1 **Combining sky and Earth: Desert ants (*Melophorus bagoti*) show weighted**

2 **integration of celestial and terrestrial cues**

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6 Eric L. G. Legge[1]*, Antoine Wystrach[2]*, Marcia L. Spetch[1], Ken Cheng[3]

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8 [1]Department of Psychology, University of Alberta, Edmonton, Canada

9 [2]School of Life Sciences, University of Sussex, Brighton, U.K.

10 [3]Department of Biological Sciences, Macquarie University, Sydney, Australia

11 *These authors contributed equally to the work.

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13

14 Corresponding author:

15

16 Eric Legge

17 Department of Psychology

18 P217 Biological Sciences Building

19 University of Alberta

20 Edmonton, Alberta, Canada T6G 2E9

21

22 Email: elegge@ualberta.ca

23 Phone: 1 780 492 3822

24 FAX: 1 780 492 1768

25

26 Running head: Integration of celestial and terrestrial

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28   **ABSTRACT**

29   Insects typically use celestial sources of directional information for path integration, and

30   terrestrial panoramic information for view-based navigation. Here we set celestial and

31   terrestrial sources of directional information in conflict for homing desert ants

32   (*Melophorus bagoti*). In Experiment 1, ants learned to navigate out of a round

33   experimental arena with a distinctive artificial panorama. On crucial tests, we rotated

34   the arena to create a conflict between the artificial panorama and celestial information.

35   In Experiment 2, ants at a feeder in their natural visually-cluttered habitat were

36   displaced prior to their homing journey so that the dictates of path integration (feeder-

37   nest direction) based on a celestial compass conflicted with the dictates of view-based

38   navigation (release point-nest direction) based on the natural terrestrial panorama. In

39   both experiments, ants generally headed in a direction intermediate to the dictates of

40   celestial and terrestrial information. In Experiment 2, the ants put more weight on the

41   terrestrial cues when they provided better directional information. We conclude that

42   desert ants weight and integrate the dictates of celestial and terrestrial information in

43   determining their initial heading, even when the two directional cues are highly

44   discrepant.

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49   Using multiple sources of information can improve decision making (Cheng et

50 al., 2007; Körding, 2007). Because each source of information comes with its associated

51 error, averaging multiple sources of information usually reduces the error, thus

52 improving the decision. In some conditions, the optimal weighting of various sources of

53 information can be specified, under what has been called a Bayesian analysis (Cheng et

54 al., 2007; Deneve and Pouget, 2004; Körding, 2007). Roughly speaking, the smaller the

55 variability or unreliability associated with a source, the more it should be weighted.

56   Many examples come from human perception and perceptual-motor actions

57 (Cheng et al., 2007). Thus, cues from different modalities may be integrated. For

58 example, Alais and Burr (Alais and Burr, 2004) showed that when both an auditory

59 source and a visual source specify the direction to an object, the dictates of the two

60 sources are averaged, and given approximately optimal weighting. Visual and haptic

61 sources of information are also integrated (Ernst and Banks, 2002; Gepshtein and

62 Banks, 2003), the cues again given approximately optimal weighting. Multiple sources

63 of dynamic perceptual information may also be integrated. For example, when a visible

64 beacon and a pattern of optical expansion provided in virtual reality each indicates a

65 direction to walk in, humans use both sources (Warren et al., 2001), although it is not

66 clear whether the integration was optimal in this case.

67   Examples of cue integration in other species are harder to come by, but some

68 recent evidence is provided by studies of insect navigation. Reid and colleagues (Reid et

69 al., 2011) presented homing night-active bull ants (*Myrmecia pyriformis*) with a conflict

70 between celestial and terrestrial cues. This was achieved by using a polarising filter that

71 rotated the pattern of polarisation by 45°, thus creating a discrepancy between this cue

72 and the unmanipulated terrestrial panorama. The ants struck a compromise direction that

73 reflected an average of the dictates of the two kinds of cues. In another myrmeciine ant

74 (the diurnal *Myrmecia croslandi*), Narendra and colleagues (Narendra et al., 2013)

75 displaced them to examine the range of their familiar territory. The authors found that

76 near the edge of their familiar terrain, *M. croslandi* struck a compromise between the

77 dictates of celestial and terrestrial cues. Collett (Collett, 2012) tested North African

78 desert ants (*Cataglyphis fortis*) on their outbound journey to a feeder via a two-legged

79 path. Tests on displaced ants produced results suggesting a compromise between the

80 dictates of path integration and those of the surrounding panorama, cues set in conflict

81 by the displacement. Collett (Collett, 2012) discussed the possibility that the ants were

82 integrating cues in a Bayesian fashion.

83   Our study continues this line in the Australian desert ant *Melophorus bagoti*, the

84 red honey ant (Cheng et al., 2009, 2014). The past decade's research has shown that this

85 ant possesses a suite of navigational capacities (Wystrach et al., 2013) like other well

86 studied insects such as *Cataglyphis* ants (Wehner, 2003, 2009) or honeybees (Cheng,

87 2000; Srinivasan, 2011). *Melophorus bagoti* integrates a path (Narendra, 2007a, 2007b;

88 Wehner et al., 2006), keeping track of the distance and direction travelled from its

89 starting point (Collett and Collett, 2000; Wehner and Srinivasan, 2003). It uses

90 panoramic terrestrial landmarks for navigation (Graham and Cheng, 2009; Wystrach et

91 al., 2011a, 2012), often using the many trees, bushes, and tussocks found in its central

