1 **Title: Walking kinematics in the polymorphic seed harvester ant *Messor barbarus:***

2 **influence of body size and load carriage**

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4 **Running title: Kinematics of load carriage in ants**

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15 Summary statement: Differences in body geometries do not explain the differences observed

16 in load carrying performances in different sized individuals in the polymorphic ant species

17 *Messor barbarus*.

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Abstract

Ants are famous in the animal kingdom for their amazing load carriage performances. Yet, the mechanisms that allow these insects to maintain their stability when carrying heavy loads have been poorly investigated. Here we present a study of the kinematics of loaded locomotion in the polymorphic seed-harvesting ant *Messor barbarus*. In this species big ants have larger heads relative to their size than small ants. Hence, their center of mass is shifted forward, and the more so when they are carrying a load in their mandibles. We tested the hypothesis that this could lead to big ants being less statically stable than small ants, thus explaining their lower load carriage performances. When walking unloaded we found that big ants were indeed less statically stable than small ants but that they were nonetheless able to adjust their stepping pattern to partly compensate for this instability. When ants were walking loaded on the other hand, there was no evidence of different locomotor behaviors in individuals of different sizes. Loaded ants, whatever their size, move too slowly to maintain their balance through dynamic stability. Rather, they seem to do so by clinging to the ground with their hind legs during part of a stride. We show through a straightforward model that allometric relationships have a minor role in explaining the differences in load carriage performances between big ants and small ants and that a simple scale effect is sufficient to explain these differences.

Key words

Biomechanics, Kinematics, Locomotion, Load transport, Ants, Allometry

Introduction

The locomotion of animals can be described as a succession of strides that follows a specific inter-leg coordination pattern called gait (Alexander, 2003). In hexapod animals such as insects the most common gait is the alternating tripod (Delcomyn, 1981), in which the animal walks by alternating the movement of two distinct sets of legs (the ipsilateral front and hind leg and the contralateral mid leg, e.g. L1, L3, R2 and R1, R3, L2 respectively, with L for left and R for right), each of which forms a tripod supporting the body. In its ideal form, the two tripods perfectly alternate: all the legs in one tripod group simultaneously lift-off while all the legs of the other tripod group are still on the ground. However, depending on various features of their locomotion, insects can also adopt more complex gait. For example, the pattern of leg coordination can change with locomotory speed (Bender et al., 2011; Wosnitza et al., 2012; Mendes et al., 2013; Wahl et al., 2015), walking curvature (Zolliköfer, 1994a) and direction of movement, i.e. forward or backward movement (Pfeffer et al., 2016). Insects also adapt their gait according to the features of the terrain on which they are moving, e.g. when they walk on a non-level substrate (Seidl and Wehner, 2008; Reinhardt et al., 2009; Grabowska et al., 2012; Ramdya et al., 2017; Wöhrl et al., 2017) or when they climb over obstacles (Watson et al., 2002). Another factor that is known to have an effect on leg coordination during locomotion in terrestrial vertebrates (Jagnandan and Higham, 2018) but that has been less studied in insects is the change in the total mass an individual has to put in motion. Changes in total mass can be progressive or sudden and can occur in a variety of situations. For example, it happens in female insects during egg development and after oviposition, autotomy, i.e. the voluntary shedding of a body segment (Fleming and Bateman, 2007; Lagos, 2017), or, in the most common case, when insects are transporting food, either internally, after ingesting liquid, or externally, in their mandibles. In all these situations the change in total mass induces a shift in the center of mass of the insect which can profoundly affect its locomotion.

Ants offer a very good model to study the effect of changes in total mass on walking kinematics for at least three reasons. First, they are famous for their load carriage performances and can routinely carry loads (prey items, seeds, nest material, nestmates and brood) weighing more than ten times their own mass over tens of meters (Bernadou et al., 2016). In addition, the food they collect can be transported internally or externally. The shift in their center of mass can thus vary both in intensity and direction, which is likely to disrupt their walking kinematics in different ways. Second, due to the high number of species in their

72   taxon (Hölldober & Wilson, 1990), the size and shape of ant bodies is extremely variable,

73   which probably affects differently the kinematics of their locomotion. And third, ants live in

74   very diverse environments and can be subterranean, ground-living, or arboreal (Hölldobler

75   and Wilson, 1990), which is bound to constrain their movements and affect their locomotion

76   differently (Gravish et al., 2013; Seidl and Wehner, 2008; Reinhardt et al., 2009; Wöhrl et al.,

77   2017).

78   The effects of changes in total mass due to load carriage on the walking kinematics of ants

79   have been poorly explored in the ant literature. The main effect of carrying a load in the

80   mandibles is to induce a forward shift of the center of mass of the system formed by the ant

81   and the load they carry. However, according to Hughes (1952), insects could counter this

82   effect and achieve balanced locomotion by using static stability, i.e. by keeping the planar

83   projection of their center of mass within the polygon formed by the legs simultaneously in

84   contact with the ground (called polygon of support). In fact, this is what loaded ants do. For

85   example, *Cataglyphis fortis* workers ensure static stability by placing their front legs in a

86   more forward position when loaded than when unloaded and by reducing their stride length

87   (Zollikofer, 1994b). In the species *Atta vollenweideri,* whose foraging workers carry

88   elongated pieces of grass over their head, ants increase their mechanical stability by

89   increasing the number of legs simultaneously in contact with the ground. They do so by

90   increasing over consecutive steps the overlap between the stance (retraction) phase of the

91   supporting tripod and some of the legs of the other tripod (mostly the front leg) and by

92   dragging their hind legs during the swing (protraction) phase (Moll et al., 2013). These ants

93   also adjust the angle of the load they carry so that the planar projection of their center of mass

94   remains within the polygon of support (Moll et al., 2013).

95   In this paper, we studied the effect of load carriage on the walking kinematics of the seed-

96   harvesting ant *Messor barbarus*, an ant species that is characterized by a highly polymorphic

97   worker caste, i.e. a high variability in the size of individuals within the same colony. In

98   addition, this polymorphism is characterized by allometric relationships between the different

99   parts of the body (Bernadou et al., 2016), which means that big workers are not an enlarged

100   copy of small workers but that the growth of some of their body parts during development is

101   different from that of small workers (Bonner, 2006). In fact, relative to their mass their legs

102   are shorter and their head bigger than those of small workers. Here, we hypothesized that this

103   allometry could lead to differences in unloaded and/or loaded locomotion in different sized

104   workers and thus could explain the differences observed in their load carriage performances

105 (Bernadou et al., 2016). To test this, we ran an experiment in which we compared the

106 kinematics of workers tested first unloaded and then loaded with loads whose relative mass

107 we varied in a systematic way across different sized ants

108 **Materials and methods**

109 *Studied species and rearing conditions*

110 We used workers from a large colony of *M. barbarus* collected in April 2018 at St Hippolyte

111 (Pyrénées Orientales) on the French Mediterranean coast. *Messor barbarus* is a seed harvester

112 ant whose mature colonies can shelter several tens of thousands individuals (Cerdan, 1989).

113 Its workers display a polymorphism characterized by a continuous monophasic allometry

114 between head mass and thorax length (Heredia and Detrain, 2000; Bernadou et al., 2016).

115 Individuals range from 1 to 40 mg in mass and from 2 to 15 mm in length. The colony was

116 kept in a box (LxWxH: 0.50x0.30x0.15 m) with Fluon® coated walls to prevent ants from

117 escaping. Ants nested inside test tubes covered with opaque paper. They had access *ad libitum*

118 to water and to seeds of various species (canary grass, niger, oats). The experimental room

119 was kept at a constant temperature of 28°C and under a 12:12 L:D regime.

120 *Experimental setup*

121 The setup we used in our experiment was designed and built by a private company (R&D

122 Vision, France. http://www.rd-vision.com). It consisted in a walkway (160 x 25mm) covered

123 with a piece of black paper (Canson®, 160g/m^2) on which the ants were walking during the

124 experiment. The walkway was surrounded by five synchronized high speed video cameras

125 (JAI GO-5000M-PMCL: frequency: 250Hz; resolution: 30µm/px for the top camera,

126 20µm/px for the others), one placed above the walkway and four placed on its sides (Figure

127 1). Four infrared spots (λ=850nm), synchronized with the cameras, were used to illuminate

128 the walkway from above, allowing a better contrast between the ants and the background on

129 the videos. The temperature on the walkway was monitored with an infrared thermometer

130 (MS pro, Optris, USA, http://www.optris.com). Over the course of the experiment the mean

131 temperature was (mean ± SD) 28 ± 1.4 °C.

