

## Alternative strategies by thermophilic ants to cope with extreme heat: individual versus colony level traits

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*Cataglyphis* is a fairly homogeneous ant genus which is widespread over the arid regions of the Old World. All *Cataglyphis* species are thermal specialists which are adapted to extreme environments where they forage at nearly lethal temperatures. This study focusses on two *Cataglyphis* species which differ considerably in their physical caste systems. These species have developed two alternative mechanisms facing extreme heat. In *C. velox*, foraging at high surface temperatures is clearly dependent on size: large *C. velox* workers forage at midday and are able to withstand higher temperatures than small workers. On the other hand, *C. rosenhaueri* has not developed great physical specialization, but the workers of this species have achieved physiological (such as low cuticular transpiration and metabolic rate), and behavioural adaptations (such as raising their abdomen to protect the vital organs contained in it from high temperatures) to tolerate thermal stress. The result is that small *C. rosenhaueri* workers may withstand extreme heat conditions in a similar way to large *C. velox* workers, and much better than small *C. velox* workers. The different mechanisms used by these two species to withstand extreme heat could reflect fundamental patterns of independent evolution. In some situations, selection may act to promote a relatively narrow size range of adult workers, all of them able to withstand thermal extremes, while in others it may act by producing different worker sizes with different tolerance to environmental conditions.

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The evolution of polymorphic worker castes is one of the major thresholds determining the complexity of caste systems in social insects (Wheeler 1991). The size range of workers will be subject to natural selection if the range is a heritable variable within species. Ant taxa with polymorphic workers have almost certainly evolved several times from monomorphic ancestors (Wilson 1953), presumably in ecological contexts in which the presence of a physically specialized group of soldiers or foragers increases colony survival or efficiency (Hölldobler and Wilson 1990, Frumhoff and Ward 1992). The empirical evidence suggests that the major determinants of caste evolution are the natural environmental contingencies with substantial size variance, such as prey captured by the colony foragers or

soil particles moved by the excavators, for which the responses of castes are presumed to represent adaptations (Oster and Wilson 1978). The addition of large castes (a generalization applied to ants is that physical polymorphism has usually been created by the addition of a major caste, Oster and Wilson 1978) may be limited by both developmental (high energy costs of manufacture and maintenance) and ecological factors (decreased flexibility in response to environmental and demographic variability). For this reason, in certain cases ant colonies follow strategies other than the production of major workers to face these selective pressures.

Extreme temperatures are considered to be the most serious form of environmental stress to ectotherms in

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dry regions (Edney 1977, Cloudsley-Thompson 1989, Huey and Kingsolver 1989, Lighton and Feener 1989). For small-bodied ectotherms such as ants, temperatures outside the range between 10 and 45°C may be considered stressful, because they are often near or beyond the ants' critical thermal limits (Frumhoff and Ward 1992, Cerdá et al. 1998). Nevertheless, in the very hot, dry areas of the world, there are different genera of thermophilic ants that seek high temperatures and increase their foraging activity as temperatures rise above 45°C (Marsh 1985, Christian and Morton 1992, Wehner et al. 1992, Cerdá et al. 1997). How have these thermophilic ants been able to penetrate into this dangerously hot environment? During the course of evolution, these thermal specialists have developed alternative mechanisms to ensure that metabolism occurs at acceptable levels over the range of environmental temperatures. The most obvious one is a large size: different studies state that variables such as desiccation (Hood and Tschinkel 1990, Kaspari 1993), respiratory rate (Lighton et al. 1987, Bartholomew et al. 1988), running speed (Bartholomew et al. 1988), maximum foraging time (Lighton and Feener 1989) and foraging temperature (Rissing and Pollock 1984, Porter and Tschinkel 1987; but see Christian and Morton 1992) vary with body size. Other mechanisms to tolerate high temperatures and to conserve body water include both physiological (Lighton and Bartholomew 1988, Lighton and Feener 1989, Lighton et al. 1993), biochemical (Gilby 1980, Gehring and Wehner 1995) or behavioural adaptations, with responses such as making frequent pauses at thermal refuges (Marsh 1985, Christian and Morton 1992, Wehner et al. 1992), or raising the abdomen over the hot substrate (Wehner 1983, 1989).