92 Australian semi-arid habitat to travel stereotypical routes (Kohler and Wehner, 2005;

93 Sommer et al., 2008; Wehner et al., 2006; Wystrach et al., 2011b). It sometimes also

94 uses the well-studied celestial compass (Wehner 1994, 1997; Wystrach et al., 2014) to

95 navigate routes outside of the context of global path integration for finding the direct

96 path to the nest, including finding the path to an exit (Legge et al., 2010), heading in the

97 last direction travelled (Schwarz et al., 2011; Wystrach et al., 2013), or compensating

98 for displacement by wind (Wystrach and Schwarz, 2013). As a last resort for finding

99 places, it engages in systematic search (Schultheiss and Cheng, 2011, 2013; Schultheiss

100 et al., 2013). Like Alais and Burr's (Alais and Burr, 2004) and Warren et al.'s (Warren

101 et al., 2001) studies on humans, the current study investigated the integration of

102 directional sources of information, both terrestrial and celestial sources, in these ants.

103   In one experiment, we set up an artificial terrestrial panorama in the form of a

104 round arena with a 'skyline' of varying height (Fig. 1). Ants were trained to enter the

105 arena and find a feeder at its centre. They had to enter and exit in a different direction

106 (at 90° discrepancy) from the beeline direction connecting feeder and home. The

107 entrance and the exit were in the same direction from the feeder. On crucial tests, the

108 arena was rotated, putting terrestrial panorama and celestial cues in conflict.

109   **Insert Fig. 1 about here**

110

111   To be clear about what these cues are, the arena was uniformly coloured. The

112 terrestrial cues consisted of the pattern of skyline formed by the arena wall. Celestial

113 cues are cues with a celestial origin, sometimes reflected off terrestrial objects. They

114 consist of the pattern of polarised light, which is known to be used by insects in path

115 integration (Wehner, 1994, 1997), the position of the sun (Wehner and Müller, 2006),

116 and spectral cues (Wehner 1994, 1997). Shadows in the arena are determined by the

117 direction of the sun. The side opposite the direction of the sun has higher intensity (from

118 the sun shining on it), and also reflects relatively more UV light.

119   The key results came from testing ants that had returned home from a foraging

120 trip, but were caught just before they entered their nest. These ants have run off the

121 vector according to path integration, and are thus called zero-vector ants. In the setting

122 of an arena, we have evidence that *M. bagoti* can and sometimes do use celestial cues

123 for orientation in the absence of path integration information (Legge et al., 2010). We

124 also have evidence that they use the terrestrial skyline panorama formed by an arena

125 (Graham and Cheng, 2009). Thus, we suspected that given a conflict between celestial

126 and terrestrial panorama cues, ants without path integration information might integrate

127 the two sources of information. We also tested ants taken directly from the feeder, who

128 still have their vector to home based on path integration (full-vector ants). We present

129 the results for the full-vector ants in supplementary materials because they showed no

130 significant effect of the arena rotation and did not provide clear evidence for cue

131 integration.

132   In a second experiment, we displaced full-vector homing ants in their natural

133 habitat before their journey home to create conflicts between celestial and terrestrial

134 cues. Narendra (Narendra, 2007b) had effected such a manipulation, and found

135 suggestive evidence that the ants used both terrestrial and celestial cues for orientation.

136 We now provide a thorough and systematic assessment, using different release points

137 that degraded the familiar view to different extents. Full-vector ants were displaced to

138 three different release points (Fig. 2). The furthest release point from the feeder was at a

139 location with little resemblance in the terrestrial panorama to the scenery at the feeder.

140 This provided a control condition in which the ants were expected to use solely celestial

141 information for homing, an expectation that was borne out by the results about to be

142 reported. The other two release points, which are closer to the feeder, can then be

143 compared with the control condition to estimate the extent to which terrestrial and

144 celestial sources of information were used for homing. At these release points, the path

145 integration system based on celestial compass cues points in the feeder-nest direction,

146 while terrestrial cues based on the panoramic scenery points in the release-point-nest

147 direction. Zero-vector ants were also tested to ensure that the terrestrial cues at the two

148 closer release points could be and would be used. Past research has shown that when

149 trained in natural conditions, the navigation of zero-vector *M. bagoti* ants is dominated

150 by terrestrial cues (Graham and Cheng, 2009; Wystrach et al., 2012).

151 **Insert Fig. 2 about here**

152

153 **RESULTS**

154 *Experiment 1*

155 In Experiment 1, zero-vector ants had to navigate their way out of a circular

156 arena based on the irregular skyline of the arena wall, with the exit at approximately 90°

157 discrepancy with the beeline direction home. When the test arena was in the same

158 orientation as the training arena, zero-vector ants were significantly oriented ($r = 0.74$,

159 $N = 43$, $P < 0.05$, Fig. 3a). Interestingly, ants did not choose a direction that would

160 bring them directly to the arena exit (270°). Instead, they showed a systematic bias

161 towards a point of the arena wall centered at 298.0°. Full-vector ants also headed

162 initially in a similar direction (mean 308.8°, Fig. S1).

163 **Insert Fig. 3 about here**

164

165 When the arena was rotated by 180˚, celestial and arena cues indicated

166 diametrically opposite directions of 298.0° and 118.0°, respectively. In this condition,

167 ants were not significantly oriented ($r = 0.14$, $N = 45$, $P > 0.05$, Fig. 3c), and the

168 distribution appeared to be random with no sign of bimodality (Rayleigh test for

169 bimodality: $r = 0.08$, $N = 45$, $P > 0.05$).

