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Leg or antenna injury in *Cataglyphis* ants impairs survival but does not hinder searching for food

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Abstract

Injury is common in nature resulting, for example, from fighting, partial predation, or the wear of body parts. Injury is costly, expressed in impaired performance, failure in competition, and a shorter life span. A survey of the literature revealed the frequent occurrence of injury in ants and its various causes. We examined whether leg or antenna injury impacts food-discovery time and reduces the likelihood of reaching food in workers of the desert ant *Cataglyphis niger*. We examined the search-related consequences of injury in groups of either 4 or 8 workers searching for food in a short arena, a long arena, and a maze. We conducted a small field survey to evaluate the prevalence of injury in the studied population. Finally, we compared the survival rates of injured versus uninjured workers in the laboratory. Injury was common in the field, with almost 9% of the workers collected out of the nest, found to be injured. Injured workers survived shorter than uninjured ones and there was a positive link between injury severity and survival. However, we could not detect an effect of injury on any of the searching-related response variables, neither in the arenas nor in the mazes tested. We suggest that workers that survive such injury are only moderately affected by it.

Keywords: binary-tree maze, desert ants, foraging, injury, movement, review, survival

Injuries result from various factors, including fights with conspecifics over food, territory, or mates, partial predation, or are even self-inflicted (Huntingford and Turner 1987, ch. 3; Juanes and Smith 1995; Bateman and Fleming 2009; Bowerman et al. 2010). Animals should avoid injuries for their multiple costs. Costs could be separated into mechanistic costs, trade-off costs, and ultimate costs. The mechanistic costs are directly inflicted on injury. They include the loss of body fluids, such as hemolymph in insects, impaired performance, such as suboptimal movement, lower capacity to carry food, or inferior ability to compete for mates and resources (Berzins and Caldwell 1983; Taylor and Jackson 2003; Maginnis 2006; Krause et al. 2017). In parallel, the process of healing or regenerating requires energy (Vitt et al. 1977; Cole et al. 2011). This energy is diverted from other requirements, such as the maintenance of a functioning immune system or investment in growth and reproduction, leading to various trade-off costs induced by injury (Walters and Pawlik 2005; Archie 2013). Owing to the mechanistic and trade-off costs, injury has long-term, ultimate costs, expressed in lower survival and reproduction (Alcock 1996; Sepulveda et al. 2008; Carey et al. 2009; Moroń et al. 2012).

That said, in some species, such as spiders, injury is quite common: even up to 40% of adults collected in the field have a leg missing. Surprisingly, there are no to only minor negative consequences of the injury for their competitive ability, foraging, development, or survival (Johnson and Jakob 1999; Brueseke et al. 2001; Dodson and Schwaab 2001; Pasquet et al. 2011). Several authors have, therefore, suggested that some spider species have "spare legs," as an explanation to why losing 1 has only minor consequences (Guffey 1998; Brueseke et al. 2001). It is also common to detect some but not comprehensive costs of injury and not all the response variables measured in the different studies are equally affected. For example, leg loss did not affect longevity and reproduction although it had a negative effect on adult body size in ground crickets, and while leg loss impaired escape speed, it did not affect the competitive success in male field crickets (Bateman and Fleming 2005; Matsuoka and Ishihara 2010).

Social insects, and ants, in particular, offer an interesting framework to study the costs of injuries. A keystone characteristic of social insects is a division of labor. In many social Hymenopteran colonies, the queen reproduces, young workers tend the brood, and old workers leave the nest to forage (Robinson 1992). Foraging workers, therefore, require much more food than their own consumption. As workers rely mostly on inclusive rather than direct fitness, they are relatively "disposable" compared with the queen or the brood (Porter and Jorgensen 1981; Shorter and Rueppell 2012). Workers, especially old ones, are involved in risky tasks, such as foraging, to feed the brood. The workers that leave the nest face many dangers (Tripet and Nonacs 2004; Tofilski 2009), including injury (Palmer 2004; Frank et al. 2017). Furthermore, the brood inside the colony is helpless and could provide an excellent source of food (or workforce) for predators or parasites (LaPolla et al. 2002; Grüter et al. 2018).

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Injuries, although rarer, might also happen inside the colony when workers fight against invaders. Social insect workers are, therefore, possibly susceptible to injury more than other insects of a similar size.