The ant genus *Cataglyphis* is widespread over the arid regions of the Old World (Agosti 1990). All the species of the genus show a strictly diurnal activity and high thermophily concentrated in the summer months (Cros et al. 1997). Species with very different degrees of worker polymorphism are found within this genus, from monomorphic (Cerdá et al. 1996) to very polymorphic species (Cerdá and Retana 1997), with all intermediate levels of polymorphism within them (Wehner 1983, Agosti 1990). This fact is especially interesting, because *Cataglyphis* is one of the rare ant genera that display this striking variation in the degree of worker polymorphism (according to Frumhoff and Ward 1992, only 4% of all ant genera, 11 out of 297, are known to contain both monomorphic and polymorphic species). If the worker caste has indeed been shaped by strong colony-level selection (Oster and Wilson 1978, Frumhoff and Ward 1992), and the species face a common problem, in this case to forage at high temperatures, we can reasonably infer that the size-frequency distributions of workers are adaptive. Nevertheless, the aim of this paper is to show that adaptation to extreme heat conditions may have fol-

lowed different routes in phylogenetically closely related species. In some situations there may be strong evolutionary pressure to maintain monomorphism even under conditions that otherwise would favour the evolution of polymorphism. In particular, we will study the way two *Cataglyphis* species, which coexist in many locations of the Southern Iberian Peninsula and occupy a similar thermal niche but differ considerably in their physical caste systems, withstand extreme heat conditions.

## Material and methods

### The ant species

The two *Cataglyphis* species in this study belong to different species groups: *C. rosenhaueri* is included in the *albicans* group, and *C. velox* in the *altisquamis* group (Agosti 1990). *C. rosenhaueri* is not found near the coast, and is limited to the Guadalquivir Depression and closed areas. *C. velox* is considered an endemic species of the Iberian Peninsula, limited to La Mancha and the Guadalquivir Depression, in an elevation range between 0 and 2400 m (Tinaut 1990). The two species display a similar thermophilic diurnal activity and an individual foraging strategy. The diet of both species is mainly zoonecrophagous, and they also collect similar prey sizes (98% of prey collected by *C. rosenhaueri* and 88% of prey collected by *C. velox* weigh less than 10 mg, although large *C. velox* workers can exceptionally retrieve large items to their nest). The two species differ considerably in their degree of worker polymorphism. *C. velox* is one of the most polymorphic *Cataglyphis* species in the Iberian Peninsula, with a continuous 22-fold range in body mass and worker length ranging between 4.5 and 12 mm (Cerdá and Retana 1997). On the other hand, *C. rosenhaueri* shows a rather lower worker polymorphism, with a continuous four-fold range in body mass and worker length ranging between 4.5 and 7.5 mm. Following Cerdá and Retana (1997), in this study workers of both species were categorized according to total body length as either very small (< 6 mm), small (6–8 mm), medium (8–10 mm) or large (> 10 mm). The last two categories were only present in *C. velox* colonies.

### Field sampling

Field study was conducted in Llanos de Palomares (Sierra Sur de Jaén), and in the backfire of Fuente del Oso (Sierra de Cazorla), both in the Jaén province (southeastern Spain). The climate of the region is Mediterranean, characterized by cold rainy winters and hot dry summers. These two sites were open areas, with a clear understorey of scattered herbs surrounded by

large portions of bare soil. At both sites, *C. velox* and *C. rosenhaueri* nests were quite abundant. To analyse worker size distributions, three nests of each species were excavated, and size (total body length and head width) of all individuals was measured later in the laboratory.

Diel patterns of workers of the different size categories were determined on midsummer sampling days in Llanos de Palomares. The observations were carried out on four *C. velox* and four *C. rosenhaueri* nests. The number of workers of each size category leaving or entering each nest was sampled during 20 min per hour throughout the whole daily activity period (from 06.00 to 18.00, solar time). Live workers were classified directly in the field into one of the four previously described size categories by reference to a standard array of preserved specimens (see Wilson 1980, or Cerdá and Retana 1997, for a similar recording technique of size-frequency data). The surface temperature at the nest entrance of each nest and the surface temperature in the shade were measured at each hourly sampling with a digital thermometer. The relationship between ground surface temperature and external activity of each species was established by dividing the whole range of temperatures registered in the field into 2°C classes. The mean activity value of each species in each temperature class was calculated considering together the data from all nests and all sampling days.

Foraging trips of different-sized workers of both species were monitored in the field following two different methodologies in order to obtain different sets of data:

1. To evaluate duration and distance of the foraging trips, 20 foragers of each size category of the two *Cataglyphis* species were followed when leaving their nest in the Llanos de Palomares area. The maximum distance from the nest that they reached was considered as a measurement of trip length, while the time taken to reach this point was considered to be the duration time of the foraging trip. Speed was calculated as a quotient between maximum distance travelled and duration time.
2. To obtain detailed information about the time spent by different-sized ants of both species at thermal refuges, the activity of ten foragers of each size category and species was carefully monitored by pairs of observers in the Fuente del Oso site. All trips were monitored at midday (from 10.00 to 13.00). During each trip, the foraging behaviour of the ant was investigated by sequentially recording the different microhabitats run over (walking in the sun, pausing in the shade or climbing up grass stalks) and the time spent on each of them. Temperatures in the sun, in the shade and at grass height (5 mm over the ground surface) were registered every 15 min during the whole sampling period. Since

temperatures were significantly different (ANOVA,  $F = 109.5$ ,  $p = 0.0001$ ,  $N = 17$  for each microhabitat) in the sun ( $46.0 \pm 0.6^\circ\text{C}$ ) than in the shade ( $35.9 \pm 0.4^\circ\text{C}$ ) or at grass height ( $34.7 \pm 0.4^\circ\text{C}$ ), only two types of microhabitat were identified: unshaded and shaded (including both shade and grass microhabitat patches).

## Laboratory measurements

Thermal resistance of workers of each size category and species was measured at the laboratory by means of an electric Plactronic Selecta hot plate of 5–200°C temperature range and 1°C accuracy. At each temperature, 10–15 individuals of each size and species were placed on the surface of the hot plate in open containers with Fluon-painted inner walls which prevented ants escaping. The time at which each ant died or lost muscular coordination was noted. The critical thermal maximum (CTM) of each size class was defined as the temperature at which at least 50% of workers died after 10 min of exposure (see a more detailed description of the method in Cerdá and Retana 1997, and Cerdá et al. 1998).

Transpiratory water loss is composed of two components (Edney 1977, Lighton and Feener 1989): transcuticular water loss, which is believed to take place across the cuticle, and respiratory water loss, which is associated with the exchange of gases in the respiratory spiracles. We have considered differences in oxygen consumption rates as an indirect measure of water loss through the respiratory system, since the rate of respiratory water loss is likely to depend on the metabolic rate: other things being equal, an increase in metabolic rate necessarily increases respiratory water loss (Edney 1977, Lighton and Bartholomew 1988).

Cuticular transpiration was measured in ten randomly selected workers of each of the different-sized groups of the two species. Ants were HCN-killed, placed in open containers, and weighed at different time intervals with a precision balance (Sartorius MC1) to the nearest 0.01 mg. Weighings were carried out at 0, 1.5, 3.0, 4.5, 6.0, 7.5, 9.0, 12.0 and 24.0 h after the start of each experiment. All measurements were made at an ambient temperature of 25°C and a relative humidity in the air of 10–20%. For each ant, the percentage of water lost after 12 and 24 h, and the transpiration rate were determined. The transpiration rate was calculated as the negative slope of the relation between  $\ln(M_t/M_0)$  and elapsed time, where  $M_0$  is the initial mass and  $M_t$  is mass at time  $t$  (Lighton and Feener 1989).

Oxygen consumption rates were determined with a Warburg V166 respirometer, where ten different jars (= samples) could be measured at the same time. Two individuals of each size group and species were placed in each jar, together with a piece of filter paper moistened with a 10% solution of KOH as a carbon dioxide

trap. Two empty jars were run concomitantly with the others so that possible variations could be corrected. Oxygen consumption was measured 4 h after the ants were placed in the respiration chamber. Following each run, the ants were killed, dried at 60°C and weighed. Measurements were made at an ambient temperature of 25°C within two–three days of the collection of the ants. Five different measurements were taken of each of the different-sized groups of the two species. The oxygen consumption rate ( $VO_2$ ) was calculated in relation to the dry body weight of the ants as:  $VO_2 = \text{oxygen consumption}/(\text{ant dry weight} \times \text{time})$ , in  $\mu\text{l O}_2 \text{ mg}^{-1} \text{ h}^{-1}$ .

Owing to their small size, it was not possible, using conventional methods, to obtain direct measurements of ant body temperature under natural conditions. An indirect estimate of temperatures experienced at the head and the gaster level by the different-sized ants of the two species was obtained by measuring temperatures at heights at which these body parts were situated in a natural position. The height of the lowest site of head and gaster of the different types of workers was measured in the laboratory to the nearest 0.1 mm. The ant posture was reconstructed using recently dead ants as models and based on field photographs and detailed observations of the ants in laboratory colonies. Two different ant postures were defined: the ant with the gaster in a horizontal position (the typical posture of *C. velox* workers, which was very rarely seen in *C. rosenhaueri*) and the ant with the gaster in a vertical position (the natural position for *C. rosenhaueri* workers, especially when temperatures were hot, see Wehner 1983, for a similar upright position of the gaster in another *Cataglyphis* species).