170 When the arena was rotated by 90°, ants were significantly oriented (Fig. 3b; r

171 $= 0.71$, $N = 48$, $P < 0.001$). We compared the distribution in this condition against the

172 distribution found on the control test. Their mean directions differed significantly

173 (Watson-Williams test, $F_{1,89} = 5.80$, $P = 0.018$), indicating a significant influence of the

174 rotated arena. When the arena was rotated by 270°, ants were once again significantly

175 oriented (Fig. 3d; $r = 0.55$, $N = 45$, $P < 0.001$). Comparing the headings in this

176 condition against the headings found on the control test, their mean directions also

177 differed significantly (Watson-Williams test, $F_{1,86} = 26.11$, $P < 0.001$). These

178 comparisons show that both these groups were not using solely celestial cues for

179 orientation, and that terrestrial cues had an effect on their navigation.

180 To test whether the unrotated cues, the celestial cues, also influenced the initial

181 orientation of the ants in the 90° and 270° conditions, we then compared the distribution

182 in each of these conditions against the headings in the Control condition rotated by the

183 same amount, thus 90° against control distribution rotated by 90°, and 270° against

184 control distribution rotated by 270°. Both comparisons indicated significant differences

185 in mean direction from the respective rotated controls (Watson-Williams test, 90°

186 condition: $F_{1,89} = 45.73$, $P < 0.001$, 270° condition: $F_{1,89} = 5.91$, $P = 0.017$). Thus, in

187 both cases the celestial cues had an effect on initial orientation as well. The ants used

188 both terrestrial and celestial sources of information to determine a direction of travel.

189 *Experiment 2*

190   In Experiment 2, ants were attracted to a feeder in their natural habitat with an

191 unobstructed view. Both full-vector and zero-vector ants were displaced to 3 different

192 release point (RP) locations for testing, with one location (RP3) a long way away (64m)

193 where the ants were not expected to be able to use terrestrial cues.

194   Examination of ants tested at RP3 showed that full-vector ants were significantly

195 oriented ($r = 0.88$, $N = 72$, $P < 0.0001$, Fig. 4f) but zero-vector ants were not ($r =$

196 0.05, $N = 68$, $P > 0.05$, Fig. 4c). This shows that at this location the ants were only

197 using celestial information to orient. It also shows that zero-vector ants tested in these

198 conditions did not use the celestial cues to orient in the absence of directional dictates

199 from path integration. We used the distribution of the full-vector RP3 group as a

200 reference direction for the use of celestial cues.

201

202   **Insert Fig. 4 about here**

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204   We released zero-vector ants at RP1 (4m from the nest) and RP2 (32m from the

205 nest) to test if ants could use terrestrial cues to orient when information from path

206 integration, and its associated sky compass, was not available. We found that at both

207 RPs, ants were significantly oriented (RP1: $r = 0.79$, $N = 108$, $P < 0.001$; RP2: $r =$

208 0.47, $N = 75$, $P < 0.001$) towards the true nest direction, indicating that ants could use

209 terrestrial cues for orientation at these two locations. We used the distribution of these

210 groups as reference directions for the use of terrestrial cues at each release point.

211   We conducted tests on full-vector ants at RP1 and RP2 to test the ants'

212 orientation when celestial and terrestrial cues are placed in conflict. Celestial cues

213 would point in the feeder-nest direction, while terrestrial cues would point in the release

214 point-nest direction. Ants were significantly oriented at both release points (RP1: $r =$

215   0.66, $N = 111$, $P < 0.001$; RP2: $r = 0.81$, $N = 81$, $P < 0.001$). At RP1, the

216   distribution of headings differed significantly from both the full-vector RP3 group

217   (representing the use of celestial information) and the zero-vector RP1 group

218   (representing the use of terrestrial information) (Watson Williams test; vs. full-vector

219   RP3: $F_{1,181} = 112.45$, $P < 0.001$; vs. zero-vector RP1: $F_{1,217} = 134.68$, $P < 0.001$).

220   Likewise at RP2, the distribution of headings differed significantly from both the full-

221   vector RP3 group (representing the use of celestial information) and the zero-vector

222   RP1 group (representing the use of terrestrial information) (Watson Williams test; vs.

223   full-vector RP3: $F_{1,151} = 13.81$, $P < 0.001$; vs. zero-vector RP2: $F_{1,154} = 119.72$, $P <$

224   0.001). Thus, at both release points, ants with a conflict between celestial and terrestrial

225   information headed in an intermediate direction that differed significantly from the

226   dictates of either celestial cues or terrestrial cues.

227   Finally, we can estimate the weight placed on terrestrial vs. celestial cues at RP1

228   and RP2 by using the mean directions of those groups, and calculating the proportion of

229   angular distance they are from the reference directions representing the use of celestial

230   and terrestrial cues respectively. The full-vector ants at RP1 (4m distance from the nest)

231   placed double the weight on terrestrial cues as did the full-vector ants at RP2 (32m from

232   the nest; 0.36 vs. 0.18). Thus, at the nearer release point, more weight was placed on

233   terrestrial cues. Indeed, results with zero-vector ants show that the ants were more

234   accurate at using the terrestrial cues at RP1 than RP2 (circular Wilcoxon test on scatter

235   of zero-vector ants at RP2 vs. those at RP1: $Z = 4.43$, $N = 183$, $P < 0.001$).

236   **DISCUSSION**

237   In two experiments, we provided homing desert ants with conflicts between the

238   dictates of terrestrial and celestial cues for direction. In Experiment 1, this was done by

239   rotating the arena that constituted the terrestrial cues for zero-vector ants. In Experiment

240   2, this was done by displacing the ants from the feeder (full-vector ants) before letting

241   them return home. In both experiments, we found evidence that the ants' mean heading

242   differed significantly from the dictates of both the celestial and the terrestrial cues,

243   being in an intermediate direction. In Experiment 1, however, an intermediate mean

244   heading was only found with rotations of 90° (the 90° and 270° conditions). It was not

245   found when the terrestrial cues (the arena) were rotated 180°; in that case, the ants

246   showed a random distribution of headings.

