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Influence of caste and subcaste characteristics on locomotion in the ant *Camponotus fellah*

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ABSTRACT

Worker polymorphism in ants has evolved repeatedly, with considerable differences in the morphometry of worker subcastes. Such body size differences and especially caste- and subcaste-specific characteristics might significantly influence locomotion. Therefore, we performed a comprehensive locomotion analysis along gradients in both body size and walking speed of *Camponotus fellah* worker subcastes, and of males, which have rarely been studied to date because of their short life spans associated with mating flights. We provide a detailed description of the morphometry and size differences of *C. fellah* castes and subcastes and analyse locomotion in the different polymorphic groups in terms of absolute and relative walking speeds (mesosoma lengths per second). Our results reveal that body size and shape affect locomotion behaviour to different extents in the worker subcastes (minor workers, medias, major workers) and in males. Nevertheless, *C. fellah* ants use the same overall locomotion strategy, with males and major workers reaching considerably lower walking speeds than minors and medias. Body size thus mainly affects walking speed. Minor workers reach the highest relative velocities by high relative stride lengths in combination with large vertical and lateral centre of mass oscillations and clearly higher stride frequencies of up to 25 Hz. Locomotion of males was characterised by clearly lower walking speeds, wider footprint positions, significant phase shifts and a notable dragging of the shorter hind legs. However, general walking parameters of males differed less from those of the female workers than expected as a result of division of labour in the colony.

KEY WORDS: *Camponotus*, Worker polymorphism, Caste system, Walking, Inter-leg coordination

INTRODUCTION

Walking in insects is of considerable interest in the study of locomotion (e.g. Hughes, 1952; Wilson, 1966), and ants have become important model organisms, providing a source of new biomechanical findings and inspiration for legged robot design (Dupeyroux et al., 2019; Yeoh and Yi, 2021). In most recent ant studies, workers of similar size were examined – soldiers and especially males were not considered (e.g. Reinhardt et al., 2009; Wahl et al., 2015; Pfeffer et al., 2019). Just a few insect locomotion studies have investigated the influence of body size (Duncan and

Crewe, 1993; Morehead and Feener, 1998; Hurlbert et al., 2008; Tross et al., 2021), and in a number of these studies no significant effect of body size was observed (e.g. Lighton et al., 1987; Duncan and Crewe, 1993). However, partly as a result of small sample size and impact of factors such as temperature (Morehead and Feener, 1998; Lighton and Duncan, 2002), body size effects may have been obscured in many of these studies (see Hurlbert et al., 2008). In addition, body shape and allometric relationships between body parts often do not change uniformly with size. Such morphometric differences exist, for example, between castes and worker subcastes in several ant species. Size and shape variations within the worker caste occur in about 13% of ant species and about 16% of ant genera and thus are not uncommon (Hölldobler and Wilson, 1990; Fjerdingstad and Crozier, 2006; Wills et al., 2018). These variations enable division of labour within the colony and are probably under strong selection. Therefore, they should influence colony fitness through colony maintenance, reproduction and survival (Billick, 2002; Powell, 2009; Wills et al., 2018), and might also affect locomotion performance. The present study examined intraspecific differences in walking behaviour in the polymorphic ant species *Camponotus fellah* under precisely this perspective, as effects of caste and subcaste characteristics have rarely been investigated.

Camponotus fellah has evolved a polymorphic worker caste with subcastes of minor and major workers (Boulay et al., 1999; Laciny et al., 2019) and a spectrum of transitions, often called media workers (medias) (Boulay et al., 2000; Lenoir et al., 2001). The worker subcastes differ significantly in their morphometry (Laciny et al., 2019), showing considerable distinction in mesosoma and head shapes (Fig. 1). In some studies, the category 'medias' is seen to be developmentally undefined (Tschinkel, 1988), but it is specified here as a subcaste because of discrete morphological differences compared with the minor and major characteristics (see also Powell and Franks, 2006; Wills et al., 2018). Minors and medias are described as primarily engaged in foraging, brood care and nest cleaning, while majors (often called 'soldiers') are characterised as specialised in nest defence (Hölldobler and Wilson, 1990; Laciny et al., 2018, 2019). Contrary to this outline, three worker groups are described in *C. fellah* by Mersch et al. (2013): nurses, foragers and cleaners; surprisingly, without consistent size differences between groups (Mersch et al., 2013). This division of tasks is relevant for smooth functioning of the colony, whereby division can be the result of polyethism or polymorphism (Espadaler et al., 1990; Galbán et al., 2021). In addition to the workers, the queen and the winged males form the reproductive caste (Hölldobler and Wilson, 1990). The queen is the largest individual, with large eyes and a well-developed gaster and reproductive system (Peeters and Ito, 2015), but was not examined in this study to preserve viability of the colony. Males usually have considerably smaller heads and bodies, fragile legs, and wings (Hölldobler and Wilson, 1990; Laciny et al., 2018, 2019). Their short lifespans associated with mating flights complicate their

List of abbreviations

AEP	anterior extreme position
BL	body length
BM	body mass
COM	centre of mass
HL	head length
HM	head mass
HW	head width
L1–3	left front, middle and hind leg
LL	leg length
MD	maximal eye distance
ML	mesosoma length
MM	mesosoma mass
MW	mesosoma width
PEP	posterior extreme position
R1–3	right front, middle and hind leg
RM	mass of gaster and legs
TCS	tripod coordination strength

investigation (Heinze, 2016; Laciny et al., 2019), making the present analysis special.

Locomotion analyses have rarely been performed in this polymorphic ant species, which seems perfectly suitable for analysing influences of caste and subcaste characteristics. In the present study, we thus performed an extensive locomotion analysis along gradients in both body size and (relative) walking speed for *C. fellah* worker subcastes and males, and emphasis was placed on morphometry and allometric relationships within the species; that is, on caste- and subcaste-specific characteristics.

MATERIALS AND METHODS
Animals and experimental sites

Camponotus fellah (Dalla Torre 1893) ants were obtained from their natural habitat near Mahrés, Tunisia (34.53°N, 10.49°S) in 2017, and have since been kept at Ulm University, Germany (48.42°N,

9.95°S). Ants were reared at 27°C in two connected nests made of plaster (to maintain constant humidity); the nests were connected to a foraging arena (50×50×20 cm) with water and honey water *ad libitum*, and locusts were supplied twice a week. To analyse the locomotion of *C. fellah* castes and subcastes, high-speed videos were recorded during 2020 at Ulm University (around noon), under both laboratory conditions and open sky, whereby no notable differences could be observed between the different environmental conditions. Ants were collected from the foraging arena, directly filmed, killed by freezing and dissected afterwards (body parts were kept in numbered tubes with 70% ethanol).

We analysed the walking behaviour in the worker subcastes of *C. fellah* (minor workers: $N=32$ ants, $n=130$ top-view runs; medias: $N=29$ ants, $n=93$ runs; major workers: $N=28$ ants, $n=116$ runs) and compared it with the males' locomotion ($N=11$ ants, $n=25$ top-view runs) to examine subcaste- and caste-specific differences. Locomotion was additionally analysed by lateral recordings ($n=15$ runs per subcaste and caste). The experiments were conducted in compliance with the current laws, regulations and ethical guidelines of Ulm University.

Morphometric analysis

For morphometric analysis, ants (Fig. 1) were dissected by cutting the head off, severing the six legs at the coxa–thorax joints and removing the gaster. Photos were taken through a dissection microscope (Stemi SV 6, Zeiss Microscopy GmbH, Jena, Germany) and measurements on the photos were performed with ImageJ (National Institutes of Health, Bethesda, MD, USA). We determined mesosoma length (ML) and width (MW), length of the leg segments (femur, tibia, basitarsus) (front legs: LL1, middle legs: LL2, hind legs: LL3), head length (HL), head width (HW), maximum eye distance (MD: the longest distance between the lateral eye margins), and total body mass (BM) of *C. fellah*. In addition to the ants used for high-speed recordings ($N=89$ workers: $N=32$ minors, $N=29$ medias, $N=28$ majors; $N=11$ males), we dissected $N=30$ workers (10 individuals per worker subcaste) and additionally determined mesosoma mass (MM), head mass (HM)
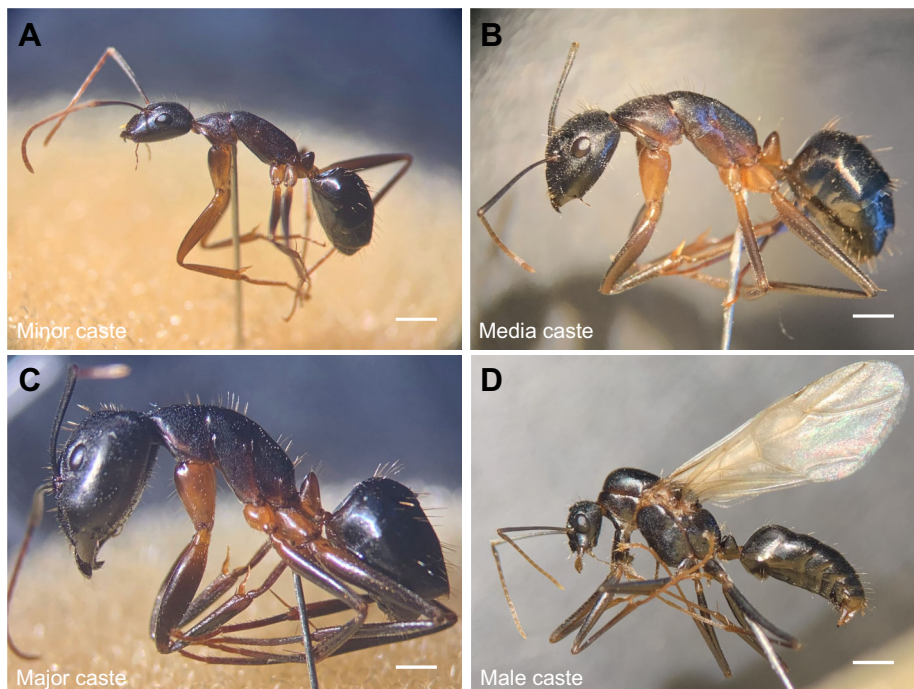

Fig. 1. *Camponotus fellah.* Morphology of minors (A), medias (B), majors (C) and males (D), in lateral view. Scale bar: 1 mm.

and the (residual) mass of gaster and legs (RM) of those ants and of the *N*=11 males used for high-speed recordings (total: *N*=119 workers, *N*=11 males).

The respective length values of the femur, tibia and basitarsus were added to arrive at leg length (LL), and means were calculated for the three leg pairs [mean dispersion measure of 0.21 mm between left and right leg (s.d.)]. Mesosoma length was used to characterise body size (see fig. 8 in Andersen et al., 2020) and was one of the main characteristics to sort workers into subcastes of different body sizes and shapes. Total body length is in the range of around 2 to 3 times mesosoma length, depending on the subcaste and the state of the gaster. To define the subcastes of minor workers, medias and major workers accurately, we further used head size and body mass (Table 1, Fig. 2).

To describe the relationship between mesosoma and leg length and mesosoma length and head size within the worker caste and in males, we used the allometric equation $y=a\times x^{b}$. Scaling factor *a* and exponent *b* were estimated for the male caste, the worker caste and the different worker subcastes (see also Sommer and Wehner, 2012; Tross et al., 2021).

High-speed videos and experimental procedures

High-speed recordings were made with two high-speed cameras (MotionBlitz EoSense Mini1 and Mini1-1, Mikrotron, Unterschleissheim, Germany; Nikon 105 mm 1:2.8 DG macro lens, Tokyo, Japan; and Tokina 100 mm 1:2.8 D macro lens, Tokyo, Japan) at sampling rates between 400 and 600 frames s^{-1}. Different frame rates were due to walking speed differences and recording window dependencies. Top-view recordings and lateral recordings were recorded separately.

