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1 **Route-following ants respond to alterations of the view sequence**

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19 **Keywords:** *Cataglyphis*, desert ants, homing, navigation, route-following, view sequence

20

21 **ABSTRACT**

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

40

41

42 **INTRODUCTION**

43 Solitary foraging desert ants are expert navigators that seek and retrieve food morsels under
44 extreme heat conditions. When foragers locate a bountiful food source they will shuttle
45 rapidly back and forth along idiosyncratic visually-guided routes (Kohler and Wehner, 2005;
46 Mangan and Webb, 2012; Wystrach et al., 2011b). That is, each ant will follow a fixed path
47 to the feeder before returning home by a similarly fixed but different path. These paths are
48 unique to each ant despite their journeys sharing the same start and end points,
49 demonstrating a lack of pheromone guidance. Instead visual information provided by the
50 ants' surroundings is sufficient for route following and individuals can even recover their
51 normal route direction following a displacement either by an experimenter (Kohler and
52 Wehner, 2005; Mangan and Webb, 2012; Sommer et al., 2008) or a wind gust (Wystrach and

53 Schwarz, 2013), suggesting that visual memories (here termed ‘views’) can be accessed
54 independently of the animal’s recent experience.

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

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

85 This simple scene-action control hypothesis is parsimonious , however, data from
86 behavioural studies suggest that the picture may not be so simple (Wehner et al., 1996).
87 Specifically, (Wystrach et al., 2013) showed that ants displaced from their nest to an
88 unfamiliar location do not immediately engage in a systematic search but instead backtrack
89 along their just travelled route bearing. This effect is only present in ants that have been
90 captured at the nest indicative of ants possessing some memory of recent visual
91 experiences. Furthermore, (Collett, 2014; Wystrach et al. 2019) demonstrated that ants
92 forced to retrace their homeward routes twice in succession (moved from the feeder back to
93 the start of their inward route) display a period of confusion where they do not seem to
94 recognise their familiar path. Graham and Mangan (Graham and Mangan, 2015) postulate a
95 series of possible explanations for such behaviours including the use of temporal
96 information about their routes such as the sequence in which views were experienced. This
97 study aims to address this possibility directly by assessing whether ants have knowledge
98 about the sequence of views encountered along their familiar foraging route. It is already
99 known that ants and bees can be trained to learn sequences of patterns (Schwarz and
100 Cheng, 2011) and motor actions (Collett et al., 1993; Macquart et al., 2008) or to act
101 accordingly to the cue they have just previously encountered (Giurfa et al., 2001; Zhang et
102 al., 2005). However, these feats requires many trials of experimental conditioning, and
103 although some can be parsimoniously explained (Cope et al., 2018), it remains unknown
104 whether these insects spontaneously learn information about the sequence of views
105 experienced along their familiar foraging routes (Riabinina et al., 2011).

106 In our study, ants were trained to home along a route through semi-natural terrain.
107 The route started with a short section through a channel providing a unique visual
108 experience as ants begin their homeward journey. During tests, the familiar channel was
109 moved to a different location at the middle of the route so that ants exiting the channel
110 were exposed to a familiar view that is not the one they usually experience immediately
111 after the channel. To assess whether breaking the normal sequence of views in such a way
112 impacted the ants, the number of scanning behaviours that ants displayed after exiting the
113 channel in several tests and control conditions was quantified. Scanning behaviour typically
114 provides a proxy for assessing the ants' current navigational uncertainty (Wystrach et al.,
115 2014). The results showed a strong effect of changing the sequence, which we discuss in the
116 light of insect behaviour and neurobiology.