To register temperatures at different heights above ground level, we used five T-type thermocouples connected to an Electronic Controls Design model 50 data logger. The thermocouples were placed on the above-mentioned electric Plactronic Selecta hot plate at 0, 1.5, 3, 4.5 and 6 mm above ground level. Temperatures at these heights were registered in the data logger for ground temperatures in the range of 30 to 70°C. Different morphological measurements were also taken. Since long legs may allow ants to stilt above the hot substrate, the length of femora and tibia of the hind legs was measured in 15 individuals of each size class and species. Both measurements were highly correlated ( $r^2 = 0.95$  and  $0.96$  for *C. rosenhaueri* and *C. velox*, respectively;  $p = 0.001$  in both cases), and only analyses with tibia data are shown hereafter.

### Data analysis

Since the factorial design with two factors (size and species) was not complete, and the main interest of this study was to compare both between and within species,

a summarizing variable, worker type, was used. For this variable, each combination of size and species was considered a different level, and thus, there were six different worker types (two *C. rosenhaueri* and four *C. velox* worker sizes). One-way ANOVAs were used to investigate differences among worker types for the different variables examined. A Fisher's protected least significant difference test was used to separate means. Inspection of residuals was carried out to check for normality and homoscedasticity. Data were  $\ln(x + 1)$  or arcsine square-root transformed when necessary. Values in this paper are given as mean  $\pm$  SE.

## Results

### Diel patterns of forager size

The general shape of the curves of temperature versus foraging activity of the two *Cataglyphis* species considered was very similar (Fig. 1): *C. velox* started at lower ground temperature than *C. rosenhaueri*, but both species showed similar maximum activity temperatures (42–46°C); both ants decreased their activity at higher temperatures, but none of them ceased activity at the upper ground temperature registered in the field (52°C).

Nevertheless, there were important differences in the proportion of different-sized workers of the two species at different times of the day, i.e. at different surface temperatures (Fig. 2). Very small and small workers of *C. rosenhaueri* were outside the nest during the whole day, especially at midday, when temperatures were at their hottest. *C. velox* colonies, on the other hand,

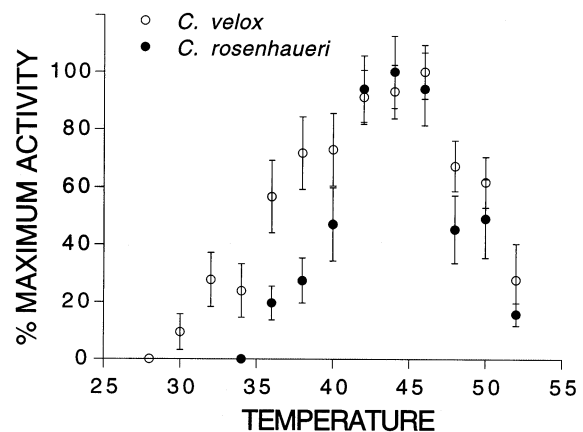


Fig. 1. Relationship between ground surface temperature (°C) and percentage of maximum activity of *Cataglyphis velox* (open dots) and *Cataglyphis rosenhaueri* (solid dots). Each point represents the mean ( $\pm$  SE) activity value of all cases with the same surface temperature from all nests and all sampling days. Data of each species have been standardized by defining the maximum mean activity value observed as 100% of activity.

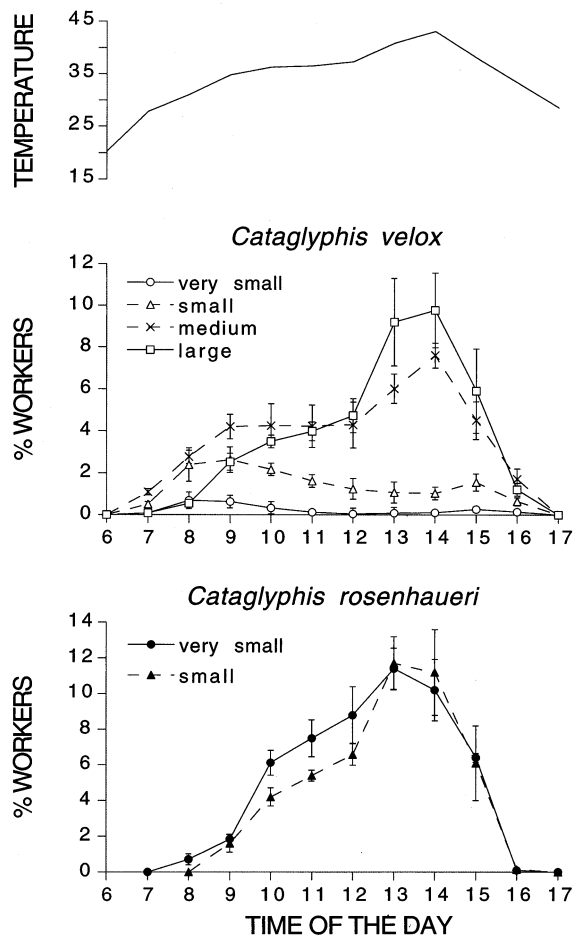


Fig. 2. Diel patterns (solar time) of sun surface temperature, and proportion of different-sized workers of *C. velox* and *C. rosenhaueri* in a representative summer day. The proportion of each worker size in each hour has been calculated by dividing all values by the total number of workers registered in all size categories and hours in each nest. Each dot represents the mean value ( $\pm$  SE) of four nests.

