video camera for filming the ants. It is not so unreasonable to imagine that you could borrow or rent a high-speed video camera for a small fraction of the cost of buying one. Moreover, notice that they also measured walking speed by recording the time taken to cover a distance of 3 m using a stopwatch. I do not know whether measurement of walking speed with a stopwatch would have been sufficient in this case, but I wish to make a general argument about the precision of measurements. How precisely should we measure something in any experiment? My answer would be, as precisely as is necessary for answering the question at hand. Unfortunately, many people insist that it should be as precise as possible. I find this unconvincing, counterproductive and dangerous.

I find utterly unconvincing, the secondary argument that greater precision than needed today may be useful for others tomorrow. If the cost of doing the experiment minus the unnecessarily great precision is a small fraction of the extra effort to achieve more precision than necessary today, it makes more sense to repeat the experiment with greater precision whenever required. The real danger of demanding greater precision than necessary for answering the question at hand just because greater precision is possible at extra cost is that it privileges better-funded scientists and deprives poorly-funded scientists and students with bright ideas but without a research grant. If we are to democratize the production of scientific knowledge, then we, especially journal editors and reviewers, must adopt the principle that precision should be based on necessity, not capability.

If the cost of doing the experiment minus the unnecessarily great precision is a small fraction of the extra effort to achieve more precision than necessary today, it makes more sense to repeat the experiment with greater precision whenever required.

Finally, we must reflect on the fact that the authors of this study were not easily satisfied. Recall “caution in coming to conclusions” in Medawars’ description of ethology, mentioned in the first article in this series [8]. They did not stop after obtaining the remarkable result that as expected from the pedometer hypothesis, ants on stilts overshot their target during the homeward run and that ants on stumps undershot. Even though it was not possible to count the number of steps, they attempted to examine if the extent of overshoot and undershoot were consistent with the extent of change in leg length. Even here, they were unsatisfied with a general agreement and specifically focussed on a tiny discrepancy between the observed and predicted values, even though the predicted values were in the correct direction. This permitted them to understand the reason for the discrepancy and to show that the discrepancy increased confidence in the pedometer hypothesis rather than cast doubt on it. In order to emulate the authors in this regard, we must guard against falling in love with the hypothesis we are testing, even if it is our own. We must be prepared to let ugly facts demolish beautiful theories. An important reason that often prevents people from being more detached from their hypotheses is that we consider a positive result supporting a hypothesis as a success worthy of a reward and a negative result as a failure, not even worthy of publication, let alone a reward. Here again, publishing policies must change. After all, a negative result can lead you to the correct hypothesis, and even if not, it spares future researchers repeating those 'unsuccessful' experiments. There is a great deal we can do to elevate the enterprise of science through simple changes in our mindset. And that is much more likely to happen if we make the practice of science more inclusive and open it up to as wide and diverse a circle of practitioners as possible.

Acknowledgement

I thank Rüdiger Wehner, Harald Wolf and T N C Vidya for many helpful comments on this manuscript.

Suggested Reading

[1] B Hölldobler and E O Wilson, *The Ants*, Belknap Press of Harvard University Press, USA, 1990.

[2] R Wehner, Arthropods in *Animal Homing*, Ed. F Papi, Chapman and Hall, London, 1992.

[3] M Wittlinger, R Wehner and H Wolf, An Ant Odometer: Stepping on Stilts and Stumps, *Science*, Vol.312, pp.1965–1967, 2006.
[4] R Gadagkar, How to Design Experiments in Animal Behaviour: 4. How Do Bees Estimate the Distance Flown? *Resonance – journal of science education* Vol.24, No.7, pp.739–751, 2019.
[5] M Wittlinger, R Wehner and H Wolf, The Desert Ant Odometer: A Stride Integrator that Accounts for Stride Length and Walking Speed, *J. Exp. Biol*, 210, Vol.198–207, 2007.
[6] S E Pfeffer and M Wittlinger, Optic Flow Odometry Operates Independently of Stride Integration in Carried Ants, *Science*, Vol.353, Issue 6304, pp.1155–1157, 2016.
[7] H Wolf, M Wittlinger and S E Pfeffer, Two Distance Memories in Desert Ants – Modes of Interaction, *PLoS ONE*, 13(10), 2018: e0204664. https://doi.org/10.1371/journal.pone.0204664.
[8] R Wehner, The *Cataglyphis Mahrésienne*: 50 years of *Cataglyphis* Research at Mahrés, *Journal of Comparative Physiology A*, Published online, 12th July, 2019, https://doi.org/10.1007/s00359-019-01333-5
[9] R Gadagkar, How to Design Experiments in Animal Behaviour: 1. How Wasps Find Their Nests, *Resonance – journal of science education*, Vol.23, No.8, pp.871–884, 2018.

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