Table 1 presents selected examples of injuries in ants, and Supplementary Table S1 presents the results of a larger literature survey including >70 papers referring to injury in ants. The most common injuries are either partial or full loss of the appendages, mostly 1 or more legs (Jutsum 1979; Steck et al. 2009; Adgaba et al. 2014) but also antennae (Bhatkar et al. 1972; Carlin and Hölldobler 1987). Other injuries involve wear of mandibles, and damage to, or even loss of the gaster (Waloff 1957; van Wilgenburg et al. 2005). It is probable that other, more subtle injuries also exist but are rather invisible to direct observation. Other than the erosion of the mandibles, caused by frequent usage (Schofield et al. 2011), most injuries in ants are inflicted by live opponents. The most common opponents are either non-nestmate conspecifics (Halliday 1981; Heinze et al. 1994) or other ant species (Powell and Clark 2004; Adgaba et al. 2014). The main contributor to such injuries is competition over limited resources, such as food, territories, or nests (Johnson et al. 1987; Palmer 2004; van Wilgenburg et al. 2005), while the minority of injuries stem from within-colony conflicts, such as those between founding queens or fighting males (Rissing and Pollock 1987; Izzo et al. 2009; Jacobs and Heinze 2017). Encounters with predators or parasites can also result in injury (Steck et al. 2009; Levenets et al. 2019), and in the case of slave-making or predatory ants, resistance by the host or prey species may lead to its injury (Foitzik et al. 2001; Frank et al. 2017). The consequences of injuries vary. Some injuries are fatal, even if not immediately so (e.g. loss of gaster; Waloff 1957; Harkness 1977). Injured workers might also survive less well because injury makes them more susceptible to attack by predators/parasites or infection (Moffett 1986; Brown and Feener 1991; Silveira-Costa and Moutinho 1996; Frank et al. 2018). Other injuries severely impair performance, such as workers that had lost 2 legs keep falling and move more slowly than uninjured ones (Frank et al. 2018). Treatment by nestmates can significantly reduce mortality after injury (Frank et al. 2017, 2018). Moderate impairment, such as that of olfactory learning in a T-maze after an antenna loss or inefficient use of mandibles after their wear, are also known (Vowles 1964; Porter and Jorgensen 1981; Schofield et al. 2011). Finally, other studies report no impairment of the worker's regular performance after injury (Ettershank and Ettershank 1982).

Although ant injuries seem to be common, we are not familiar with any up-to-date review of its prevalence across species. Our first goal was, therefore, to summarize existing literature on injuries in ants, which we did above. Second, we examined how an injury might affect the chance of discovering food in the desert ant *Cataglyphis niger*. *Cataglyphis niger* ants are solitary foragers, which do not use pheromone communication for recruiting. Unlike many other ant species, *C. niger* cannot rely on tracks left by earlier workers or on massive recruitment to sites rich with food while foraging. Therefore, this species is not able to compensate for injuries with the recruitment of more foragers to discovered food sources. Our general hypothesis is that injuries would be important for worker ants and incur a mechanistic cost (impaired foraging) and an ultimate one (survival). We predicted that food-discovery times would increase disproportionately for injured ants foraging in more complex experimental arenas. We also expected a stronger effect of injury in small groups of workers than in large groups. Third, we conducted a survival experiment. We inflicted injuries of increasing severity and documented the workers' survival. We expected a lower survival rate of the injured workers than those in a control, uninjured group and a negative association between injury severity and survival. Fourth, we conducted a small-scale field survey to estimate the injury prevalence in *C. niger*. The goal was to determine whether injuries are common in nature and which injury types are characteristic of the studied population.

Materials and Methods

A small-scale survey in the field

Our literature review revealed that there are only a few and mostly anecdotal reports on the prevalence of injury in ants in the field (but see, e.g. Adgaba et al. 2014; Yusuf et al. 2014). We, therefore, estimated the prevalence of injuries in the population under study: *C. niger* ants in the Tel-Baruch sand dunes, Israel (32°12'83N, 34°78'67E). *Cataglyphis* species are diurnal, thermophilic, and individually foraging ants (Harkness and Wehner 1977; Lenoir et al. 2009). The Tel-Baruch dunes present an enclave of disturbed natural habitat, surrounded by the Mediterranean Sea to the west, the city of Tel Aviv to the south, and other suburbs to the east and north. Other common ant species in this habitat comprise several *Messor* and *Camponotus* species (Saar et al. 2018). We (3 observers) surveyed seven sites for roaming *C. niger* workers in their natural habitat for a total of 6 h spread throughout all sites in May 2020. See the Supplementary Material for a map with the surveyed sites. When we encountered a *C. niger* worker, we documented any observable injury. All encountered workers were removed and kept aside until the end of the survey in order not to count the same worker twice.

