1 **Computer vision and deep learning automates nocturnal rainforest ant tracking to provide insight**

2 **into behavior and disease risk**

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11

12 **Abstract**

13 Determining how ant colonies optimize foraging while mitigating disease risk provides insight into how

14 the ants have achieved ecological success. Fungal infected cadavers surround the main foraging trails of

15 the carpenter ant *Camponotus rufipes,* offering a system to study how foragers behave given the persistent

16 occurrence of disease threats. Studies on social insect foraging behavior typically require many hours of

17 human labor due to the high density of individuals. To overcome this, we developed deep learning based

18 computer vision algorithms to track foraging ants, frame-by-frame, from video footage. We found

19 foragers can be divided into behavioral categories based on how straight they walk across the trail. Eighty

20 percent of ants walk directly across the trail, while 20% wander or circle when crossing the trail.

21 Departure from the main trail encourages exploration of new areas and could enhance discovery of new

22 food resources. Conversely, results from our agent-based model simulations suggest deviation from a

23 straight path exposes foragers to more infectious fungal spores. Consistency in walking behavior may

24 protect most ants from infection, while the foragers with increased exposure due to their mode of walking

25 could be a sufficient number of new hosts to sustain disease in this environment.

26

Introduction

27

28   Resource acquisition drives animals into new territories, while threat avoidance limits where animals

29   move. A consistent threat is the presence of infectious propagules of parasites and these are hypothesized

30   to be major determinants of the distribution of animals in the wild[1]. Examples of animals avoiding

31   pathogen contaminated areas span diverse taxa, from mammals to insects, implying anti-parasite behavior

32   is widespread[1–5]. Central place foragers are interesting in the context of parasite avoidance as they must

33   obtain food while avoiding threats with the additional constraint of returning to a defined location after

34   each trip. For volant central place foragers, like wasps, bees, bats and birds, much of the trip is through

35   the air likely reducing contact with infectious material. However, for taxa which walk on the ground (e.g.

36   ants), encounters with parasite propagules are presumably higher[6]. To effectively study such pressure, it is

37   crucial to use systems where we can study foragers in nature, surrounded by their naturally occurring

38   pathogens.

39   The foraging strategies of ants range from workers searching and retrieving food entirely

40   independently to obligately in a group[7]. Chemical trails commonly facilitate group foraging, and in some

41   cases, these chemical trails develop into semi-permanent trails known as 'trunk trails'[8]. Trunk trails

42   stimulate research interest largely from the perspective of the self-organization behavior of ants, such as

43   how ants regulate traffic[9–11]. Trunk trails have also been studied from the perspective of their temporal

44   and spatial dynamics as well as their energetic value in terms of efforts expended and resources

45   obtained[12,13]. Yet, studies have not investigated how utilizing the same trails day after day impacts the

46   exposure of ants to parasitism. Moreover, studies on ant foraging have largely occurred in a laboratory

47   setting, and of the work that took place in the field, most studies relied on human observation or

48   manipulated the environment in some way (see references in Supplementary Table S1). An ant species

49   that forages collectively and predictably in time and space would be useful to assess the relationship

50   between trail behavior and disease risk.

51   A potential system is the carpenter ant *Camponotus rufipes* in southeastern Brazil, which forms

52   trunk trails lasting for multiple months[14,15]. Colonies of this ant were recorded as having a chronic

53   infection by the fungal parasite *Ophiocordyceps camponoti-rufipides* across 20 months[16,17]. This fungus

54   manipulates foragers to leave the nest and die biting the underside of a leaf [17,18]. To complete its lifecycle,

55   the fungus must grow out of the ant cadaver and form a fruiting body that releases spores onto the ground

56   below that will infect other ants[18]. Cadavers are found attached to leaves surrounding the ant nest[17]. The

57   chronic nature of infection at the colony level means the spores of the pathogen are continuously in the

58   environment from the perspective of the foragers. The spores are curved and large (80-95 microns[16])

59   implying they do not travel far and land on the nearby trails once released from ant cadavers that hang

60   above trails. Spores germinate to produce infectious secondary spores on hairs (capilliconidia) which

61   attach to ants as they walk over them[19]. Thus, infection does not require a spore to hit an ant as it walks on

62   a trail below a cadaver. Instead, the trail substrate itself serves as the source of contamination.

63   Foragers of the carpenter ant *C. rufipes* mostly collect nectar from hemipteran secretions and

64   extrafloral sources[14,20]. The exploitation of a stable resource suggests that all foragers will emerge to walk

65   directly to the food source, utilizing trails near the colony entrance as a highway[15]. Evidence from other

66   systems demonstrate trunk trails as well organized for traffic flow[10]. Traffic is bi-directional on the trunk

67   trails of *C. rufipes*. Thus, we expect an even mixture of inbound and outbound ants as this is hypothesized

68   to increase flow[21]. If colonies can regulate the number of foragers on the trail to create a steady flow, we

69   expect forager speed to remain approximately constant throughout the foraging period as foragers are not

70   limited by the density of ants on the trail. Lastly, we are interested to see how the individual walking

71   behavior observed influences the likelihood of an ant encountering an infectious spore.

72   We set out to study trails of seven *C. rufipes* colonies in their undisturbed rainforest habitat with

73   both food sources and pathogens occurring at natural levels. We devised a system of recording trails using

74   infrared lights and modified cameras to contend with the nocturnal foraging of this species. To overcome

75   observer bias and ensure a larger body of data from which patterns may emerge, we used machine

76   learning to automate ant tracking. This provided us with a powerful dataset from which the movement

77   pattern of ants throughout a foraging period can be examined. We then characterized the forager

78   trajectories on speed, straightness, and direction. Based on these measurements, we were able to classify

79   ants on the trail into behavioral groups. Using an agent-based model based on our data, we suggest a

80   mechanism for the maintenance of disease in this system.