132 *Experimental protocol*

133 All experiments were performed between April and July 2018.

134 On the first day of a week in which we tested ants, we installed a bridge between the colony

135 and a box in which a few seeds were placed. We then collected during one hour one ant out of

136 three that carried a seed back to the colony. These ants were kept apart and used for the

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137   experiment in the following days. We also collected a few ants (weighing between 10-15 mg)

138   to dissect their Dufour gland in order to create an artificial pheromone trail in the middle of

139   the walkway (Heredia and Detrain, 2000). Since ants tended to follow the trail this increased

140   the chance to obtain a straighter path in the middle of the walkway, which allowed us to

141   neglect the effect of path curvature on ant kinematics (Zollliköfer, 1994a). In order to extract

142   the Dufour gland, ants were first anesthetized by placing them in a vial plunged in crushed

143   ice, then killed by removing their head and fixed on their back with insect pins under a

144   binocular microscope. Their gaster was opened transversally with a scalpel following the first

145   sternite and the ventral part was pulled away. The poison gland and the fat gland were then

146   gently removed until the Dufour gland became visible. This latter was then collected and

147   placed in a hexane solution to extract the trail pheromone. We used a concentration of 1 gland

148   / 20µl which has been shown to be sufficient to elicit a clear trail following response in *M.*

149   *barbarus* workers (Heredia and Detrain, 2000).

150   Each time an ant was tested, it was picked from the group of ants that had been separated on

151   the first day of the week, then weighed and isolated in a small box with access to water. The

152   ant was first tested unloaded and then loaded with lead fishing weights whose mass ranged

153   from 2 to 100mg. Every five tested ant we made an artificial trail on the walkway by

154   depositing every centimeter with a 10 µl syringe a droplet of 1 µl of the solution of Dufour

155   gland extract. To reduce stress, ants were transferred from their individual box to the walkway

156   by letting them climb up and down on a pencil. Once on the walkway, the movement of the

157   ant was recorded as soon as it started to walk along a more or less straight path. The ant was

158   then captured at the end of the walkway and anesthetized by placing it in a vial plunged in

159   crushed ice. It was then fixed dorsally with Plasticine under a binocular microscope with its

160   head maintained horizontally. This allowed us to put a drop of superglue (Loctite,

161   http://www.loctite.fr) on the top of its mandibles and to glue a fishing weight. The same

162   procedure as for unloaded ants was then used to film loaded ants. At the end of the

163   experiment the ant was killed and we weighed its head, thorax (with the legs), and gaster

164   separately to the nearest 0.1 mg with a precision balance (NewClassic MS semi-micro,

165   Mettler Toledo, United Sates). These measures were used to compute the displacement of the

166   center of mass (CoM) of the ants on the videos (see SI for details of the procedure).

167   Whether unloaded or loaded, we filmed all ants for at least three strides. We defined a stride

168   period as the time elapsed between two consecutive lift off of the right mid leg. For our

169   analysis, we cropped our videos to a whole number of strides.

170   *Data extraction and analysis*

171   In order to compute the horizontal movement of the ants' main body parts (gaster, thorax,

172   head or head and load if one was carried, see SI for details) and of its overall center of mass

173   (CoM) we used the software Kinovea (version 0.8.15, https://www.kinovea.org) to semi-

174   automatically track points of interest on the videos of the top camera (Figure 2). When several

175   video shootings of the same ant had been made, we selected the video in which the ant had the

176   straighter path. As a criteria for path straightness we calculated the ratio of the distance

177   actually traveled by the ant (based on the horizontal trajectory of its center of mass) on the

178   straight line distance between the first and last point of the trajectory and considered that the

179   path was sufficiently straight when this ratio was lower than 1.2.

180   Using the software Kinovea and ImageJ (version 2.0.0, FIJI distribution, https://fiji.sc/) we

181   then accessed the information corresponding to the stepping pattern of the ant. For each video

182   frame we visually determined the state of each leg during locomotion (i.e. in stance phase,

183   swung or dragged) on the lateral view of the ant and recorded the spatial position of the leg

184   tarsus during the stance phases on the dorsal view. These positions were expressed in a

185   coordinate system centered on the neck of the ant, with the X axis corresponding to the

186   longitudinal axis of its body and the Y axis to the transverse axis (Figure 2). In order to

187   compare ants of different sizes, all distances were normalized to the body length of the ant

188   (calculated from the tip of the gaster to the tip of the mandibles).

189   We computed the duty factor for each leg as the fraction of the stride the leg was in contact

190   with the ground (Ting et al., 1994; Spence et al., 2010). For each leg, we also computed the

191   mean relative position at lift off (Posterior Extreme Position: PEP) and at touch down

192   (Anterior Extreme Position: AEP) by averaging the relative positions of the leg over the

193   strides we filmed. Since the path followed by the ant was straight, we also averaged the values

194   of the right and left leg of each pair of legs when computing the duty factors and relative leg

195   positions. Following Wosnitza et al. (2012) and Wahl et al. (2015) we calculated step

196   amplitude rather than stride length. For each leg we computed step amplitude by averaging

197   the distances between PEP and AEP positions in the ant coordinate system. Again, because

198   the path followed by the ant was straight, we averaged the values of the right and left leg of

199   each pair of legs when computing step amplitude.

200   We studied inter-leg coordination by comparing the time of lift off of every leg to the time of

201   lift off of the right mid leg (Wosnitza et al., 2013; Wahl et al., 2015). More precisely, we

202   computed, for each leg, the time lag between the leg lift off and the last lift off of the right

203 mid leg. We then divided the value of the time lag by the time elapsed between two

204 successive lift off of the right mid leg. This value was expressed as a phase shift between $-\pi$

205 and π for each leg. Finally, we used circular statistics (Batschelet, 1981) to compute the mean

206 vector of the distribution of the phase shifts for specific groups of ants. As an indication of

207 how ant gait was close to an ideal alternating tripod locomotion we also computed the Tripod

208 Coordination Strength (TCS) (Wosnitza et al., 2012; Wahl et al., 2015; Ramdya et al., 2017).

209 This index can take values between 0 and 1. A TCS of 1 corresponds to a perfect alternating

210 tripod gait while a TCS of 0 means that the ant performed a completely different type of gait.

211 Finally, following Moll et al. (2013), we also computed for each ant the change over time of

212 the static stability margin (SSM) during locomotion. For every video frame, the SSM was

213 defined as the minimum distance between the horizontal projection of the ant CoM and the

214 edges of the polygon formed by all legs in contact with the ground, including the dragged

215 legs. The SSM is positive if the projection of the CoM lies inside the polygon, negative

216 otherwise. We considered that the locomotion was statically stable when the ant managed to

217 maintain static stability during locomotion (i.e., when the SSM was positive) and that it was

218 statically unstable when it was not the case (i.e., when the SSM was negative).

219 We performed all data analysis and designed all graphics with R (version 3.5.1) run under

220 RStudio (version 1.0.136). For unloaded condition, we expressed all kinematic variables as a

221 linear function of the decimal logarithm of ant mass. For loaded condition, because the same

222 ants were tested loaded and unloaded, we calculated the difference in the value of each

223 kinematic variable between loaded and unloaded conditions and expressed it as a linear

224 function of both the decimal logarithm of ant mass and the decimal logarithm of load ratio

225 which was defined as 1 + (load mass/ant body mass) (Bartholomew et al., 1988).

226 **Results**

227 *Unloaded ants: influence of body mass (Table 1)*

228 Stride frequency ($F_{1,43}= 64.82$, $P<0.001$) and step amplitude for every leg (front leg: $F_{1,43}=$

229 4.1, $P=0.049$; mid leg: $F_{1,43}= 13.0$, $P<0.001$; hind leg: $F_{1,43}= 30.0$, $P<0.001$) decreased with

230 ant mass. As a result, the speed decreased with ant mass as well ($F_{1,43}= 109.2$, $P<0.001$;

231 Figure 3). Thus, relative to their size, big ants move more slowly than small ants. However,

232 absolute speed did not depend on ant mass (mean ± SD: 29.1 ± 4.5 mm.s^{-1})

233 The duty factor of all legs increased with increasing ant mass, particularly for the front ($F_{1,43}=$

234 36.7, $P<0.001$) and mid ($F_{1,43}= 22.6$, $P<0.001$) legs (Figure S1A-C). Therefore, compared to

8

235 small ants big ants tend to have more legs in contact with the ground during a stride ($F_{1,43} =$

236 36.4, $P<0.001$). The front and mid legs were almost never dragged by the ants. However,

237 independent of ant mass, hind legs were dragged during 23 % of a stride on average.

238 In an ideal alternating tripod gait, all legs of a tripod lift off simultaneously. In actual

239 locomotion however, there is no such perfect synchronization. Nevertheless, the alternating

240 tripod gait model still holds if the time interval between the lift off of the three legs of the

241 same tripod is small. Figure 4A shows that the ants' gait is very close to an ideal tripod gait

242 (see also Fig. S2A). However, for bigger ants, the front legs tended to lift off slightly after the

243 mid and hind legs of the same tripod. As a result, the TCS slightly decreased for bigger ants

244 ($F_{1,43} = 6.3$, $P= 0.016$).