General procedure and injury treatments

We collected 65 *C. niger* colonies (277 ± 193 workers; mean ± 1 SD) from Tel-Baruch between October 2019 and May 2020. Colonies were kept for 1 week under laboratory conditions (~28°C; 12:12 L:D) in self-constructed Plexiglas cages of 20 × 20 × 5 cm (l × w × h), each colony in a separate cage. Colonies were provided with water in tubes sealed with cotton wool but no food. Next, from each colony, we separated 3 groups of either 4 or 8 workers (hereafter, 4-worker groups and 8-worker groups), which were placed in round plastic boxes (11 cm diameter × 7.5 cm high), with access only to water. Each group was allocated to 1 of 3 treatments: 1) leg injury—removal of the hind right leg, chosen because its removal has the strongest effect on movement speed in a congeneric species (Wittlinger and Wolf 2013); 2) antenna injury—removal of the right antenna. No difference in food-discovery time had been found for ants in which either the right or left antenna was removed in preliminary tests; 3) an uninjured, control treatment. In treatments 1 and 2, all ants were similarly injured. Regarding the injury procedure, the ants were separated from their colony either by hand or soft forceps and were held by an appendage while either a leg (the cut was done between the femur and the trochanter) or an antenna was removed by surgical scissors. *Cataglyphis niger* ants, although highly polymorphic, are big enough for this procedure to be carried

Table 1. Examples from the literature of common injury types in ants, the causes of such injuries, their consequences, and their prevalence

	Species	Reference	Comments
Common injuries			
Leg	*Formica cunicularia* *Dorylus quadratus* *Megaponera analis*	Le Moli and Parmigiani (1981); Adgaba et al. (2014); Frank et al. (2017)	Sometimes more than a single leg
Antenna	*Solenopsis invicta*	Bhatkar et al. (1972); Balas and Adams (1996)	Both workers and queens
Gaster	*Lasius flavus* *Cataglyphis bicolor*	Waloff (1957); Harkness (1977)	Both workers and queens
Mandible	*Iridomyrmex purpureus* *Atta cephalotes*	van Wilgenburg et al. (2005); Schofield et al. (2011)	Wearing of mandibles due to usage or fights
More than 1 body part	*Camponotus floridanus* *Crematogaster* sp.	Carlin and Hölldobler (1987);Prestwich et al. (1977)	Mostly leg and antenna
Who inflicts the injury?			
Nestmates	*Leptothorax gredleri* *Veromessor pergandei* *Cardiocondyla venustula*	Heinze et al. (1994); Rissing and Pollock (1987); Jacobs and Heinze (2017)	Nestmate queens, workers, or males
Non-nestmate conspecifics	*Cataglyphis iberica* *Oecophylla smaragdina*	Dahbi et al. (1996); Hölldobler (1983)	Fights between pairs or larger groups
Other ant species	*Nomamyrmex esenbeckii*, *Atta* sp.*Formica cunicularia*	Powell and Clark (2004); Le Moli and Parmigiani (1981)	During fights or raids
Predators/parasites	*Cataglyphis fortis* *Formica rufa*	Steck et al. (2009); Levenets et al. (2019)	Injury by rodents or flies
Dangerous prey/host	*Temnothorax americanus* *Megaponera analis*	Foitzik et al. (2001); Frank et al. (2017)	Injury by ant or termite species
Reason for injury			
Fights between queens	*Veromessor pergandei* *Solenopsis invicta*	Rissing and Pollock (1987); Balas and Adams (1996)	
Fights between males	*Cardiocondyla* spp.	Kinomura and Yamauchi (1987); Jacobs and Heinze (2017)	
Fights over nests	*Lasius flavus* *Crematogaster* spp.	Waloff (1957); Palmer (2004)	Fight after colony founding
Fights over territories	*Iridomyrmex purpureus* *Oecophylla smaragdina*	van Wilgenburg et al. (2005); Hölldobler (1983)	
Mating	*Atta* spp.*Formica japonica*	Baer and Boomsma (2006); Kamimura (2008)	Injury to the queen0's genitalia
Injury consequences			
Minor	*Iridomyrmex purpureus* *Cataglyphis fortis*	Ettershank and Ettershank (1982); Steck et al. (2009)	
Major/lethal	*Lasius neoniger* *Solenopsis saevissima* *Pseudomyrmex Ferruginea*	Bhatkar et al. (1972); Mintzer (1982)	The effect is not always immediate
Movement impairment	*Tetramorium caespitum* *Megaponera analis*	Kay et al. (2010); Yusuf et al. (2014)	Some workers must be carried
Lower survival	*Paraponera clavata* *Pheidologeton diversus*	Brown and Feener (1991); Moffett (1986)	Exposure to predators/ parasites
Prevalence			
Low	*Temnothorax longispinosus*	Jongepier and Foitzik (2016)	0<10% injured workers
Intermediate	*Cephalotes atratus*	Yanoviak et al. (2010)	0~40% injured workers
High	*Myrmecina graminicola*	Grasso et al. (2020)	0>100%
Variable	*Solenopsis invicta*	Plowes and Adams (2005)	10–1000%