247   Consistent with Collett (Collett, 2012), we interpret the results to mean that the

248   ants were using both kinds of cues in these test situations. Their headings were based on

249   a weighted average of the dictates of the two kinds of cues. The findings extend the

250   integration of multiple cues for navigation to another ant species tested in different

251   conditions. Integration of multiple cues has now been shown in *M. pyriformis* (Reid et

252   al., 2011), *M. croslandi* (Narendra et al., 2013), *C. fortis* (Collett, 2012) and *M. bagoti*

253   (current results), across a range of cue manipulations.

254   The 90° and 270° conditions in Experiment 1 differed in that in the former

255   condition, the ants placed more reliance (weight) on the unrotated (celestial) cues while

256   in the latter condition, they placed more reliance (weight) on the rotated (terrestrial)

257   cues. It is possible that this difference in weighting occurred due to the pattern of light

258   reflected off the arena wall by the sun. Perhaps by rotating the arena by 90°, the visual

259   appearance of the arena changed more than when the arena was rotated by 270°. If this

260   was the case, the informative value of the visual panorama, and hence the weight

261   assigned to it, was altered asymmetrically by the arena rotation. Future experiments will

262   be required to determine if this is indeed the case.

263   Experiment 2 also shows that the weight assigned to terrestrial vs. celestial cues

264   can vary, but in this case, a clear, a-priori explanation can be provided. At the release

265   point closer to the feeder, with presumably a panorama that is more familiar and more

266   similar to that at the feeder, the ants put more weight on terrestrial vs. celestial

267   directional cues. Evidence concerning the familiarity or similarity of the release sites

268   comes from the scatter of headings of zero-vector ants released at these two locations

269   (Fig. 4): the group released closer to the feeder had smaller directional scatter. If the

270   more familiar view is associated with a more reliable directional signal (Wystrach and

271   Graham, 2012), this pattern accords with Bayesian principles (Cheng et al., 2007;

272   Friedman et al., 2012; Körding 2007), at least qualitatively: the weight assigned to a cue

273   is in accord with its reliability.

274   In Experiment 1, when cues conflicted by 180°, the zero-vector ants were not

275   oriented. Two interpretations are plausible. One is that the ants were not engaged in any

276   systematic heading at all, but started a systematic search pattern, which might start in

277   any direction. This would presumably be caused by the large mismatch between the

278   dictates of celestial and terrestrial cues. The other interpretation is that the ants were

279   actually averaging the highly discrepant cues. But variable weights across animals

280 accorded to the two types of cues resulted in a random distribution. We can report that

281 under some conditions, *M. bagoti* ants do integrate different celestial cues at 180°

282 discrepancy (Wystrach et al., 2014). This took place in full-vector ants when the

283 position of the sun, one of the celestial cues, was mirror-reflected by 180°. According to

284 theoretical derivations based on Bayesian principles, however, cues that are too

285 discrepant should not be averaged, because the chances are that they are associated with

286 two different objects or sources rather than one (Körding et al., 2007). Perhaps this

287 species of ants integrate multiple cues no matter how discrepant they are, large

288 discrepancies being rare in their lives.

289   The way in which celestial cues are used may differ between the artificial arena

290 environment used in Experiment 1 and the natural environment used in Experiment 2.

291 In the arena, rotation of terrestrial cues provided by the panorama had no significant

292 effect on full-vector ants (see supplementary materials), but resulted in cue integration

293 by zero-vector ants. We assume that, in this case, the artificial terrestrial cues were

294 relatively weak and the path integration system based on celestial cues dominated

295 search for the full-vector ants. When the path integration system was inactivated for the

296 zero-vector ants, both celestial and terrestrial cues were used by the view-based system

297 and the information was integrated when the cues conflicted. In the natural environment

298 used in Experiment 2, full-vector ants integrated celestial-based path integration and

299 terrestrial information, suggesting that the natural terrestrial cues were sufficiently

300 salient to exert some control even when path-integration information was present.

301 However, celestial cues were not used to orient in the absence of path integration (zero-

302 vector ants), presumably because the natural terrestrial cues were salient and had

303 dominated during learning. Similar dominance of terrestrial cues for zero-vector ants

304 that had learned a natural panorama was found in Graham and Cheng's (Graham and

305 Cheng, 2009) study. That study suggested that it was the learning conditions that

306 mattered. Graham and Cheng replaced the natural panorama on tests with a black arena

307 approximating the skyline at the feeder. This impoverished test panorama had full

308 control of the zero-vector ants' orientation when it was rotated. Further research is

309 needed to verify these hypotheses.

310   The integration of cues is found across diverse taxa (Cheng et al., 2007),

311 reflecting perhaps some convergent evolution driven by functional Bayesian principles.

312 Among hymenopterans, various pieces of evidence suggest that honeybees integrate

313 multiple kinds of cues, for instance in their dance behaviour (von Frisch, 1948, 1949),

314 and in striking a heading after displacement (Menzel et al., 1998). Recent models of

315 insect navigation accommodate cue integration well (Collett, 2012; Cruse and Wehner,

316 2011; Wystrach et al., 2013). While these models posit a great deal of modularity, with

317 different subsystems devoted to processing different cues for particular tasks, they

318 suggest that the different 'modules' feed into a common centre that integrates the cues

319 and directs motor output. Computationally, this provides a locus at which weights may

320 be assigned to different cues. This process of assigning weights, however, should not be

321 interpreted too mentalistically as some decision process strategically parcelling out

322 weights. It might somehow emerge from more basic neurobiological processes of

323 information processing. Indeed, much work is needed to discover the neurobiological

324 bases of cue integration, and to elucidate how widespread it is in insect navigation.

