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RESEARCH ARTICLE

Route-following ants respond to alterations of the view sequence

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ABSTRACT

Ants can navigate by comparing the currently perceived view with memorised views along a familiar foraging route. Models regarding route-following suggest that the views are stored and recalled independently of the sequence in which they occur. Hence, the ant only needs to evaluate the instantaneous familiarity of the current view to obtain a heading direction. This study investigates whether ant homing behaviour is influenced by alterations in the sequence of views experienced along a familiar route, using the frequency of stop-and-scan behaviour as an indicator of the ant's navigational uncertainty. Ants were trained to forage between their nest and a feeder which they exited through a short channel before proceeding along the homeward route. In tests, ants were collected before entering the nest and released again in the channel, which was placed either in its original location or halfway along the route. Ants exiting the familiar channel in the middle of the route would thus experience familiar views in a novel sequence. Results show that ants exiting the channel scan significantly more when they find themselves in the middle of the route, compared with when emerging at the expected location near the feeder. This behaviour suggests that previously encountered views influence the recognition of current views, even when these views are highly familiar, revealing a sequence component to route memory. How information about view sequences could be implemented in the insect brain, as well as potential alternative explanations to our results, are discussed.

KEY WORDS: *Cataglyphis*, Desert ants, Homing, Navigation, Insect memory, Insect learning

INTRODUCTION

Solitary foraging desert ants are expert navigators that seek and retrieve food morsels under extreme heat conditions. When foragers locate a bountiful food source they will shuttle rapidly back and forth along idiosyncratic visually guided routes (Kohler and Wehner, 2005; Mangan and Webb, 2012; Wystrach et al., 2011b), i.e. each ant will follow a fixed path to the feeder before returning home by a similarly fixed but different path. These paths are unique to each ant despite their journeys sharing the same start and end points, demonstrating a lack of pheromone guidance. Instead, visual information provided by the ants' surroundings is sufficient for route following and individuals can even recover their normal route direction following a displacement either by an experimenter (Kohler and Wehner, 2005; Mangan and Webb, 2012; Sommer

et al., 2008) or a wind gust (Wystrach and Schwarz, 2013), suggesting that visual memories (here termed 'views') can be accessed independently of the animal's recent experience.

This memory feature is embedded in recently developed computational models of visual route following. A key insight was that if retinotopy is maintained in the view encoding (Baddeley et al., 2012, 2011; Collett et al., 2017; Möller, 2012; Wystrach et al., 2013; Zeil et al., 2003), the correct direction to move at any point along a route can be recovered by finding the viewing direction that produces the best match, or least novelty, when compared with the complete set of visual memories stored in a previous traversal of the route. By simply moving along the direction with the least novelty the animal would repeatedly align with the direction it previously travelled and retrace its path. Ardin and colleagues (Ardin et al., 2016a) demonstrated that the circuitry of the insect mushroom body (MB) is ideally suited to measure the novelty of the current 'view' against those previously experienced. Each view is assumed to create a unique sparse activation pattern in the MB Kenyon cells and can be stored as 'familiar' by reducing the output weights of those cells. Views from novel locations or from familiar locations when facing the wrong direction will produce novel activation patterns and thus still activate the network's output, to trigger steering corrections. Computational models using such novelty-driven MB networks have produced realistic route-following behaviours in simulated environments (ants: Ardin et al., 2016a; bees: Müller et al., 2018) and on a mobile robot in a real ant habitat (Kodzhabashev and Mangan, 2015). Both experimental data (Freas et al., 2018; Narendra et al., 2013; Wehner and Räber, 1979; Wystrach et al., 2011a) and neurobiological data (Ardin et al., 2016b; Webb and Wystrach, 2016) in ants accommodate the mentioned models, although some other processes may also be at work during visual navigation (Cartwright and Collett, 1983; Mangan and Webb, 2009; Möller, 2012; Wystrach et al., 2012).

As noted, a key feature of these models is that 'memory of a route' does not include any information about the sequence in which views are encountered. The agent can tell whether a given view is familiar, but cannot tell whether it corresponds to the beginning, the end or any other location along the route. Consequently, the agent has no information about whether two views should be experienced in succession, nor any expectation that any particular view will occur after another. In theory, one could present all the views from a familiar route in a random order with no difference in the agents' behaviour.