For additional information, see Supplementary Table S1. The studied species, *C. niger*, suffers from almost all the injury types presented here (leg, antenna, gaster, and mandible; with sometimes more than a single body part injured).

out with relative ease (head width ranging between 1.15 to 3.03 mm measured in the experiment "The effect of injury on survival in the laboratory" described below). The experiments commenced 48 h after injury. No permits were required because neither the ant species nor their natural habitats are protected.

Experiments in short and long arenas

We examined whether injury impairs the discovery of food in a simple habitat, simulated by a short arena. 4- or 8-worker groups were placed at one end of the short arena (54 × 10 × 6 cm) and 0.5 g of honey was placed at the opposite end (Figure 1A). The workers' behavior was recorded for 20 min, and we documented the time taken for the first worker to discover the food (hereafter, food-discovery time) and the number of ants arriving at the food. Note that this number could be larger than the number of workers in the group, as workers that left the food, returned to the nest, and again arrived at the food were counted twice. This could be perceived as a general measure of activity. We used grouped rather than isolated ant workers, because ants may change their behavior under isolation, and examining their response to isolation is out of the scope of this study. Next, we examined whether injury produced a greater effect in a longer arena (162 × 10 × 6 cm) that required the ants also to turn twice (after 54 and 108 cm; Figure 1B). It was possible that moving over longer distances as well as turning would be more energetically costly for injured workers than uninjured ones. Because the longer arena was 3-fold the length of the shorter ones, we recorded the experiment for 60 min and documented the time taken to food-discovery and the number of arrivals at the food. If the workers did not arrive at the food, the test was given the maximal time for each experiment (20 min in short arenas, 60 min in long arenas, and 60 min in mazes, see below). The experiments in the short and long arenas comprised 20 and 22 colonies, respectively. Half of the colonies provided workers for the 4-worker groups and the other half for the 8-worker groups.

Maze experiment

We examined whether the negative consequence of injury is enhanced further when searching for food in the complex habitat of a maze. We used a binary-tree maze (dimensions of 25 × 20 cm; Figure 1C; Saar et al. 2017; Reznikova 2020), which required 3 correct turns to reach the food (Figure 1C, marked with "H"). We considered this a complex habitat due to the dead ends, which forced the worker to turn back. A 4- or 8-worker group was placed in the nest area, connected to the maze by a corridor, and 0.5 g of honey was placed at the far side of the maze. The test began by removing the doors to the corridor connecting the nest part to the maze. We then recorded the workers' behavior for 60 min and documented the time taken to food-discovery and the number of arrivals at the food. The experiment comprised 23 colonies, 13 of which provided workers for the 4-worker groups and the rest provided workers for the 8-worker groups.

The effect of injury on survival in the laboratory

Because injury affected neither the food-discovery time nor the number of workers arriving at the food reward in any of the arenas or mazes (see Results), we examined whether injuries also had no or little effect on survival in the lab. 10 *C. niger* colonies were collected in the field in October 2020 and 24 ants from each were allocated to 1 of 6 treatments, grouped in 4 similarly treated workers ($n = 40$ per treatment): 1) *Light leg injury*: half the tarsus of the rear right leg was removed. 2) *Removal of 1 leg*: the rear right leg (femur, tibia, and tarsus) was removed. (3) *Removal*