245 The front legs tended to be positioned at a larger distance from the longitudinal body axis (Y

246 position) in big ants compared to small ants both at touch down (AEP) ($F_{1,43} = 17.5$, $P<0.001$)

247 and lift off (PEP) ($F_{1,43} = 15.5$, $P<0.001$). All legs, especially the hind legs, tended to be

248 positioned in a more forward position (X position) at lift off (PEP) in big ants compared to

249 small ants (front legs: $F_{1,43} = 20.4$, $P<0.001$; mid legs: $F_{1,43} = 16.6$, $P<0.001$; hind legs: $F_{1,43}$

250 $= 50.5$, $P<0.001$) (Figure 5A).

251 The static stability margin decreased during a stride and reached a local minimum value just

252 before touch down of one of the front legs (Figure 6A & 6B). The minimum value of the

253 static stability margin decreased with increasing ant mass ($F_{1,43} = 4.7$, $P= 0.036$). Moreover,

254 the proportion of time an ant moved in statically unstable locomotion increased with ant mass

255 ($F_{1,43}= 5.0$, $P= 0.030$, compare Figures 6A & 6B). Therefore, small ants have a more balanced

256 locomotion than big ants.

257 *Loaded ants: influence of ant body mass and load ratio (Table 2)*

258 Figure 7 shows the values of load ratio tested for ants of different body masses. Depending on

259 the value of the load ratio, ants exhibited two kinds of behaviors when loaded. They could

260 either keep the load lifted above the ground during locomotion or they could maintain their

261 head in a very slanted position and push the load in front of them (see SI Movies 1-3 for

262 illustrations). We called the first behavior "carrying" and the second "pushing". The criteria

263 we used to distinguish between the two behaviors is based on whether or not the load glued on

264 the ant mandibles was in contact with the ground during locomotion. Pushing generally

265 occurred for load ratio higher than five for ants above 10mg, while for ants of lower body

266   mass both carrying and pushing could be observed for load ratio higher than four (Figure 7).

267   We will only consider ants that carry their load in the following analyses.

268   Independent of ant mass, stride frequency decreased with increasing load ratio ($F_{2,42} = 19.7$,

269    $P<0.001$). Step amplitude was independent of load ratio for front and mid legs but decreased

270   for hind legs ($F_{2,42} = 3.2$, $P=0.051$). Consequently, ant speed decreased with increasing load

271   ratio ($F_{2,42} = 23.6$, $P<0.001$). However, for ants transporting equivalent loads there was no

272   effect of body mass on both stride frequency and step amplitude.

273   Independent of ant mass, the duty factor increased for the front ($F_{2,42} = 62.2$, $P<0.001$), mid

274   ($F_{2,42} = 27.1$, $P<0.001$) and hind legs ($F_{2,42} = 14.9$, $P<0.001$) for increasing load ratio and,

275   independent of load ratio, the duty factor increased with ant mass, confirming the results

276   obtained on unloaded ants (Figure S1D-F). The mean number of legs simultaneously in

277   contact with the ground increased for increasing load ratio independent of ant size and, to a

278   lesser extent, increased for increasing body mass independent of load ratio ($F_{2,42} = 57.7$,

279    $P<0.001$). Similar to what occurred when ants were unloaded, the front and mid legs were

280   almost never dragged during locomotion when ants were loaded.

281   When the ants were walking loaded their mid and hind legs tended to be more distant from

282   their longitudinal body axis (Y position) with increasing load ratio, both during lift off (mid

283   legs: $F_{2,42} = 10.0$, $P<0.001$; hind legs: $F_{2,42} = 5.3$, $P= 0.009$) and touch down (mid legs: $F_{2,42} =$

284   6.1, $P=0.005$; hind legs: $F_{2,42} = 5.5$, $P= 0.007$) (Figure 5B).

285   While performing loaded locomotion, ants did not exhibit the same inter-leg coordination

286   pattern than during unloaded locomotion (Figure 4). First, there was more dispersion in phase

287   shift between legs for loaded ants, especially for the hind legs and for high values of load ratio

288   (> 3.5, see Figure 4B2). Second, the three legs of the same tripod tended to lift off in a

289   specific order (i.e. mid leg -> front leg -> hind leg). This was especially clear for the hind leg,

290   which was the last to lift off in each tripod. This order seems to be more strictly followed for

291   higher load ratio and for bigger ants (Figure 5B2). As a result, the value of TCS decreased for

292   increasing load ratio ($F_{2,42} = 8.7$, $P<0.001$).

293   Independent of ant size, the proportion of time ants were performing statically unstable

294   locomotion increased with increasing load ratio in loaded ants ($F_{2,42} = 65.4$, $P<0.001$).

295   **Discussion**

296   In this study, we investigated the kinematics of locomotion of unloaded and loaded ants of the

297   polymorphic species *M. barbarus*. We found that, relative to their size, small ants were able to

298   carry larger loads than big ants. Small ants also walked faster and were more stable than big

299   ants; all ants, whatever their size, reduced their speed when carrying loads of increasing mass.

300   The locomotion of unloaded ants was very close to an ideal alternating tripod gait. This

301   allowed them to perform a rather statically stable locomotion. On the other hand loaded ants

302   were most of the times statically unstable and their gait changed to more tetrapod-like

303   locomotion, wave gait locomotion and hexapodal stance phases (Figure S2B).

Unloaded ants

305   In *M. barbarus* big ants have, relative to their size, bigger heads than small ants (Heredia and

306   Detrain, 2000; Bernadou et al., 2016). This means that their center of mass is located in a

307   more anterior position compared to small ants. Big ants are thus more likely to be off balance

308   than small ants. Therefore, one should expect static stability to decrease with increasing ant

309   mass, which is what we actually observed. Nevertheless, the question arises of whether the

310   decrease in stability we observed is the same as the decrease one should observed

311   mechanically because of the forward shift of the center of mass of the body, or whether this

312   decrease is less than the one expected because big ants adjust their gait in order to maintain

313   their stability. In order to answer this question we modeled an ideal tripod gait for ants of

314   different sizes. Our model took into account the difference in morphology across ants (for

315   both mass repartition between body parts and relative leg lengths) but assumed ants had the

316   same stepping pattern (based on the mean value of the leg positions observed in our

317   experiment for all ants and then corrected for leg length, see SI for details). Following the

318   method described in the method section, we then computed the minimum static stability

319   margin (SSM) and the proportion of statically unstable locomotion for these ants. The slope of

320   the regression line describing the relationship between the minimum static stability margin

321   and $\log_{10}$(ant mass) and the proportion of statically unstable locomotion and $\log_{10}$(ant mass)

322   was -0.142 and 0.183, respectively. These values should be compared to the values we found

323   in our experiment, i.e., (mean $\pm$ CI$_{0.95}$) -0.041 $\pm$ 0.036 and 0.029 $\pm$ 0.026, respectively (see

324   Table 1). Thus, if ants were walking with an ideal tripod gait, the influence of ant mass would

325   be more important than what we observed in our data. This means that big ants adjust their

326   locomotion in order to increase their static stability. That this is indeed the case is suggested

327   by the differences observed between small and big ants in both leg positioning and in gait

328   parameters (Table 1). In big ants the front legs lifted off in a more anterior position, so that

329   the minimum SSM (which occurs just before the front leg touch down, Figure 6) was less

330   critical. This led to both an increase in duty factor and a decrease in step amplitude for front

11

331   and mid legs. In conclusion, the differences in morphology between ants of different sizes do

332   induce a less statically stable locomotion in big ants but this effect is reduced by the fact that

333   they are able adjust their stepping pattern to compensate for this instability.

334   *Carrying capacity*

335   Small ants were able to carry relatively heavier loads than big ants. This is concordant with

336   the results obtained by Bernadou et al. (2016) in the same species and with those obtained by

337   Burd (2000) in the leaf cutting ants *Atta colombica and A. cephalotes*. This difference in load

338   carrying capacity can be accounted for by two non-exclusive explanations. The first is related

339   to the well-known scale effect, while the second is related to differences in the locomotion

340   and/or the morphology (induced by allometric relationships) of ants of different sizes.

341   The scale effect is due to the fact that the section of the muscles of an animal (which is

342   directly related to the mechanical power they can develop) increases as the square of its body

343   length while body mass increases as the cube (Schmidt-Nielsen, 1984; Dial et al., 2008). This

344   would lead to a reduction in relative load capacity in big ants compared to small ants.