This simple scene action control hypothesis is parsimonious; however, data from behavioural studies suggest that the picture may not be so simple (Wehner et al., 1996). Specifically, Wystrach et al. (2013) showed that ants displaced from their nest to an unfamiliar location do not immediately engage in a systematic search but instead backtrack along their just-travelled route bearing. This effect is only present in ants that have been captured at the nest indicative of ants possessing some memory of recent visual experiences. Furthermore, Collett (2014) and Wystrach et al. (2019, 2020) demonstrated that ants forced to retrace their homeward routes twice

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in succession (moved from the feeder back to the start of their inward route) display a period of confusion where they do not seem to recognise their familiar path. Graham and Mangan (2015) postulate a series of possible explanations for such behaviours, including the use of temporal information about their routes such as the sequence in which views were experienced. This study aims to address this possibility directly by assessing whether ants have knowledge about the sequence of views encountered along their familiar foraging route. It is already known that ants and bees can be trained to learn sequences of visual patterns (Schwarz and Cheng, 2011) and motor actions (Collett et al., 1993; Macquart et al., 2008) or to act according to the cue they have just previously encountered (Giurfa et al., 2001; Zhang et al., 2005). However, these feats require many trials of experimental conditioning, and although some can be parsimoniously explained (Cope et al., 2018), it remains unknown whether these insects spontaneously learn information about the sequence of views experienced along their familiar foraging routes (Riabinina et al., 2011).

In our study, ants were trained to home along a route through semi-natural terrain. The route started with a short section through a channel providing a unique visual experience as ants begin their homeward journey. During tests, the familiar channel was moved to a different location at the middle of the route so that ants exiting the channel were exposed to a familiar view that is not the one they usually experience immediately after the channel. To assess whether breaking the normal sequence of views in such a way had an impact on the ants, the number of scanning behaviours that ants displayed after exiting the channel in several tests and control conditions was quantified. Scanning behaviour typically provides a proxy for assessing the ants' current navigational uncertainty (Wystrach et al., 2014). The results showed a strong effect of changing the sequence, which we discuss in the light of insect behaviour and neurobiology.

MATERIALS AND METHODS
Species and study site
All experiments were carried out on the desert ant *Cataglyphis velox* Santschi 1929 at a field site in the periphery of Seville, Spain. *Cataglyphis velox* is a thermophilic ant species common in the area that exhibits behavioural traits typical for desert ants (Cerda, 2001). Instead of following pheromone trails, *C. velox* foragers venture out solitarily to search for food during the heat of the day and develop idiosyncratic routes relying on visual terrestrial and celestial navigational cues (Mangan and Webb, 2012; Schwarz et al., 2017).

Ethics
This work did not require any ethical approval. Ants were free to forage and return to the nest at will in our set-up. Only self-motivated foragers (holding their cookie crumbs) were tested. No ants were killed.

General experimental set-up
Two experiments were conducted over two field seasons in June 2016 and June 2017. In both experiments, ants were trained to run along a defined route to collect food items at a feeder location (Figs 1A,C and 2A,B). The routes were enclosed by slippery white plastic planks (approximately 5 cm high) submerged in a 5 cm ditch. This enabled the ants to perceive the surrounding natural scenery during route-following while preventing them from foraging elsewhere (Wystrach et al., 2012). The foraging routes were cleared of clutter and vegetation to ease the movements of the foragers on the ground. Small plastic bowls (15×15×15 cm) sunk into the ground so that their top edges aligned with the ground

surface served as feeders. The upper rim of the feeder walls was covered with transparent tape to prevent the ants from escaping. Foraging ants eventually jumped or fell into the feeder and picked up a biscuit crumb or meal worm piece and were then individually marked with acrylic or enamel modelling paint. In both experiments, foragers that had picked up a food item started their homing journeys by travelling through an open-topped, 50×5×10 cm white plastic channel that directly connected the feeder to the start of the homeward route. Thus, all ants across conditions experienced views within the white channel for the first 50 cm of their homeward route. Only well-trained individuals with high familiarity of the visual surroundings were tested (see detail for each experiment below). For tests, homing ants were captured just before entering their nest so that their current path integration homeward vector (accumulated during the outbound trip) had returned to zero (hence termed zero-vector ants, ZV). For proper homing motivation, only ants holding a food item were tested. Once captured, the ant was transferred in a darkened plastic vial and released at one of the test locations along the route, either within a 'test channel' or directly on the ground (see details below). The transition between the capture point at the nest and the release at either the feeder or mid-route location caused an additional alteration of the view sequence and hence could trigger scanning behaviour. In all conditions, ZV ants were likely to scan a few times upon release from the carrying tube. However, ZV ants were always released 50 cm before the actual test areas, giving the foragers enough time and space to recover their bearings and resume visual homing before data recording started at the designated test areas (Figs 1A,B and 2A). Furthermore, the test channel was always placed at the exact location where the ant homed during her previously displayed homing path. This procedure helped minimise changes in visual familiarity during tests. To avoid differences in the ground substrate across the different test locations, the immediate area after the channel exit (50×50 cm) was covered with a layer of sand (Figs 1 and 2; grey areas). In all tests, a GoPro Hero3+ camera was mounted on the top end of the test channel and the behaviour of the tested ant was recorded on the 50×50 cm area after the channel exit. Panoramic images shown in the figures were taken with a Sony Bloggie camera and unwarped with PhotoWarp2 software.