For top-view recordings, a high-speed camera was mounted above an aluminium channel (30 cm long, 7 cm wide, and walls 7 cm high) and the ants were filmed while walking through that channel. The channel floor was coated with white paper to provide a slip-free ground and allow normal walking behaviour. For lateral-view recordings, the ants were filmed while walking through a channel with one transparent sidewall. For indoor illumination under laboratory conditions, two fibre optic cold light sources (Schott KL 1500LCD, 150 W, Schott AG, Mainz, Germany) were used, while no additional light source was necessary under the open sky. Ants were filmed at different controlled temperatures

Table 1. Morphometric analysis

	Minor workers	Medias	Major workers	Males
ML (mm)	3.07±0.14	3.59±0.26	4.36±0.19	3.54±0.25
MW (mm)	1.14±0.09	1.41±0.16	1.86±0.14	1.63±0.14
ML/MW	2.71±0.19	2.55±0.17	2.36±0.11	2.18±0.16
LL1 (mm)	4.87±0.20	5.70±0.42	6.83±0.37	5.73±0.35
LL2 (mm)	5.23±0.25	6.03±0.43	7.12±0.34	5.83±0.38
LL3 (mm)	7.11±0.33	8.21±0.61	9.52±0.46	6.73±0.50
HL (mm)	2.17±0.13	2.69±0.42	3.76±0.10	1.52±0.20
HW (mm)	1.34±0.09	1.80±0.33	2.97±0.30	1.17±0.09
MD (mm)	1.32±0.10	1.72±0.27	2.56±0.21	1.34±0.09
HL/HW	1.62±0.08	1.51±0.15	1.27±0.10	1.29±0.12
BM (mg)	9.29±2.80	17.81±7.82	37.96±5.27	10.28±1.23
MM (mg)	0.86±1.09	2.51±0.12	3.56±0.09	5.17±0.69
HM (mg)	1.40±0.15	4.65±0.23	11.87±0.29	0.64±0.27
RM (mg)	7.12±1.35	13.24±2.34	25.78±3.45	4.47±0.63

Calculated values (means±s.d.) for mesosoma length (ML), mesosoma width (MW), ML/MW; lengths of front (LL1), middle (LL2), hind legs (LL3); head length (HL), head width (HW), maximum eye distance (MD), HL/HW; total body mass (BM), mesosoma mass (MM), head mass (HM) and (residual) mass of gaster and legs (RM) are shown.
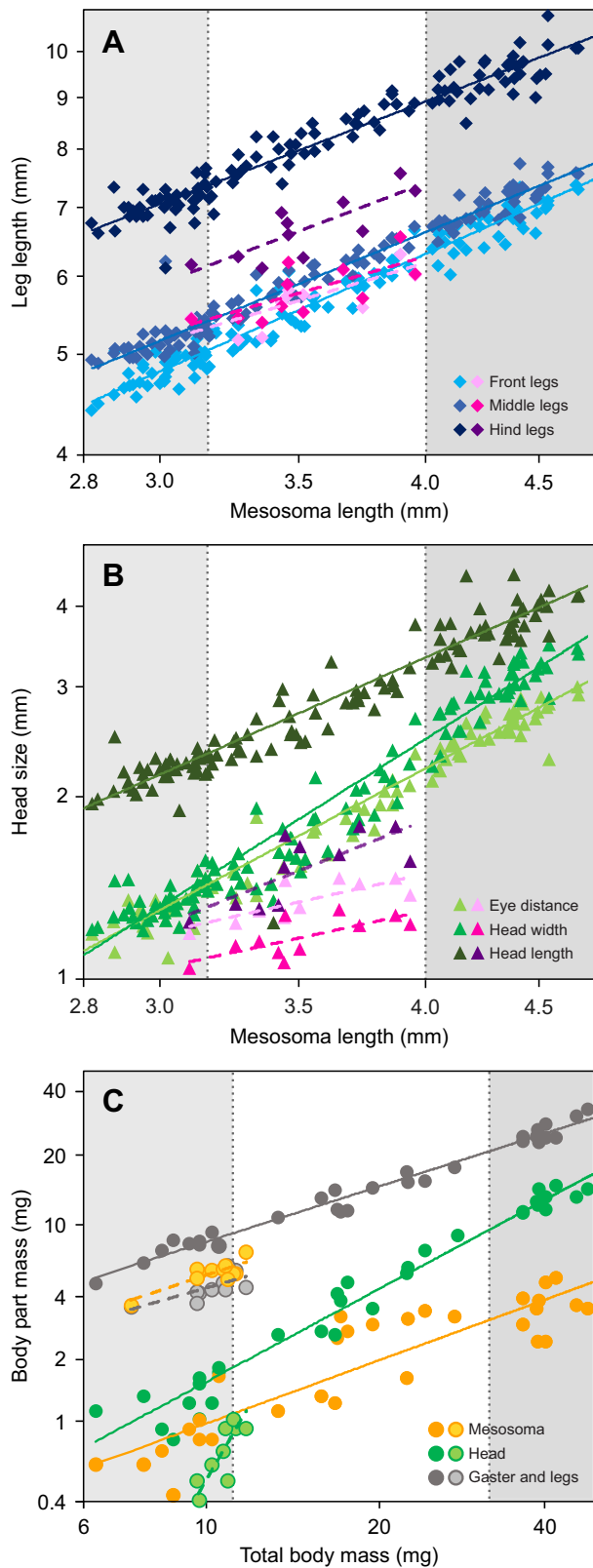
(10–40°C) to cover a broad walking speed range, as an increase in activity and speed could be achieved easily by increasing temperature (Fig. 3A). Channel temperature was regulated with both cooling pads and a heating light source and constantly measured with an infra red thermometer. Highest walking speeds were usually recorded at temperatures around 30°C; higher temperatures for more than a few minutes led to a decreased activity and lower walking speeds. Ants were not foraging and were not positively or negatively motivated. Sometimes, individuals were additionally spurred on by touching them with a fine paint brush (for activation), and thus we did not start the recording directly afterwards (random walks, no escape or foraging runs). Ants were filmed several times and up to five recordings of each individual were evaluated (each ant was filmed at low, medium and high temperatures, and we never used recordings of an ant with the same speeds). Furthermore, only straight and smooth running trajectories were analysed. Walking sequences with stops, decelerations, slipping steps or ants touching the channel walls were disregarded, and each evaluated high-speed video had to consist of a minimum of three complete step cycles per leg (and more steps for analysis of inter-leg coordination patterns). Only videos with nearly constant speed were evaluated (ensured by manual frame-by-frame analysis of speed and walking parameters during three consecutive steps). A piece of millimetre grid was used for calibration. All measurements were performed in a manual frame-by-frame video analysis with ImageJ (National Institutes of Health, Bethesda, MD, USA).

Analysis of the high-speed videos and data evaluation
General walking parameters

To compare the walking behaviour in *C. fellah* castes and subcastes, general walking parameters were analysed (Fig. 3; Figs S1, S2). Mean walking speed was calculated as the distance covered from the beginning of the first step cycle to the end of the third step cycle, divided by the time needed to cover this distance. Relative walking speed was defined as mean walking speed divided by mesosoma length. The timing of every lift-off and touch-down of the six tarsal tips was measured to obtain swing and stance phase durations. These values formed the basis for most further evaluations. Swing phase duration was calculated as the time between tarsal lift-off and touch-down. Stance phase duration was defined as the time when the tarsal tip touched the ground and did not move relative to the ground (Reinhardt and Blickhan, 2014; Wahl et al., 2015). Stride length was calculated for the respective leg as the distance between the tarsal lift-off and touch-down positions on the ground (no absolute stride length within the animal's own coordinate system). To calculate stride frequency, we divided mean walking speed by stride length. Despite small differences between leg pairs, all parameters described above were averaged for all six legs to allow better comparison (see Fig. S2, single leg pair analysis).

Footfall geometry

In a frame-by-frame analysis, the *x*- and *y*-coordinates of tarsal lift-off (posterior extreme position, PEP) and tarsal touch-down (anterior extreme position, AEP) were measured for each leg with respect to the centre of mass [COM, assumed to reside in the petiole (Reinhardt and Blickhan, 2014) and graphically determined (Weihmann et al., 2015)] to quantify footfall geometry (see Seidl and Wehner, 2008; Mendes et al., 2013). The *x*-axis is lateral (left and right side) to the COM (origin) and the *y*-axis is parallel to the animal's longitudinal axis in the walking direction. Footprint positions relative to the COM were normalised to total body length

Fig. 2. Morphometric analysis. (A) Correlation between mesosoma length and leg length, with separate analysis for front legs (power functions: light blue: $y=1.68x^{0.95}$, $R^2=0.96$; lilac: $y=2.08x^{0.80}$, $R^2=0.51$), middle legs (power functions: blue: $y=1.95x^{0.88}$, $R^2=0.96$; pink: $y=2.27x^{0.75}$, $R^2=0.74$) and hind legs (power functions: dark blue: $y=2.81x^{0.83}$, $R^2=0.93$; dark purple: $y=2.09x^{0.93}$, $R^2=0.74$) of *C. fellah* workers ($N=119$, shades of blue) and males ($N=11$, shades of purple). (B) Correlation between mesosoma length and maximum eye distance (power functions: light green: $y=0.16x^{1.87}$, $R^2=0.96$; lilac: $y=0.26x^{1.32}$, $R^2=0.98$), head width (power functions: green: $y=0.11x^{2.22}$, $R^2=0.95$; violet: $y=0.22x^{1.35}$, $R^2=0.99$) and head length (power functions: dark green: $y=0.38x^{1.55}$, $R^2=0.87$; dark purple: $y=0.19x^{1.60}$, $R^2=0.92$) of *C. fellah* workers ($N=119$, shades of green) and males ($N=11$, shades of purple). (C) Correlation between total body mass and mesosoma mass (power functions: orange: $y=0.10x^{0.99}$, $R^2=0.79$; light orange: $y=0.64x^{0.89}$, $R^2=0.68$), head mass (power functions: green: $y=0.05x^{1.46}$, $R^2=0.96$; light green: $y=1E-05x^{4.69}$, $R^2=0.91$) and combined mass of gaster and legs (power functions: grey: $y=1x^{0.87}$, $R^2=0.98$; light grey: $y=0.73x^{0.78}$, $R^2=0.53$) of *C. fellah* workers ($N=30$) and males ($N=11$), respectively. Ranges of minors (mesosoma length, ML: ~2.8–3.2 mm), medias (ML: ~3.2–4.0 mm) and majors (ML: 4.0–4.9 mm) are marked with dotted lines, and minor and major are indicated by the grey bars. Detailed allometric comparison in Table S1.

Corporation, Remond, WA, USA), Sigma Plot 11.0 (Systat Software Inc., San Jose, CA, USA) and Inkscape (Inkscape 1.0).

COM movement and lateral tarsus tracking (including lateral footfall geometry)

Tracking of COM movement was performed in the horizontal (Fig. 5A, top view) and lateral planes (Fig. 5B, lateral view) in a frame-by-frame analysis using ImageJ (National Institutes of Health). We analysed COM movement in *C. fellah* worker subcastes and males during one step cycle (beginning of swing phase in one tripod until end of swing phase of the following tripod; $n=30$ top-view and $n=15$ lateral-view step cycles per group) at the same walking speeds (average foraging speed range at 25°C, ~90–125 mm s^{-1}). In Fig. 5, the *x*-axis represents one step cycle, and the *y*-axis shows lateral (Fig. 5A) and vertical (Fig. 5B) COM oscillations towards the longitudinal axis in the walking direction.

The *x*- and *y*-coordinates of six tarsal positions during one step were measured for the front, middle and hind legs with respect to the COM in a frame-by-frame analysis of the lateral high-speed videos (compare method of footfall geometry) (Fig. 5C): tarsal lift-off (PEP), tarsal touch-down (AEP), highest tarsus position (Max.) and three additional coordinates of tarsus position (P1, P2 between PEP and Max., and P3 between Max. and AEP; with equal distances in each case). Tarsus positions relative to the COM were normalised to mesosoma length (ML) and standard deviations illustrate the spread of tarsus positions (Fig. 5C). The *x*-axis is parallel to the animal's longitudinal axis in the walking direction; the *y*-axis is perpendicular to the *x*-axis, representing tarsus positions relative to the COM.