81

82   **Methods**

83   *Study site*

84   Fieldwork took place at the Research Station of Mata do Paraíso, Universidade Federal de Viçosa, Minas

85   Gerais, Southeast Brazil (20°48'08 S 42°52'31 W) between 10 and 25 January 2017. The carpenter ant

86   *Camponotus rufipes* is abundant in this area, forming trails lasting multiple months[14,15]. Trails of *C.*

87   *rufipes* are typically found on 'bridges' composed of woody debris, lianas and tree branches and are

88   rarely directly on the forest floor[15]. Ants forage at night and activity peaks in the early evening[15].

89

90   *Trail filming*

91   Trails from seven different *C. rufipes* nests were filmed between 10 and 25 January 2017. Nests were

92   selected based on their location and structure. Only nests found above the ground with nest material

93   clearly visible were used. Trails were filmed before a branching point from the main trail so that ants

94   were filmed coming directly from or towards the nest. In the case where multiple trails came from one

95   nest, the busiest trails were selected. The width of the branches filmed ranged from 0.8 cm to 7 cm (mean

96   ± standard deviation; 2.97 cm ± 2.53) and the length of the area filmed for all branches was

97   approximately 15 cm.

98   GoPro cameras (model: HERO 3+, GoPro, Inc., San Mateo, USA) with a modified infrared filter

99   (RageCams.com, Michigan, USA) were used for filming. Stakes were placed 30 centimeters from the

100   trails and 30 cm medium trigger clamps (DWHT83140, DeWalt, Towson, USA) were attached to the

101   stakes. Cameras were attached to clamps so that cameras were approximately 30 centimeters above the

102   trails looking down at the ants walking on the trails (Supplementary Fig. S1). An additional camera was

103   placed on the stake, looking sideways at the ants, to allow another perspective for behavioral analysis.

104   Filming lasted from 19:30 to 00:00 for 4-7 nights for each trail (Supplementary Table S2). Timing of

105   filming was based on previous work showing activity begins around 19:30 and peaks around 21:00[15].

106   Infrared lights (IR30, CMVision, Houston, USA) were connected 12-Volt 7Ah batteries (UP1270,

107   UniPower, São Paulo, Brazil) to allow illumination of the trail without disturbing the behavior of the ants.

108   The camera batteries lasted for approximately 1.5 hours, so the battery was changed once in the middle of

109   a filming period. Slight adjustments in where the trail was positioned in the video view would sometimes

110   occur at this time. Figure 1a shows an example image of a trail filmed and images of the remaining trails

111   filmed are found in Supplementary Figure S2.

112

113   *Automated ant tracking*

114   A total of 78 hours and 56 minutes of video were recorded for seven colonies across four nights

115   (Supplementary Table S2). We developed a machine learning approach to process and analyze these

116   videos using a deep learning based segmentation model that identified ants as they came onto the screen

117   and tracked them as they moved across the screen.

118   Our automatic ant tracking method contains two main processes: (1) detecting ants in each image

119   frame of all videos, and (2) building ant trajectories for every video based on the detected ants.

120   Commonly, deep learning schemes require a large amount of labeled ground truth data for model training.

121   Since our dataset is quite large (> 8 million image frames), we aimed to generate sufficient labeled data

122   for training our deep learning model without incurring excessive human labeling effort. Also due to the

123   large size of our dataset, common active learning based sample selection methods (e.g.[22]) are not efficient.

124   The goal of ant detection is to build ant movement trajectories and since ant trajectories normally span

125   multiple consecutive frames in videos, detected ant positions in earlier frames assist with ant detection in

126   later consecutive frames. That is, while ant detection forms a basis for building ant trajectories,

127   trajectories of detected ants may also help ant detection. Hence, we designed our trajectory building

128   procedure such that it not only can track detected ants but also can provide cues to indicate where (which

129   frames and locations) there might be inconsistencies in ant trajectories and difficult scenarios for ant

130   detection (e.g. densely clustered ants). We used such cues to select difficult cases from the frames for

131   labeling to improve the deep learning detection model as well as the ant detection results. Therefore, our

132   detection-tracking method consists of two rounds (with the second round improving the detection and

133   tracking results of the first round), and each round performs two major steps, ant detection and trajectory

134   building, as described below.

135

136   (1) Ant detection. This aims to detect ants in all the frames of the videos. We applied a novel object

137   detection and segmentation model, Mask R-CNN [23], to automatically detect ants in every frame.

138

139   (2) Ant trajectory building. Given the detected ants in each frame, the next step is to form ant trajectories

140   that connect detected ants frame-by-frame in videos. We formulated this ant trajectory building problem

141   as a *transportation problem*, that is, between every two consecutive frames in each video, we find an

142   optimal transportation (for ants) that corresponds to real movement of ants. In this transportation

143   formulation, each detected ant in frame K can be viewed as a 'supplier' and each detected ant in frame

144    $K+1$ can be viewed as a 'receiver'. The dissimilarity (based on spatial distance and appearance

145   difference) between ants in two consecutive frames is a measure of how much 'cost' it would take to

146   transport (move) one ant in frame K to another in frame $K+1$. The objective is to transport detected ants

147   (as many as possible) in frame K to frame $K+1$ with the minimum total cost. Optimal transportation based

148   tracking methods are known to be effective for tracking sets of moving and changing objects in image

149   sequences[24,25].