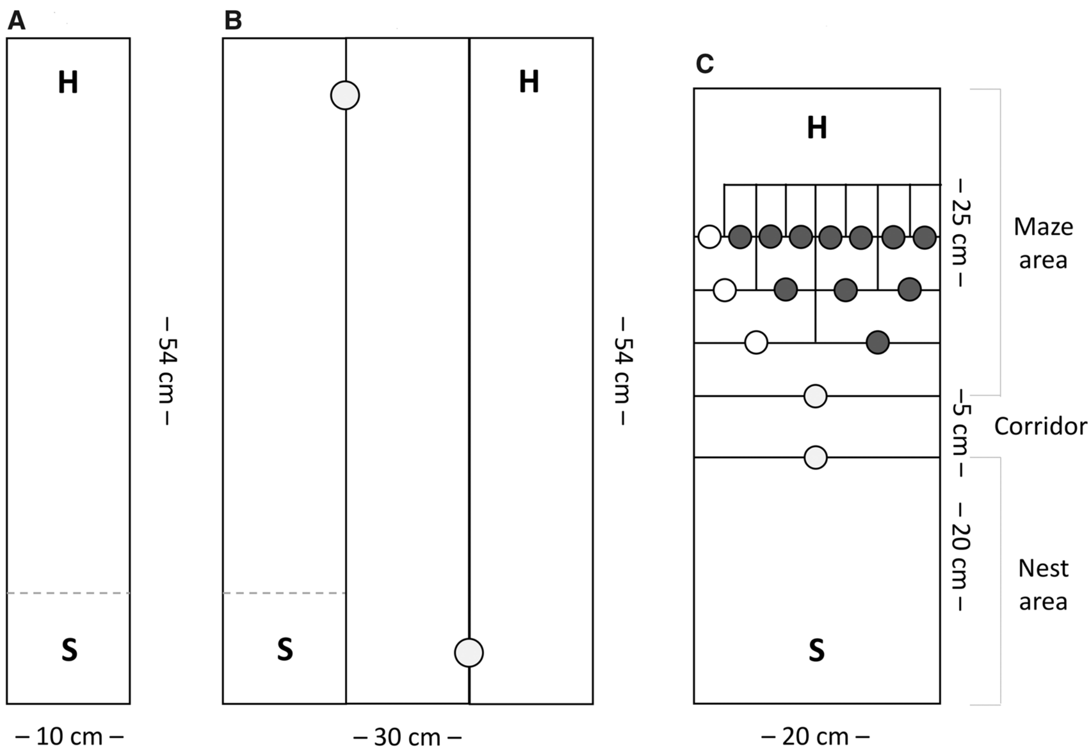

Figure 1. A scheme of the (A) short arena, (B) long arena (3 short arenas connected with doors requiring 2 turns), and (C) maze in the experiments. Walls and temporary barriers are marked with black lines and gray-dashed lines, respectively. The food reward (honey), placed on a small plastic surface, is marked with "H." The small circles stand for doors. White doors lead to the food reward and gray doors lead to dead ends. *Cataglyphis niger* groups of either 4 or 8 workers were placed in the "nest area" (lower part of each scheme, marked with "S"). The experiment started by removing the barrier (dashed line in the short and twhe long arenas) or opening the door to the corridor, through which the ants can enter the maze.

of 2 legs: the rear right and mid left legs were removed. 4) *Antenna removal*: the right antenna was removed. 5) *Sham control*: the ants were picked up with forceps and treated in a similar way to the limb-removal procedure but without the removal of an actual limb. 6) *Control group*: ants underwent no treatment. The ants were kept in groups of 4 in round plastic boxes (11 cm diameter × 7.5 cm high) and had ab libitum access to water but no food. Boxes were kept at 28°C and 12:12 L:D conditions and we checked mortality daily. After the worker's death, we photographed its head and measured its width, which is a common proxy of body size (Tschinkel et al. 2003; Modlmeier et al. 2013). 16 of 240 workers were lost/damaged, their heads were not photographed, and they were not included in the statistics.

Statistical analysis

Experiments in short and long arenas and the Maze experiment

We analyzed food-discovery time using the Cox proportional-hazards model. This test fits well data with censored values (Hosmer et al. 2008), which we required since not all groups arrived at the food. Arrival at the food (y/n) was used as the censoring variable, and injury type (coded in 2 dummy variables), group size (4 versus 8 workers), and arena length (short versus long) as the explanatory variables. We used a robust jackknife variance estimated, grouped according to tests per colony, to avoid pseudoreplications (Lin and Wei 1989). The number of worker arrivals at the food was analyzed using a hierarchical generalized linear model with a Poisson distribution, with colony as a random variable, and injury type, group size, and arena length as explanatory variables. The maze experiment was analyzed similarly, excluding arena length. The 2-way interactions (injury × group size and injury × arena length) were not included in any of the analyses because they were never significant ($P > 0.15$).

The effect of injury on survival in the laboratory

We used the Cox proportional-hazards model, with survival time as the dependent variable, and injury type (coded using five dummy variables) and head width as explanatory variables. Colony was accounted for as described above.