Phase analysis

The onset of swing phase in the left hind leg (L3) was taken as the reference in phase analyses (Fig. 6). Phase plots show the coordination of the six legs in a circular step cycle diagram and were created with the 'CircStat' Toolbox in MATLAB (MathWorks, Inc., Natick, MA, USA) (Berens, 2009).

To evaluate the quality of tripod coordination, we calculated tripod coordination strength (TCS) (Wosnitza et al., 2013). For that, we measured t_1, the time period with all three legs of one tripod in swing phase, and t_2, the time period from the first swing onset in any of the legs in one tripod to the last swing termination. The ratio t_1/t_2

and the standard deviation of the footfall positions was used to illustrate the spread of footfall positions (Fig. 4). To track the movement of the legs and to analyse the footfall geometry, we used ImageJ (National Institutes of Health), Excel (Microsoft
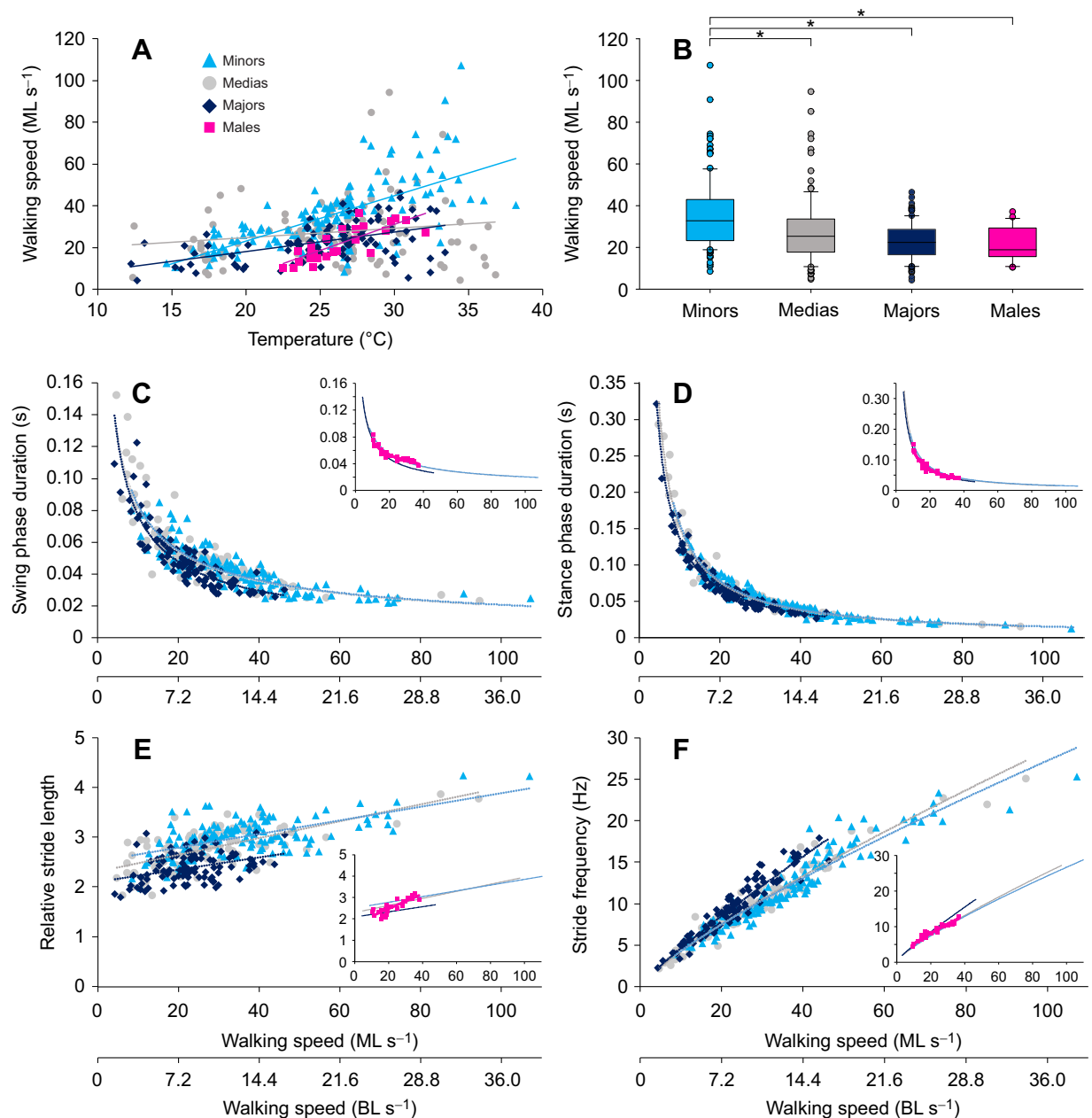
Fig. 3. Walking parameters. (A) Relationship between temperature and speed for minors (light blue triangles), medias (grey circles), majors (dark blue diamonds) and males (pink squares) of *C. fellah*. ML, mesosoma length. (B) Speed range of worker subcastes and males. Significant differences (ANOVA on ranks, H_3=52.083, $P<0.001$) occurred between minors and medias (Q=4.152, *$P<0.05$), minors and majors (Q=6.695, *$P<0.05$), and minors and males (Q=4.268, *$P<0.05$). (C–F) The most significant walking parameters in minors, medias, majors and males, plotted as functions of relative speed. BL, body length. Each data point represents the mean value of the three leg pairs in a video sequence (minors: n=130, medias: n=93; majors: n=116; insets: males: n=25). (C) Swing phase duration (power functions: minors: $y=0.34x^{-0.61}$, R^2=0.77, medias: $y=0.34x^{-0.61}$, R^2=0.81, majors: $y=0.39x^{-0.70}$, R^2=0.81, males: $y=0.21x^{-0.46}$, R^2=0.87). (D) Stance phase duration (power functions: minors: $y=1.64x^{-1.02}$, R^2=0.95, medias: $y=1.58x^{-1.01}$, R^2=0.96, majors: $y=1.42x^{-1.02}$, R^2=0.95, males: $y=1.24x^{-0.97}$, R^2=0.93). (E) Relative stride length (linear regressions: minors: $y=0.01x+2.51$, R^2=0.38, medias: $y=0.02x+2.30$, R^2=0.41, majors: $y=0.01x+2.10$, R^2=0.14, males: $y=0.04x+1.78$, R^2=0.72). (F) Stride frequency (power functions: minors: $y=0.68x^{0.80}$, R^2=0.92, medias: $y=0.63x^{0.83}$, R^2=0.94, majors: $y=0.60x^{0.88}$, R^2=0.93, males: $y=0.91x^{0.72}$, R^2=0.92).

was taken as the TCS. Higher TCS values indicate a strong synchronisation of the three legs of a tripod group (L1–R2–L3 and R1–L2–R3), whereas lower values indicate that the temporal relationship of swing phases shifted to other coordination patterns (TCS values between 0 and 1, with a value of 1.0 indicating perfect tripod coordination) (Wosnitza et al., 2013).

We calculated duty factor, the ratio of stance phase to step cycle period (stance plus swing of a given stride) that describes the

transition from walking to running in bipeds but is also a common parameter in insect locomotion studies. Considering a particular pair of legs, a duty factor of 0.5 signifies the point where aerial phases appear in the leg coordination pattern to achieve higher walking speeds (Alexander, 2003). Note that in animals walking on more than one pair of legs, phase shifts between leg pairs can still avoid aerial phases for the whole animal (Wilson, 1966; Hildebrand, 1985).
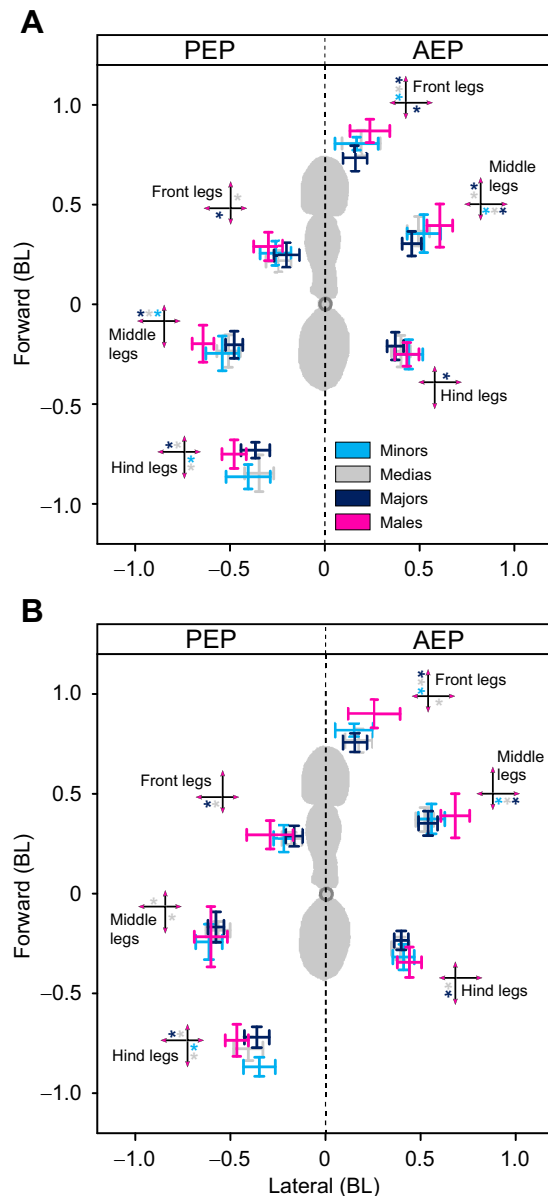
Fig. 4. Footfall geometry. Average relative footfall position of the legs of minors (light blue), medias (grey), majors (dark blue) and males (pink) of *C. fellah* with respect to the petiole at low (0–20 ML s^{-1}, 0–7.2 BL s^{-1}; A) and medium (20–40 ML s^{-1}, 7.2–14.4 BL s^{-1}; B) speeds. For each footfall position, 10 videos (six videos for males) with three step cycles each were evaluated. Posterior extreme positions (PEP) are shown for the left body side and anterior extreme positions (AEP) for the right body side. Axes were normalised to body length (BL). Error bars represent standard deviation. Significant differences in forward/backward and lateral shifts between males and the worker subcastes are illustrated by coloured asterisks (ANOVA on ranks, *P<0.05). Significant differences between subcastes only occurred in individual cases and are therefore not reliable. Speeds above 40 ML s^{-1} (not shown) were reached by minors and medias only, and no notable differences in footfall positions were observed compared with medium speeds.

Qualitative and quantitative analyses of inter-leg coordination patterns

Inter-leg coordination patterns were assessed using a frame-by-frame video analysis for qualitative and quantitative leg coordination analyses. Podograms were created to visualise the sequence of swing and stance movements of the six legs. They illustrate the patterns of the six legs, with black bars indicating swing phases and white bars indicating stance phases. We further quantified the inter-leg coordination by classifying each frame according to its momentary leg coordination pattern (Mendes et al., 2013; Pfeffer et al., 2019). These data were compared quantitatively for different walking speed ranges in the three worker subcastes and in males of *C. fellah*. To this end, an index number and a respective index colour, corresponding to the momentary inter-leg coordination, were assigned to each frame. We considered the momentary coordination patterns hexapod (brown, −1), pentapod (dark purple, 1), tetrapod (lilac, 2), tripod (dark green, 3), bipod (yellow, 4), monopod (orange, 5) and aerial phase (red, 6), depending on specific inter-leg coordination patterns (Fig. S4). Other leg coordination patterns were classified as undefined (grey). For a more detailed description, see legends of Fig. 7 and Fig. S5, regarding particular inter-leg coordination patterns, Fig. S4. To calculate the podograms and inter-leg coordination index, we used a MATLAB environment (MathWorks, Inc., Natick, MA, USA).