325

326 **METHODS**

327 *Field site and study species*

328   Experimentation took place ~10km south of Alice Springs town centre, at a site

329 filled with tussocks, mostly of the invasive buffel grass (*Cenchrus ciliaris*), with a

330 scattering of bushes, trees, and some buildings. Nests of red honey ants (*M. bagoti*) are

331 found in the area, and one nest was used for each experiment (N = 181 in Experiment 1

332 and N=132 in Experiment 2). The species is highly thermophilic (Christian and Morton,

333 1992): when tested in a closed flask with 3mm of soil, its critical thermal maximum was

334 determined to be ~56.7°C. It forages solitarily in the heat of the day during the summer

335 months on a range of dead animals and plant materials (Muser et al., 2005; Schultheiss

336 and Nooten, 2013).

337 *Training set up for Experiment 1*

338   Ants were trained to visit a feeder provisioned with pieces of cookies and

339 mealworm. To encourage the ants to visit the feeder, we set up an enclosure that

340 surrounded both nest and feeder. The enclosure was constructed of plastic-coated

341 particle boards of 10 cm width, with the plastic side inwards. This prevented most ants

342 from exiting the enclosure and encouraged them to visit the feeder. We sank the boards

343 into the ground so that the tops were at ground level. We dug a trench on the inside and

344 filled it with debris. This allowed ants unobstructed views of the panorama as they

345 travelled to and from the feeder. Otherwise, we cleared the enclosure of debris and

346 vegetation regularly during experimentation.

347   The ants had to enter a circular arena to get to the feeder (Fig. 1). The outside

348 entrance to the arena was marked with two short black cylinders. These cylinders were

349 not visible from inside the arena (see Fig. 1B). The training arena (diameter $= 1.5m$,

350 maximum height $= 50cm$) was made out of 1mm Lexan™ polycarbonate resin

351 thermoplastic (colour: flat green). We placed it in the field so that its centre was 9.1m

352 south from the nest (compass bearing to the nest: 350°). The location of the arena

353 entrance/exit was located at a compass bearing of 270° relative to the nest entrance,

354 which was dug under the wall (Fig. 1A). The arena had irregular height in its walls (see

355 Figs. 1B and 1C). The feeder was a plastic box (~15 cm square and ~9 cm deep). We

356 coated it with fluon to prevent ants from climbing out when we wanted to test them.

357 During training, we placed sticks in the feeder as exit ramps for the ants. Around the

358 feeder, we placed a goniometer made out of the same Lexan material (30 cm radius).

359 This goniometer was transparent and lightly dusted with sand to mimic the natural

360 substrate.

361   We set up a second circular arena with the same pattern of wall height for

362 testing. This arena had no exit and no feeder. The goniometer in the test arena did not

363 have a hole where the feeder would be. It was divided into 24 sectors of 15° each. We

364 drew a circle of 30 cm radius on the goniometer.

365 *Procedure for Experiment 1*

366   On first arrival at the feeder, we marked each ant with a dob of acrylic paint on

367 the thorax or abdomen to mark the day on which it started training. We left ants to

368 forage naturally for at least one day before testing them. On a test, we allowed the tested

369 ant to enter the feeder, take a bit of food, and head home. Just before the ant entered the

370 nest, we captured it and placed it in the centre of goniometer in the testing arena. In

371 four different testing conditions, we rotated the test arena 0°, 90°, 180°, or 270°

372 relative to the training arena. We tested each ant only once, always in the

373 afternoon between 1300 and 1800 local time. We only tested ants that held on to a

374 piece of food, to ensure motivation for homing. Data gathered consisted of the sector

375 that the tested ant crossed at 30 cm after being released at the centre.

376 *Training set up for Experiment 2*

377   We again made an enclosure using particle boards, this time consisting of a

378 circle around the nest (1.5 m diameter) with a long extension down one side (Fig. 2A).

379 We removed the board joining the circle to the long extension when training ants.

380 Otherwise, we kept the ants within the circle surrounding their nests to limit their visual

381 experience. We used the same kind of feeder as in Experiment 1, this time placed 11m

382 south of the nest (direction from feeder to nest: 0°).

383 *Procedure for Experiment 2*

384   We again marked ants at the feeder with one dot of paint to identify the day at

385 which they started training. After at least one full day of training, we tested ants in one

386 of six conditions. In three of the conditions, we captured ants directly from the feeder

387 for testing on the three release points (full-vector ants). In the other three conditions, we

388 captured ants very close to the nest entrance for testing on the three release points (zero-

389 vector ants). All tested ants were loaded with food to ensure homing motivation.

390   We tested full-vector and zero-vector ants at the same three different RPs, whose

391 panoramic views are displayed in Fig. 2B. RP3 is a long way from the feeder, making

392 its view unfamiliar, and we expected the ants to rely completely on celestial information

393 for navigation at this site. RP1 and RP2 are closer, and at these release points, celestial

394 information based on path integration and terrestrial information based on view-

395 matching were in conflict. At each release site, we set up a wooden goniometer divided

396 into 24 sectors, and we released the ant at the centre of the goniometer (marked with an

397 ✕). Again, we noted the sector at which the ant crossed at 30 cm from the starting point.