345   However, this reasoning would hold only if big ants were a simple enlargement of small ones,

346   i.e. if their body parts grew isometrically. As mentioned before, this is not exactly the case in

347   *M. barbarus*: compared to small ants, big ants not only have larger heads (Bernadou et al.,

348   2016, Fig. S5) but they have also relatively shorter legs (Fig. S6). Nonetheless, the scale

349   effect could still apply to some extent. To assess its importance we compared our data of load

350   carrying capacity in ants of different sizes to those that would be expected if the predictions of

351   the scale effect were computed on ants of different sizes but with same morphology. We

352   considered as the basis for the computation of our predictions the carrying capacity we

353   observed for an ant weighing 1.5mg. As can be seen in Figure 8, the predictions of the

354   carrying capacity for ants of different sizes is close to the curve based on our experimental

355   data representing the 50% probability of carrying a load vs pushing it. It is also close to the

356   curve representing the 50% probability of carrying a load vs dragging it from the field

357   experiments by Bernadou et al. (2016) in which the ants transported food items of various

358   sizes deposited on their foraging trails. Therefore, it seems that ants start pushing in our

359   experiment for about the same load ratio values as they start dragging in Bernadou et al.

360   (2016). Pushing instead of dragging probably occurs as a result of the load being glued on the

361   mandibles.

362   Nonetheless, one cannot exclude that the differences observed in locomotor behavior between

363   small and big ants could also partly explain the differences in carrying capacity. Table 2

364   indeed points out some differences in the kinematics of ants of different sizes, independent of

365   load ratio. However, these differences do not follow any particular logic and are difficult to

366   interpret. Most of the observed differences in carrying capacities in ants of different sizes can

367   thus be explained by a scale effect.

368   *Influence of load ratio on locomotion*

369   The main effect of carrying a load for an ant is to shift its CoM forward. As a consequence the

370   CoM is located closer to the front edge of the polygon of support, or even lies out of it, and

371   the SSM decreases or becomes negative, leading the ant to perform less statically stable or

372   statically unstable locomotion. Moll et al. (2013) showed that loaded *Atta vollenweideri* ants

373   can reduce this effect by changing the way they carry their load: by carrying the pieces of

374   grass blade they hold in their mandibles in a more upward, backward-tilted position they can

375   shift their CoM in a somewhat backward position. This is of course impossible in our

376   experiment because ants cannot adjust the position of the load glued on their mandibles. This

377   would not happen either in the field because most of the seeds collected by *M. barbarus* are

378   not elongated enough to be carried in the same way as pieces of grass blades in grass-cutting

379   ants. The CoM of loaded *M. barbarus* workers is thus shifted forward and the proportion of

380   time their locomotion is statically unstable during a stride increases for increasing load ratio

381   (Table 2), reaching up to 90% for the highest load ratios.

382   Such statically unstable locomotion has already been reported for insects in the literature. For

383   instance, the cockroach *Blaberus discoidalis,* when moving at very high speed, often performs

384   statically unstable locomotion and thus maintains its balance through dynamic stability (Ting

385   et al., 1994; Koditschek et al., 2004). Dynamic stability refers to individuals keeping their

386   balance when statically unstable by only briefly "falling" forward before new supporting legs

387   contact the ground (Moll et al., 2013). Statically unstable locomotion has also been observed

388   by Moll et al. (2013) in loaded workers of the grass cutting ant *A. vollenweideri*. These

389   authors have suggested that loaded ants could use dynamic stability in order to avoid falling

390   over during the statically unstable part of their locomotion (Moll et al., 2010, 2013).

391   However, loaded *M. barbarus* workers move too slowly (Table 2) to maintain their balance

392   through dynamic stability: they would fall forward before the front leg catches up. Rather, we

393   assume that they maintain their balance by clinging to the ground with the tarsal claws located

394   at the end of their mid and hind legs. Consequently, they tend to keep more legs in contact

395   with the ground for increasing load ratio. This leads to a decrease of their stride frequency and

396   TCS and to an increase of the duty factor of all legs (Table 2). Hind legs are of particular

397   importance in keeping the ant balanced because they have a higher lever-arm effect. In our

398   experiment the percentage of time the hind legs were dragged decreases as soon as the ant

399   was loaded independent of ant mass and load ratio (mean $\pm$ CI$_{0.95}$: -12,1 $\pm$ 8,2 %), probably

400   because, due to the position of the claws on the pretarsus (Fig; S3), the hind legs can better

401   cling to the ground when they are not moving. In this respect, it would be interesting to

402   investigate how ants maintain their balance when their adhesive prestarsal structures are

403   blocked or when they walk on a slipping substrate (see Ramdya et al., 2017 for an example in

404   *Drosophila melanogaster*). The tendency for the hind legs to lift off after the front legs

405   touched down also increased for increasing load ratio (Figure 4B1 & 4B2). This is coherent

406   with the balance strategy used by ants, as the SSM is maximal at front leg touch down (Figure

407   6C & 6D) and thus it is less risky to lift off the hind leg at this time. Finally, as a result of the

408   stride frequency diminution (and because step amplitude remains constant), the speed

409   decreases with increasing load ratio, which is concordant with most studies in other load

410   carrying ants, e.g. *Atta colombica* (Lighton et al., 1987), *A. vollenweideri* (Röschard and

411   Roces, 2002) and *Veromessor pergandei* (Rissing, 1982).

412   Reinhardt and Blickhan (2014a) showed that, during steady state locomotion, *Formica*

413   *polyctena* uses mainly its hind legs in order to generate propulsion forces while Wöhrl et al.,

414   (2017) showed in *Cataglyphis fortis* that it is the mid legs that are mainly used for propulsion.

415   In both cases however, the front legs have a brake effect on locomotion. To our knowledge,

416   there are no study so far that measured the ground reaction forces (GRF) in loaded ants.

417   Nonetheless, it is possible to infer the propulsion behavior of the legs in our experiment based

418   on the position of their tarsi. Indeed, as shown by Endlein and Federle (2015), depending on

419   the GRFs, the tarsi attach differently to the substrate. The morphology of the tarsal attachment

420   of *M. barbarus* (Figure S3) is comparable to that of other ants (Federle et al., 2001; Endlein

421   and Federle, 2008). It seems thus fair to assume that they cling to the substrate in a similar

422   way. As Endlein and Federle (2015), we observed in our videos two positions for the hind leg

423   tarsi during the stance phase: on "heels", during the first part of the stance phase, and on

424   "toes", during its second part (Figure S4). This would suggest that hind legs have a

425   "compression and pushing" action in the first part of the stance phase, i.e. participate to

426   propulsion, and then have a "tension and pulling" action on the last part of the stance phase,

427   acting as a holding point for the ants not to fall over. For mid legs, the tarsi were usually in

428   the "heel" position and were thus likely to participate in propulsion. These observations are

429 purely qualitative as the resolution of our videos makes a quantitative analysis of these data

430 tricky. The use of a miniature force plate (Bartsch et al., 2007; Reinhardt & Blickan, 2014b)

431 to compare the GRFs of unloaded and loaded ants would provide crucial insights on how the

432 different legs of the ants contribute to the stability and propulsion of loaded locomotion.

433 *Conclusion*

434 We have shown in this study that unloaded *M. barbarus* workers display different gaits

435 depending on their body mass. For big ants, these differences seem to be mainly explained by

436 a compensation for the imbalance caused by their disproportionally bigger head. Small ants

437 are able to carry proportionally heavier loads than big ants and scale effect provides a simple

438 and satisfactory explanation for this difference. Moreover, our results show that loaded ants

439 are often statically unstable during locomotion and that they maintain their balance by

440 clinging to the ground. Further studies are required to determine the contribution of each leg

441 to both stability and propulsion.

442 Big ants are more costly to produce than small ants. So why do colonies produce them if they

443 are less efficient in transporting loads? One answer to this question is that, although big ants

444 have lower load carriage performances than small ants, they are nonetheless able to carry on

445 average loads of higher masses than small ants and to seize and transport items of larger

446 diameters with their large and powerful mandibles (Fig. 3 in Bernadou et al., 2016). This

447 could allow colonies to increase the size range of the food items retrieved to the nest so that

448 they can enlarge their diet breadth and better match the size distribution of the food resources

449 available in their environment (Davidson, 1978). Big ants may also play other roles than

450 foraging in seed-harvesting ant colonies, such as removing the obstacles encountered on

451 foraging trails, cutting thick plant stalks or milling the seeds inside the nest to prepare them

452 for consumption. The significance of our results for the foraging ecology and division of labor

453 in *M. barbarus* remains therefore to be investigated.