Experiment 1
In June 2016, ants were trained to follow a curved outbound route to a feeder located approximately 8 m away from the nest and then a zigzagged shaped inbound route back to the nest (Fig. 1A). The homeward paths of ants started inside the plastic channel, which had an approximate slope of 30 deg linking the entrance at the dug-in feeder to the channel exit at ground level (Fig. 1B). Hence the channel exit pointed up towards the sky and ants could see no terrestrial cues from inside. For each individually marked forager, training continued at least until they were able to negotiate a straight homebound route without colliding with the baffles or the surrounding planks enclosing the zigzag route (Fig. 1A). ZV ants were tested in one of the following conditions.

Test channel to feeder (start of the route)
Ants were transferred into a test channel that was identical to the training channel and placed alongside the training channel (Fig. 1A) at the feeder. Thus, in the 'Feeder test', the sequence of visual memories experienced was unaltered from training.

Test channel to mid-route
Ants were transferred into the same test channel as at the feeder but this time the channel was placed in the middle of the third leg of the
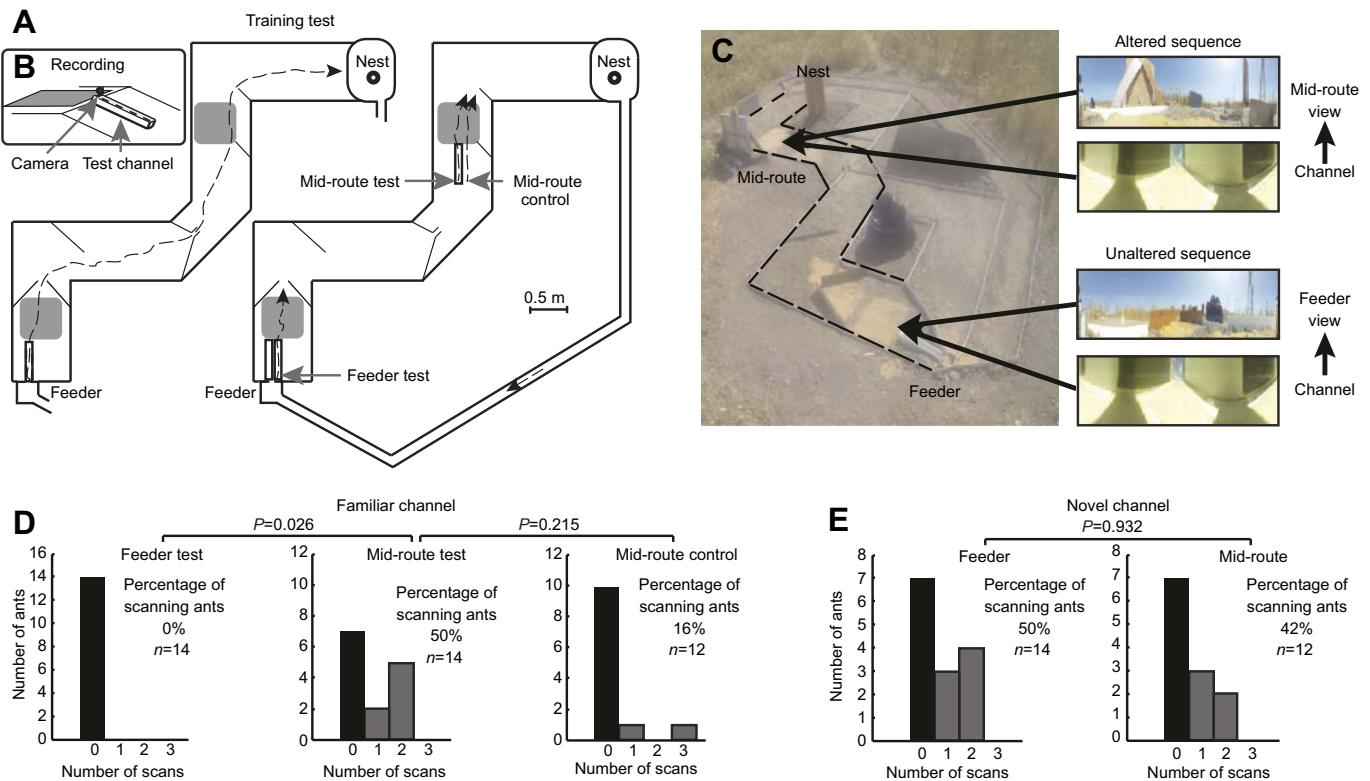
Fig. 1. Summary of Experiment 1. (A) Schematic aerial view of the experimental set-up with training and testing conditions. Ants were limited to follow a one-way foraging route between the nest and feeder. The feeder was connected to a channel that all ants had to pass through before they could return to the nest along a zigzag-shaped inbound route. During tests, zero-vector ants were transferred to one of three release points (Feeder test, Mid-route test, Mid-route control) and their scanning behaviour was recorded in the designated test areas (grey quadrants). Dashed arrows indicate example paths of training and testing, and black lines within the route depict baffles. (B) Schematic of test channel and data recording. All channels in training and testing were lopsidedly placed onto the ground with an approximately 30 deg slope. Tested ants were released in the channel and their subsequent scanning behaviour in the test area (grey quadrant) was recorded with a small camera at the top end of the channel. (C) Photographs of the experimental set-up with panoramic images from within the test channel, the feeder view (unaltered view sequence) and the mid-route view (altered view sequence). Dashed line framing the set-up indicates the part of the route used in the experiment. (D) Results of the Feeder test, Mid-route test and Mid-route control. Ants scanned significantly more at the Mid-route test compared with the Feeder test (GLM: $P=0.026$, $Z=-2.357$) but did not reach a significant difference when compared with Mid-route control (GLM: $P=0.215$, $Z=-1.272$). (E) Results of the Novel channel test. Ants showed no difference in scanning behaviour between feeder and mid-route release points (GLM: $P=0.932$, $Z=0.097$).