showed a very clear diel pattern of forager size, with very small and small workers foraging preferentially in the morning hours and decreasing their relative proportion at midday, while the greatest foraging force at midday was mainly composed by medium and large workers (Fig. 2). Moreover, the proportion of workers of different sizes foraging outside the nests and in the entire excavated nests differs statistically in *C. velox* ( $\chi^2 = 541$ ,  $p = 0.0001$ ), but not in *C. rosenhaueri* ( $\chi^2 = 2.13$ ,  $p = 0.16$ ). Thus, the proportions of very small and small *C. rosenhaueri* workers outside the nests (52.1 and 47.9%, respectively) were similar to those in the entire nests (57.4 and 42.6%), but the proportion of very small and small *C. velox* foragers (3.6 and 16.2%, respectively) was lower than in the entire nests (23.4% and 30.0%, respectively), while that of medium and, especially, large workers increased (39.3 and 40.9% outside

the nest versus 35.6 and 11.0% in the entire nests, respectively, for medium and large workers).

### Physiological differences

Thermal resistance (measured as the mean survival time of ants at each temperature) decreased with increasing temperature at the hot plate for all groups of workers (Fig. 3), although there were differences according to size: medium and large *C. velox* workers showed greater survival time at all temperatures tested (Fisher LSD  $F$ -test,  $p < 0.05$ ) than very small or small workers of both *C. velox* and *C. rosenhaueri*. The critical thermal maximum (CTM) also depended on size class but not on species: CTM of very small workers of both species was 50°C, that of small workers was 52°C, while CTM of medium and large *C. velox* workers was, respectively, 54 and 56°C.

The three variables calculated to evaluate cuticular transpiration in the different groups of workers showed a different pattern: water loss across the cuticle was higher and quicker in very small and small *C. velox* foragers, and lower and slower in the other worker groups, including very small and small *C. rosenhaueri* ants (Table 1). Similarly, very small and small *C. velox* workers respired more quickly per mg of tissue than medium and large *C. velox* workers and also very small and small *C. rosenhaueri* workers, which did not show significant differences in their metabolic rates compared with the larger *C. velox* ants (Table 1). Thus, from the point of view of water loss, the behaviour of very small and small *C. rosenhaueri* workers was completely different from that of their equivalents in size of *C. velox*, but very similar to that of medium and large workers of the latter species.

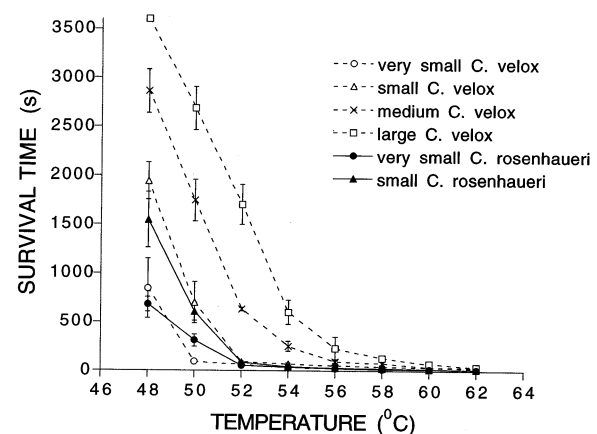


Fig. 3. Survival time (defined as the time until an ant subjected to heat stress dies or loses muscular coordination) of *Cataglyphis rosenhaueri* (solid lines) and *Cataglyphis velox* (broken lines) of different sizes in relation to temperature at the hot plate. Mean ( $\pm$  SE) values are given for each temperature.

Table 1. Variables describing the physiological traits of the different worker types. The cuticular transpiration rate was calculated as the negative slope of the relation between  $\ln(M_t/M_0)$  and elapsed time, where  $M_0$  is the initial mass and  $M_t$  is mass at time  $t$ . The results of the one-way ANOVAs used to investigate differences among worker types for the different variables examined are given. Those groups of workers that were not significantly different based on Fisher's protected LSD post hoc test share a common letter. Data of percentage of water loss after 12 and 24 h were arcsine transformed, and data of cuticular transpiration rate were  $\ln$  transformed before performing the ANOVA tests.  $N = 5$  for the metabolic rate and  $N = 10$  for the other three variables.