454 **Acknowledgements**

458 **Competing interests**

459 The authors have no competing interests to declare.

Author contributions

H.M., P.M. and V.F. conceived and designed the experiments and interpreted their results; H.M. conducted the experiments and performed the analyses; V.F. and H.M. did the statistical analyses of the data; V.F. and H.M. wrote the paper and P.M. made essential contributions to the text; G.L helped for the collection of ant colonies and was in charge of their maintenance.

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Data availability

Dataset is available from http://doi.org/10.5281/zenodo.2646485

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Table 1: Influence of body mass (in mg) on the kinematics of unloaded ants (N = 45).

	Variable		Model prediction for mean(ant mass) ± CI	Slope for $\log_{10}$(ant mass) ± CI	Adj R²
Gobal kinematics	Speed (BL.s^{-1})		3.918 ± 0.206	-2.831 ± 0.546 ***	0.71
	Stride frequency		4.578 ± 0.196	-2.004 ± 0.520 ***	0.57
	Step amplitude front leg (BL)		0.471 ± 0.015	-0.040 ± 0.040 *	0.07
	Step amplitude mid leg (BL)		0.559 ± 0.018	-0.083 ± 0.046 ***	0.21
	Step amplitude hind leg (BL)		0.425 ± 0.014	-0.103 ± 0.038 ***	0.4
Gait	Duty factor	Front legs	0.635 ± 0.008	0.063 ± 0.021 ***	0.45
		Mid legs	0.675 ± 0.010	0.060 ± 0.025 ***	0.33
		Hind legs	0.615 ± 0.015	0.042 ± 0.040 *	0.08
	Proportion of time the hind legs are dragged		0.232 ± 0.026	-0.015 ± 0.070 ns	0
	Mean number of legs in contact with the ground		4.306 ± 0.039	0.309 ± 0.103 ***	0.45
	Tripod Coordination Strength (TCS)		0.620 ± 0.035	-0.114 ± 0.092 *	0.11
Leg positionning	AEP (BL)	Front legs X	0.391 ± 0.010	-0.011 ± 0.027 ns	0
		Front legs Y	0.218 ± 0.011	0.058 ± 0.028 ***	0.27
		Mid legs X	-0.015 ± 0.014	-0.006 ± 0.037 ns	0
		Mid legs Y	0.575 ± 0.009	0.006 ± 0.024 ns	0
		Hind legs X	-0.680 ± 0.012	0.010 ± 0.032 ns	0
		Hind legs Y	0.383 ± 0.009	0.008 ± 0.025 ns	0
	PEP (BL)	Front legs X	-0.085 ± 0.011	0.066 ± 0.029 ***	0.31
		Front legs Y	0.228 ± 0.013	0.068 ± 0.035 ***	0.25
		Mid legs X	-0.569 ± 0.014	0.076 ± 0.037 ***	0.26
		Mid legs Y	0.545 ± 0.009	0,002 ± 0.024 ns	0
		Hind legs X	-1.115 ± 0.014	0.129 ± 0.037 ***	0.53
		Hind legs Y	0.426 ± 0.010	0.020 ± 0.027 ns	0.03
Static	Minimum static stability margin		-0.058 ± 0.014	-0.041 ± 0.036 *	0.09

| stability | Proportion of statically unstable locomotion | 0.044 ± 0.010 | 0.029 ± 0.026 * | 0.08 |

Each line gives the results of a linear model describing the influence of $\log_{10}$(ant mass) on each kinematics variables studied. The first column corresponds to the model prediction $\pm 95\%$ confidence interval for the mean value of ant mass (11.8 mg). The second column gives the effect of ant mass expressed by the slope $\pm 95\%$ confidence interval for $\log_{10}$ (ant mass) (with the level of significance given by the t value: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; . $p < 0.1$; n.s. $p \geq 0.1$). The third column gives the adjusted R^2 for the linear model. BL= body length.

577

Table 2: Influence of body mass (mg) and load ratio on the changes in kinematics between unloaded and loaded locomotion (N = 45).

|	Variable		Model prediction for mean(ant mass) and LR=1 ± CI	Slope for $\log_{10}$(ant mass) ± CI	Slope for log(load ratio) ± CI	Adj R²
Gobal kinematics	Δ Relative speed (BL.s^{-1})		-0.199 ± 0.741	0.938 ± 0.817 *	-3.519 ± 1.573 ***	0.51
	Δ Stride frequency		-0.327 ± 0.595	0.562 ± 0.656 .	-2.762 ± 1.263 ***	0.46
	Δ Step amplitude front leg (BL)		0.011 ± 0.057	-0.052 ± 0.063 ns	-0.063 ± 0.121 ns	0.02
	Δ Step amplitude mid leg (BL)		0.003 ± 0.072	0.006 ± 0.079 ns	-0.062 ± 0.152 ns	0
	Δ Step amplitude hind leg (BL)		0.027 ± 0.060	0.004 ± 0.066 ns	-0.134 ± 0.126 *	0.09
Gait	Δ Duty factor	Front legs	-0.013 ± 0.029	0.029 ± 0.032 .	0.319 ± 0.061 ***	0.74
		Mid legs	-0.004 ± 0.038	0.054 ± 0.042 *	0.293 ± 0.081 ***	0.54
		Hind legs	0.018 ± 0.057	0.064 ± 0.062 *	0.322 ± 0.120 ***	0.39
	Δ % of time the hind legs are dragged		-0.121 ± 0.082	0.010 ± 0.090 ns	-0.081 ± 0.174 ns	0
	Δ Mean number of legs in contact with the ground		-0.246 ± 0.155	0.318 ± 0.171 ***	1.734 ± 0.330 ***	0.72
	Δ Tripod Coordination Strength (TCS)		0.036 ± 0.160	-0.108 ± 0.177 ns	-0.688 ± 0.340 ***	0.26
Leg positionning	Δ AEP (BL)	Front legs X	-0.017 ± 0.039	0.008 ± 0.042 ns	0.040 ± 0.082 ns	0
		Front legs Y	-0.019 ± 0.032	-0.034 ± 0.035 .	-0.028 ± 0.067 ns	0.04
		Mid legs X	0.055 ± 0.053	0.009 ± 0.058 ns	0.045 ± 0.112 ns	0
		Mid legs Y	-0.034 ± 0.030	0.014 ± 0.034 ns	0.108 ± 0.065 **	0.19
		Hind legs X	0.018 ± 0.058	0.040 ± 0.064 ns	-0.119 ± 0.123 .	0.16
		Hind legs Y	0.015 ± 0.031	0.053 ± 0.034 **	0.084 ± 0.066 *	0.17
	Δ PEP (BL)	Front legs X	-0.030 ± 0.042	0.030 ± 0.047 ns	0.125 ± 0.091 **	0.12
		Front legs Y	0.001 ± 0.042	-0.075 ± 0.048 **	-0.060 ± 0.092 ns	0.15
		Mid legs X	0.060 ± 0.063	0.003 ± 0.070 ns	0.101 ± 0.134 ns	0.02
		Mid legs Y	-0.029 ± 0.026	0.033 ± 0.028 *	0.120 ± 0.055 ***	0.29
		Hind legs X	0.008 ± 0.039	0.029 ± 0.042 ns	0.030 ± 0.082 ns	0
		Hind legs Y	-0.030 ± 0.037	0.056 ± 0.041 **	0.110 ± 0.079 **	0.16
Static	Δ Minimum static stability margin		-0.144 ± 0.063	0.136 ± 0.071 ***	-0.127 ± 0.135 .	0.43

| stability | Δ Proportion of statically unstable locomotion | -0.037 ± 0.110 | 0.018 ± 0.110 ns | 0.919 ± 0.212 *** | 0.68 |