398 In this experiment, we tested each ant twice, at 2 of the 3 release points, chosen at

399 random and balanced across ants.

400 *Data analysis*

401   We conducted circular statistics following Batschelet (1981), using either the

402 software Oriana (Kovach Computing Services, Anglesey, Wales, UK) or MATLAB

403 (MathWorks, Natick, MA, USA). We used Rayleigh tests to examine if ants were

404 significantly oriented in any direction in a condition, and the Watson-Williams test to

405 examine if the mean direction in two conditions differed. We set the alpha level at $P =$

406 0.05.

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410 **Acknowledgements**

425 **Competing Interests**

426　The authors declare that they have no conflict of interests.

427

428 **Author Contributions**

429　All authors made significant contributions to the conceptualization of this project,

430　including the precise question of study, design of the experiments, and analysis and

431　interpretation collected data. All authors also made significant contributions to the

432　preparation and editing of this manuscript. EL and AW conducted the two

433　experiments presented in this paper, and collected all relevant research data.

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596

597 **Figure captions**

598 **Fig. 1**. (A) A diagram depicting ants' nest relative to the training arena used in

599 Experiment 1. (B) An image of the training arena used in Experiment 1. The

600 channel outside of the arena extends from the nest to the arena wall to prevent

601 ants from foraging naturally. (C) 360° panoramic images taken from the centre of the

602 arena (bottom image). The top image is a 360° panoramic image taken at the same

603 location without the arena present. Note that the arena completely obscures

604 terrestrial cues from the natural skyline, but is open at the top so that natural

605 celestial cues remain available.

606 **Fig. 2**. (A) Diagram of the set up used in Experiment 2, showing the relative

607 locations of the nest, feeder, and release points (RP). Inset: the set up around the

608 nest and feeder. Channels were filled with brambles to discourage foragers from

609 leaving the main training path. (B) 360° panoramic images taken at the nest

610 entrance, and at the centre of each of the three release points used in

611 Experiment 2. Note that the images are ordered from top to bottom according

612 to their Euclidian distance from the nest entrance. Euclidian distance from the

613 nest entrance is indicated in parentheses for each image.

614 **Fig. 3**. Zero-vector ant directional bearings for each test in Experiment 1. Grey

615 circles indicate ants' directional bearings after crossing a marked goniometer line

616 30cm from a central release location. Open triangular markers indicate the exit

617 direction according to a learned route (celestial local vector). Closed triangular

618 markers indicate the exit direction according to ants' view-based navigational

619 system (terrestrial panorama). These were obtained by taking the closed triangle in

620 the no-rotation condition and rotating it by the amount of rotation in each of the

621 rotated conditions. Thus, these theoretical directions were determined by the

622 performance of the control group rather than specified a priori. The true feeder-nest

623 direction is 0° while the true feeder-exit direction is 270°. Headers above each

624 circular plot indicate the degree the testing arena was rotated relative to the

625 orientation of the training arena. When ants were significantly oriented, the 95%

626 confidence interval of the mean is displayed as a curved marker on the inside of
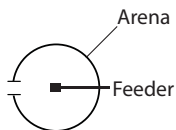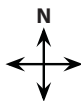
627 the circular plot.