150

151   In the first round, we randomly selected frames to label as training data. This allowed us to quickly and

152   unbiasedly obtain data samples for training a decent detection model. We then applied the trained model

153   to all of the frames to produce ant detection results. Next, we conducted trajectory building on detected

154   ants to form the ant trajectories. Besides tracking ant movement, our trajectory building procedure in the

155   first round also provided cues for identifying inconsistencies in ant trajectories and difficult cases in the

156   frames for ant detection. In the second round, we applied training data selection to those difficult cases to

157   find additional frames for labeling, and the enlarged training dataset thus obtained was used to re-train the

158   Mask R-CNN detection model. The re-trained detection model was then applied to all the frames to

159   produce the final ant detection results, which were used to build the final ant trajectories in the videos.

160   To identify difficult cases for additional training data selection, we used the following set of

161   measures to capture possible errors in ant detection and trajectory results. (i) Ant speed: At a place where

162   ants usually do not move very fast but a fast movement is suggested by the optimal transportation

163   solution, this instance might indicate an error in ant detection. (ii) Missing ants in the middle part of a tree

164   branch: When the optimal transportation solution does not find a corresponding ant instance in the next

165   frame in the interior section of a tree branch, it might suggest a missing data point in ant detection. (iii)

166   Ant identification (ID) switching: Each detected ant was assigned an ID number; when multiple ants are

167   seen at spatially close interaction and slight changes on the dissimilarity scores among these ants give

168   largely different solutions for the optimal transportation problem, this might suggest an ant ID switch

169   error. Based on these observations and measures, our trajectory building process can help identify

170   difficult detection and tracking cases for additional training data selection to improve model performance.

171   Overall, our automatic ant detection and tracking method extracted the x and y coordinates in

172   pixels of detected ants in every frame and assigned each ant an identification number (Fig. 1a;

173   Supplementary Video S1). Ant identification numbers were used to form ant trajectories used in further

174   analysis.

175

176   *Error assessment*

177   To assess the accuracy of the computer model, we watched a subset of videos and determined the error

178   rate. GoPro cameras automatically divide footage into 26-minute-long videos, so one night of footage at a

179   single trail has 6 to 10 videos. This provides a way of checking the accuracy of the computer tracking at

180   random points throughout a night. We first error checked videos from the middle of the night (when the

181   trails should be busiest) to determine if the data from that colony was high enough quality to use in our

182   analysis. If the error rate was sufficiently low, we continued to error check all videos and nights for that

183   colony. To error check, we counted the number of ant trajectories with errors out of the first 15-30 tracked

184   ants. The number of ant trajectories checked varied because videos from early in the foraging period

185   sometimes had fewer ants.

186   To ensure consistency in the type of ant trajectories that were analyzed, trajectories beginning in

187   the middle of the field of video view were removed. This created uniformity between all colonies and

188   nights in the type of ants that were compared as it focused on the ants that made it from one end of the

189   trail to the other completely in the view of the video.

190

191   *Trajectory analysis*

192   We used R version 3.4.4 and RStudio version 1.1.447 for all analyses[26,27]. Ant location data was frame-

193   by-frame, so we used the native frame rate of the cameras (29.97 or 25 frames per second; the default

194   setting of the cameras varied) to convert the time in frames to seconds and then used the start times of

195   each video to convert it to real time (Supplementary Table S2). To convert ant location data from pixels

196   to centimeters, we placed a ruler in each video to determine the conversion factor (Supplementary Fig. 2).

197   To determine how individual ants were moving, we calculated the following variables: average

198   speed, overall direction, time on the trail, and straightness. Average speed was taken as the total distance

199   an ant travels while in the video over the time it takes for them to travel that distance. Overall direction

200   was whether the ant headed away from or towards the nest which we determined based on where the ant

201   entered and exited the video view. A variety of measures are used to determine the straightness or

202   tortuosity of an animal's movement path [28,29]. Ant movement on trunk trails is expected to move in an

203   oriented direction, and not be a random search path, thus we used the simplest measure, the straightness

204   index [29]. The straightness index (ST) is a ratio between the net displacement and total path length:

205        $$ST = d/L;$$

206   where d = the distance between the beginning and end of the path and L = total

207   path length.

208

Agent-based model

To assess the influence of foraging style on disease risk, we developed an agent-based model in NetLogo 6.0.2[30] based on the walking style of the ants in our videos (full details in Supplementary Materials). This model tested how walking straight influences the hypothetical number of spores an ant picks up. Spore density varied from 10% to 100% of the patches in the environment covered in spores. We varied spore density in 10% increments, leading to 10 different spore density conditions. The straightness of an ant varied from 0 to 1 in 0.01 increments, leading to 101 different straightness scores. The model was run 30 times for each combination of parameters (1010 total combinations) leading to a total of 30300 runs.

Statistical analysis

A linear mixed-effects models fit was used to assess whether the speed of ant changes over a foraging period. The model was generated using the lmer function in the R package' lme4'[31], with speed as the fixed effect and colony and date as the random effects. The package 'lmerTest'[32] was used to generate p-values. We checked the plotted residuals to ensure homoscedasticity prior to utilizing the results of the model. We used linear regression to analyze the results of the agent-based model, with the straightness value as the predictor of proportion of spores picked up in the environment with a log transformation to control for skew.

Data Availability

The original videos and data analyzed in this study will be accessible through ScholarSphere (https://scholarsphere.psu.edu/) upon publication of this study.

Results

Automated tracking performance

235
236
237 The automated tracking of ants in video frames resulted in 20,230,585 data points on ant movement. The

238 model had two types of accuracy against which it can be judged, relative to a human. The first is species

239 accuracy (detection accuracy) which is a measure of how well the model recognized the correct species of

240 ant. The model correctly detected *C. rufipes* ants with an accuracy of 97.86%. The model picked up other

241 insects or species of ants on the trail (false positive) or failed to detect a *C. rufipes* ant as it went across

242 the trail 2.14% of the time.