578

579 *Each line gives the result of a linear model describing the influence of $\log_{10}$(ant mass) and $\log_{10}$(load ratio) on the changes of kinematics*
580 *variables. The first column corresponds to the model prediction for the mean value of ant mass (11.8 mg) and a load ratio of 1 (unloaded ant).*
581 *The second column gives the coefficient for $\log_{10}$(ant mass) and the third column the coefficient for $\log_{10}$(load ratio) (with the level of*
582 *significance given by the t value: *** p< 0.001; ** p < 0.01; * p < 0.05; . p < 0.1; n.s. p ≥ 0.1). The last column gives the adjusted R^2 for the*
583 *linear model. BL= body length.*
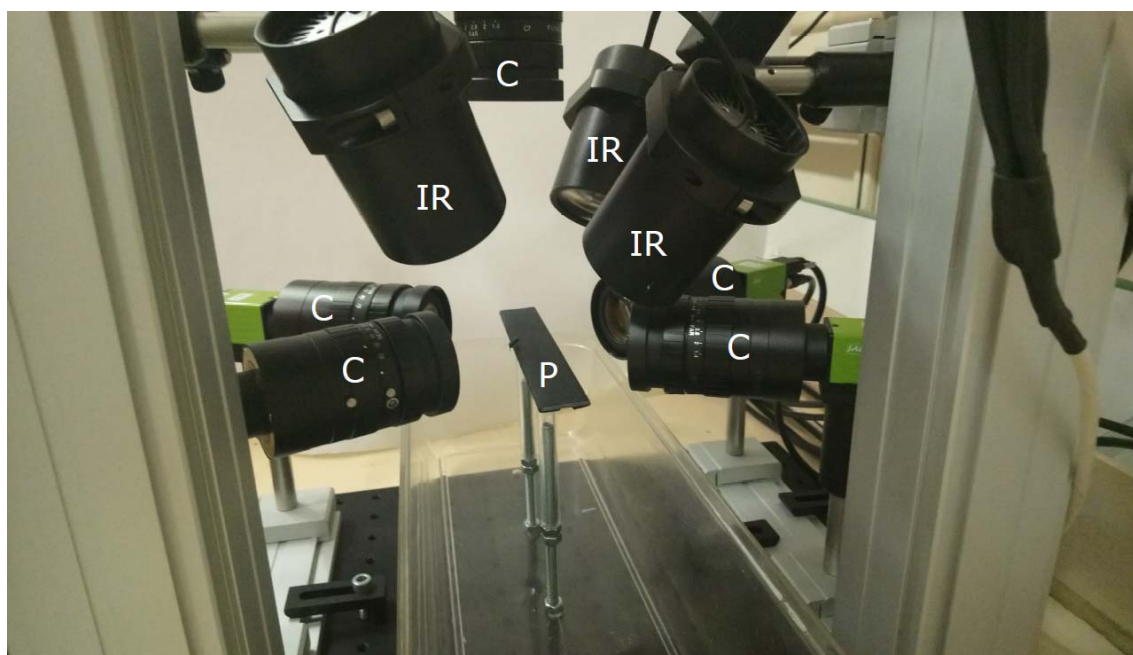
*Figure 1:* **Video acquisition system**. *C: cameras; IR: infra-red spots; P: 25mm wide walkway.*
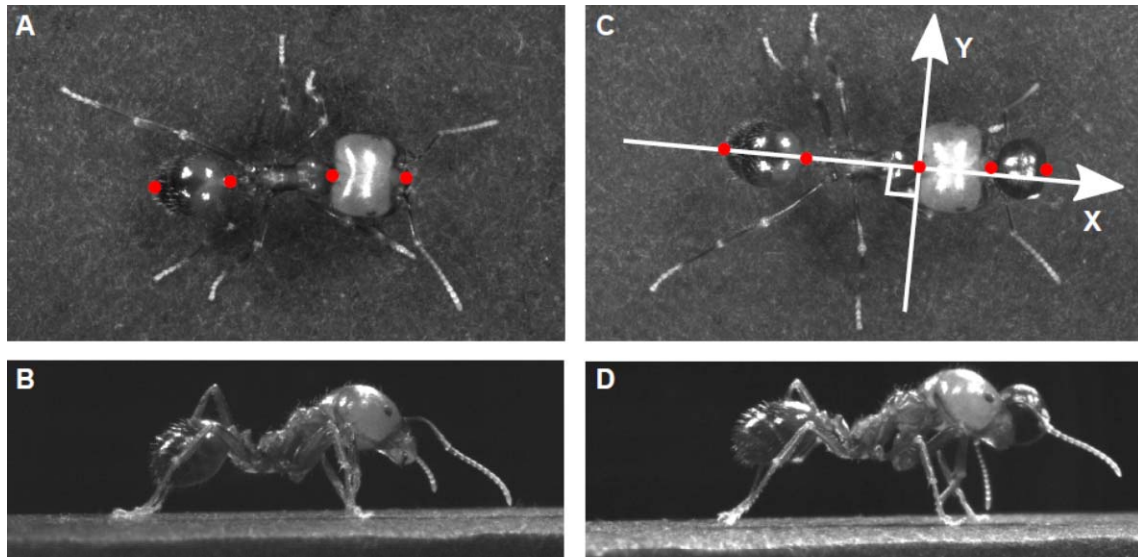
*Figure 2: **Location of the points tracked on each ant.** The snapshots show a top view (**A**, **C**) and a side view (**B**, **D**) of the same ant (ant mass = 32.5mg) tested in unloaded (**A**, **B**) and loaded condition (**C**, **D**) (load mass = 63mg). In **C**) the X axis corresponds to the longitudinal body axis while the Y axis corresponds to the transverse body axis. The position of the track points are represented in red (See SI for details).*
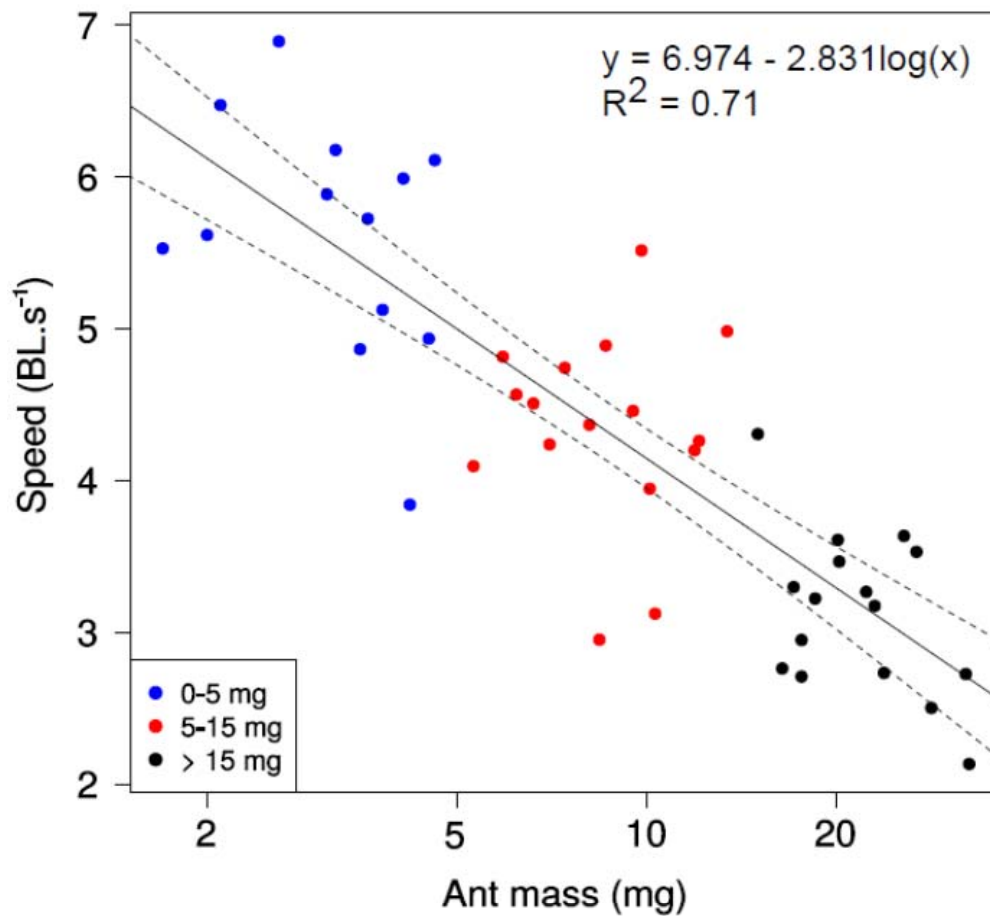