zigzag route with the same compass orientation as at the feeder (Fig. 1A). The visual surrounding of the 'Mid-route test' differed greatly from the one of the Feeder test as it contained several big, nearby artificial objects (Fig. 1A,C). Thus, this is the crucial experimental condition in which the sequence of familiar visual memories was altered compared with a normal homing journey.

Novel channel to start or mid-route

A control for the potential difference in visual familiarity between the feeder- and mid-route release was the 'Novel channel test'. Ants were transferred into an unfamiliar channel and released at either the feeder or the mid-route location (Fig. 1A). The novel channel was a modified version of the normal test channel. The walls and the ground were covered with thin beige cardboard and hence provided a different substrate material and colour.

Mid-route no channel (control)

To control for the possibility that ants might always scan when released at the mid-route location, irrespective of the sequence alteration, a 'Mid-route control' was conducted. The test channel was placed as for the Mid-route test location but the ants were released on the ground, right beside the beginning of the test channel (Fig. 1A).

Channel to unfamiliar location (control)

To verify that scanning behaviour is evoked by visual unfamiliarity, ants were released in the familiar test channel after it had been placed so that they would emerge in completely unfamiliar visual surroundings approximately 25 m away (Fig. S1).

Each ant was tested only once, in one of the test conditions.

Experiment 2

To account for the large individual variance observed in Experiment 1, a second experiment was conducted in June 2017 in which each ant was tested in all conditions and therefore provided paired data across trials. Ants were trained to follow a straight foraging route (approximately 8 m long and 1.2 m wide; Fig. 2A) and similar training procedures to Experiment 1 were followed, whereby foragers always returned from the feeder via a 50 cm long channel before continuing their homebound trip. The training and test channels were augmented with black stripes attached to the walls to enhance visual contrast and optic flow as well as to lower potential reflections from the sun. An additional channel at the middle of the route (Mid-route test location) was present during all training trials (approximately 6 m in feeder-nest direction; Fig. 2A,B) to diminish differences of the views due to the test channel during Mid-route test and Mid-route control. Ants were individually marked and considered trained after