Results

A small-scale survey in the field

In total, we collected 600 *C. niger* workers, of which 53 were perceptibly injured (8.8%; see Supplementary Figure S1 for typical examples). Because 5 of the workers had 2 injured body parts, the total number of detectable injuries was 58. This is the prevalence of injury specifically among foraging workers, and not among all workers, including nurses, for which injury is probably rare. See the Supplementary Material for the injury type and prevalence in each of the 7 sites from which we collected *C. niger* workers. The most common injury was either partial or full loss of a front leg (25 injuries). Injuries to the mid or hind legs were detected in 13 additional cases, making leg injury the most common injury by far. Nine workers had another ant (or an unidentified arthropod) attached to a hind leg, which although perhaps not being considered an injury, might hinder movement. Other less common injuries are damaged abdomen (7 cases), partial or full loss of an antenna (3 cases), and a single case of a partial loss of the mandible. While not statistically significant, right legs tended to be more injured than left ones (15/10, 4/2, and 5/2 injuries for the right/left front, mid, and hind leg, respectively).

Experiments in short and long arenas

Results are summarized in Table 2

Food-discovery time. Injury had no effect on food-discovery time ($z = 0.39$ and 0.27, $P = 0.700$ and 0.786 for the antenna removal and leg removal treatments vs. the control). Workers discovered the food faster in the short arena than in the long one ($z = -7.16$, $P < 0.001$) and 8-worker groups discovered

Table 2. Summary of results of experiments in short and long arenas and mazes

		Mean	SD	Mean	SD	Mean	SD	N[a]
Arena	Group	Control		Leg injury		Antenna injury		
		Food-discovery time (s)						
Long	4	2833.2	1122.7	2285.2	1340.1	2373.2	1056.7	11
Long	8	2072.6	1308.3	1892.8	1123.3	1585.9	1153.5	11
Short	4	565.2	391.6	795.7	408.7	716.0	361.6	10
Short	8	434.9	328.0	406.7	248.0	505.5	380.1	10
Maze	4	3012.1	935.0	3203.7	671.9	2172.1	1554.9	13
Maze	8	2071.9	1019.9	1650.8	1021.7	2631.4	1134.2	10
		Number of worker arrivals at the food						
Long	4	1.00	1.10	1.18	1.33	1.36	1.36	11
Long	8	3.00	3.29	2.36	1.57	2.09	1.76	11
Short	4	1.40	0.70	1.30	1.34	1.70	0.95	10
Short	8	1.70	1.06	2.40	1.17	2.10	1.20	10
Maze	4	0.69	1.03	0.69	1.03	0.85	0.99	13
Maze	8	2.10	1.97	2.30	1.42	1.60	2.17	10

[a]Sample size per treatment combination and injury type.