Statistical analysis

Box-and-whisker plots, normality tests, and statistical pairwise and multiple comparisons were generated in SigmaPlot 11.0 (Systat Software Inc.) and in Excel (Microsoft Corporation). Box-and-whisker plots show the median as the box centre, the 25th and the 75th percentiles as box margins and the 10th and 90th percentiles as whiskers. Data were tested for normal distribution with the Shapiro–Wilk test and Levene's mean test was used to assess equal variance. The *t*-test was used for pairwise comparison of normally distributed data. For multiple comparisons of normally distributed data, we used a one-way ANOVA with Holm–Šidák's method as *post hoc* test, and for non-normally distributed data, we used an ANOVA on ranks with Dunn's method as *post hoc* test. For comparison of circular data, we employed the MATLAB 'CircStat' Toolbox (Berens, 2009). Differences between phase relationships were tested for significance using the Kuiper two-sample test (circular analogue of Kolmogorov–Smirnov test). Tests for correlation were performed in Excel (statistical analysis of, for example, walking parameters, TCS values and duty factor). Final editing of the figures and tables was performed in Inkscape (Inkscape 1.0).

RESULTS
Morphometric analysis

For morphometric analysis, ML, MW, HL, HW, MD and LL were measured. Furthermore, the ratios ML/MW and HL/HW, and BM were determined (Table 1). With regard to body size, the entire size range was covered, although the queen was not measured to preserve viability of the colony. The queen caste was the largest by eye, with large eyes and ocelli and a well-developed reproductive system, and to our knowledge the largest body mass and size (see Peeters and Ito, 2015). We followed the division into reproductive caste (queen and males) and (non-reproductive) worker caste, with further division into subcastes of minor, media and major workers, based on our data and the following pre-established criteria. Animals with a clear head modification and ML of at least 4.0 mm were classified as majors. Ants with ML below 3.2 mm or BM lower than 10 mg were considered minors. The class of medias consists of a multitude of transitions between minors and majors (ML: 3.2–4.0 mm, no head modification). In comparison, males exhibited markedly different body size and shape, with small heads, fragile legs, a different mesosoma shape and wings (Fig. 1).

ML of workers ranged from about 2.8 to 5.0 mm (minors: 3.07±0.14 mm, medias: 3.59±0.26 mm, majors: 4.36±0.19 mm),
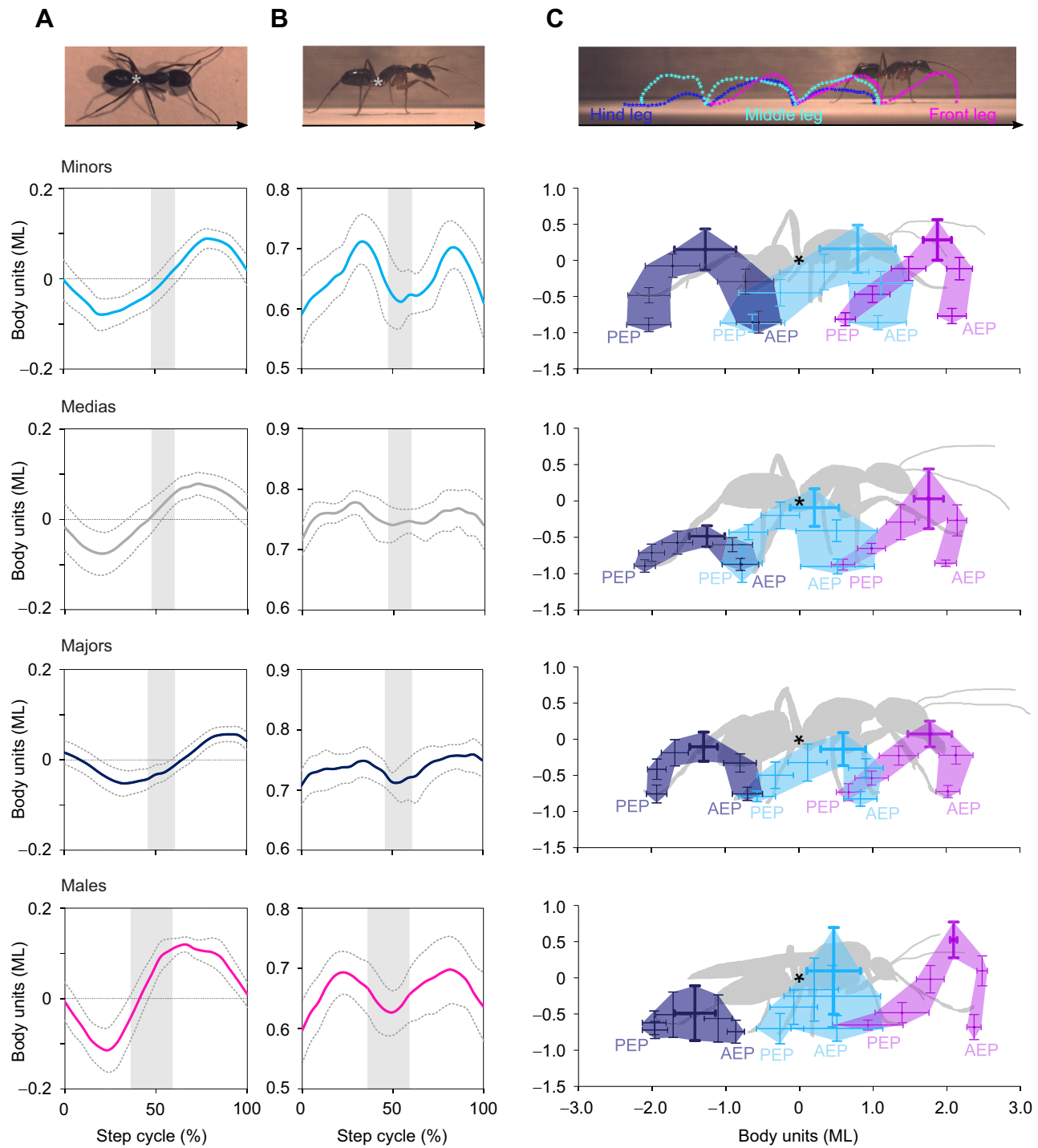
Fig. 5. Movement of the centre of mass (COM), lateral tarsus tracking and footfall geometry. Top images illustrate the recording situation. (A) Lateral COM movement (ML) in (from top to bottom) minors, medias, majors and males of *C. fellah* during one step cycle (horizontal plane view, *n*=30 step cycles each). Dotted lines represent 95% confidence intervals of the mean. Grey bars mark the beginning of stance of the first tripod. (B) Vertical COM movement in minors, medias, majors and males during one step cycle (vertical plane view, *n*=15 step cycles each). (C) Average relative tarsus positions of front (violet), middle (light blue) and hind legs (dark blue) of one body side in minors, medias, majors and males, plotted with respect to the petiole (COM, asterisk) at average foraging speeds between 90 and 125 mm s^{-1}. Tarsal positions during swing movements were traced from PEP to AEP in the right set of legs (*n*=15 steps each, lateral view). Maximum tarsus elevations are indicated in bold, as well as three equidistant tarsus positions before and after maximum elevation (means and s.d. error bars). Coloured areas indicate maximum movement ranges. Axes were normalised to mesosoma length (ML).

with head size (HW) increasing out of proportion towards the majors (minors: 1.34±0.09 mm, medias: 1.80±0.33 mm, majors: 2.97±0.30 mm). Positive allometric relationships were observed when considering ML in relation to HL, HW and MD, illustrating this change in head shape and especially the increase in HW. Majors

had a larger and relatively wider head while medias and minors had more elongated head shapes (Table 1, Fig. 2). Allometric relationships between ML and LL in workers were slightly negative, with *b*-values for front, middle and hind legs <1. That is, with increasing body size, LL increased at a slower rate than ML,
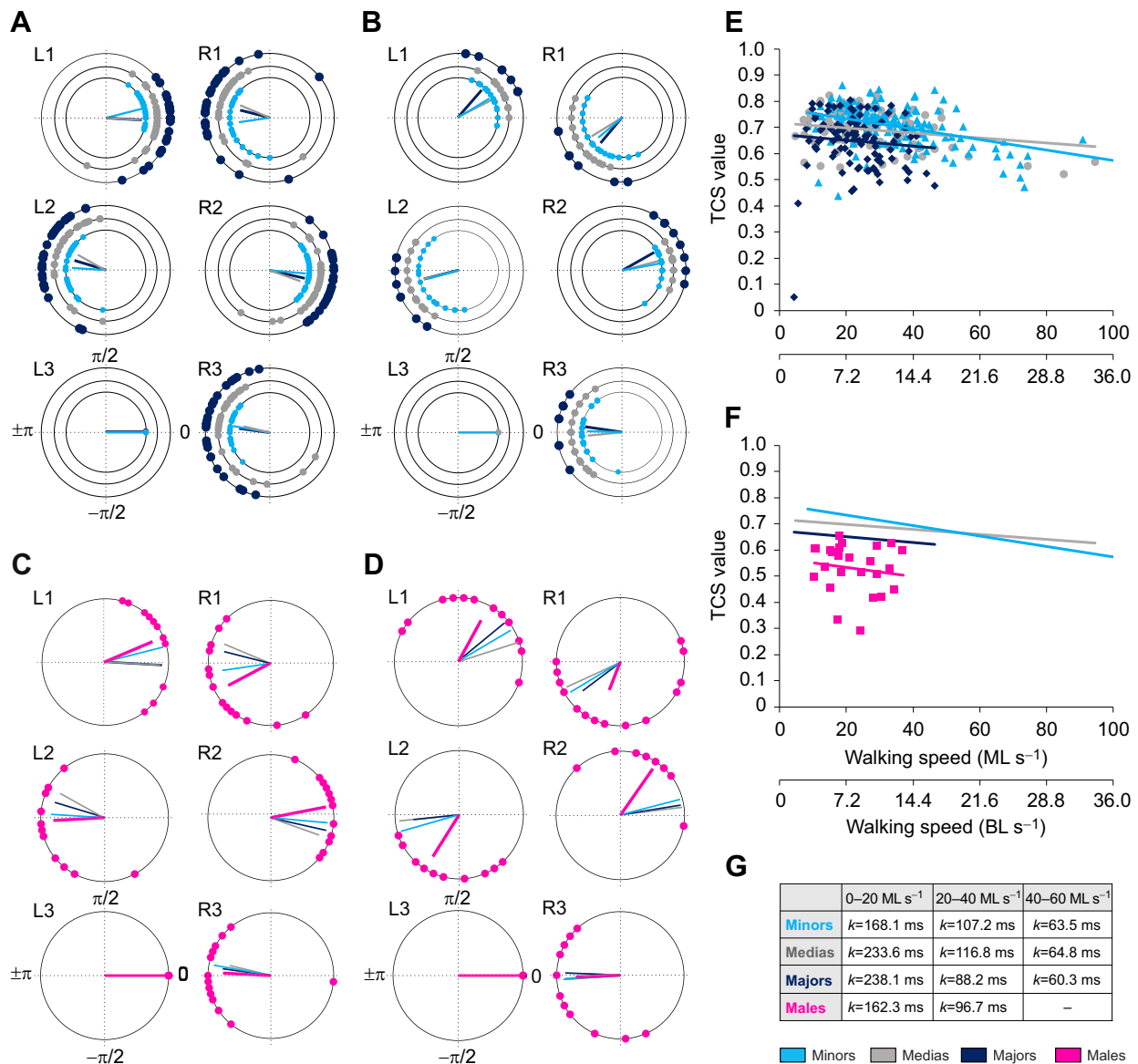
Fig. 6. Phase analysis. (A–D) Circular step cycle diagrams of the six legs, L1–R3, analysed for minors (light blue), medias (grey), majors (dark blue) (A,B), and males (C,D). The left hind leg (L3) was taken as the reference to plot swing phase onset. Phase plots of minors, medias and majors are shown in sequence from centre to periphery (A,B), with mean phase vectors scaled to the inner circle. Vector length indicates the variance of data points. Vectors of the subcastes are also plotted in the phase plots of males (C,D) for comparison. Ten videos with three step cycles each were analysed for the different speed bins (only three videos for majors at high speeds): (A) low speeds 0–20 ML s^{-1}, (B) high speeds 40–60 ML s^{-1}, (C) low speeds 0–20 ML s^{-1} and (D) medium speeds 20–40 ML s^{-1}. (E,F) Tripod coordination strength (TCS). Values for worker subcastes (E; linear regression: minors: $y=-0.002x+0.77$, $R^2=0.17$, medias: $y=-0.0009x+0.72$, $R^2=0.02$, majors: $y=-0.001x+0.67$, $R^2=0.01$) and males (F; linear regression: $y=0.0012x+0.537$, $R^2=0.014$) are plotted as a function of relative speed (ML s^{-1} and BL s^{-1}). Regression lines for the worker subcastes are plotted for comparison. (G) Circumference (k), representing step cycle duration, for phase plots of minors, medias, majors and males.

with majors possessing relatively shorter legs (Fig. 2A; for a detailed analysis, see Table S1). In comparison, males had a different body shape with ML comparable to that of medias (3.54±0.25 mm) but with very small heads (HW: 1.17±0.09 mm), longer gasters, fragile legs and noticeably shorter hind legs (Fig. 2; Table S1).