117

118 **MATERIALS AND METHODS**

119 **Species and study site**

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

126

127 **General experimental set-up**

128 Two experiments were conducted over two field seasons in June 2016 and June 2017. In
129 both experiments ants were trained to run along a defined route to collect food items at a
130 feeder location (Figs. 1A, C; 2A, B). The routes were enclosed by slippery white plastic planks
131 (approx. 5 cm high) submerged in a 5 cm ditch. This enabled the ants to perceive the
132 surrounding natural scenery during route-following while preventing them to forage
133 elsewhere (Wystrach et al., 2012). The foraging routes were cleared of clutter and
134 vegetation to ease the movements of the foragers on the ground. Small plastic bowls
135 (15×15×15 cm) sunk into the ground so that their top edges aligned with the ground surface
136 served as feeders. The upper rim of the feeder walls was covered with transparent tape to
137 prevent the ants from escaping. Foraging ants eventually jumped or fell into the feeder and
138 picked up a biscuit crumb or meal worm piece and were then individually marked with
139 acrylic or enamel modelling paint. In both experiments, foragers that had picked up a food
140 item started their homing journeys by travelling through an open-topped, 50×5×10 cm white
141 plastic channel that directly connected the feeder to the start of the homeward route. Thus,
142 the visual route memories of all ants across conditions for the first 50 cm of their route was
143 inside of the white channel. Only well-trained individuals with high familiarity of the visual
144 surroundings were tested (see detail for each experiment below). For tests, homing ants
145 were captured just before entering their nest so that their current path integration
146 homeward vector (accumulated during the outbound trip) had returned to zero; hence
147 termed zero-vector ants (ZV). For proper homing motivation, only ants holding a food item
148 were tested. Once captured, the ant was transferred in a darkened plastic vial and released

149 at one of the test locations along the route, either within a ‘test channel’ or directly on the
150 ground (see details below). The transition between the capture point at the nest and the
151 release at either the feeder or mid-route location caused an additional alteration of the view
152 sequence and hence could trigger scanning behaviour. In all conditions, ZV ants were likely
153 to scan a few times upon release from the carrying tube. However, ZV ants were always
154 released 50 cm before the actual test areas, giving the foragers enough time and space to
155 recover their bearings and resume visual homing before data recording started at the
156 designated test areas (Figs. 1A, B; 2A). Furthermore, the test channel was always placed at
157 the exact location where the ant homed during her previously displayed homing path. This
158 procedure helped minimise changes in visual familiarity during tests. To avoid differences in
159 the ground substrate across the different test locations the immediate area after the
160 channel exit (50×50 cm) was covered with a layer of sand (Figs. 1, 2; grey areas). In all tests,
161 a GoPro Hero3+ camera was mounted on the top end of the test channel and the behaviour
162 of the tested ant was recorded on the 50x50 cm area after the channel exit. Panoramic
163 images shown in figures were taken with a Sony™ Bloggie camera and unwarped with
164 PhotoWarp2™.

165

166 **Experiment 1**

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

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

- 180 2. Test channel to mid-route. Ants were transferred into the same test channel as at
181 the feeder but this time the channel was placed in the middle of the third leg of
182 the zigzag route with the same compass orientation as at the Feeder (Fig. 1A).
183 The visual surrounding of the *Mid-Route Test* differed greatly from the one of the
184 Feeder test as it contained several big, nearby artificial objects (Fig. 1A, C). Thus,
185 this is the crucial experimental condition in which the sequence of familiar visual
186 memories was altered as compared to a normal homing journey.
- 187 3. Novel channel to start or mid-route. A control for the potential difference in
188 visual familiarity between the feeder- and mid-route release was the *Novel*
189 *Channel Test*. Ants were transferred into an unfamiliar channel and released at
190 either the feeder or the mid-route location (Fig. 1A). The novel channel was a
191 modified version of the normal test channel. The walls and the ground were
192 covered with thin beige cardboard and hence provided a different substrate
193 material and colour.
- 194 4. Mid-route no channel (control). To control for the possibility that ants might
195 always scan when released at the mid-route location, irrespective of the
196 sequence alteration, a *Mid-Route Control* was conducted. The test channel was
197 placed as for the Mid-Route Test location but the ants were released on the
198 ground, right beside the beginning of the test channel (Fig. 1A).
- 199 5. Channel to unfamiliar location (control). To verify that scanning behaviour is
200 evoked by visual unfamiliarity, ants were released in the familiar test channel
201 after it had been placed so that they would emerge in completely unfamiliar
202 visual surroundings approx. 25 m away (Fig. S1).