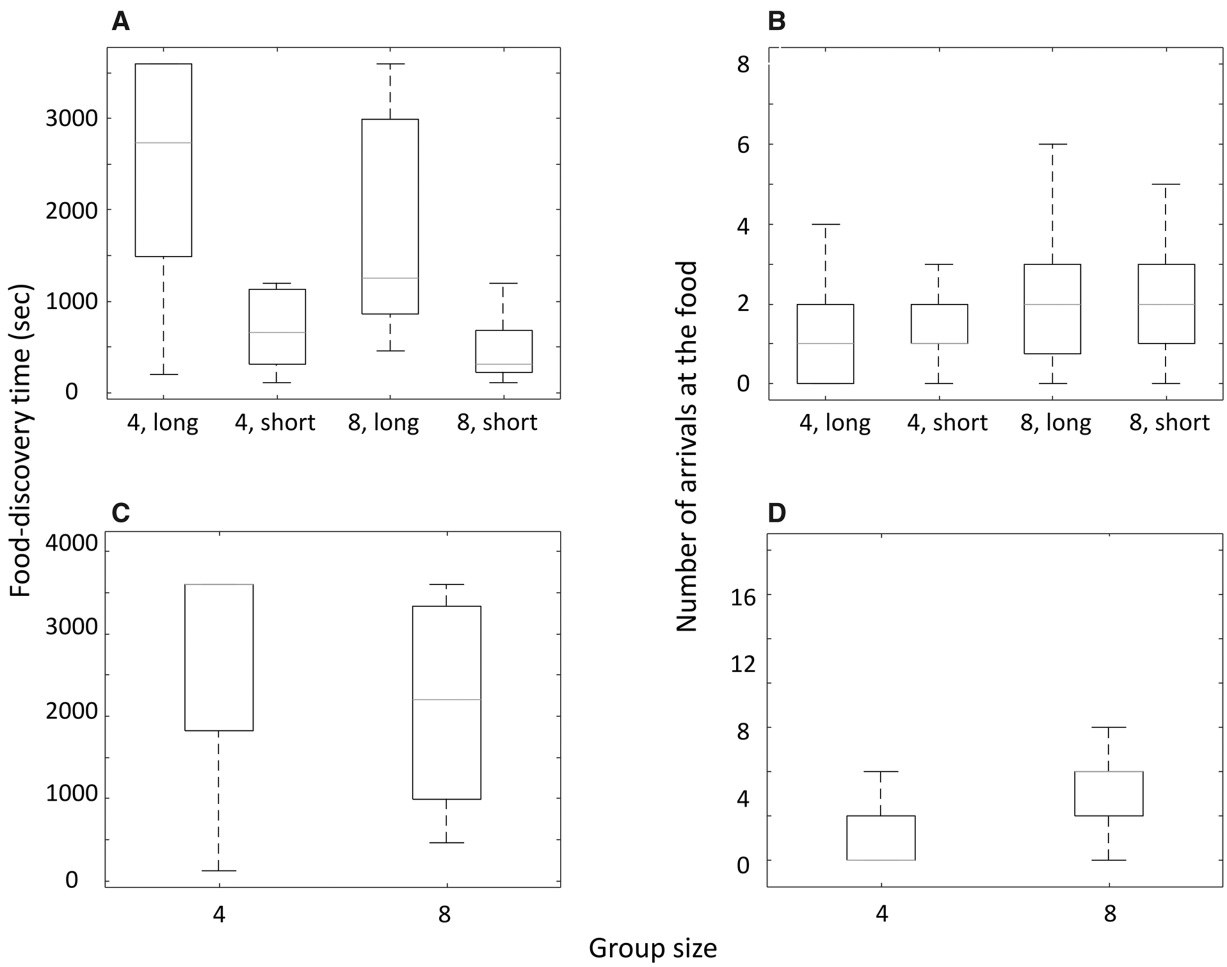

Figure 2. Food-discovery time and the number of arrivals at the food reward in (A, B) the short and the long arenas and in (C, D) the maze, and of groups of either 4 or 8 *C. niger* workers. The boxplots include the medians, the 25th, and 75th percentiles, and the data range, except for outliers.

the food faster than 4-worker groups (z = 3.16, P = 0.002; Figure 2A). Model statistic: Wald χ^2_4 = 61.7, P < 0.001.

Number of arrivals at the food. The only significant effect was that of group size—more arrivals were documented in 8-worker groups than in 4-worker groups (z = 3.72, P < 0.001; Figure 2B). Injury did not affect the number of arrivals at the food (z = 0.08, P = 0.935) as well as arena length (z = 0.20, P = 0.844). Model statistic: Wald χ^2_4 = 13.92, P = 0.008. See the Supplementary Material for full statistical tables.

Maze experiment

Results are summarized in Table 2

Food-discovery time. 8-worker groups discovered the food in the maze faster than 4-worker groups (z = 3.02, P = 0.003; Figure 2C). There was no effect of injury on food-discovery time (z = −0.16 and 0.43, P = 0.871 and 0.667 for the antenna and leg injury treatments, respectively). Model statistic: Wald χ^2_3 = 10.38, P = 0.016.

Number of arrivals at the food. More workers reached the food when 8-worker groups were searching in the maze than when 4-worker groups searched (z = 4.27, P < 0.001; Figure 2D). There was no effect of injury on food-discovery time (z = −0.40 and 0.25, P = 0.691 and 0.800 for the antenna and leg injury treatments, respectively). Model statistic: Wald χ^2_3 = 18.62, P < 0.001.

The effect of injury on survival in the laboratory

Removal of either 1 or 2 legs resulted in lower survival than the noninjured control (z = 2.59 and 6.23, P = 0.010 and P < 0.001, respectively; Figure 3). Neither light injury (partial leg removal), nor the antenna removal, nor the sham control differed in survival from the noninjured control (z = 1.06–1.49; P = 0.138–0.288). Head width was positively linked with survival time (z = 4.12, P < 0.001). Means ± 1 SD of survival days for the control, sham control, antenna removal, light leg injury, leg removal, and 2-leg removal treatments were 13.0 ± 14.0, 11.5 ± 11.6, 9.8 ± 7.2, 9.8 ± 9.4, 6.8 ± 7.2, and 5.3 ± 5.0, respectively. Model statistic: Wald χ^2_6 = 175.23, P < 0.001.