*Figure 3: **Speed as a function of ant mass for unloaded ants.** The straight line gives the prediction of a linear regression model (F1,43 =115.7, P<0.001) and the dashed lines gives the 95% confidence interval of the slope of the regression line (N= 45 ants).*
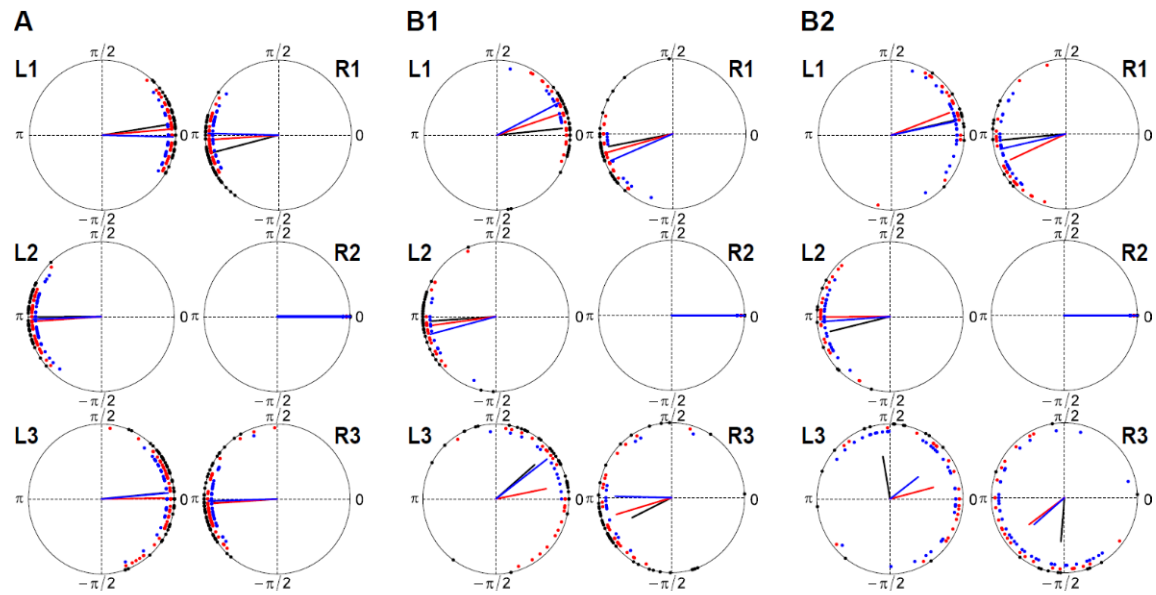
*Figure 4 : **Phase plots of lift off onset of all legs with respect to the right mid leg (R2) A -** unladen ants (N_{0-5mg} = 13; N_{5-15mg} = 16; $N_{>15mg}$ = 15); **B** – laden ants; **B1** – Load Ratio ≤ 3.5 (N_{0-5mg} = 4; N_{5-15mg} = 9; $N_{>15mg}$ = 12)); **B2** – Load Ratio > 3.5 (N_{0-5mg} = 9; N_{5-15mg} = 7; $N_{>15mg}$ = 3). R, right; L, left; blue: data for small ants (0-5mg); red: data for intermediate ants (5-15mg); black: data for big ants (>15mg); lines: mean vector – length is inversely proportional to dispersion.*
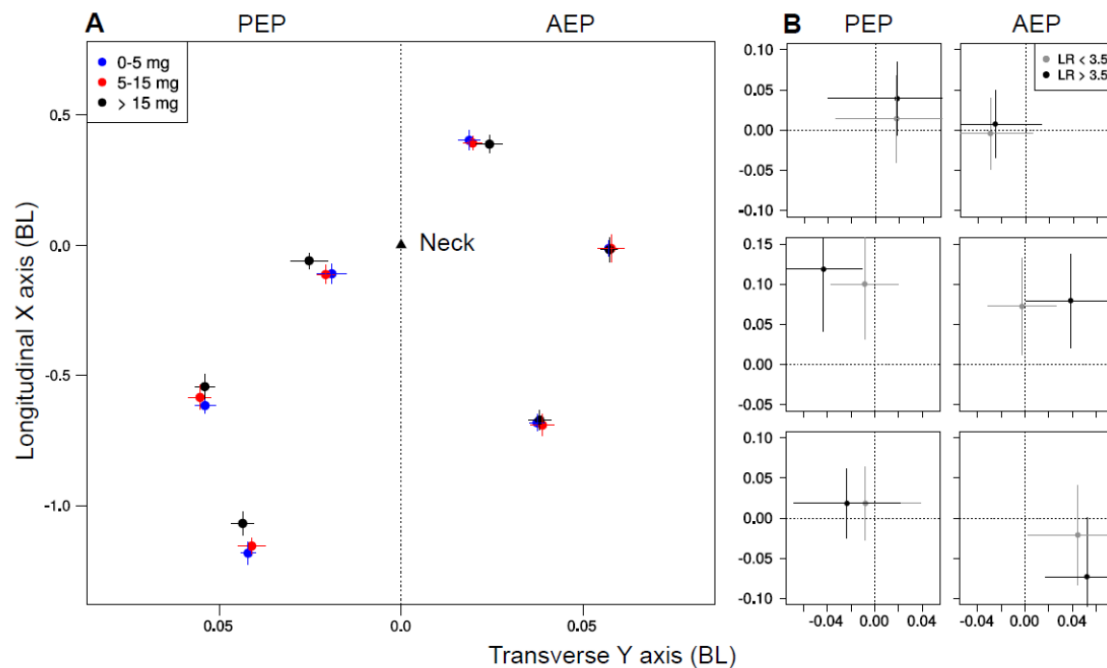
*Figure 5: **Footfall geometry of ants during locomotion**. **A**: Unloaded ants; the mean position of the front, mid, and hind legs during lift off (PEP) and touch down (AEP) along with their standard deviation is shown for different ranges of ant body masses ($N_{0-5mg} = 13$, $N_{5-15mg} = 16$, $N_{>15mg} = 16$). **B**: Loaded ants; changes in leg positions at lift off (PEP) and touch down (AEP) when ants were walking loaded compared to when they were walking unloaded. The origin corresponds to the leg position for unloaded ants. The average change in position over three strides along with their standard deviation is shown. Ants were categorized in two groups depending on load ratio ($N_{LR<3.5} = 26$, $N_{LR>3.5} = 19$). The scale is in units of body length.*