243   The second accuracy measurement is tracking accuracy. The computer had to detect *C. rufipes*

244 ants and follow them as they moved across the screen. If an ant moved in a straight line this required the

245 computer to recognize and track that ant for about 4 seconds or 120 frames. The computer assigned

246 identification numbers to individual ants to follow an ant as it travelled across the screen. The machine

247 learning model sometimes made errors in doing this. The computer may switch identification numbers

248 when ants walked too closely together (Supplementary Video S2). The average tracking accuracy for all

249 colonies was 78.70%. The tracking accuracy was the lowest for MP2 (40.0%), MP11 (31.7%), and MP17

250 (50.6%). Identification number switches commonly happened in colonies MP2 and MP11. These trails

251 were very thin and introduced more challenges in determining the trajectories of individual ants, so they

252 were removed from further analysis. We have additionally removed MP17 as an obstruction in the trail

253 led to ants departing from the branch and walking underneath leaves (Supplementary Video S3). Ants

254 disappearing under leaf debris made it difficult to track an individual ant. We have made all videos and

255 data available as we expect improved future machine learning models can make use of them.

256   The exclusion of these colonies brought the size of the dataset to 8,505,784 data points on ant

257 movement from four colonies: MP1, MP6, MP10, and MP16. The large reduction of the number of data

258 points from the elimination of 3 colonies can be attributed to the errors in these branches, where the

259 density of individuals in congested areas lead to a false inflation of the number of ants and overall data

260 points. The data points from the 4 included colonies represents the movement data for 64,499 ants. The

261   average tracking accuracy of the remaining colonies was 81.39% (MP1: 72.0%; MP6: 82.1%; MP10:

262   77.2%; MP16: 92.1%). Most errors were due to an identification number switching to a different ant

263   (8.28%). The high error rate for MP1 could be attributed to the darkness of the videos causing the model

264   to miss part of an ant's trajectory or failing to detect an ant in the dark areas of the trail. If we consider

265   only the errors where a number is on a wrong ant or a number is not on an ant, the accuracy improves

266   greatly (overall: 90.94%; MP1: 91.5%; MP6: 88.8%; MP10: 86.6%; MP16: 96.3%). We are mainly

267   concerned with the direction and shape of trajectories, and the main error that impacts an individual ant's

268   trajectory is when ants switch to the wrong identification number, so the second calculation of accuracy

269   rate is more reflective of this.

270

271   *Collective movement pattern*

272   Most ants walk on the same area of the available trail space (Fig. 1). Ants often follow each other,

273   walking across the same area (Supplementary Video S4). The trail usage pattern is consistent between

274   nights (Fig. 1c). The mean speed of all ants from all colonies and nights was 5.19 cm/s ± 1.61 (standard

275   deviation). The average speed of the colonies ranged from 4.74 cm/s to 5.62 cm/s and within colony

276   variability in speed was similar between colonies (mean (cm/s) ± standard deviation; MP1: 4.99±1.69;

277   MP6: 5.62±1.60; MP10: 4.88±1.53; MP16: 4.74±1.41). The results of the linear mixed effects model

278   showed that ant speed decreases by 0.50 cm/s ± 0.07 (standard error) throughout the night ($t_{(96.45)}$ = -7.12,

279   p < 0.0001) (Supplementary Fig. S3).

280

281   *Individual movement pattern*

282   Although most ants walked on the same area of the branch (Fig. 1b-c), there was a subset of ants that

283   walked differently based on the straightness score (Fig. 2). Based on the behavioral analysis of videos,

284   ants that had a straightness score of close to one walked straight across the trail as was expected (Fig. 2b;

285   Supplementary Video S5). We found that 80.8% of ants had a straightness score from 0.75 to 1 (n =

286   50,813). We labelled these ants as 'direct walkers'. Ants with an intermediate straightness score typically

287   made it from one end of the trail to the other, but spent time wandering and covering more area of the trail

288   (Fig. 2b; Supplementary Video S5). We labelled these ants as 'wanderers'. They represent 13.0% of ants

289   and had a straightness score from 0.25 to 0.75 (n = 8,194). By contrast, 6.2% of ants had a straightness

290   score of less than 0.25 (n=3,869). These ants with a very low straightness score typically circled on the

291   trail consequently entering and exiting on the same side of the video view (Fig. 2c; Supplementary Video

292   S5). We labelled these ants as 'circlers'.

293   The wanderers and circlers constituted the minority of records (13% and 6.2% respectively). We

294   observed these two behavioral phenotypes regardless of whether there were other ants in the area

295   (Supplementary Video S5). These ants often stopped and groomed or antennated the trail or air

296   (Supplementary Video S6). However, direct walkers were also observed stopping and grooming their

297   antennae (Supplementary Video S7). There was a significant effect of straightness group on time spent on

298   the trail for all three groups (Fig. 3e; one-way ANOVA; $F_{(2, 64495)}$ =14350, p < 0.0001). Post hoc

299   comparisons using the Tukey Test indicates circlers did not spend more time on the trail (mean=8.1

300   seconds, SD=6.85) than wanderers (mean=7.58 seconds, SD=3.59), but both spent significantly more

301   time on the trail than direct walkers (mean=3.88 seconds, SD=1.42).

302

303   *Temporal movement pattern*

304   The flow of all three groups of ants (direct walkers/wanderers/circlers) in and out of the nest was

305   approximately the same throughout the night (Fig. 3). There is a large increase in the number of direct

306   walkers on the trail throughout the night, while the number of wanderers and circlers throughout the night

307   is relatively constant.