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

204

205 **Experiment 2**

206 To account for individual variance in Experiment 1, a second experiment was conducted in
207 June 2017 in which the same ant was tested in all conditions and therefore provided paired
208 data across trials. Ants were trained to follow a straight foraging route (approx. 8 m long and
209 1.2 m wide; Fig. 2A) and similar training procedures to Experiment 1 were followed, whereby
210 foragers always returned from the feeder via a 50 cm long channel before continuing their
211 homebound trip. The training and test channels were augmented with black stripes attached

212 to the walls to enhance visual contrast and optic flow as well as to lower potential
213 reflections from the sun. An additional channel at the middle of the route (Mid-Route Test
214 location) was present during all training trials (approx. 6 m in feeder-nest direction; Fig. 2A,
215 B) to diminish differences of the views due to the test channel during Mid-Route Test and
216 Mid-Route Control. Ants were individually marked and considered trained after performing
217 at least five straight homing bounds. Trained ZV ants were subjected to the following test
218 conditions.

- 219 1. Test channel to feeder (start of the route). In the *Feeder Test*, ants were released in a
220 test channel (identical to the training channel) and placed alongside the training
221 channel (Fig. 2A, B). As in the equivalent condition of Experiment 1, the sequence of
222 views experienced by the ant was unaltered from training.
- 223 2. Test channel to mid-route. In the *Mid-Route Test*, ants were transferred to the same
224 test channel as for Feeder Tests, but this time the channel was placed exactly on the
225 location of the mid-route channel, thus replacing it (Fig. 2A, B). Here too, visual
226 differences between Mid-Route- and Feeder Test location were emphasised by the
227 additional visual objects (Fig. 2B). As in the equivalent condition of Experiment 1, the
228 usual sequence of views experienced by the ant was thus altered.
- 229 3. Mid-route no channel (control). As in Experiment 1, for the *Mid-Route Control* ants
230 were released on the ground, right beside the beginning of the mid-route channel
231 (Fig. 2A, B) to test whether this location might appear less familiar than the Feeder
232 location irrespective of the sequence.
- 233 4. Familiarity Control with altered visual surrounding at the Feeder Test was
234 additionally conducted to test whether the increase of scans during Mid-Route tests
235 could have been caused by a drop of familiarity just as the ants is exiting the test
236 channel (Fig. S2). This exact view (at the border between the channel exit and the
237 Mid-Route test surrounding) has never been encountered by the ants and might have
238 triggered the scan increase in Experiment 1 and 2 instead of the altered sequence of
239 views.

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

243

244 **Data recording and analysis**

245 The number of scans performed by the ants on the 50×50 cm test areas was assessed in two
246 ways. Firstly, scans were observed and recorded directly in the field by two experimenters.
247 Scans were defined by the following criteria: the ant stops forward motion and rotates at
248 least in one direction on the spot before resuming forward motion. Given the rotational
249 component, such a behaviour is usually obvious and hence unambiguous. Experimenters
250 agreed upon the number of scans on each test and the data-point was recorded. This was
251 supported by video recording of all tests using a GoPro Hero3+ (1920×1080 pixel; 60 fps) and
252 a Panasonic Lumix camera (DMC FZ200) for Fig. S2. Some video files were corrupted
253 (Experiment 1: 17 out of 76; Experiment 2: 2 out of 66; Fig. S2: 12 out of 44) and the number
254 of scans were solely based on live observations of two experimenters.