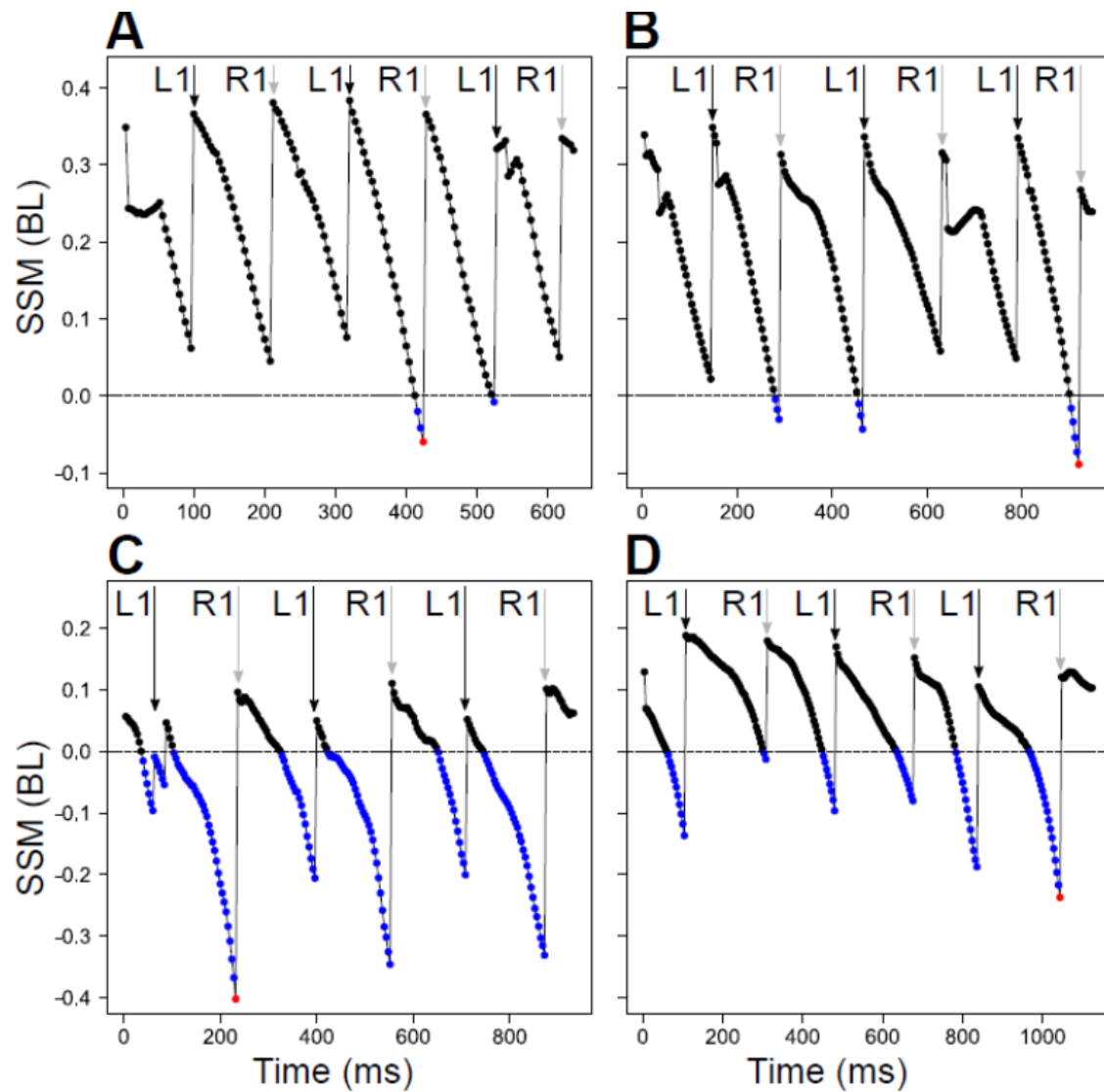
*Figure 6: **Time variation of the Static Stability Margin (SSM).** The value of SSM for unloaded (**A-B**) and loaded (**C-D**) ants, normalized by body length, is shown during three consecutive strides for a small ant (**A-C**) (ant mass = 4.2 mg, LR = 5.0) and a big ant (**B-D**) (ant mass = 32.1 mg, Load Ratio = 2.0). Black and grey arrows represent R1 and L1 touched down, respectively, blue dots correspond to negative SSM and red dots correspond to the minimum SSM.*
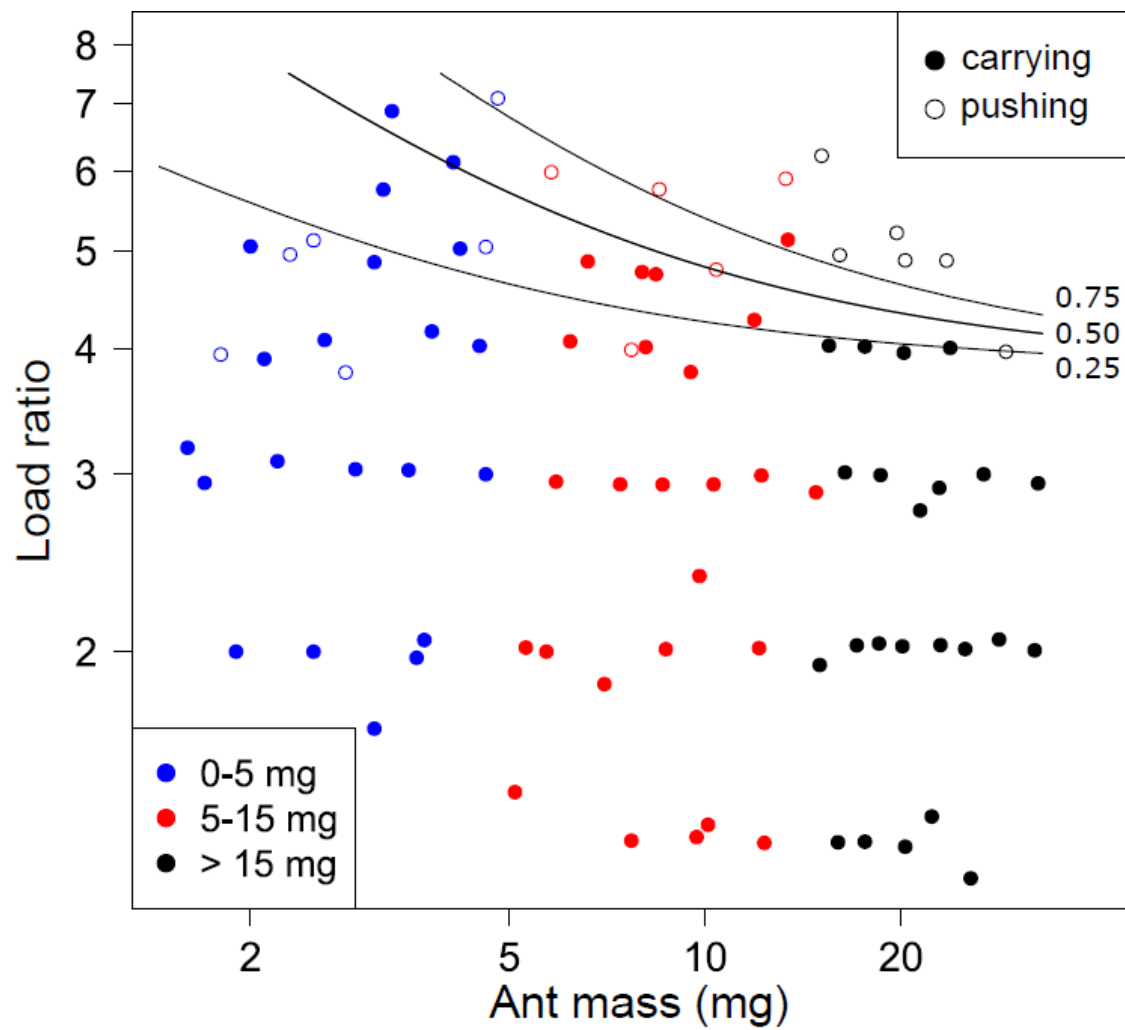
*Figure 7: **Transportation method used by ants during locomotion.** Probability of pushing a load as a function of ant mass and load ratio. The lines of equal probability were calculated by a logistic regression model (N = 86 ants).*
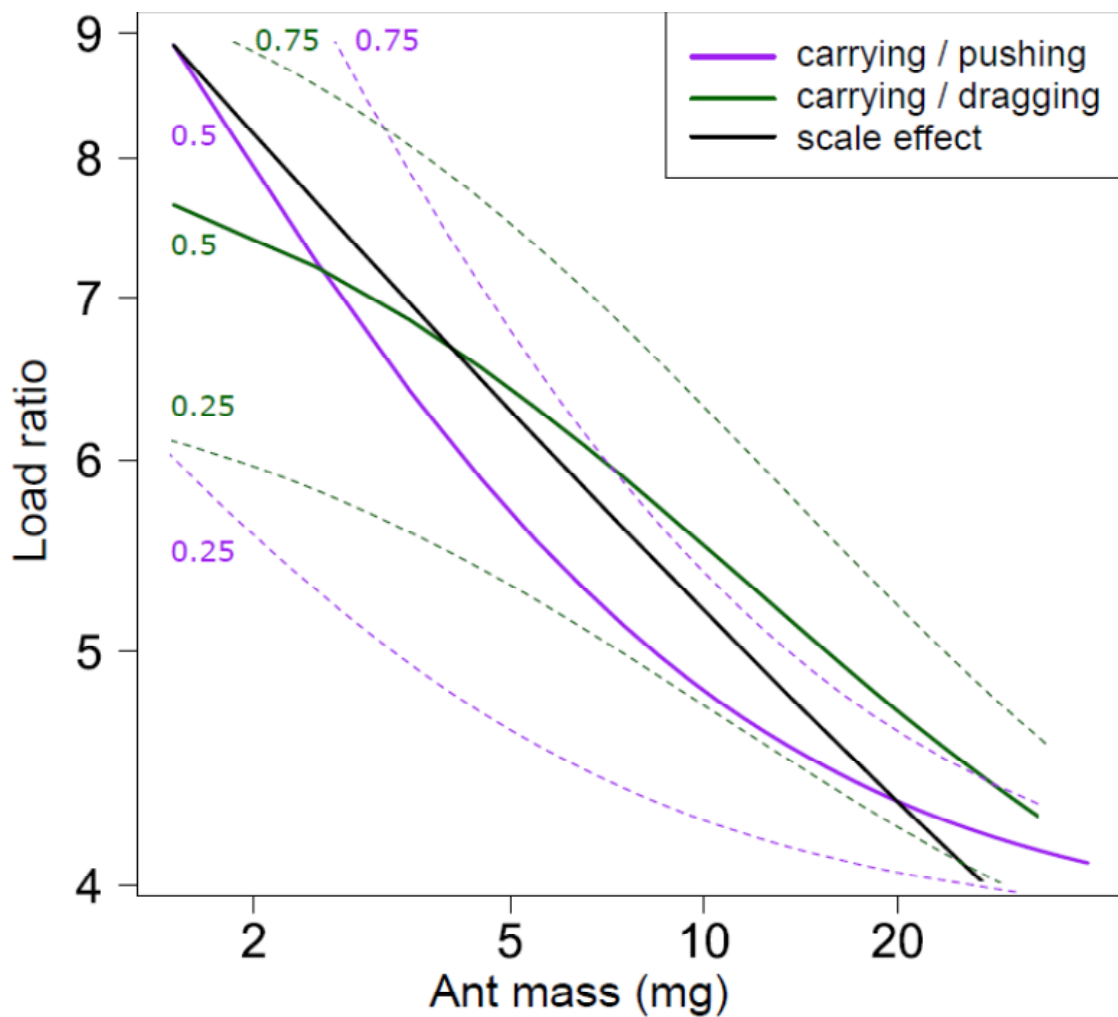
*Figure 8: **Ant carrying capacities and scale effect prediction**. The purple line represents 50% probability of carrying the load versus pushing it; The green line represents 50% probability of carrying the load versus dragging it (data from (Bernadou et al., 2016)); in both cases, the dashed lines represent 25% and 75% probabilities; the black line represents the prediction of load carrying capacity based on scale effect and a 1,5mg ant reference.*