308

309   *Agent-based model*

310   Based on the results obtained from our agent based model, walking straight significantly decreases the

311   proportion of potential spores an ant picks up in an environment (linear regression: $F_{(1, 30298)}$=5,458, p <

312   0.0001; Fig. 4a). The three different groups of foragers differed in the number of spores they pick up in

313   the environment regardless of spore density ($F_{(2, 30297)}$=21,208, p < 0.0001; Fig. 4b). Circlers pick up

314   significantly more spores than wanderers and wanderers may pick up significantly more spores than direct

315   walkers (Tukey Test; p < 0.0001).

316

317   **Discussion**

318   Our study utilized an unobtrusive filming set-up to record behavioral data on more than 64,000 ants

319   moving in a rainforest at night in an area of high disease pressure. The study design facilitated the capture

320   of natural ant behavior unaffected by either a laboratory environment or proximity to human observers.

321   Combining this approach with computer vision techniques increases the scale at which we can study

322   animal behavior. Using computer vision and deep learning we collected approximately 20 million ant

323   movement data points from 80 hours of nighttime video. A previous study, using humans to score the

324   positions of ants in each frame, required approximately 1,600 hours of human work to create a dataset of

325   6.9 million data points (Modlmeier et al., in review). Advances in camera technology improving our

326   nighttime recording capabilities along with increased computing power allowing machine learning to

327   identify individuals promotes research on natural animal behavior.

328   For our study on ant behavior in the context of disease transmission, the scale of this data

329   detected higher level patterns likely unobservable with a less detailed dataset. Our data shows ants

330   flowing in and out of the nest at approximately the same rate (Fig. 3). Work on harvester ants

331   (*Pogonomyrmex barbatus*) has shown that the feedback from returning foragers stimulates inactive

332   foragers to leave on a new trip[33]. Our even flow rate also validates work on Argentine ants (*Linepithma*

333   *humile*) showing ants exiting and entering the nest at approximately the same rate in the summer[34]. Ant

334   colonies operate through local interactions and without centralized control, so there is no authority

335   controlling when ants leave and return to the nest[35,36]. The lack of centralized control combined with the

336   even flow rate gives insight into the processes occurring within a nest, with returning foragers likely

337   stimulating new foragers to leave the nest.

338   Given that there is no centralized plan for foraging, it is impressive that the same foraging pattern

339   arises on different days (Fig. 1c). This consistent trail usage pattern, along with most ants walking straight

340   across the trail (Fig. 2a) likely emerges from the use of a chemical trail, which this species of ant (*C.*

341   *rufipes*) is known to use[37]. For ants to walk on the same area of the trail on different nights, the trail

342   pheromone must either persist between foraging periods or foragers repeatedly reinforce the fastest route

343   across the branch each night. While rare, we observed some ants on these trails during the daytime, and

344   other studies have observed *C. rufipes* foraging during the day[20]. This could allow the trail to be

345   reinforced around the clock. Alternatively, laboratory studies have demonstrated ants as preferentially

346   selecting the shortest route to food[38,39]. The path that receives more pheromone will be reinforced

347   quicker[40]. Thus, each night the portion of the trail that ants walk on fastest could reach a higher

348   concentration of trail pheromone quicker, leading to the pattern observed.

349   The texture of the tree branch could also drive the space usage pattern, as substrate and landscape

350   features impact ant locomotion[41,42]. Loreto et al. (2013) demonstrated, in the same population we studied,

351   that *C. rufipes* foragers in this environment prefer to walk on woody debris because they walk faster on

352   this material than on the forest floor (see Supplementary Video S8 for an example of how ants are

353   impeded on the forest floor). The type of wood could also make a difference, with ants preferring to walk

354   on areas of the trail that are least restrictive to their movement. Another pattern emerged through

355   investigation of the straightness index of the ants. As expected, the straightness index of most ants was

356   close to one (80.8%; n=50,813), indicating that they walked directly across the trail (Fig. 2). Ants may

357   prefer on the path that deviates the least from their original direction of travel[43]. Straighter individual

358   paths enhance information spread and increase the chance that an ant will find food[44,45], perhaps making

359   this pattern beneficial to the collective colony in resource acquisition.

360   Despite the dominance of ants walking straight across the trail, a proportion of foragers wandered

361   before making it across the trail and another group circled the trail and headed back in their original

362   direction (Supplementary Video S5). Other species of ants provide evidence of different roles within

363   foragers, such as patrolling, trail maintenance, and defense. Patrollers in harvester ants are the first to

364   leave the nest in a foraging period and determine which trails the colony will use that day[46]. However, if

365   the subtypes in this study were patrollers, we would expect there to be more of them at the beginning of

366   the night which is not the case (Fig. 3). The leaf cutting ant *Atta cepahlotes,* which also forms consistent

367   trails, has a special class of foragers involved in trail maintenance[13,47]. Ants were observed carrying leaves

368   (Supplementary Video S9), although this could be for nest material and not trail cleaning. Another role

369   could be maintaining the pheromone trail. For example, *Atta sexdens* minims help with the pheromone

370   trail instead of food transport[47]. Ants were observed dragging their gaster on the trail likely depositing

371   trail pheromone (Supplementary Video S10). However, it seems unlikely wanderers and circlers were

372   involved in maintaining the pheromone trail, as they should walk straight across the trail to ensure the

373   pheromone trail was on the most direct path.

374   The subtypes could also be involved in defense. Wanderers and circlers spent more time on the

375   trail (Fig. 2c) and were observed stopping and antennating (Supplementary Video S8). Smaller workers

376   hitchhike on leaf fragments carried by larger workers in *Atta colombica* leaf-cutting ants, and this likely

377   serves as a defense against parasitoid Phorid flies[48]. Flies, that could possibly be parasitoids, were

378   observed closely following ants on the trail and in some cases appearing to land which may indicate

379   laying eggs on the ants which later become endoparasitoids (Supplementary Video S11) although the

380   prevalence of parasitoid flies attacking *C. rufipes* is unknown. We have observed adult ants infected by

381   decapitating phorid flies in our study area (Supplementary Video S12).