255 Differences between the number of scans across tests were analysed with a General-
256 linear-model (GLM) for count data (quasipoisson distribution). For Experiment 2 with paired
257 data, we used the GLM for mixed effects with conditions as fixed effect and individual ants
258 as random effect. In both experiments, the key Mid-Route Test condition – where the
259 sequence of view is altered – was compared to both other conditions (Feeder Test and Mid-
260 Route Control) simultaneously in the model.

261

262 **RESULTS**

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

270

271 **Experiment 1**

272 In Feeder Tests, that is without altered visual sequence, not a single ant (0%, 0/14) scanned
273 in the test area (Fig. 1D). In contrast, in the Mid-Route Test, where ants experienced an
274 altered visual sequence, 50% of ants (7/14) scanned at least once in the test area (Fig. 1D),
275 indicating some degree of navigational uncertainty. There is a significant increase of scans in

276 the Mid-Route Test when compared to the Feeder Test (GLM: $P=0.026$, $Z=-2.357$). In the
277 Mid-Route Control, with the ants released beside of the mid-route-channel, only two out of
278 twelve ants (16%) scanned (Fig. 1D) suggesting that the increased scanning number in the
279 Mid-Route Test is not due to unfamiliarity of the absolute position, although this difference
280 did not reach significance (GLM: $P=0.215$, $Z=-1.272$; Fig. 1D). An additional control confirmed
281 that the increase of scans was due to the altered sequence of views from test channel to the
282 visual surrounding at the exit of the test channel and not caused by a lack of visual route
283 knowledge. Ants from the Novel Channel Tests showed no significant difference between
284 the feeder and mid-route release points (GLM: $P=0.932$, $Z=0.097$; Fig. 1E). Both tests bore
285 unfamiliarity due to the novel test channel and produced scans in 50% (7/14) and 42% (6/12)
286 of ants respectively.

287

288 **Experiment 2**

289 In Experiment 2, each ant was tested in all three conditions (Feeder Test, Mid-Route Test
290 and Mid-Route Control), providing paired data accounting for individual differences. As
291 before, in the Feeder Test few ants (14%, 3/22) scanned in comparison to 77% (17/22) of
292 ants in the Mid-Route Test condition (Fig. 2C). Also, in accordance with data from
293 Experiment 1, only 9% (2/22) of ants in the Mid-Route Control scanned, which is in line with
294 data observed in the Feeder Test (Fig. 2C). We observed a significant increase in scans during
295 the Mid-Route Test as compared to the Feeder Test (GLM: $P=0.001$, $Z=-3.502$) and Mid-
296 Route Control (GLM: $P=0.002$, $Z=-3.166$). This effect was not due to a few ants scanning
297 many times as most of the ants (31/44) displayed a higher number of scans in the Mid-Route
298 Test (Fig. 2D) and only one single ant decreased her number of scans between the Feeder-
299 and Mid-Route Test. The sequence across test conditions was balanced across individual and
300 had no detectable effect on the results (GLM: $P=0.463$, $Z=-0.734$).

301

302 **DISCUSSION**

303 A reliable sign of navigational uncertainty in ants is the occurrence of scanning behaviour
304 (Wystrach et al., 2019; Wystrach et al., 2014). In the current study, 90% of ants leaving a
305 familiar channel from a feeder and finding themselves in a completely novel location
306 exhibited repeated scanning (Fig. S1). This behaviour was used as an assay to investigate
307 whether experiencing familiar views in an altered, novel sequence also produces uncertainty

308 in ants, indicating that their memory of routes includes some information about the
309 sequence of views experienced. If so it would challenge, or require augmentation of the
310 current prevailing models of ant route memory. The main finding of this study is that an
311 alteration of the sequence of views along a familiar route reliably increases the probability of
312 a scanning response in ants.