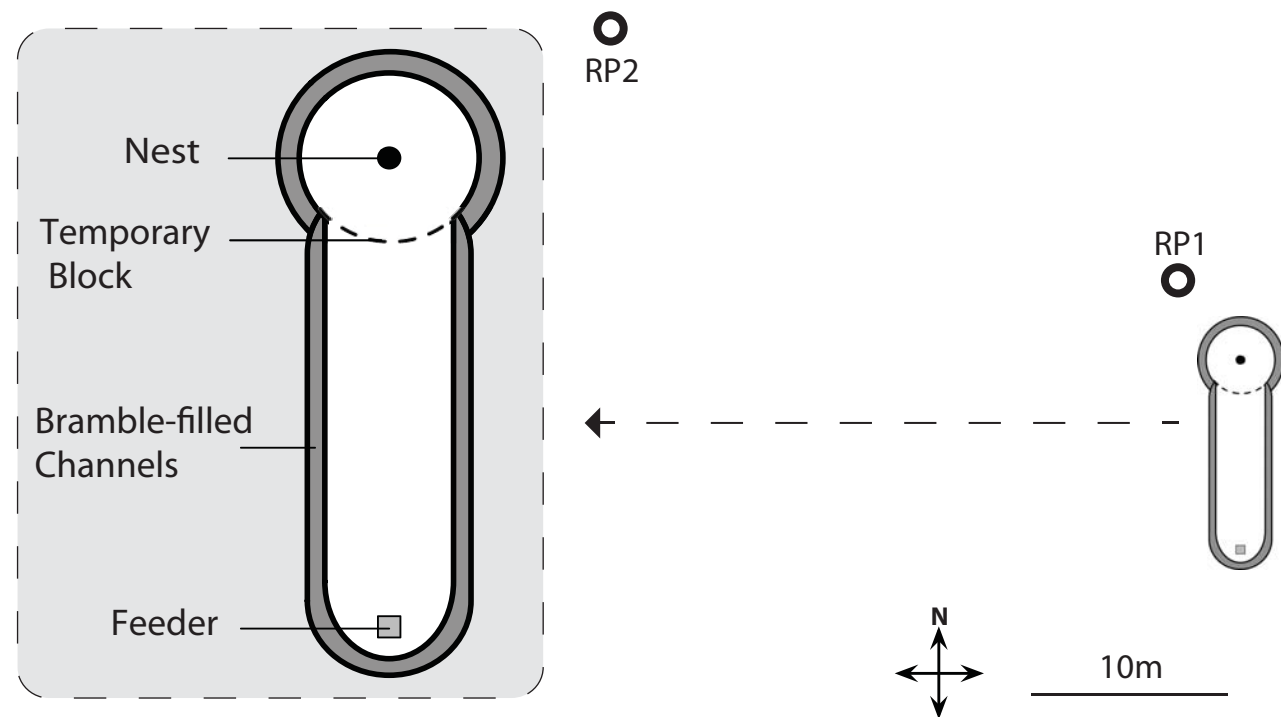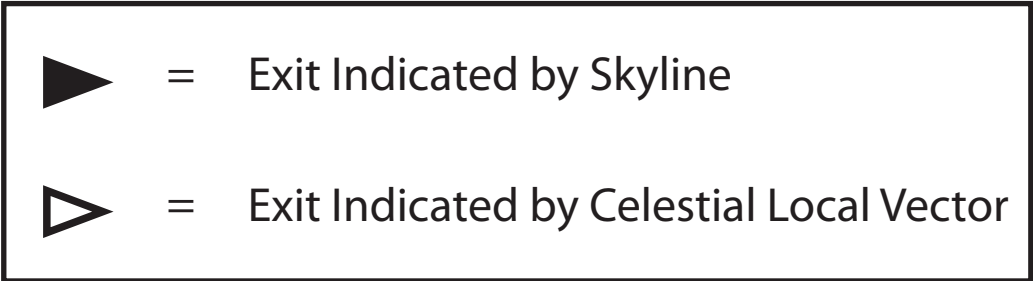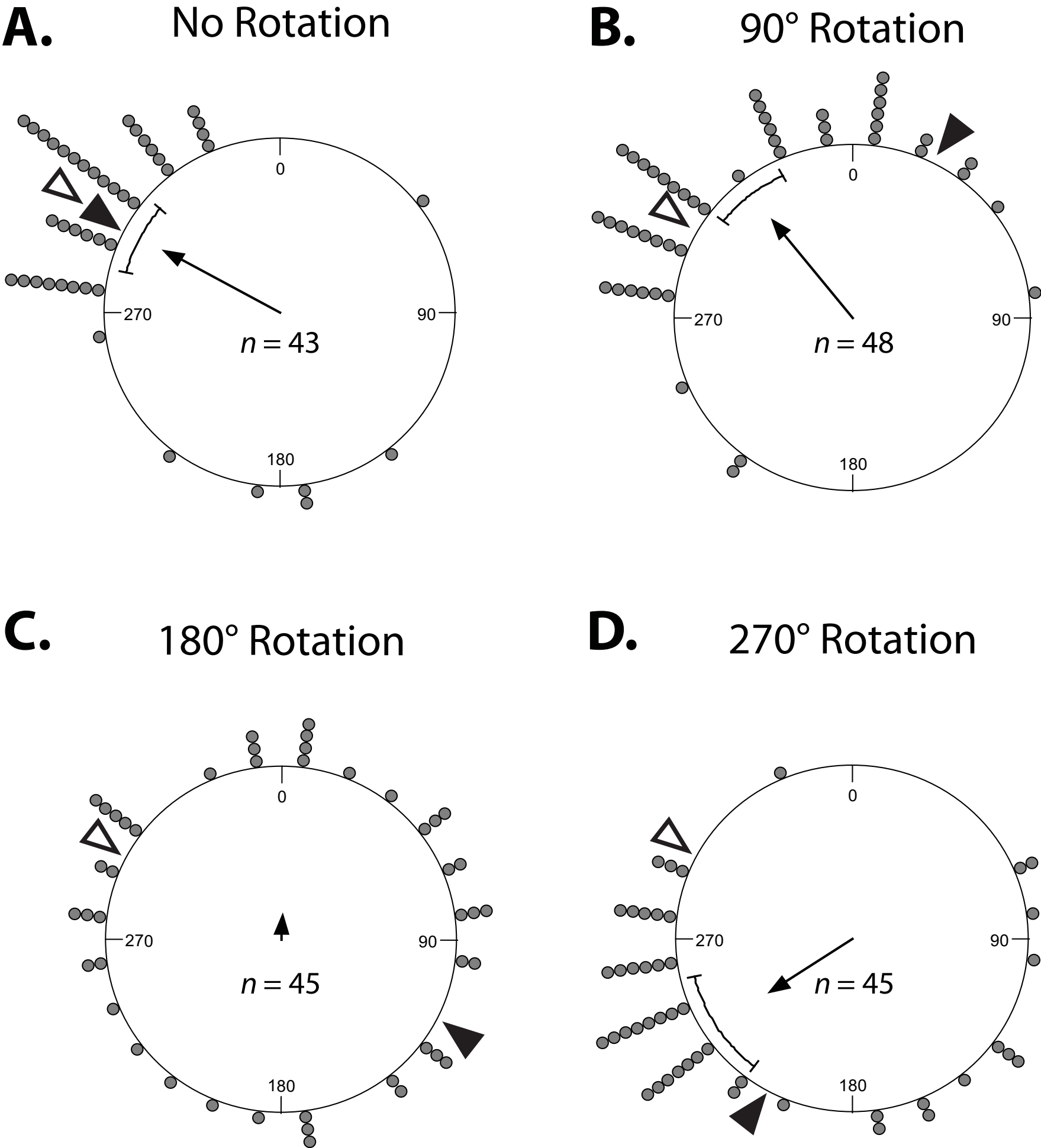628 **Fig. 4**. Ant directional bearings for each test in Experiment 2. Grey circles indicate

629 an ant's directional bearings after crossing a marked goniometer line 30cm from a

630   central release location. Open triangular markers indicate the nest direction

631   according to ants' path integration system (The true feeder-to-nest direction is 0°).