382   Conversely, the forager variation in walking straight may not indicate different roles within the

383   colony and instead demonstrate differences in response plasticity, as individuals may differ in their

384   detection of the pheromone trail. Bumblebees (*Bombus terrestris*) vary in their antennal sensitivity to

385   odors and different behavioral thresholds have been found for castes of the ant *Pheidole pallidula*[49,50].

386   Heterogeneity can be beneficial to the collective colony in tasks such as selecting a new nest[51]. In the

387   context of trunk trail foraging, it could encourage exploration and increase the chance of discovery of

388   high value food items. *Camponotus rufipes* typically uses trunk trails to exploit dependable food

389   resources such as hemipteran secretions or extrafloral secretions. If the colony always remains on the

390   trunk trails, they might deplete their dependable source of food and not have a suitable alternative.

391   Argentine ants are able to adapt trails in response to resource availability[52]. We suggest wandering and

392   circling as a mechanism for *C. rufipes* ants to similarly respond to changes in resource availability. We

393   filmed only a small area of the foraging trails, providing a brief snapshot of an ant's behavior. To know

394   whether wanderers or circlers are more likely to wander from the trail and discover new food resources,

395   one would need to follow individual ants during an entire foraging trip, which was beyond the scope of

396   this study.

397   Following individual ants for their entire foraging trip would also clarify whether the straightness

398   groups represent fixed behavioral groups or if they just demonstrate variation in individual behavior over

399   time. Campos et al. (2016) studied the activity patterns of *Aphaenogaster sensilis* ants and found foraging

400   trajectories to be descriptively similar with individual temporal activity patterns showing greater

401   variation. In the context of our study, perhaps all ants engage in wandering or circling behavior on these

402   trails, and it is related to their temporal activity pattern and not their behavioral role.

403   Regardless of whether these are fixed subtypes within the colony, variability in walking behavior

404   could impact the maintenance of disease in this environment. Fungal infected cadavers surround these

405   trunk trails, likely dropping spores directly onto the trails below[17]. It is not possible to quantify the

406   abundance and distribution of micron sized spores on trails in a forest, but the long term tracking of

407   cadaver abundance and the proximity to the trails implies spore presence on the foraging trails[17].

408   According to our simulations, walking in a straight line reduces a forager's risk of picking up spores (Fig.

409   4). If all ants walked in exactly the same straight line, this could prevent the disease cycle from

410   continuing, especially since the first ants would initially clear all of the spores off. Yet, this does not

411   occur as the circlers and wanderers deviate from the straight path increasing their probability of picking

412   up spores and maintaining a chronic infection of the colony.

413   If the risk of infection is larger for the circlers and wanderers (Fig. 4), why do these subgroups

414   still exist? Social insects have members of the colony known as scouts that assist in discovering and

415   recruiting the colony to new food sources[54–57]. The wandering and circling behavior observed in this study

416   could reflect the individual ant's role in food discovery, instead of food retrieval. Colonies with this

417   variability in forager behavior are perhaps better able to obtain resources, improving their reproductive

418   success and maintaining the diversity in forager behavior. Simultaneously, it allows persistence of the

419   fungal parasite in the system, but the loss these ants is likely not enough to have a selective impact on the

420   colony, as it is only a small percentage of foragers as suggested by Loreto et al. (2014). In the harvester

421   ant *Pogonomyrmex owyheei*, less than 10% of the colony foraged and it was usually less than 6% at one

422   time[58]. Reproductive success was also hardly impacted when harvester ant foragers were restricted from

423   foraging[59] implying colonies usually gather more than enough food and fitness would likely not take a

424   huge hit from the loss of a few foragers. Scharf et al (2017) additionally demonstrated that colony fitness

425   (as measured through the number of reproductive individuals produced) remained unchanged from

426   parasitic infection. In our system, relatively few foragers appear to be infected and killed by the parasite[17].

427   In addition, the density of spores in the trails could be very low, since the trail network occupies less than

428   2% of the nest surrounding area[15]. Understanding the direct relationship between ant cadavers (from

429   where spores are shot) and the trails (where new hosts are found) would improve our agent-based model

430   predictions and help to understand the importance of wanderers and circlers for colony disease risk

431   management.

432   Although these behavioral subtypes are only a small proportion of the colony, that small

433   proportion could be more than enough ants to sustain a parasitic fungus. Understanding how variation in

434   behavior influences pathogen risk provides information on the factors that shape the distribution of

435   animals in time and space. Computational techniques serve as a way to collect large datasets on animal

436   behavior, where one can begin to unearth the complex interactions between an animal and its habitat.