313 Specifically, in experiments conducted across two field seasons and with different
314 nests, ants were trained along a homing route that started with a 50 cm channel, providing a
315 unique and well-controlled visual experience, before exiting into the open route
316 surroundings which they followed home. During tests, trained ants were captured close to
317 their nest (to prevent the use of PI) and released in an identical-looking test channel. Upon
318 release, these foragers dashed along the correct homing direction and out of the channel
319 showing that they recognised the familiar channel scenery. If they then found themselves
320 close to the unaltered (training) Feeder Test location they scanned rarely if at all (0% and
321 14% of ants scanned). However, if the channel had been relocated to the middle of the
322 route, creating an altered visual sequence, foragers emerging from the channel typically
323 stopped and displayed one or two scans (Mid-Route Test, 50% and 77% of ants scanned)
324 before resuming their normal motion and completing the route at their usual pace (Figs. 1D,
325 2C). Mid-Route Control ants, released 50 cm before the test area beside the beginning of the
326 test channel showed little scanning behaviour in the actual test area (Mid-Route Control,
327 16% and 9%; Figs. 1D, 2C). On the other hand, using a novel channel tended to equally
328 induce scans in both the feeder and mid-route locations (Novel Channel Test, 50% and 42%;
329 Fig. 1E). Overall, results suggest that it is the change in sequence, rather than anything about
330 the mid-route location or the displacement from the nest back to the route, that causes
331 navigational uncertainty.

332 Interestingly, ants exiting an unfamiliar looking channel (Novel Channel Tests) also
333 displayed a high number of scan. This suggest that the novelty component of the channel
334 (new wall colour and substrate) extended from inside to outside the channel, putting
335 forward the idea that the unfamiliarity experienced at a given moment may have a sustained
336 impact on behaviour.

337

338 **Alternative explanations to sequence encoding**

339 The overall experimental designed aimed to contrast the hypothesis of sequence of
340 views vs. previous models of ant navigation. Results in all conditions validate the a priori
341 predictions of the use of view sequence, and thus favour this novel hypothesis. However,
342 alternative explanations may also explain the results.

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

354 It should be also noted that both the ants from the Mid-Route Test and the Mid-
355 Route Control walked 50 cm before testing occurred (Fig. 1A, 2A). In both conditions, tested
356 ants typically scanned upon release, that is, 50 cm before the test area. Scans before the test
357 area were not recorded which in hindsight would have allowed further scrutiny about
358 potential differences between test and control. Yet, in the actual test area most scans
359 occurred during Mid-Route Tests and hardly ever during Mid-Route Controls (Fig. 1D, 2C).

360 When ants emerge from the (familiar) channel into (familiar) mid-route
361 surroundings, they must experience for a very brief moment a composite view (channel in
362 the rear-view and mid-route scene in the frontal visual field) which must be unfamiliar. It
363 may be argued that this short moment where the overall scene must appear unfamiliar
364 induced the scanning response observed in the Mid-Route Tests. In a follow-up control
365 experiment (Fig. S2), we altered the rear-ward portion of the view as ants emerged from the
366 channel into the (expected) start of the route (Feeder Test) and found that ants indeed
367 would scan at a similar rate to those exiting the channel in the (unexpected) middle of the
368 route (Mid-Route Test). However, this alternative explanation remains uncertain for several
369 reasons. First, the artificial change created in this follow-up experiment exerted a much
370 longer-lasting visual unfamiliarity than the one experienced at the exit line of the test route

371 channel (Fig. S2). Second, Feeder tests and Mid-Route Controls also contained elements of
372 unfamiliarity because ants were released on a location slightly offset compared to their
373 usual route, but this did not trigger scans. Finally, the test channels were lopsidedly dug into
374 the ground so that ants could not see the visual surroundings before exiting the channel (Fig.
375 1B), and hence the unfamiliar composite view would be experienced for no more than a
376 split-second when passing the exit line (Fig. S2). Short moments of unfamiliarity must be
377 experienced regularly when ants navigate through grassy environments or new fallen debris
378 (not to mention bystanding experimenters). Yet ants do not trigger scans in these cases
379 (pers. observ. SS, MM, BW, AW). Visual recognition in grassy environments must be noisy
380 and responding to brief drops in the familiarity signal would lead to regular stops and scans,
381 which seem counterproductive in these rapid runners.