BM largely supported division into minors (9.29±2.80 mg), medias (17.81±7.82 mg) and majors (37.96±5.27 mg). BM of males was 10.28±1.23 mm. The relationship between HM, MM and residual BM was also determined by BM measurements. While the ratio of MM to total BM in workers and males was nearly isometric, HM exhibited a positive allometry in all ants (Fig. 2C).

In summary, the strong intraspecific size polymorphism was remarkable, with different allometric relationships between body parts, concerning in particular head size and leg length (Fig. 2; Table S1). Considering the entire colony, there were clearly more minors and even more medias than majors.

General walking parameters

Highest walking speeds were achieved by minors and a few medias (319 mm s^{-1} or 107 ML s^{-1} and 330 mm s^{-1} or 95 ML s^{-1}, respectively), with highest relative speeds in minors. Majors achieved considerably lower speeds (199 mm s^{-1} or 46 ML s^{-1}) and males achieved maximum speeds as low as 120 mm s^{-1} or
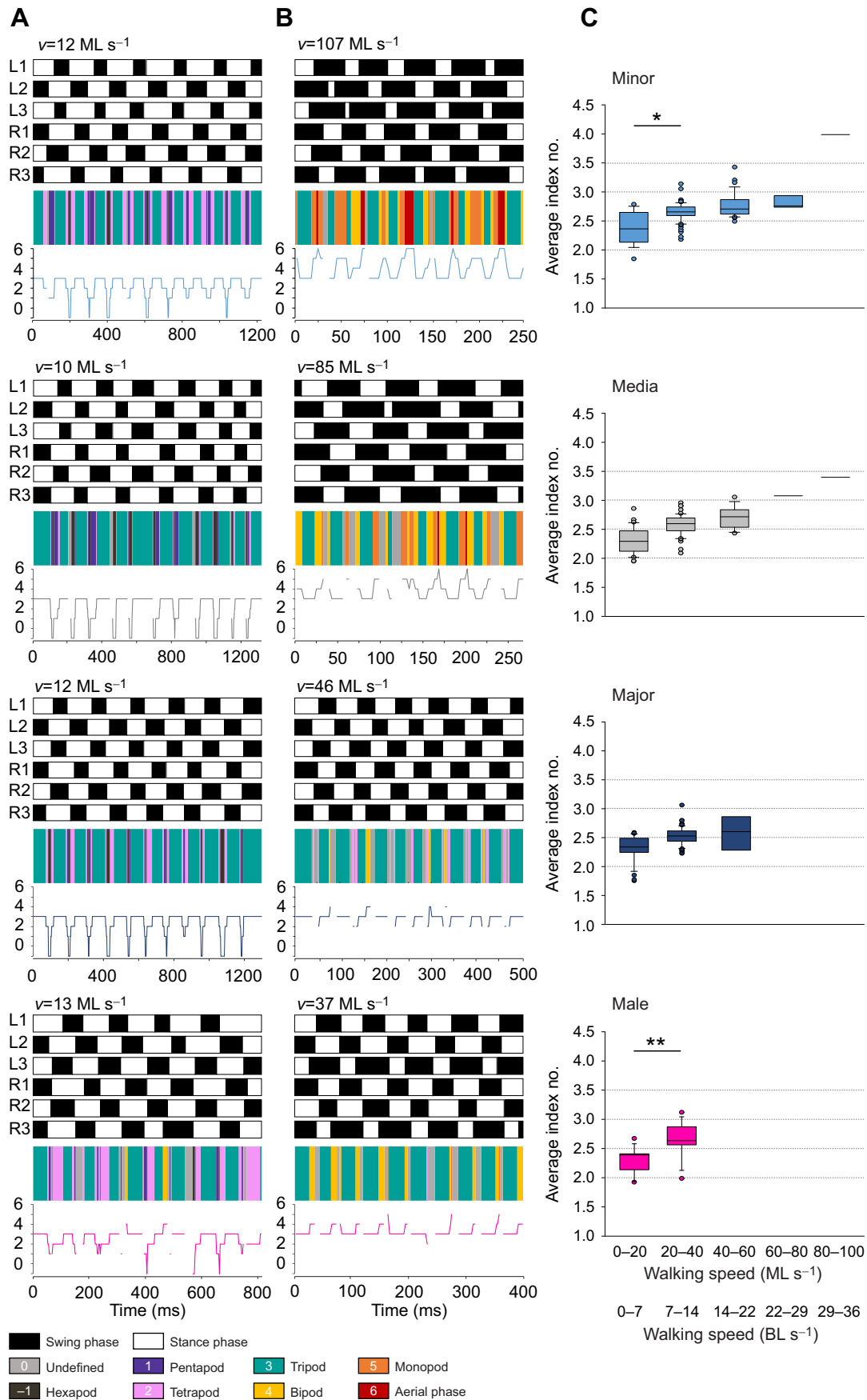
Fig. 7. See next page for legend.

Fig. 7. Qualitative and quantitative analysis of inter-leg coordination patterns. (A,B) Qualitative analysis. Podograms recorded in minors, medias, majors and males at the lowest (0–20 ML s⁻¹; A) and highest (40–110 ML s⁻¹; B) relative speeds, with corresponding colour and number indices plotted along the same axes. Podograms illustrate the patterns of the six legs, with black bars indicating swing and white bars indicating stance phases (L: left leg, R: right leg; 1, 2, 3: front, middle, hind leg). Each video frame was assigned an index number and a corresponding colour code according to the specific leg coordination in that frame (see colour key). For an exact definition of coordination patterns, see Fig. S4. (C) Quantitative analysis according to speed class (0–20 to 80–100 ML s⁻¹ or 0–7 to 29–36 BL s⁻¹) for minors (n=119), medias (n=97), majors (n=99) and males (n=25). Data are shown as averaged index numbers for the respective speed bins. No data were available for speeds >40 ML s⁻¹ in males and only n=3 majors, n=2 medias and n=5 minors reached relative speeds higher 40, 60 and 60 ML s⁻¹, respectively (data illustrate trend but are not included in further comparisons). Statistically significant differences in the mean index occurred between the 0–20 and 20–40 ML s⁻¹ speed bins in minors and males (ANOVA on ranks, *P<0.05 and **P=0.001, respectively). When comparing number indices of minors, medias, majors and males for the above speed bins, there were statistically significant differences for the 20–40 ML s⁻¹ speed bins (ANOVA on ranks, **P<0.01).

37 ML s⁻¹. All further parameters were evaluated in relation to both relative (Fig. 3) and absolute speeds (Fig. S1). Medias reached speeds above 200 mm s⁻¹ in only a few cases and therefore cannot be compared meaningfully with minors in higher speed classes.

It is remarkable that minors and medias reached considerably higher absolute and relative walking speeds than majors. With increasing speed, stride length and stride frequency increased in all worker subcastes. Majors tended to achieve the largest stride lengths (up to 13 mm) and showed higher absolute stride lengths than the other subcastes over the entire speed range (Fig. S1). In individual cases, minors and small medias increased stride length by the insertion of aerial phases at the highest speeds (Fig. 7). Aerial phases started to emerge at speeds around 250 mm s⁻¹ or 80 ML s⁻¹. Shorter stride lengths at similar speeds were compensated for by higher stride frequencies in smaller ants. Maximum frequencies were 25 Hz in minors and medias and 18 Hz in majors.

With increasing speed, swing phase and stance phase durations levelled off with a power function. Swing phase duration roughly plateaued towards 22 ms in all worker subcastes at speeds of around 150 mm s⁻¹ or 40 ML s⁻¹, and higher speeds resulted in hyperbolically decreased stance phase durations. Stance phase duration was as short as 12 ms in minors, at speeds around 300 mm s⁻¹ or 100 ML s⁻¹, and was slightly higher in medias and majors (minimum values 15 ms and 26 ms, respectively). Stance phase duration was longer than swing phase duration at low speeds, and this relationship reversed at higher speeds, though only in minors (∼139 mm s⁻¹) and medias (∼156 mm s⁻¹).

Overall, our relative walking speed (ML s⁻¹) data suggest that majors have relatively shorter legs than their smaller kin, as demonstrated by the slightly negative leg allometry of the worker caste (see morphometric analysis above; Fig. 2). At the same relative speeds, majors showed lower swing and stance phase durations than minors and medias; their relative stride lengths were lower and their stride frequencies were higher (Fig. 3).

Surprisingly small differences were observed in general walking parameters between subcastes and males, with respect to both absolute and relative speeds. However, significantly higher swing and shorter stance phase durations of the males' hind legs, a stronger increase in stride length with speed, and clearly lower maximum speeds of males were noticeable (Fig 3; Figs S1, S2).

Footfall geometry

Footfall patterns of the tarsi in PEP, shortly before lift-off at end of stance, and in AEP, shortly after touch-down at the beginning of stance, were analysed for worker subcastes and males. Compared with minors and medias, majors consistently showed footfall positions of all six legs, in both AEPs and PEPs, that were closer to the body over the entire speed range (Fig. 4), in agreement with their relatively shorter legs (Fig. 2A). Surprisingly, males showed footfall positions clearly more distant from the COM, compared with the worker subcastes. A significant influence of speed on footfall position could not be observed in the different groups, even if points of tarsal ground contact tended to be minimally closer to the body at low speeds.

COM movement and lateral tarsus tracking (lateral footfall geometry)

In one step cycle, the COM covered distances between 8 and 13 mm in the anterior–posterior direction in all ants (minors: 9.53±0.80 mm, medias: 11.01±0.86 mm, majors: 10.38±1.03 mm, males: 10.98±1.59 mm; average foraging speed at 25°C). As described by Reinhardt and Blickhan (2014), forward COM speed decreased in the double support phase and increased during the rest of the step cycle. In the lateral direction (top-view videos) COM oscillated around zero, with maximum lateral displacements on a relative scale in minors (∼0.25 mm, ∼0.08 ML), followed by medias (∼0.26 mm, ∼0.07 ML) and majors (∼0.24 mm, ∼0.05 ML) (Fig. 5). In comparison, maximum lateral displacement of the petiole in males was around 0.40 mm (0.1 ML). Maximum values were reached at the beginning of swing phase in L1 and L3, or R1 and R3, respectively, and stance initiation in R2 or L2. On average, COM was at least 1.9±0.2 mm (0.64±0.07 ML), 2.6±0.3 mm (0.72±0.09 ML), 3.2±0.3 mm (0.73±0.06 ML) and 2.2±0.2 mm (0.61±0.07 ML) above the ground at the beginning of swing in a tripod in minors, medias, majors and males, respectively. The highest oscillation amplitude was observed in minors and males, with maximum values of 0.38 and 0.34 mm (0.12 and 0.10 ML), compared with 0.21 and 0.22 mm (0.06 and 0.04 ML) in medias and majors, respectively (Fig. 5).

Regarding body posture, minors walked with a more erect posture with their heads held higher than for medias and majors (Table 2; Movie 1). Lateral recordings showed that all ants, and especially males, were repeatedly out of balance for short time periods, stumbling, which affected the results of vertical COM movement and lateral tarsus tracking.