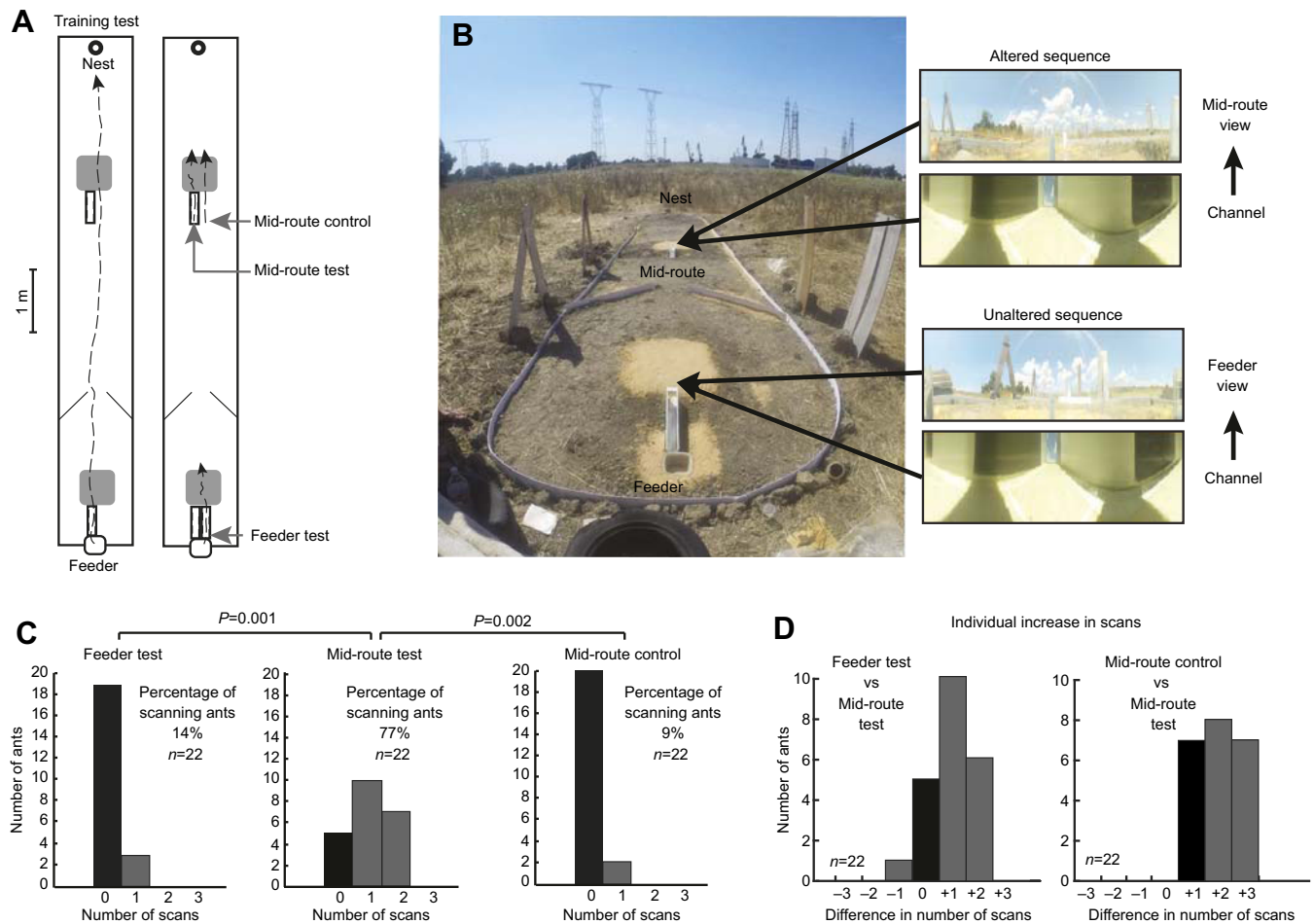
Fig. 2. Summary of Experiment 2. (A) Schematic aerial view of the experimental set-up with training and testing conditions. Ants were trained to forage on a route between the nest and feeder. The feeder was connected to a channel that all ants had to pass through before they could return to the nest. During tests, zero-vector ants were transferred to one of three release points (Feeder test, Mid-route test, Mid-route control) and their scanning behaviour was recorded in the designated test areas (grey quadrants). Dashed arrows indicate example paths of training and testing, and black lines within the route depict baffles. (B) Photographs of the experimental set-up with panoramic images from within the test channel, the feeder view (unaltered view sequence) and the mid-route view (altered view sequence). (C) Results of the Feeder test, Mid-route test and Mid-route control. Each ant was tested at all three release points. Ants scanned significantly more at the mid-route release compared with the feeder (GLM: $P=0.001$, $Z=-3.502$) and mid-route control (GLM: $P=0.002$, $Z=-3.166$) release points. (D) Increase of scans of individual ants compared between feeder and mid-route release as well as mid-route control and mid-route release points.

performing at least five straight homing bounds. Trained ZV ants were subjected to the following test conditions.

Test channel to feeder (start of the route)
In the Feeder test, ants were released in a test channel (identical to the training channel) and placed alongside the training channel (Fig. 2A,B). As in the equivalent condition of Experiment 1, the sequence of views experienced by the ant was unaltered from training.

Test channel to mid-route
In the Mid-route test, ants were transferred to the same test channel as for the Feeder tests, but this time the channel was placed exactly on the location of the mid-route channel, thus replacing it (Fig. 2A,B). Here too, visual differences between Mid-route test and Feeder test location were emphasised by the additional visual objects (Fig. 2B). As in the equivalent condition of Experiment 1, the usual sequence of views experienced by the ant was thus altered.

Mid-route no channel (control)
As in Experiment 1, for the Mid-route control ants were released on the ground, right beside the beginning of the mid-route channel

(Fig. 2A,B) to test whether this location might appear less familiar than the Feeder location irrespective of the sequence.