632   Closed black triangular markers indicate the nest direction according to ants'

633   view-based navigation system, while the true release-point-to-nest direction is

634   indicated by grey triangles. Note that there are no open triangular markers in panels

635   (A)-(C), as all ants tested here were zero-vector, and did not have access to path

636   integration information. Note that there are no closed triangular marker in panels

637   (F) and (C), as ants could not use view-based navigational cues to orient

638   themselves at this release point. When ants were significantly oriented, the 95%

639   confidence interval of the mean is displayed as a curved marker on the inside of the

640   circular plot.

A. Nest ●
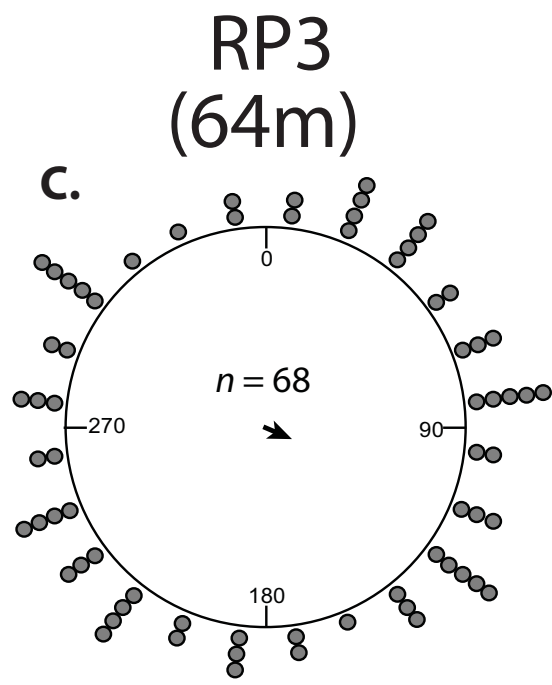
1m

N

Arena

Feeder

B.

C.

A.

Nest

Temporary Block

Bramble-filled Channels

Feeder

RP2

RP1

RP3

N

10m
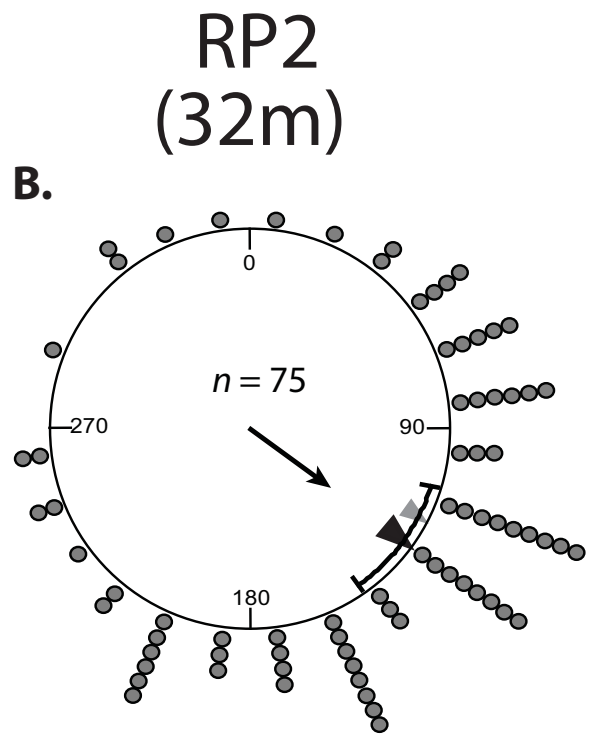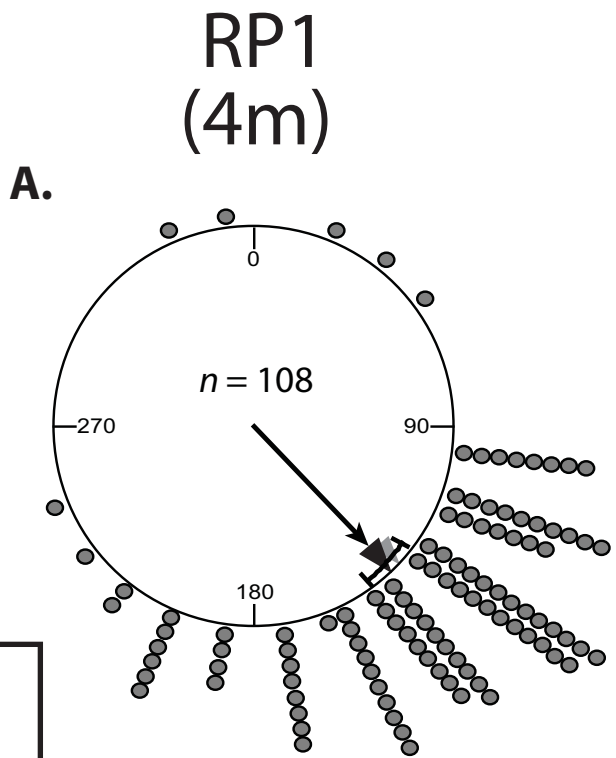
B. 360° Panoramic Site Images

View from nest

View from RP1 (4m)

View from RP2 (32m)

View from RP3 (64m)

Zero Vector Ants

A. No Rotation

n = 43

B. 90° Rotation

n = 48

C. 180° Rotation

n = 45

D. 270° Rotation

n = 45

▶ = Exit Indicated by Skyline

▷ = Exit Indicated by Celestial Local Vector