Tarsus trajectories of the front, middle and hind legs differed during a step, with maximum relative tarsus movement in minors (Fig. 5C). Maximum tarsus elevation tended to be highest in minors and males (0.57 and 0.76 ML above COM in front legs), while medias and majors rarely showed maximum relative tarsus elevation above the COM. Variance (s.d. bidirectional) was highest in minors

Table 2. Body posture

	Minors	Medias	Majors	Males
Body to surface angle (deg)	21.83±4.99	8.88±4.80	11.37±4.81	12.25±8.62

Estimated body-to-surface angle (means±s.d.) of minors, medias, majors and males of *C. fellah* at intermediate speeds (90–120 mm s⁻¹). Angles were measured at the end of swing phase (touch down) in the front legs of six steps of five ants per caste and subcaste each (total: n=30 angles per group). Differences in body posture were significant (*P<0.05, one-way ANOVA) except for differences between medias and majors, and majors and males.

and males and lowest in medias and majors. In males, dragging of the hind legs across the ground was noticeable.

Phase analysis

TCS provides a measure of tripod gait quality, with a value of 1 characterising exact synchrony in the movements of the legs in a tripod. TCS values of workers were mostly between 0.5 and 0.8 and decreased with higher speeds (Fig. 6E). TCS values were higher in minors and medias than in majors (Fig. 6; test for correlation, $P<0.05$), and highest values were achieved at speeds of 50–100 mm s^{-1} (20–40 ML s^{-1}). It is noticeable that TCS values of minors and medias rose again at the highest speeds, as relative swing phase duration increased, in conjunction with the occurrence of aerial phases. By contrast, TCS values were lowest in males and nearly constant at around 0.5 over their speed range (compare phase deviations, duty factor).

Phase plots illustrate variability of inter-leg coordination. They show the onset of swing phase during the step cycle in a circular diagram, with the left hind leg (L3) serving as reference. For all groups, a clear antiphase relationship between the two tripods L1–R2–L3 and R1–L2–R3 was evident. This tripod coordination was observed over the entire speed range, with small but consistent phase shifts between the tripod legs (significant shifts only in males: L3–R2 ($P<0.001$), R3–L2 ($P<0.02$, Kuiper two-sample test).

At low speeds (0–20 ML s^{-1}), phase shifts within one tripod group were smaller compared with those at higher speeds (20–40, 40–60 ML s^{-1}), which is consistent with the TCS values above. With increasing speed, an inter-leg coordination sequence hind leg–middle leg–front leg within a tripod group was established, with shifts between these legs of about 10% in minors (leg 3–2: 3.8%, 2–1: 6.3%), 8% in medias (leg 3–2: 3.1%, 2–1: 4.4%) and up to 15% (leg 3–2: 7.3%, 2–1: 7.8%) in majors (with no significant differences between subcastes) (Fig. 6A,B). Males, by comparison, exhibited the largest phase shifts of around 18% (leg 3–2: 15.4%, 2–1: 2.4%) with highest variance in the front legs (Fig. 6C,D). Furthermore, contralateral shifts between leg pairs (L3–R3, L2–R2, L1–R1) were around 50%, while ipsilateral shifts (L3–L2–L1, R3–R2–R1; consistent with shifts within tripod groups) varied between 42% and 65%, with higher phase shifts between leg 2 and leg 1, than between leg 3 and leg 2 in all subcastes (Table 3).

Duty factor characterises the transition from walking to running, which might occur at values around 0.5 (strictly for bipedal locomotion only; Alexander, 2003). With increasing speed, duty factor decreased non-linearly for all leg pairs. Only in the case of minors and medias did all three leg pairs fall below a duty factor of 0.5 (minors: front legs: 148 mm s^{-1}, middle legs: 206 mm s^{-1}, hind legs: 119 mm s^{-1}; medias: front legs: 165 mm s^{-1}, middle legs:

255 mm s^{-1}, hind legs: 131 mm s^{-1}) and these ants may thus be considered to run (Fig. S3). As the middle legs were the last to reach a value below 0.5 (middle legs with shortest swing and longest stance phases; Fig. S2), they determined the threshold at which the transition from walking to running might occur (minors: 206 mm s^{-1} or 65 ML s^{-1}, medias: 255 mm s^{-1} or 71 ML s^{-1}). All three leg pairs of minors fell below the value of 0.5 earlier in relation to absolute and relative speeds (test for correlation, $P<0.05$; Fig. S3). In comparison, majors and males did not make the transition from walking to running in their observed speed range.

Inter-leg coordination patterns

We analysed inter-leg coordination patterns of worker subcastes and males by assigning an index number and colour to each video frame, according to its momentary leg coordination (number, colour codes of specific coordination patterns in Fig. S4). Podograms, with colour and number indices of worker subcastes and males at lowest and highest relative speeds were created (qualitative analysis, Fig. 7A,B) and the proportion of each coordination pattern was determined by indexing in adjacent speed bins (quantitative analysis, Fig. 7C; Fig. S5), for both relative (ML s^{-1}) and absolute speed (mm s^{-1}).

At low speed, coordination patterns with three or more legs in stance phase dominated in all groups, and phases with all six legs on the ground occurred. Fractions of tripod coordination increased in all groups up to medium speeds of 100–150 mm s^{-1} (20–40 ML s^{-1}) and decreased thereafter (Fig. S5). Patterns with four or five legs in stance were replaced at medium and high speeds by bipod and monopod situations. Majors exhibited a remarkably high fraction of tetrapod coordination (150–200 mm s^{-1}: 6.7%; 40–60 ML s^{-1}: 9.6%) (compare ipsilateral phase shifts), while minors showed distinctly smaller fractions [<1% above 150 mm s^{-1} (60 ML s^{-1})].

Speeds above 200 mm s^{-1} (60 ML s^{-1}) were achieved only by minors and few medias. Tripod coordination decreased notably at highest speeds (200–250 mm s^{-1}: 54.7%, >250 mm s^{-1}: 40.6%) and specific combinations with fewer than three legs in stance increased correspondingly (200–250 mm s^{-1}: 29.1%, >250 mm s^{-1}: 58.3%). A rare but special feature observed in only a few minors and medias was the incorporation of aerial phases at speeds above 250 mm s^{-1} (minors: 14.4%, medias: 1.2%). In males, a comparatively high fraction of bipod coordination (14.4%) at speeds between 50 and 100 mm s^{-1} was striking (compared with <1% in worker subcastes). This may be related to their short hind legs, with bipod fractions representing combinations of front and middle legs of a tripod.

In summary, coordination patterns of majors changed only little over the entire speed range compared with those of minors, medias

Table 3. Phase shift analysis

	Minors		Medias		Majors		Males	
	Contralateral (%)	Ipsilateral (%)	Contralateral (%)	Ipsilateral (%)	Contralateral (%)	Ipsilateral (%)	Contralateral (%)	Ipsilateral (%)
Speed (ML s^{-1})	L3-R2-L1/ R3-L2-R1	L3-L2-L1/ R3-R2-R1	L3-R2-L1/ R3-L2-R1	L3-L2-L1/ R3-R2-R1	L3-R2-L1/ R3-L2-R1	L3-L2-L1/ R3-R2-R1	L3-R2-L1/ R3-L2-R1	L3-L2-L1/ R3-R2-R1
0–20	−0.9; 4.4	49.0; 54.3	−4.9; −1.3	44.9; 53.3	−3.0; 1.5	47.1; 51.7	2.4; 3.6	52.4; 55.1
20–40	3.7; 4.5	53.7; 54.3	1.3; 4.1	51.3; 54.2	2.3; 8.6	52.3; 58.6	15.4; 2.4	65.4; 52.4
40–60	3.8; 6.3	53.8; 56.4	3.1; 4.4	53.1; 54.4	7.3; 7.8	57.4; 57.7	–	–

Individual phase shift values (%) of contralateral shifts within one tripod group (leg 3–2, 2–1, mean value of L3–R2–L1 and R3–L2–R1) and (corresponding) ipsilateral shifts (leg 3–2, 2–1, mean value of L3–L2–L1 and R3–R2–R1) for worker subcastes and males. L1–3, left front, middle and hind leg; R1–3, right front, middle and hind leg.

and males. Further, the constantly high tetrapod fraction in majors and the occasional incorporation of aerial phases in minors and medias are notable (Fig. 7; Fig. S5).

DISCUSSION

Camponotus fellah is a species characterised by a distinct intraspecific size polymorphism with different relationships between body parts in the worker subcastes and in males (Fig. 2). Such an investment in different castes and especially more than one worker caste may influence colony fitness, reproduction and the competitive ability of *C. fellah* and has evolved repeatedly in a number of ant species (Bershers and Traniello, 1994; Powell, 2009; Wills et al., 2018). In this context, we investigated the walking behaviour of *C. fellah* and the influence of caste and subcaste characteristics, as efficient locomotion is relevant for colony survival. Concerning allometric relationships between body parts, in summary, majors have relatively shorter legs and wider heads than minors and medias (Fig. 2). In comparison, the male reproductive caste is distinctly different, equipped with wings, a small head and wispy legs with comparatively short hind legs. Significant differences in body size, associated leg length and head shape should influence walking performance between subcastes, and in comparison to males.

We observed species-specific locomotor behaviour with comparably small differences in walking parameters, though notable differences in speed, COM movement, walking posture and coordination pattern between castes and subcastes. Surprisingly, males showed quite similar walking behaviour to female workers, but with lower speeds, wider footprints and deviating phase relationships.

Walking speed related to colony structure and division of labour

Regarding the influence of caste and subcaste characteristics on speed, one could assume that larger ants with longer legs (majors) reach higher speeds through larger steps than their smaller kin, minors and medias. Contrary to this expectation, highest speeds were achieved by minors and medias (318 mm s^{-1} or 107 ML s^{-1} and 330 mm s^{-1} or 95 ML s^{-1}, respectively), but it should be noted that medias achieved such high speeds rarely. By contrast, majors reached distinctly lower maximum speeds (199 mm s^{-1} or 46 ML s^{-1}), which appears to be related to their function in the colony. An investment in achieving high speeds is not required for majors that are specialised in nest defence with their massive mandibles, in contrast to minors and medias that are mainly responsible for brood care and foraging (Hölldobler and Wilson, 1990; Laciny et al., 2018, 2019). Foragers have to walk as efficiently as possible across long distances to provide food and ensure colony survival. *Camponotus fellah* males reached considerably lower speeds (120 mm s^{-1} or 40 ML s^{-1}), which nonetheless is a good performance level considering their wispy legs (Fig. 1). Compared with the workers, males have a different function and are thus equipped for a different form of locomotion (clearly not adapted towards running longer distances). They possess wings for mating flights, large eyes relative to head size, and low body mass. Nevertheless, walking is also an issue for males, as they stay in the colony while waiting for nuptial flights that are synchronised across nests (Boomsma et al., 2005).

In conclusion, division of labour is based to a large extent on different castes and subcastes, and this is reflected in locomotion behaviour: highest speeds were achieved by small ants that are responsible for foraging, while large majors, specialised for nest

defence, are much slower. Winged males were again significantly slower, as they generally do not walk much outside the nest. The observed differences in maximum speed illustrate that scaling of speed depends on body size and shape (Hurlbert et al., 2008; Hirt et al., 2017), and in this context on caste and subcaste characteristics.

Converted to body lengths per second, maximum speeds of *C. fellah* correspond to 38 BL s^{-1} (minors), 33 BL s^{-1} (medias), 17 BL s^{-1} (majors) and 14 BL s^{-1} (males). Higher speeds are achieved by, for example, *Cataglyphis* desert ants (108 BL s^{-1}) (Pfeffer et al., 2019) and a few other arthropods, including *Cicindela eburneola* (171 BL s^{-1}) (Kamoun and Hogenhout, 1996). *Camponotus fellah* workers thus cannot compete with typical *Cataglyphis* desert ants in terms of speed, even though some of them (e.g. *C. albicans*, *C. bicolor*) are observed in the same habitats. The lower speeds are probably related to nocturnal foraging activity, first, as lower night temperatures reduce walking speed in heterotherms (Hurlbert et al., 2008) and second, as there is no heat stress during the night that would require higher foraging speeds (Wehner et al., 1992). In this context, we could observe an increase in speed with temperature: highest speeds were achieved at around 30°C; even higher temperatures led to an activity and speed decrease (Fig. 3A,B) (Hurlbert et al., 2008). Further, *C. fellah*'s morphology with relatively short and muscular legs and notable differences in head shape and size would appear to affect locomotor performance (for example, compare leg length of *C. bicolor*; Tross et al., 2021). Other ant species, for example *Formica polyctena* (Reinhardt and Blickhan, 2014) or *Messor barbarus* (Merienne et al., 2020), do not achieve speeds higher than 200 mm s^{-1}, and *C. fellah* appears to be more comparable to these species with regard to locomotion.