Familiarity control
Familiarity control with altered visual surrounding at the Feeder test was additionally conducted to test if the increase of scans during Mid-route tests could have been caused by a drop in familiarity just as the ants exit the test channel (Fig. S2). This exact view (at the border between the channel exit and the Mid-route test surrounding) has never been encountered by the ants and might have triggered the scan increase in Experiments 1 and 2 instead of the altered sequence of views.

In Experiment 2, each ant was tested once in each condition, with at least two uninterrupted training trips between tests. This provided individually paired data across the three tests. The order of tests varied across individuals in a systematic fashion.

Data recording and analysis
The number of scans performed by the ants on the 50×50 cm test areas was assessed in two ways. Firstly, scans were observed and recorded directly in the field by two experimenters. Scans were

defined by the following criteria: the ant stops forward motion and rotates at least in one direction on the spot before resuming forward motion. Given the rotational component, such a behaviour is usually obvious and hence unambiguous. Experimenters agreed upon the number of scans on each test and the data point was recorded. This was supported by video recording of all tests using a GoPro Hero3+ (1920×1080 pixels; 60 frames s^{-1}) and a Panasonic Lumix camera (DMC FZ200) for Fig. S2. Owing to camera overheating, some video files were corrupted (Experiment 1: 17 out of 76; Experiment 2: 2 out of 66; Fig. S2: 12 out of 44) and the number of scans were solely based on live observations of two experimenters.

Differences between the number of scans across tests were analysed with a general linear model (GLM) for count data (quasi-Poisson distribution). For Experiment 2 with paired data, we used the GLM for mixed effects with conditions as fixed effect and individual ants as random effect. In both experiments, the key Mid-route test condition – where the sequence of view is altered – was compared with both other conditions (Feeder test and Mid-route control) simultaneously in the model.

RESULTS
To investigate whether recently experienced views affect the route-following behaviour of ants, ZV ants were tested at the feeder (unaltered sequence of views) or at the middle of their familiar route (altered sequence of views) and the number of scans displayed in the area following the channel exit was analysed. The occurrence of scans is a suitable indicator of navigational uncertainty in this experimental context: ants exiting the test channel in totally unfamiliar surroundings showed systematic scanning behaviours (90%, 9/10) and the highest numbers of scans (up to 6) across all test conditions (Fig. S1).

Experiment 1
In Feeder tests, i.e. without altered visual sequence, not a single ant (0%, 0/14) scanned in the test area (Fig. 1D). In contrast, in the Mid-route test, where ants experienced an altered visual sequence, 50% of ants (7/14) scanned at least once in the test area (Fig. 1D), indicating some degree of navigational uncertainty. There is a significant increase of scans in the Mid-route test when compared with the Feeder test (GLM: $P=0.026$, $Z=-2.357$). In the Mid-route control, with the ants released beside the mid-route-channel, only two out of 12 ants (16%) scanned (Fig. 1D), suggesting that the increased scanning number in the Mid-route test is not due to unfamiliarity of the absolute position, although this difference did not reach significance (GLM: $P=0.215$, $Z=-1.272$; Fig. 1D). An additional control confirmed that the increase of scans was due to the altered sequence of views from test channel to the visual surroundings at the exit of the test channel and not caused by a lack of visual route knowledge. Ants from the Novel channel tests showed no significant difference between the feeder and mid-route release points (GLM: $P=0.932$, $Z=0.097$; Fig. 1E). Both tests bore unfamiliarity due to the novel test channel and produced scans in 50% (7/14) and 42% (5/12) of ants, respectively.

Experiment 2
In Experiment 2, each ant was tested in all three conditions (Feeder test, Mid-route test and Mid-route control), providing paired data accounting for individual differences. As before, in the Feeder test few ants (14%, 3/22) scanned in comparison with 77% (17/22) of ants in the Mid-route test condition (Fig. 2C). Also, in accordance with data from Experiment 1, only 9% (2/22) of ants in the Mid-route control scanned, which is in line with data observed in the Feeder test (Fig. 2C). We observed a significant increase in scans

during the Mid-route test compared with the Feeder test (GLM: $P=0.001$, $Z=-3.502$) and Mid-route control (GLM: $P=0.002$, $Z=-3.166$). This effect was not due to a few ants scanning many times as most of the ants (31/44) displayed a higher number of scans in the Mid-route test (Fig. 2D) and only one ant decreased her number of scans between the Feeder test and Mid-route test. The sequence across test conditions was balanced across individuals and had no detectable effect on the results (GLM: $P=0.463$, $Z=-0.734$). Please see Movies 1–3 for examples of the Feeder test, Mid-route test and unfamiliar release point.