Type of worker	% water loss after 12 h	% water loss after 24 h	Cuticular transpiration rate	Metabolic rate ( $\mu\text{l O}_2 \text{ mg}^{-1} \text{ h}^{-1}$ )
Very small <i>C. rosenhaueri</i>	10.0 ± 0.8 ab	14.8 ± 2.1 a	0.009 ± 0.001 ab	0.09 ± 0.01 a
Small <i>C. rosenhaueri</i>	9.3 ± 0.7 a	15.1 ± 1.3 a	0.008 ± 0.001 a	0.06 ± 0.02 a
Very small <i>C. velox</i>	14.1 ± 1.4 c	22.9 ± 3.0 b	0.012 ± 0.002 c	0.27 ± 0.03 c
Small <i>C. velox</i>	13.4 ± 2.0 bc	21.8 ± 3.0 b	0.012 ± 0.002 bc	0.17 ± 0.04 b
Medium <i>C. velox</i>	7.5 ± 0.6 a	13.7 ± 1.3 a	0.007 ± 0.001 a	0.05 ± 0.01 a
Large <i>C. velox</i>	7.4 ± 0.7 a	12.2 ± 1.2 a	0.006 ± 0.001 a	0.05 ± 0.01 a
ANOVA test	$F = 6.81$ $p = 0.0001$	$F = 4.65$ $p = 0.0001$	$F = 6.23$ $p = 0.0001$	$F = 15.56$ $p = 0.0001$

### Behavioural and morphological differences

Behavioural variables measured during the foraging trips showed several differences among worker types (Table 2). ANOVA and post hoc tests showed that the maximum distance travelled and speed varied with worker size. There were two-fold or even three-fold differences between medium and large *C. velox* workers and small workers of the two species. The maximum distance an ant went from the nest on a search was 60.1 m in *C. velox* and 21.1 m in *C. rosenhaueri*, while the maximum duration of a foraging trip was 32 min in *C. velox* and 42 min in *C. rosenhaueri*. There were no significant differences among types of workers in the percentage of time spent at thermal refuges (range: 7.3–14.4% of trip duration), or in pause duration, which was usually short, on average less than 5 s for all types of workers. There were only slight significant differences in the frequency of thermal respites, which varied from 0.8 pauses/min in large *C. velox* workers to 2.0 pauses/min in very small *C. rosenhaueri* and small *C. velox* workers (Table 2).

The different types of workers did, however, show considerable morphological differences concerning leg length, an important feature because long legs allow the ants to stilt above the hot substrate and to achieve a high running speed and thus short foraging. In both species, tibia length (a measure of leg length) increased significantly with body size (relationship between head width and tibia length:  $r^2 = 0.97$ ,  $p = 0.0001$ ,  $N = 60$  for *C. velox*, and  $r^2 = 0.72$ ,  $p = 0.0001$ ,  $N = 30$  for *C. rosenhaueri*). Thus, tibia length was greater in large (mean ± SE:  $4.22 \pm 0.04$  mm,  $N = 15$ ) and medium ( $3.77 \pm 0.11$  mm,  $N = 15$ ) *C. velox* foragers, than in very small and small workers of both species (for *C. velox*:  $1.98 \pm 0.08$  and  $2.62 \pm 0.08$  mm, and for *C. rosenhaueri*:  $2.18 \pm 0.05$  and  $2.42 \pm 0.05$  mm, for very small and small workers, respectively;  $N = 15$  in all cases).

Mean height of the lowest site of head and gaster differed among the different worker types (Table 3). Large *C. velox* workers considerably raised their body over the ground due to their longer legs (see previous paragraph). Nevertheless, there were differences between workers of similar size of the two species: *C. velox* displayed their body in a horizontal position, with head and gaster at low height over the ground surface; on the contrary, *C. rosenhaueri* workers raised both head and gaster 0.2–0.4 mm compared to *C. velox* individuals of similar size. This led to lower temperatures experienced by *C. rosenhaueri* workers compared to *C. velox* ones (2–3°C for the head and 4–6°C for the gaster, Table 3). As evidence of the advantage of this behaviour for *C. rosenhaueri* workers, the difference in temperature of the gaster placed in a horizontal or a vertical position was 5.7–5.8°C at 50°C and 6.4–6.6 at 55°C at ground level.