Caste- and subcaste-dependent locomotion strategies

Different castes and subcastes of *C. fellah* use the same overall locomotion strategy, independent of their body characteristics, with a comparably strong decrease of swing phase duration in all groups (Fig. 3C). Nevertheless, there are certain differences between castes and subcastes. Minors and medias employed higher stride frequencies (up to 25 Hz), very rarely increasing stride length by the incorporation of aerial phases at the highest speeds. Majors, by contrast, achieved maximum frequencies around 18 Hz and were not able to increase stride length by aerial phases. The lower frequencies may be attributed to their larger body mass and the fact that stride frequency is limited by contraction and relaxation kinetics of leg muscles and tissue viscosity (Rubin et al., 2016). When considering the whole relative speed range, majors reached significantly lower speeds than their smaller kin with shorter relative stride lengths and higher relative frequencies in comparison, which might be partly attributed to the slightly negative leg allometry in the worker caste (majors have relatively shorter legs; Table S1). Surprisingly, despite distinct morphometric differences, general walking parameters of males were quite similar, except for significantly higher swing and lower stance phase durations of the hind legs and a stronger increase in stride length (Fig. 3). In this context, gait variability and also phase shifts do not differ significantly between sexes in stag beetles, indicating that they also move in a dynamically similar way (Goyens et al., 2015). Overall, *C. fellah* ants, like most insects, maintain a typical tripod(-like) coordination pattern without aerial phases (tripod >50%; Fig. S5) (Hughes, 1952; Full and Tu, 1991; Reinhardt and Blickhan, 2014), with partly desynchronised tripods in males. Locomotion of *C. fellah* might be characterised as a form of

'grounded running' (Reinhardt and Blickhan, 2014; Merienne et al., 2021), a statically stable type of gait in small insects with comparable high speeds and without aerial phases.

Statically stable locomotion performance

Negative leg allometry in the worker caste – more precisely, relatively shorter legs of majors – were also observed in footprint positions, as their relative footprint positions are closer to the body than for medias and minors. The most striking differences in footfall position were observed between workers and males, indicating increased static stability in males (Ting et al., 1994; Zollikofer, 1994). It should be noted, however, that footprints of worker subcastes support statically stable walking as well, and broader footfall positions of males might be related to their spindly legs. In general, legs placed in more sprawled positions around the body provide a more stable posture for resistance against disturbances (Alexander, 1971) and can offer static stability during locomotion, especially at low speeds (Ting et al., 1994). In this context, it appears that continuous static walking in *C. fellah* results in less strict tripodal leg coordination with increasing speed (partly desynchronised tripods) – as indicated by phase shifts within tripods and lower TCS values – except for the few ants that incorporated aerial phases (more dynamic walking). When considering phase relationships, phase shifts between leg pairs were apparent in all ants, with a clear tendency for front legs to lift off last and strongest phase shifts in males (Fig. 6). Phase shifts increased with speed, which is reflected in decreasing TCS values, leading to poor tripod coordination accuracy, particularly in males (see Weihmann, 2018). In animals walking on more than one leg pair (multi-legged), phase shifts can avoid aerial phases for the whole animal up to a certain point (Hildebrand, 1985), explaining the connection between missing aerial phases and phase shifts at the velocities observed. Comparable shifts for front legs occurred in *C. bicolor* (Tross et al., 2021), and even more distinct phase shifts could be observed in connection with a metachronal gait during fast runs of the cockroach *Nauphoeta cinerea* (Weihmann et al., 2017). A velocity-dependent gait continuum with tripod coordination as the major pattern (Hughes, 1952; Wilson, 1966; Schilling et al., 2013; Dürr et al., 2018; Pfeffer et al., 2019; Tross et al., 2021) was observed in all ants, with a large fraction of combinations with fewer than three legs in stance. In this context, it was striking that majors did not show considerable changes in leg coordination patterns within the continuum, and had a consistently high tetrapod fraction compared with their smaller kin.

We further report that lateral and vertical COM positions are characterised by a periodic pattern with two peaks corresponding to the two tripods in a step cycle. Highest relative and absolute lateral and vertical oscillations were observed in minors and males, and it appears that minors are walking with a clearly more erect posture compared with their larger kin (Table 2). This has been described for *Messor barbarus* and might be related to a more excited state of minors in response to manipulation and environmental conditions and to the division of labour within a colony (Merienne et al., 2021). Differences in response to a threat between differently sized ants have also been described in *Atta capiguara* ants (Hughes and Goulson, 2001). However, different postures could also result from their mass distribution – majors have large and heavy heads – and the comparatively small body mass of minors. On average, majors are about 4 times heavier than minors. Moreover, tarsus positions relative to the COM, leg and COM movement indicated a spatial consistency during walking in majors and medias (Fig. 5). Maximum relative tarsus movement was observed in minors, and

maximum tarsus positions above the COM in the front and middle legs of minors and males. Lowest variance in tarsus position was observed in majors, which also showed the lowest COM oscillations, indicating a comparatively uniform locomotion pattern with consistent leg movements (Movie 1). The noticeable dragging of the hind legs across the ground in males is related to their much shorter hind legs. In summary, *C. fellah* ants walk with relatively high speeds and periodic lateral and vertical COM oscillations, mainly to ensure ground contact and stable locomotion (Blickhan and Full, 1993; Reinhardt and Blickhan, 2014), but with the occasional bipod and monopod situations in their coordination patterns.

Species-specific similarities independent of caste and subcaste characteristics

Although the polymorphic workers and males of *C. fellah* show distinct differences in body size, shape and mass, intraspecific differences in locomotion are less pronounced than morphological differences may suggest. Comparing *C. fellah* with *Cataglyphis* desert ants, for example, the latter employ distinctly higher speeds, with a strong increase in stride length, more than quadrupling in *C. bombycina*, in combination with aerial phases (Pfeffer et al., 2019). *Camponotus fellah* do not even double stride length over their speed range and hardly ever use aerial phases. Other ant species, such as *Formica polyctena* (Reinhardt and Blickhan, 2014) or *Messor barbarus* (Merienne et al., 2021), both of which perform a form of 'grounded running' with speeds comparable to those of *C. fellah*, also show walking patterns different from those of *C. fellah*. *Formica polyctena*, for example, performs a tripod gait with no more than three legs in the air at any time (Reinhardt and Blickhan, 2014), while all *C. fellah* groups inserted bipod and monopod situations (Fig. 7; Fig. S5). Relative speeds comparable to those of *C. fellah* males are achieved by *Drosophila melanogaster* ($\sim$15 BL s^{-1}) (Wosnitza et al., 2013), which are adept at walking and flying locomotion, with tripod gait at high speeds and tetrapod and pentapod patterns at lower speeds. Studies on larger insects, such as cockroaches or beetles (Hughes, 1952), also demonstrated that tripod coordination is used at the highest speeds and main walking patterns are more variable at lower speeds, including tetrapod and pentapod patterns. In this context, a slow and fast gait (ambling and trotting) was found in *Blaberus discoidalis* (Bender et al., 2011) and a fast metachronal gait in *Nauphoeta cinerea* (Weihmann et al., 2017). In conclusion, *C. fellah* exhibit a more species-specific walking behaviour described as statically stable 'grounded running' (Szczecinski et al., 2018). Moreover, caste and subcaste characteristics mainly affect speed and, in this context, relevant walking parameters, while the general locomotion strategy is just slightly affected. Similar small intraspecific differences in locomotion were observed in male and female stag beetles (Goyens et al., 2015) and in workers of *Cataglyphis bicolor*, a species with strong variation in body size (ML 2.1–5.0 mm) but no subcaste differentiation. *Cataglyphis bicolor* show little intraspecific difference in walking performance with speed, in comparison to allometrically similar *C. albicans* ants (Tross et al., 2021).

Conclusion

Investment in different castes and especially worker subcastes influences colony survival, fitness and reproduction of ants (Bershers and Traniello, 1994; Powell, 2009; Wills et al., 2018), as matching of worker size and shape with task promotes organisational efficiency (Oster and Wilson, 1978; Powell and Franks, 2006). Such caste and subcaste characteristics are obviously

reflected in the walking behaviour of *C. fellah*. On the one hand, our results support the idea of the division into small-sized and fast-walking foragers (minors and medias) and the considerably slower majors with large heads and mandibles for nest defence (Hölldobler and Wilson, 1990; Laciny et al., 2019). However, it seems that such a strict division may not accurately reflect additional factors such as size-dependent maximum load capacity (Mersch et al., 2013; Merienne et al., 2020). On the other hand, our study reveals that *C. fellah* ants use a common locomotor strategy across all body sizes, castes and subcastes, but with minors reaching the highest speeds. Thus, body size and shape mainly affect speed, and to a comparably small extent basic walking parameters, footprint positions, phase relationships and COM movements. The walking behaviour of the allometrically special males was particularly surprising here, but supports the assumption that differences in locomotion are more species specific than size or caste specific, when comparing our data with those from other ant species (e.g. Reinhardt and Blickhan, 2014; Pfeffer et al., 2019; Merienne et al., 2021).

References

Alexander, R. M. (1971). *Size and Shape (No. 29)*. Hodder Education.

Alexander, R. M. (2003). *Principles of Animal Locomotion*. New Jersey, USA: Princeton University Press.

Andersen, A. N., Hoffmann, B. D. and Oberprieler, S. K. (2020). Megadiversity in the ant genus *melophorus*: the *M. rufoniger* Heterick, Castalanelli and Shattuck species group in the top end of Australia's Northern territory. *Diversity* **12**, 386. doi:10.3390/d12100386

Bender, J. A., Simpson, E. M., Tietz, B. R., Daltorio, K. A., Quinn, R. D. and Ritzmann, R. E. (2011). Kinematic and behavioral evidence for a distinction between trotting and ambling gaits in the cockroach *Blaberus discoidalis*. *J. Exp. Biol.* **214**, 2057-2064. doi:10.1242/jeb.056481

Berens, P. (2009). CircStat: a MATLAB toolbox for circular statistics. *J. Stat. Softw.* **31**, 1-21. doi:10.18637/jss.v031.i10

Bershers, S. N. and Traniello, J. F. (1994). The adaptiveness of worker demography in the attine ant *Trachymyrmex septentrionalis*. *Ecology* **75**, 763-775. doi:10.2307/1941733

Billick, I. (2002). The relationship between the distribution of worker sizes and new worker production in the ant *Formica neorufibarbis*. *Oecologia* **132**, 244-249. doi:10.1007/s00442-002-0976-7

Blickhan, R. and Full, R. J. (1993). Similarity in multilegged locomotion: bouncing like a monopode. *J. Comp. Physiol.* **173**, 509-517. doi:10.1007/BF00197760

Boomsma, J. J., Baer, B. and Heinze, J. (2005). The evolution of male traits in social insects. *Annu. Rev. Entomol.* **50**, 395-420. doi:10.1146/annurev.ento.50.071803.130416

Boulay, R., Quagebeur, M., Godzinska, E. J. and Lenoir, A. (1999). Social isolation in ants: evidence of its impact on survivorship and behavior in *Camponotus fellah* (Hymenoptera, Formicidae). *Sociobiology* **33**, 111-124.