382 The possibility of learning sequences in ants has been explored before in several
383 experimental contexts but the results were not clear-cut (Macquart et al., Riabinia et al.,
384 2011; 2008; Schwarz and Cheng, 2011;). Bulletproof evidence for learning a sequence of
385 views would probably require experiments in virtual reality, where the tested ants can be
386 easily and instantaneously ‘transferred’ from one part of the route to another.

387

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

389 A most ‘peripheral’ explanation to the encoding of information about the visual sequence
390 would be that ants do not store static but dynamic views, that is, how the visual input is
391 actually changing as they move forward. Altering the sequence of familiar views as we did
392 here would produce a novel – and thus unfamiliar – dynamical visual input, hence triggering
393 scanning behaviours. Past observations in ants cast doubt upon this hypothesis. First, during
394 scanning behaviours, ants actually stop and pause, exposing the visual system to a static
395 view of the world during a tenth of a second or so before resuming motion in a correct
396 direction. This behaviour is particularly apparent in fast walking desert ants such as
397 *Melophorus bagoti* (Wystrach et al., 2014). During learning walks ants display numerous
398 scan-like pauses while leaving the nest (Fleischmann et al., 2016; Fleischmann et al., 2017;
399 Müller and Wehner, 2010; Wystrach et al., 2014) or the feeder (Judd and Collett, 1998;
400 Nicholson et al., 1999), suggesting that they do learn static views of the world. Moreover,
401 dynamic views of the world would intrinsically encode information about absolute distances
402 of object, but experiments altering object configuration show that ant searches are based on

403 retinal overlap rather than absolute distance (Graham et al., 2003; Judd and Collett, 1998;
404 Wehner and R aber, 1979), suggesting that the stored views are static rather than dynamic.
405 Third, recent experiments in *C. velox* (work in preparation) and other species (Murray et al.,
406 2020) show that ants easily recognise familiar views when tethered to run on the spot on a
407 spherical air treadmill (Dahmen et al., 2017), thus proving that views can be recognised
408 without the change produced by forward motion.

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

421

422 **Ultimate considerations**

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

434 Lastly, it is worth mentioning that mechanisms for visual navigation and the neural
435 underpinning of visual memories seem to be shared across insects or at least across central
436 place foraging hymenoptera (Cheng, 2012; Warrant and Dacke, 2016; Webb and Wystrach,
437 2016; Wehner et al., 1996; Zeil and Fleischmann, 2019). Hence, it is likely that the influence
438 of the sequences of views during route-following is not only limited to *C. velox* but also
439 present in other ants and visually guided insects.

440

441 **CONCLUSION**

442 This study shows that altering the usual sequence of views triggers a transient resurgence of
443 scanning behaviours even though the ants are still in their familiar environment.

444 Functionally, learning sequences of views might improve the robustness of visual recognition
445 to environmental change. The experimental manipulations required to altered the sequence
446 of views in the real world will always enable alternative explanations to be put forward.

447 Hence, future experiments using virtual reality could provide the means to a definite proof
448 and the way to explore the mechanisms underlying visual sequence learning, which is likely
449 to be widespread among insect navigators.

450

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458

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

462

463 **Competing interests.** The authors declare no competing interest.

464

465 **Author contributions.** Conceptualisation: S.S., M.M., B.W., A.W.; Methodology: S.S., M.M.,
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467 A.W.; Writing - review & editing: S.S., M.M., B.W., A.W.