### Discussion

All *Cataglyphis* species are thermal specialists which are adapted to an extreme thermal environment where most other ants stop foraging (Wehner 1989, Wehner et al. 1992, Cerdá et al. 1998). They are forced to follow a risk-prone strategy by foraging at nearly lethal environmental conditions, because all of them behave as subordinate species in interspecific interactions, i.e., they are at the bottom of the dominance/aggressiveness hierarchy, and are forced to abandon the food resources by the other ant species (Baroni Urbani and Aktaş 1981, Cerdá et al. 1997, Cerdá and Retana 1998). Nevertheless, they benefit from this strategy by having a better foraging performance when collecting food (Cerdá et al. 1998). Although all *Cataglyphis* species fill similarly this ecological thermal niche, the results obtained in this study indicate that at least two alternative mechanisms have been developed within this genus during the

Table 2. Variables describing the foraging trips of the different worker types. The results of the one-way ANOVAs used to investigate differences among worker types for the different variables examined are given. Those groups of workers that were not significantly different based on Fisher's protected LSD post hoc test share a common letter. Data of maximum distance travelled, trip duration and speed were ln transformed, while data of percentage of time at thermal refuges were arcsine transformed. Since all trips were monitored at midday, and very small *C. velox* were not found outside the nests during this period of the day, no data for this group are available.  $N = 20$  for the maximum distance travelled and trip duration, and  $N = 10$  for the percentage of time at thermal refuges, number of pauses per min, and pause duration.

Type of worker	Maximum distance travelled (m)	Trip duration (min)	Speed (m/min)	% time in thermal refuges	$N$ pauses per min	Pause duration (s)
Very small <i>C. rosenhaueri</i>	8.7 ± 0.9 a	11.7 ± 1.4	1.0 ± 0.1 a	14.4 ± 1.8	2.0 ± 0.2 b	4.6 ± 0.5
Small <i>C. rosenhaueri</i>	10.2 ± 1.1 ab	11.6 ± 2.1	1.2 ± 0.1 a	10.9 ± 2.2	1.6 ± 0.2 b	4.3 ± 0.4
Small <i>C. velox</i>	11.5 ± 2.4 ab	8.2 ± 1.3	1.4 ± 0.2 ab	13.4 ± 2.8	2.0 ± 0.4 b	4.7 ± 0.6
Medium <i>C. velox</i>	21.4 ± 3.8 b	10.4 ± 1.8	2.1 ± 0.3 b	11.8 ± 2.3	1.5 ± 0.2 ab	4.5 ± 0.8
Large <i>C. velox</i>	24.6 ± 2.6 c	9.5 ± 1.5	3.5 ± 0.4 c	7.3 ± 2.5	0.8 ± 0.2 a	4.1 ± 1.2
ANOVA test	$F = 5.93$ $p = 0.0003$	$F = 0.22$ $p = 0.93$	$F = 12.6$ $p < 0.0001$	$F = 1.95$ $p = 0.12$	$F = 2.88$ $p = 0.032$	$F = 0.12$ $p = 0.98$

course of evolution facing the same selection pressure, in this case extreme heat.

In *C. velox*, foraging at high surface temperatures is clearly dependent on size. Polymorphism is a way to withstand the critical environmental conditions to which foragers are exposed. In this species, only medium and large workers forage at high temperatures, while very small and small workers show reduced foraging activity concentrated in the morning or the afternoon hours. Moreover, medium and large *C. velox* workers are apparently more dedicated to foraging than very small and small workers of this species, because they are much more represented outside the nests than might be expected from their proportion in the entire nests. As has been shown in other species (Porter and Tschinkel 1987, Lighton and Feener 1989), there is a close relationship between thermal biology and worker polymorphism: large *C. velox* workers may forage at higher temperatures and perform longer trips than small workers because 1) their reduced surface/mass ratio makes them more heat tolerant and less subject to desiccation, and 2) their longer legs allow them to perform longer and faster trips, and keep their body at a height of ca 1.6–2.0 mm above ground (see Table 3), where temperatures are 13.3–14.5°C lower than on the hot substrate (50°C at ground level).