Boulay, R., Soroker, V., Godzinska, E. J., Hefetz, A. and Lenoir, A. (2000). Octopamine reverses the isolation-induced increase in trophallaxis in the carpenter ant *Camponotus fellah*. *J. Exp. Biol.* **203**, 513-520. doi:10.1242/jeb.203.3.513

Duncan, F. D. and Crewe, R. M. (1993). A comparison of the energetics of foraging of three species of Leptogenys (Hymenoptera, Formicidae). *Physiol. Entomol.* **18**, 372-378. doi:10.1111/j.1365-3032.1993.tb00610.x

Dupeyroux, J., Serres, J. R. and Viollet, S. (2019). AntBot: a six-legged walking robot able to home like desert ants in outdoor environments. *Sci. Robot.* **4**, eaau0307. doi:10.1126/scirobotics.aau0307

Dürr, V., Theunissen, L. M., Dallmann, C. J., Hoinville, T. and Schmitz, J. (2018). Motor flexibility in insects: adaptive coordination of limbs in locomotion and near-range exploration. *Behav. Ecol. Sociobiol.* **72**, 15. doi:10.1007/s00265-017-2412-3

Espadaler, X., Retana, J. and Cerda, X. (1990). The caste system of *Camponotus foreli* Emery (Hymenoptera: Formicidae). *Sociobiology* **17**, 299-312.

Fjerdingstad, E. J. and Crozier, R. H. (2006). The evolution of worker caste diversity in social insects. *Am. Nat.* **167**, 390-400. doi:10.1086/499545

Full, R. J. and Tu, M. S. (1991). Mechanics of a rapid running insect: two-, four- and six-legged locomotion. *J. Exp. Biol.* **156**, 215-231. doi:10.1242/jeb.156.1.215

Galbán, A., Cuezzo, F. and Torréns, J. (2021). The pronotum of worker of *Camponotus borellii* emery (hymenoptera: formicidae): how can it affect performance of the head, work division, and development of the worker caste? *Neotrop. Entomol.* **50**, 78-89. doi:10.1007/s13744-020-00828-0

Goyens, J., Dirckx, J. and Aerts, P. (2015). Costly sexual dimorphism in *Cyclommatus metallifer* stag beetles. *Funct. Ecol.* **29**, 35-43. doi:10.1111/1365-2435.12294

Heinze, J. (2016). The male has done his work—the male may go. *Curr. Opin. Insect Sci.* **16**, 22-27. doi:10.1016/j.cois.2016.05.005

Hildebrand, D. M. (1985). Walking and running. In *Functional Vertebrate Morphology* (ed. M. Hildebrand, D.M. Bramble, K.F. Liem and D.B. Wake), pp. 38-57. Cambridge, MA: Belknap Press of Harvard University Press.

Hirt, M. R., Jetz, W., Rall, B. C. and Brose, U. (2017). A general scaling law reveals why the largest animals are not the fastest. *Nat. Ecol. Evol.* **1**, 1116-1122. doi:10.1038/s41559-017-0241-4

Hölldobler, B. and Wilson, E. O. (1990). *The Ants*. Harvard University Press.

Hughes, G. M. (1952). The co-ordination of insect movements: I The walking movements of insects. *J. Exp. Biol.* **29**, 267-285. doi:10.1242/jeb.29.2.267

Hughes, W. O. and Goulson, D. (2001). Polyethism and the importance of context in the alarm reaction of the grass-cutting ant, *Atta capiguara*. *Behav. Ecol. Sociobiol.* **49**, 503-508. doi:10.1007/s002650100321

Hurlbert, A. H., Ballantyne, F. and Powell, S. (2008). Shaking a leg and hot to trot: the effects of body size and temperature on running speed in ants. *Ecol. Entomol.* **33**, 144-154. doi:10.1111/j.1365-2311.2007.00962.x

Kamoun, S. and Hogenhout, S. A. (1996). Flightlessness and rapid terrestrial locomotion in tiger beetles of the *Cicindela L.* subgenus *Rivacindela* van Nidek from saline habitats of Australia (*Coleoptera: Cicindelidae*). *Coleopt. Bull.* **50**, 221-230.

Laciny, A., Zettel, H., Kopchinskiy, A., Pretzer, C., Pal, A., Salim, K. A., Rahimi, M. J., Hoenigsberger, M., Lim, L., Jaitrong, W. et al. (2018). *Colobopsis explodens* sp. n., model species for studies on "exploding ants" (Hymenoptera, Formicidae), with biological notes and first illustrations of males of the *Colobopsis cylindrica* group. *ZooKeys* **751**, 1-40. doi:10.3897/zookeys.751.22661

Laciny, A., Nemeschkal, H. L., Zettel, H., Metscher, B., Druzhinina, I. S. (2019). Caste-specific morphological modularity in the ant tribe Camponotini (Hymenoptera, Formicidae). *BMC Zool.* **4**, 9. doi:10.1186/s40850-019-0048-7

Lenoir, A., Hefetz, A., Simon, T. and Soroker, V. (2001). Comparative dynamics of gestalt odour formation in two ant species *Camponotus fellah* and *Aphaenogaster senilis* (Hymenoptera: Formicidae). *Physiol. Entomol.* **26**, 275-283. doi:10.1046/j.0307-6962.2001.00244.x

Lighton, J. R. and Duncan, F. D. (2002). Energy cost of locomotion: validation of laboratory data by in situ respirometry. *Ecology* **83**, 3517-3522. doi:10.1890/0012-9658(2002)083[3517:ECOLVO]2.0.CO;2

Lighton, J. R., Bartholomew, G. A. and Feener, D. H. (1987). Energetics of locomotion and load carriage and a model of the energy cost of foraging in the leaf-cutting ant *Atta colombica* Guer. *Physiol. Zool.* **60**, 524-537. doi:10.1086/physzool.60.5.30156127

Mendes, C. S., Bartos, I., Akay, T., Márka, S. and Mann, R. S. (2013). Quantification of gait parameters in freely walking wild type and sensory deprived *Drosophila melanogaster*. *Elife* **2**, e00231. doi:10.7554/eLife.00231.001

Merienne, H., Latil, G., Moretto, P. and Fourcassié, V. (2020). Walking kinematics in the polymorphic seed harvester ant *Messor barbarus*: influence of body size and load carriage. *J. Exp. Biol.* **223**, jeb205690. doi:10.1242/jeb.205690

Merienne, H., Latil, G., Moretto, P. and Fourcassié, V. (2021). Dynamics of locomotion in the seed harvesting ant *Messor barbarus*: effect of individual body mass and transported load mass. *PeerJ* **9**, e10664. doi:10.7717/peerj.10664

Mersch, D. P., Crespi, A. and Keller, L. (2013). Tracking individuals shows spatial fidelity is a key regulator of ant social organization. *Science* **340**, 1090-1093. doi:10.1126/science.1234316

Morehead, S. A. and Feener, D. H., Jr. (1998). Foraging behavior and morphology: seed selection in the harvester ant genus, *Pogonomyrmex*. *Oecologia* **114**, 548-555. doi:10.1007/s004420050479

Oster, G. F. and Wilson, E. O. (1978). *Caste and Ecology in the Social Insects.* Princeton University Press.

Peeters, C. and Ito, F. (2015). Wingless and dwarf workers underlie the ecological success of ants (Hymenoptera: Formicidae). *Myrmecol. News* **21**, 117-130.

Pfeffer, S. E., Wahl, V. L., Wittlinger, M. and Wolf, H. (2019). High-speed locomotion in the Saharan silver ant, *Cataglyphis bombycina. J. Exp. Biol.* **222**, jeb198705. doi:10.1242/jeb.198705

Powell, S. (2009). How ecology shapes caste evolution: linking resource use, morphology, performance and fitness in a superorganism. *J. Evol. Biol.* **22**, 1004-1013. doi:10.1111/j.1420-9101.2009.01710.x

Powell, S. and Franks, N. R. (2006). Ecology and the evolution of worker morphological diversity: a comparative analysis with Eciton army ants. *Func. Ecol.* **20**, 1105-1114. doi:10.1111/j.1365-2435.2006.01184.x

Reinhardt, L. and Blickhan, R. (2014). Level locomotion in wood ants: evidence for grounded running. *J. Exp. Biol.* **217**, 2358-2370. doi:10.1242/jeb.098426

Reinhardt, L., Weihmann, T. and Blickhan, R. (2009). Dynamics and kinematics of ant locomotion: do wood ants climb on level surfaces? *J. Exp. Biol.* **212**, 2426-2435. doi:10.1242/jeb.026880

Rubin, S., Young, M. H.-Y., Wright, J. C., Whitaker, D. L. and Ahn, A. N. (2016). Exceptional running and turning performance in a mite. *J. Exp. Biol.* **219**, 676-685. doi:10.1242/jeb.128652

Schilling, M., Hoinville, T., Schmitz, J. and Cruse, H. (2013). Walknet, a bio-inspired controller for hexapod walking. *Biol. Cybern.* **107**, 397-419. doi:10.1007/s00422-013-0563-5

Seidl, T. and Wehner, R. (2008). Walking on inclines: how do desert ants monitor slope and step length. *Front. Zool.* **5**, 8. doi:10.1186/1742-9994-5-8

Sommer, S. and Wehner, R. (2012). Leg allometry in ants: extreme long-leggedness in thermophilic species. *Arthropod Struct. Dev.* **41**, 71-77. doi:10.1016/j.asd.2011.08.002

Szczecinski, N. S., Bockemühl, T., Chockley, A. S. and Büschges, A. (2018). Static stability predicts the continuum of interleg coordination patterns in *Drosophila. J. Exp.Biol.* **221**, jeb189142. doi:10.1242/jeb.189142

Ting, L. H., Blickhan, R. and Full, R. J. (1994). Dynamic and static stability in hexapedal runners. *J. Exp. Biol.* **197**, 251-269. doi:10.1242/jeb.197.1.251

Tross, J., Wolf, H. and Pfeffer, S. E. (2021). Allometry in desert ant locomotion (*Cataglyphis albicans* and *Cataglyphis bicolor*) – does body size matter? *J. Exp. Biol.* **224**, jeb242842. doi:10.1242/jeb.242842

Tschinkel, W. R. (1988). Colony growth and the ontogeny of worker polymorphism in the fire ant, *Solenopsis invicta. Behav. Ecol. Sociobiol.* **22**, 103-115. doi:10.1007/BF00303545

Wahl, V., Pfeffer, S. E. and Wittlinger, M. (2015). Walking and running in the desert ant *Cataglyphis fortis. J. Comp. Physiol. A* **201**, 645-656. doi:10.1007/s00359-015-0999-2

Wehner, R., Marsh, A. C. and Wehner, S. (1992). Desert ants on a thermal tightrope. *Nature* **357**, 586-587. doi:10.1038/357586a0

Weihmann, T. (2018). Leg force interference in polypedal locomotion. *Sci. Adv.* **4**, eaat3721. doi:10.1126/sciadv.aat3721

Weihmann, T., Goetzke, H. H. and Günther, M. (2015). Requirements and limits of anatomy-based predictions of locomotion in terrestrial arthropods with emphasis on arachnids. *J. Paleontol.* **89**, 980-990. doi:10.1017/jpa.2016.33

Weihmann, T., Brun, P. G. and Pycroft, E. (2017). Speed dependent phase shifts and gait changes in cockroaches running on substrates of different slipperiness. *Front. Zool.* **14**, 54. doi:10.1186/s12983-017-0232-y

Wills, B. D., Powell, S., Rivera, M. D. and Suarez, A. V. (2018). Correlates and consequences of worker polymorphism in ants. *Annu. Rev. Entomol.* **63**, 575-598. doi:10.1146/annurev-ento-020117-043357

Wilson, D. M. (1966). Insect walking. *Annu. Rev. Entomol.* **11**, 103-122. doi:10.1146/annurev.en.11.010166.000535

Wosnitza, A., Bockemühl, T., Dübbert, M.,, Scholz, H. and Büschges, A. (2013). Inter-leg coordination in the control of walking speed in *Drosophila. J. Exp. Biol.* **216**, 480-491. doi:10.1242/jeb.078139

Yeoh, C. E. and Yi, H. (2021). Conceptual design of the combinable legged robot bio-inspired by ants' structure. *Appl. Sci.* **11**, 1379. doi:10.3390/app11041379

Zollikofer, C. (1994). III. Stepping patterns in ants-influence of load. *J. Exp. Biol.* **192**, 119-127. doi:10.1242/jeb.192.1.119