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440   **Figures**
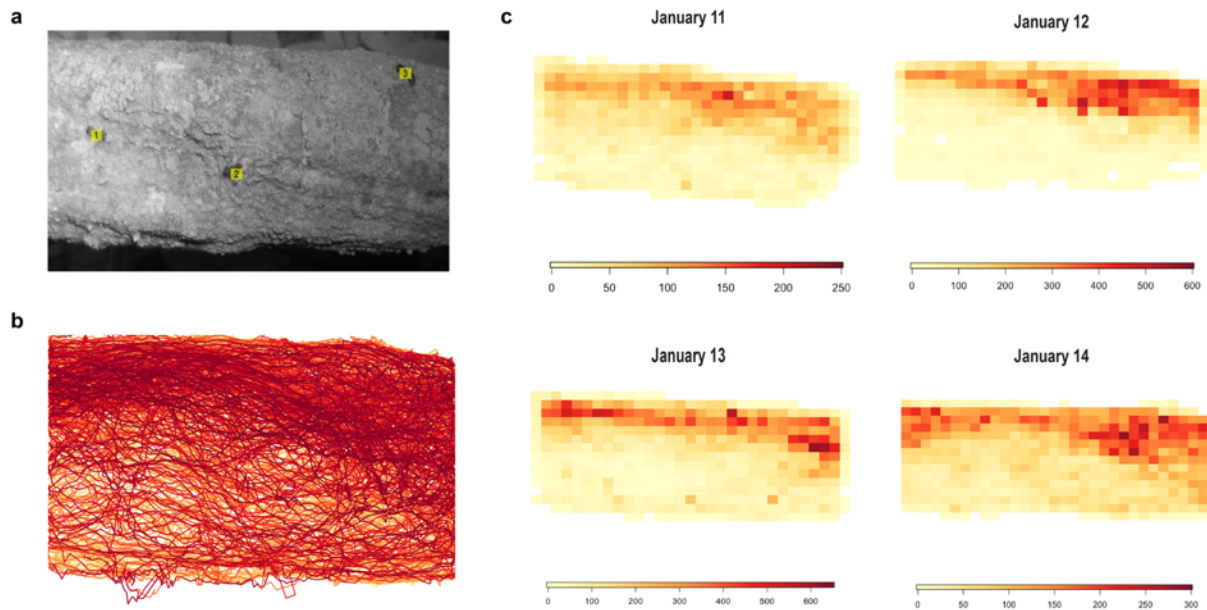
441

442   **Figure 1. Trail image, trajectory overlay, and collective movement pattern. (a)** Example image of a

443   trail filmed taken from GoPro footage from colony MP1. Ants are labeled with identification numbers.

444   **(b)** All of the trajectories from a single night of footage (January 14) at colony MP1. Each line across the

445   trail represents a different ant, with the different colors distinguishing between different ant tracks. **(c)**

446   The trail space from (a) was divided into a grid with each square representing approximately 1cm^2. The

447   number of times an ant walks into a square of the grid was calculated and the darker colors represent

448   areas of the trail that ants walked over more. Each heatmap represents a different date (January 11

449   through January 14) from approximately the middle of the night to control for differences in the timing of

450   filming. Different scales were used for each night, due to variance in the number of ants that walked

451   across the trail.

452
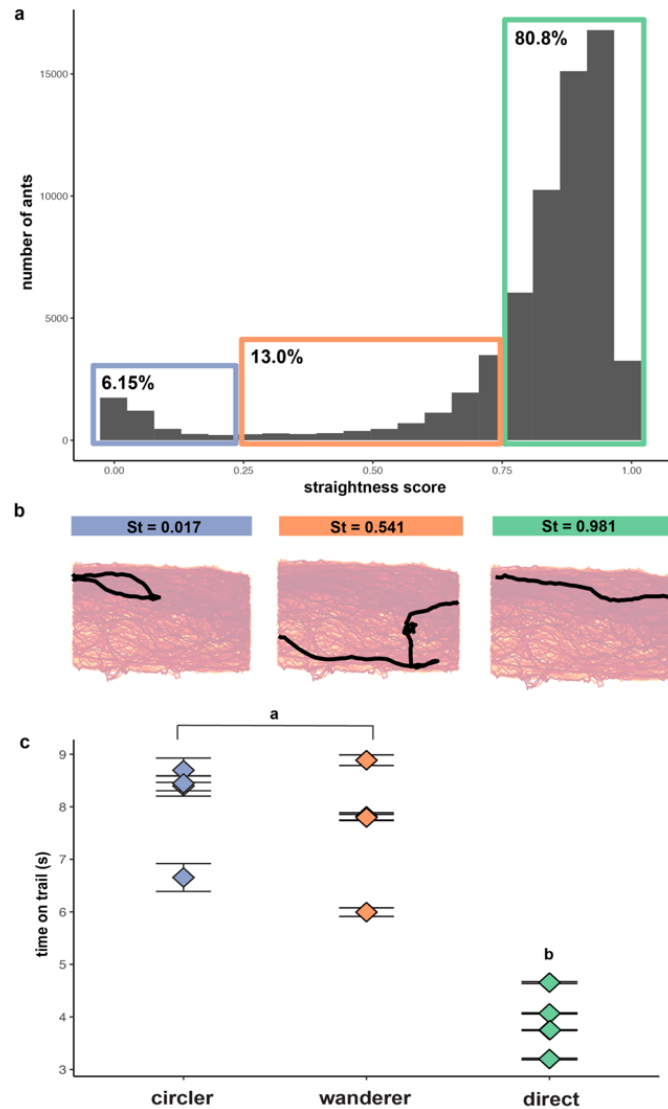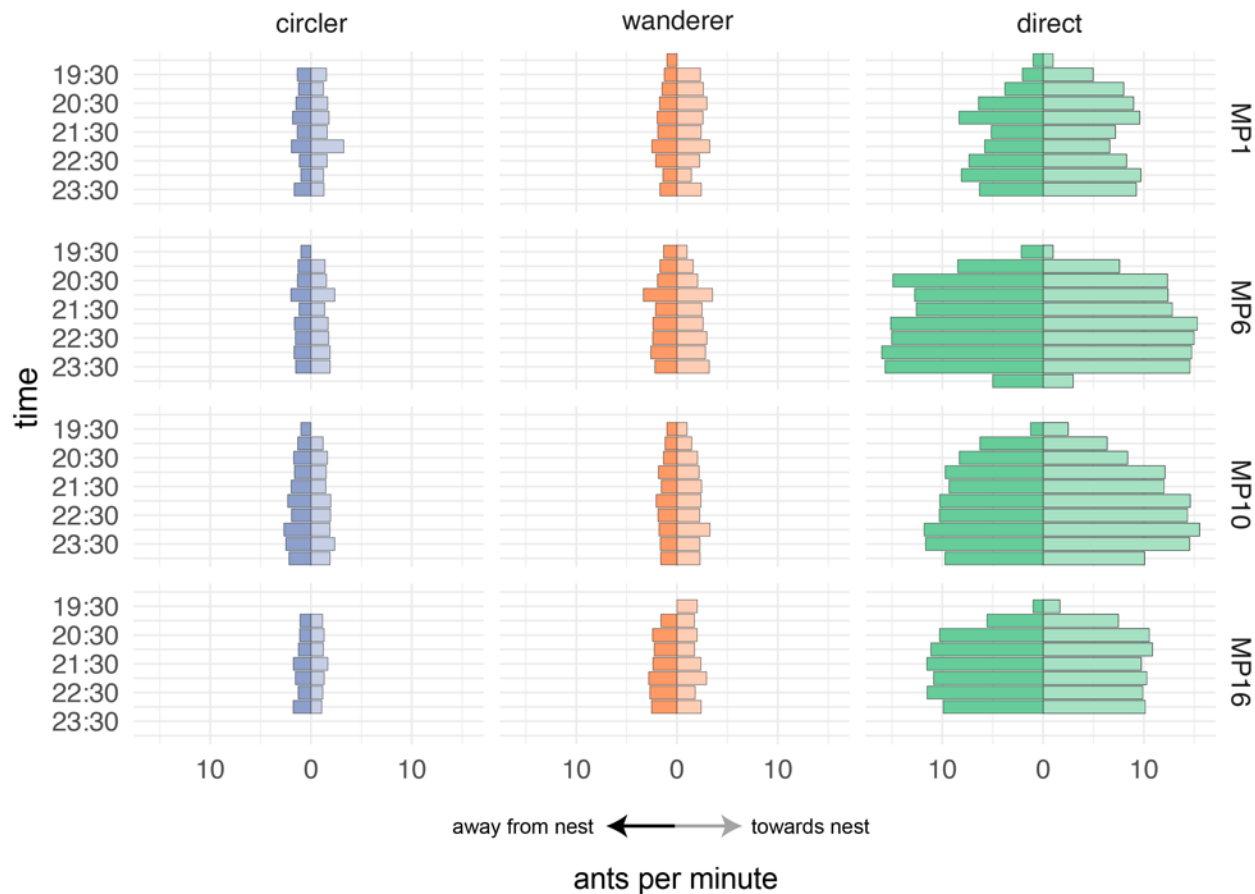
Figure 2. **Different behavioral groups based on straightness score. (a)** Histogram showing the distribution of straightness scores for all nights and colonies. **(b)** Example trajectories for a circler, wanderer and direct walker highlighted over all of the trajectories shown in Figure 1b. The straightness score (St) for that trajectory is included above. **(c)** Mean time spent moving across the trail in seconds for each different behavioral group and colony ± standard error of the mean. Different points within a behavioral group represent different colonies. Superscripts indicate groups as significantly different (p < 0.0001).