Although the results obtained for *C. velox* suggest that worker polymorphism may represent an adaptive way of achieving better exploitation of external environmental conditions by thermophilic ants, it is obvious that this is not the case of *C. rosenhaueri*. Workers of this species are small in size but forage outside the nest during the whole day, especially at midday, when they have to withstand very hot temperatures. *C. rosenhaueri* has not increased the physical specialization of its worker caste, but individuals of this species have achieved several physiological and behavioural adaptations to tolerate thermal stress. They show a lower thermal resistance in laboratory conditions than medium and large *C. velox* workers, but the different variables calculated to evaluate water loss indicate that *C. rosenhaueri* workers show similar cuticular permeability and metabolic rate to large *C. velox* workers, and considerably lower than their equivalents in size of this species. These traits involving high resistance to dehydration by low rates of water loss are considered physiological adaptations to dry and hot environments (Lighton and Bartholomew 1988, Lighton and Feener 1989), and generate great differences between the workers of similar size of the two species with regard to control of water loss. Together with these physiological adaptations, there are also behavioural/morphological mechanisms that allow *C. rosenhaueri* workers to withstand high ground temperatures. *C. rosenhaueri* workers raise their abdomen from 1.0–1.1 to 1.9–2.1 mm when temperatures are hot, and this mechanism allows them to protect the vital organs contained in the gaster

Table 3. Mean height (H) of the lowest site of head and gaster of the different worker types and temperatures (T) experienced at these levels when temperatures were 50 and 55°C at ground level. For *C. rosenhaueri* workers, measures were taken with the gaster in a horizontal (a comparable measure as the one taken in *C. velox*) and in a vertical position (the natural position for this species when temperatures were hot).

Type of worker	Head			Gaster (horizontal position)			Gaster (vertical position)		
	H (mm)	T (50°C)	T (55°C)	H (mm)	T (50°C)	T (55°C)	H (mm)	T (50°C)	T (55°C)
Very small <i>C. rosenhaueri</i>	1.3	39.0	42.3	1.0	41.7	45.2	1.9	35.9	38.6
Small <i>C. rosenhaueri</i>	1.5	37.5	40.3	1.1	40.8	44.2	2.1	35.1	37.8
Very small <i>C. velox</i>	1.0	41.7	45.2	1.0	41.7	45.2	–	–	–
Small <i>C. velox</i>	1.3	39.0	42.3	1.2	39.9	43.2	–	–	–
Medium <i>C. velox</i>	1.7	36.7	39.4	1.6	37.1	39.8	–	–	–
Large <i>C. velox</i>	2.0	35.5	38.2	2.0	35.5	38.2	–	–	–

from high temperatures (at 50 and 55°C at ground level, there is a difference of ca 6 and 6.5°C, respectively, between placing the gaster in a horizontal or in a vertical position, see Table 3). With this behavioural mechanism, *C. rosenhaueri* foragers substitute the long legs of large *C. velox* workers, and place their gaster at a similar (and ever higher) height above the ground.

The different mechanisms used by these two species to withstand extreme heat conditions could reflect fundamental patterns of independent evolution. Whether a species' size range is restricted or expanded over evolutionary time will depend on the selection pressures imposed on it (Wheeler 1991). The increase in worker size range is presumed to benefit colony fitness by allowing the colony to perform certain tasks more efficiently than it could if only one size of workers were present (Oster and Wilson 1978, Beshers and Traniello 1994, Retana and Cerdá 1994). Major workers of various ant species appear to arise as specialists for very few primary tasks, such as the transport of heavy loads (Franks 1986, Waller 1989, Wetterer 1994, Retana and Cerdá 1994), food storage (Rissing 1984) or defence (Wilson 1976). Major workers may also be advantageous thermal specialists for *C. velox* colonies, because they are able to withstand temperatures 6 to 8°C higher than smaller ones, and this means, at the colony level, a longer and more continuous daily activity period. Nevertheless, although it has been suggested that a large body size is required if overheating and water loss are not to become limiting factors when foraging (Lighton and Feener 1989), the relationship between the polymorphism and thermal biology of species is not universal among thermophilic ants. In species such as *C. rosenhaueri*, the adaptation to heat may have led to a selection of physiological, behavioural or maybe even biochemical (see Gehring and Wehner 1995) traits. The result is that small *C. rosenhaueri* workers may withstand extreme heat conditions in a way similar to large *C. velox* workers, and much better than small workers of the latter species or than workers of most ant species of larger size (Cerdá et al. 1998). Workers of both species spend similar periods of time outside the nest

while foraging, and do not show differences in the total time spent at thermal refuges (either in shaded patches or on the tops of grass stalks), or in pause duration. This means that tolerance to extreme heat conditions has been well achieved in the two species, but probably following different evolutionary ways: in some situations, selection may act to promote a relatively narrow size range of adult workers, all of them able to withstand thermal extremes, while in others, it may act by producing different worker sizes with different tolerance to environmental conditions. The generality of the patterns described in this study could be tested in future studies that should compare additional species of this genus. *C. velox* and *C. rosenhaueri* belong to two different species groups which are placed quite distant in the *Cataglyphis* cladogram (Agosti 1990). A multi-species, explicitly phylogenetic approach which included more closely related species would lead to the detection of rates and directions of evolutionary change in both thermal biology and worker polymorphism.

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