DISCUSSION
A reliable sign of navigational uncertainty in ants is the occurrence of scanning behaviour (Wystrach et al., 2019a,b; Wystrach et al., 2014). In the present study, 90% of ants leaving a familiar channel from a feeder and finding themselves in a completely novel location exhibited repeated scanning (Fig. S1). This behaviour was used as an assay to investigate whether experiencing familiar views in an altered, novel sequence also produces uncertainty in ants, indicating that their memory of routes includes some information about the sequence of views experienced. If so, it would challenge or require augmentation of the current prevailing models of ant route memory. The main finding of this study is that an alteration of the sequence of views along a familiar route reliably increases the probability of a scanning response in ants.

Specifically, in experiments conducted across two field seasons and with different nests, ants were trained along a homing route that started with a 50 cm channel, providing a unique and well-controlled visual experience, before exiting into the open route surroundings which they followed home. During tests, trained ants were captured close to their nest (to prevent the use of path integration) and released in an identical-looking test channel. Upon release, these foragers dashed along the correct homing direction and out of the channel, showing that they recognised the familiar channel scenery. If they then found themselves close to the unaltered (training) Feeder test location, they scanned rarely if at all (0 and 14% of ants scanned in Experiments 1 and 2, respectively). However, if the channel had been relocated to the middle of the route, creating an altered visual sequence, foragers emerging from the channel typically stopped and displayed one or two scans (Mid-route test, 50% and 77% of ants scanned) before resuming their normal motion and completing the route at their usual pace (Figs 1D and 2C). Mid-route control ants, released 50 cm before the test area beside the beginning of the test channel, showed little scanning behaviour in the actual test area (Mid-route control, 16% and 9%; Figs 1D and 2C). However, using a novel, unfamiliar-looking channel tended to equally induce scans in both the feeder and mid-route locations (Novel channel test, 50% and 42%; Fig. 1E). Overall, results suggest that it is the change in sequence, rather than anything about the mid-route location or the displacement from the nest back to the route, that causes navigational uncertainty.

Interestingly, ants exiting an unfamiliar-looking channel (Novel channel tests) also displayed a high number of scans. This suggests that the novelty component of the channel (new wall colour and substrate) extended from inside to outside the channel, putting forward the idea that the unfamiliarity experienced at a given moment may have a sustained impact on behaviour.

Alternative explanations to sequence encoding
The overall experimental design aimed to contrast the hypothesis of sequence of views versus previous models of ant navigation. Results in all conditions validate the *a priori* predictions of the use of

view sequence, and thus favour this novel hypothesis. However, alternative explanations may also explain the results.

The channel may exert a motor constraint by forcing the ants to keep to a fixed straight path and perhaps stereotyped movements on exiting it. It could be argued that it is these motor components rather than the scene in the channel that contributes to generating the unmet expectation that leads the ants to scan when exiting the Mid-route channel during tests. Several papers have shown sequential links between vision and motor behaviour (Chittka, 1998; Collett et al., 1993; Macquart et al., 2008; Vowles, 1965; Zhang et al., 1996). However, all these studies examine whether seeing a particular visual pattern can prime a turn in one direction, rather than the reverse. Motor constraints should have been minimal in our case because the straight and fast paths of *C. velox* held no apparent differences within the channel or not, at least to the naked eye, but this idea may still be worth investigating in other contexts.

It should be also noted that the ants from both the Mid-route test and the Mid-route control walked 50 cm before testing occurred (Figs 1A and 2A). In both conditions, tested ants typically scanned upon release, i.e. 50 cm before the test area. Scans before the test area were not recorded, which in hindsight would have allowed further scrutiny about potential differences between test and control. Yet in the actual test area, most scans occurred during Mid-route tests and hardly ever during Mid-route controls (Figs 1D and 2C).

When ants emerge from the (familiar) channel into (familiar) mid-route surroundings, they must experience for a very brief moment a composite view (channel in the rear view and mid-route scene in the frontal visual field), which must be unfamiliar. It may be argued that this short moment where the overall scene must appear unfamiliar induced the scanning response observed in the Mid-route tests. In a follow-up control experiment (Fig. S2), we altered the rearward portion of the view as ants emerged from the channel into the (expected) start of the route (Feeder test) and found that ants would indeed scan at a similar rate to those exiting the channel in the (unexpected) middle of the route (Mid-route test). However, this alternative explanation remains uncertain for several reasons. First, the artificial change created in this follow-up experiment exerted a much longer-lasting visual unfamiliarity than the one experienced at the exit line of the test route channel (Fig. S2). Second, Feeder tests and Mid-route controls also contained elements of unfamiliarity because ants were released at a location slightly offset compared with their usual route, but this did not trigger scans. Finally, the test channels were lopsidedly dug into the ground so that ants could not see the visual surroundings before exiting the channel (Fig. 1B), and hence the unfamiliar composite view would be experienced for no more than a split-second when passing the exit line (Fig. S2). Short moments of unfamiliarity must be experienced regularly when ants navigate through grassy environments or new fallen debris (not to mention bystanding experimenters). Yet ants do not trigger scans in these cases (authors' personal observations). Visual recognition in grassy environments must be noisy, and responding to brief drops in the familiarity signal would lead to regular stops and scans, which seem counterproductive in these rapid runners.