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Figure 3. Distribution of different behavioral groups over time. Mean number of ants per minute in

each behavioral group in a 30-minute period going either away from the nest or towards to the nest.

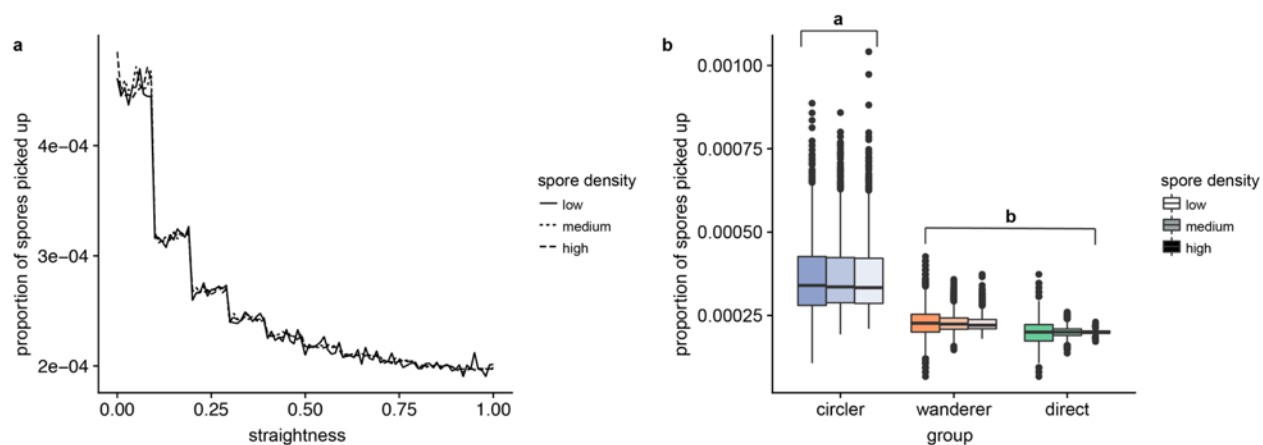Averaged across all nights for each colony. Right side numbers represent different colonies.

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Figure 4. Risk of spore exposure for different behavioral groups. (a) Mean proportion of

spores picked up as simulated ants in the agent-based model walk across the trail with different

straightness scores **(b)** Data from (a), with straightness divided into behavioral groups.

Superscripts indicate groups as significantly different (p < 0.001).

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629   **Author Contributions Statement.** D.P.H., R.G.L, and N.I. conceived and designed the study. N.I. and

630   C.K. performed the field work with technical input from R.GL.. Y.Z. and D.Z.C. created the computer

631   model and processed the data. N.I. analyzed the data and wrote the manuscript with guidance from

632   D.P.H.. All authors reviewed the manuscript.

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634   **Additional Information.** *Competing Interests:* The authors declare no competing interests.

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