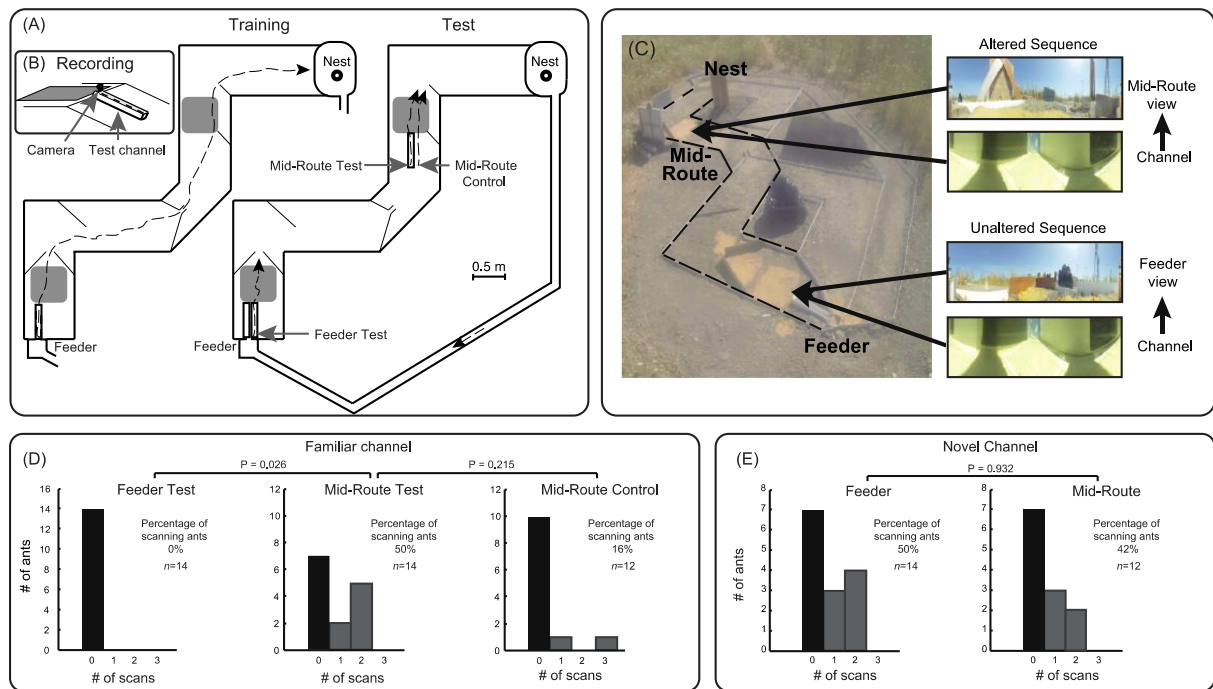
468

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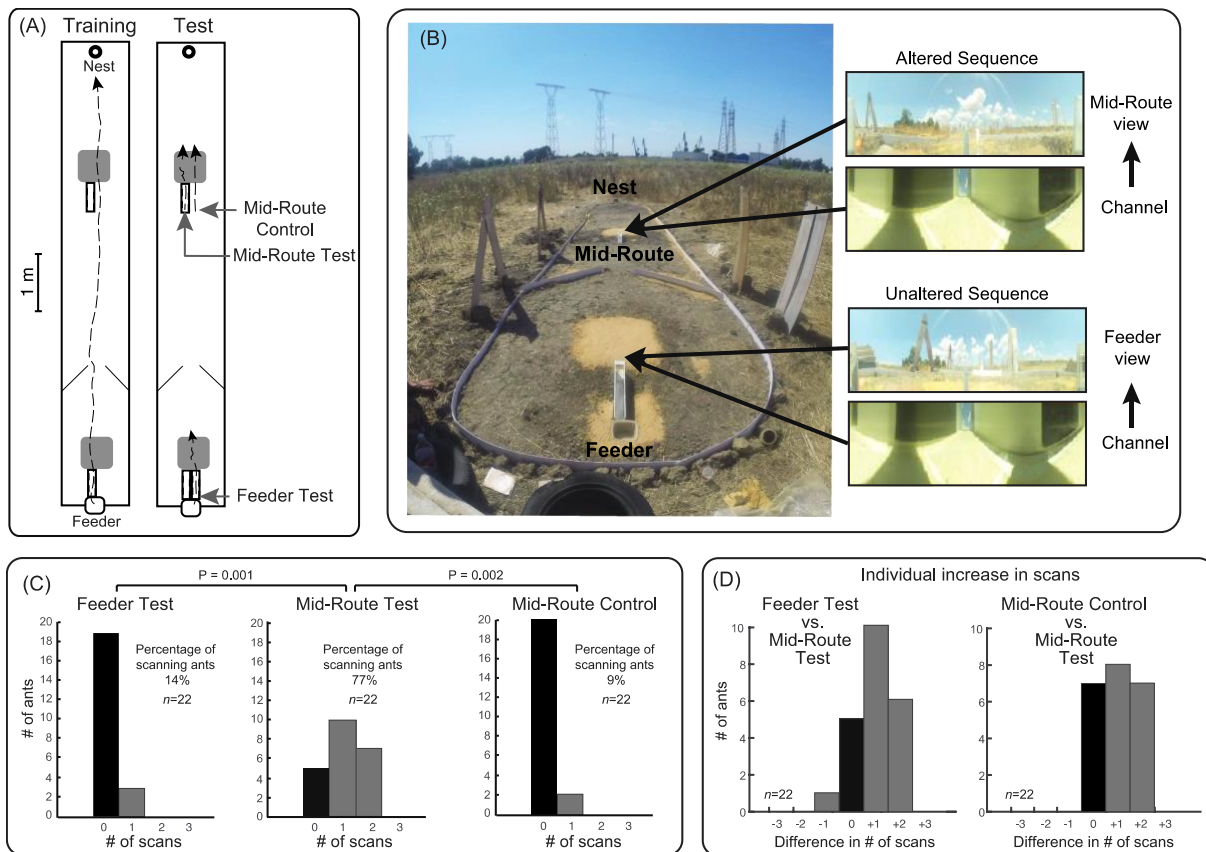
473 **Data availability.** All data generated or analysed during this study are included in this
474 published article (and its Supplementary information).

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477 **Fig. 1. Experiment 1.** (A) Schematic aerial view of the experimental set-up with training and
 478 testing conditions. Ants were limited to follow a one-way foraging route between the nest
 479 and feeder. The feeder was connected to a channel that all ants had to pass to before they
 480 could return back to the nest along a zigzag-shaped inbound route. During tests zero-vector
 481 ants were transferred to one of three release points (Feeder Test, Mid-Route Test, Mid-
 482 Route Control) and their scanning behaviour was recorded in the designated test areas (grey
 483 quadrants). Dashed arrows indicate example paths of training and testing and black lines
 484 within the route depict baffles. (B) Schematic of test channel and data recording. All
 485 channels in training and testing were lopsidedly placed onto the ground with an approx. 30°
 486 slope. Tested ants were released in the channel and their subsequent scanning behaviour in
 487 the test area (grey quadrant) was recorded with a small camera at the top end of the
 488 channel. (C) Photographs of the experimental set-up with panoramic images from within the
 489 test channel, the Feeder- (unaltered view sequence) and the Mid-Route view (altered view
 490 sequence). Dashed line framing the set-up indicates the part of the route used in the
 491 experiment. (D) Results of the Feeder Test, Mid-Route Test and Mid-Route Control. Ants
 492 scanned significantly more at the Mid-Route Test as compared to the Feeder Test (GLM:
 493 $P=0.026$, $Z=-2.357$) but did not reach a significant difference when compared to Mid-Route
 494 Control (GLM: $P=0.215$, $Z=-1.272$). (E) Results of the Novel Channel Test. Ants showed no
 495 difference in scanning behaviour between Feeder and Mid-Route release points (GLM:
 496 $P=0.932$, $Z=0.097$).



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Fig. 2. 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 to before they could return back 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 (unaltered view sequence) and the Mid-Route view (altered view sequence). (C) Results of the Feeder test, Route test and Route Control. Each ant was tested at all three release points. Ants scanned significantly more at the Mid-Route release as compared to 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.

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