The possibility of learning sequences in ants has been explored before in several experimental contexts but the results were not clear cut (Riabinina et al., 2011; Macquart et al., 2008; Schwarz and Cheng, 2011). Bulletproof evidence for learning a sequence of views would probably require experiments in virtual reality, where the tested ants can be easily and instantaneously 'transferred' from one part of the route to another.

How could sequences of views be encoded in the insect brain?

A most 'peripheral' explanation to the encoding of information about the visual sequence would be that ants do not store static but dynamic views, i.e. how the visual input is actually changing as they move forward. Altering the sequence of familiar views as we did here would produce a novel – and thus unfamiliar – dynamic visual input, hence triggering scanning behaviours. Past observations in ants cast doubt upon this hypothesis. First, during scanning behaviours, ants actually stop and pause, exposing the visual system to a static view of the world during a tenth of a second or so before resuming motion in a correct direction. This behaviour is particularly apparent in fast-walking desert ants such as *Melophorus bagoti* (Wystrach et al., 2014). During learning walks, ants display numerous scan-like pauses while leaving the nest (Fleischmann et al., 2016, 2017; Jayatilaka et al., 2018; Müller and Wehner, 2010; Wystrach et al., 2014; Zeil and Fleischmann, 2019) or the feeder (Judd and Collett, 1998; Nicholson et al., 1999), suggesting that they do learn static views of the world. Moreover, dynamic views of the world would intrinsically encode information about absolute distances of objects, but experiments altering object configuration show that ant searches are based on retinal overlap rather than absolute distance (Graham et al., 2003; Judd and Collett, 1998; Wehner and Räber, 1979), suggesting that the stored views are static rather than dynamic. Third, recent experiments in *C. velox* (work in preparation) and other species (Murray et al., 2020) show that ants easily recognise familiar views when tethered to run on the spot on a spherical air treadmill (Dahmen et al., 2017), thus proving that views can be recognised without the change produced by forward motion.

Alternatively, information about view sequence could be encoded in the mushroom bodies, which are thought to be the seat of visual memories for navigation (Webb and Wystrach, 2016). There are several hypotheses for how a succession of views could be encoded in the mushroom bodies. One is that recurrency in this circuit could be exploited for learning temporal sequences (Arena et al., 2013; Cognigni et al., 2018; Grünewald, 1999; Li and Strausfeld, 1999). Another is that connections between Kenyon cells (KCs) could adapt through Hebbian mechanisms to alter the responsiveness to repeated pattern sequences (Nowotny et al., 2003). Furthermore, it is known that KCs possess several gap junctions between each other (Wu et al., 2011), suggesting that each active KC could increase the activation probability of other KCs, given a small delay. Under this assumption, the pattern of KC activity at a given time is not only dependent on the current stimulus but also the previously active pattern of KCs, i.e. the stimulus previously experienced (Nowotny et al., 2003).

Ultimate considerations

The current study suggests that the disturbance of one transition along the sequence affects behaviour. This can be accounted for by the storage of a short sequence and does not necessarily imply that the complete sequence of experienced views is stored. From a computational perspective, there are potential advantages in storing even short sequences of view memories, as it can reduce the risk of aliasing errors (Graham and Mangan, 2015). Matching of short sequence images has been shown to be very robust in robot localisation algorithms, even with drastic changes in lighting such as sunny days versus stormy nights (Milford and Wyeth, 2012), using very low resolution images (Milford, 2013), or with substantial tilt and pitch variation (Stone et al., 2016). Robustness to visual change and reduction of memory load would obviously be

beneficial for ants that need to memorise and recognise long visual routes across their lifetime.

Finally, it is worth mentioning that mechanisms for visual navigation and the neural underpinning of visual memories seem to be shared across insects or at least across central place foraging hymenoptera (Cheng, 2012; Warrant and Dacke, 2016; Webb and Wystrach, 2016; Wehner et al., 1996; Zeil and Fleischmann, 2019). Hence, it is likely that the influence of the sequences of views during route-following is not only limited to *C. velox* but also present in other ants and visually guided insects.

Conclusions

This study shows that altering the usual sequence of views triggers a transient resurgence of scanning behaviours even though the ants are still in their familiar environment. Functionally, learning sequences of views might improve the robustness of visual recognition to environmental change. The experimental manipulations required to alter the sequence of views in the real world will always enable alternative explanations to be put forward. Hence, future experiments using virtual reality could provide the means to a definite proof and the way to explore the mechanisms underlying visual sequence learning, which is likely to be widespread among insect navigators.

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