Discussion

The most interesting result of this study is the contrast between the ultimate cost and mechanistic cost of injury tested in the ant *C. niger*. On the one hand, injury impaired survival, with a positive correlation between injury severity and survival impairment. On the other hand, injuries had no effect on foraging performance, measured either as the time to discover food or the number of worker arrivals to the food, in

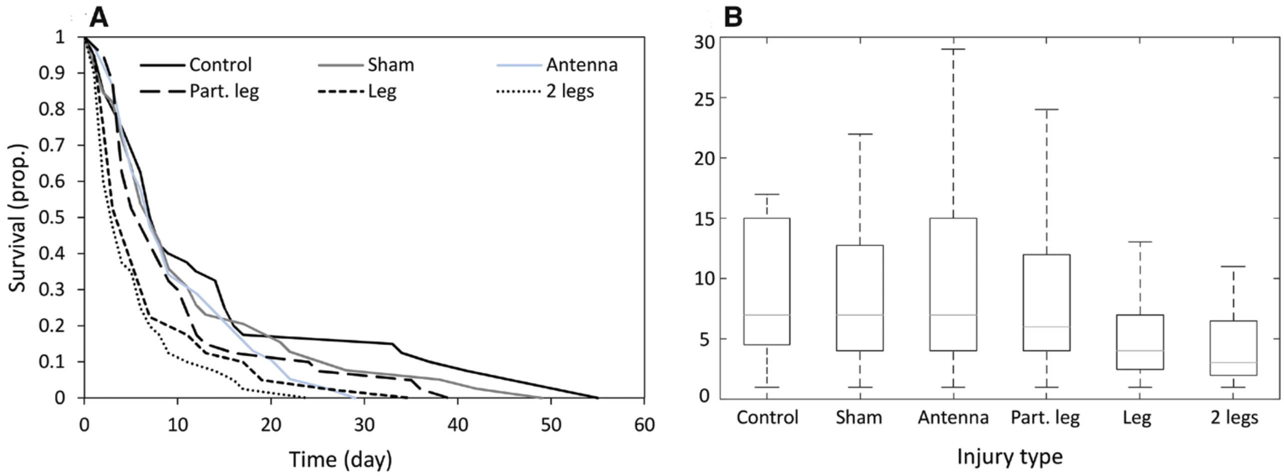

Figure 3. (A) A survival curve for the 6 injury treatments: control, sham control, light leg injury (partial leg removal), removal of 1 leg, removal of 2 legs, and antenna removal. (B) The same data presented using a boxplot.

neither short arenas, nor long arenas, nor mazes. The negative effects on injured workers might be more prominent under natural settings, due to increased fatigue and higher energy cost of moving with fewer legs (Frank et al. 2017). The prevalence of injury in the natural habitat was estimated to be ~9%, which is an intermediate value compared with reports on other ant species.

We detected no effects of injuries on foraging in any of the platforms tested. This is equivalent to some other examples, such as those often observed in spider species (see Section 1, Introduction). We suggest that similar to some spiders having "spare legs," a loss of a single leg in the studied ants has only moderate consequences. Nevertheless, both in spiders and in the studied ants, missing more than 1 leg already takes its toll (Guffey 1999; Brautigam and Persons 2003; this study). Furthermore, and similar to our contradiction between the effects on survival and foraging, other studies have also demonstrated the effects of injuries on some traits but not others (see Section 1, Introduction). Specifically, in ants, the consequences of a leg loss are species-specific and also depend on the task tested (cf. Steck et al. 2009; Yanoviak et al. 2011; Wittlinger and Wolf 2013).

The failure to detect an effect of leg or antenna injury on food-discovery time can be explained in several ways. First, it could be that the effect is undetectable in the short distances in the laboratory but that it accumulates over longer distances, as *Cataglyphis* workers forage over hundreds of meters in the field (Buehlmann et al. 2014; Huber and Knaden 2015). Indeed, shortening the legs in other studies led to space misperception and the underestimation of movement distances, at least temporarily (Wittlinger et al. 2007). However, previous studies on *C. niger* in mazes of a similar size and complexity revealed differences in food-discovery time and linked them to the type of food reward and previous exposure to food immediately before searching in the maze (Bega et al. 2020; Saar et al. 2020). Second, it is possible that focusing on other foraging-related response variables could result in a stronger effect of injury, such as injured individuals carrying smaller loads or settling for food of lower quality than healthy ones, as demonstrated in bees (Higginson and Barnard 2004; Johnson and Cartar 2014). Third, it is also possible that injury, as inflicted here, has indeed a little effect on foraging for food, despite its effect on survival. It could, therefore, be that workers have evolved some compensatory mechanisms that enable them to function almost as usual.

The prevalence of *C. niger* injury we detected in the field (~9%; Supplementary Table S5) strikes us as intermediate, compared with the findings of other field studies on ants (Supplementary Table S1). The injury rate also varies greatly among studies. For instance, less than 1% and more than 20% of ant workers raiding on termites were injured in 2 different studies, respectively (cf., Yusuf et al. 2014; Frank et al. 2017). Reports on ant injury prevalence in the field are scarce, but they may help us to understand the injury costs from the colony perspective. This is important because the colony and not the individual forager is the unit undergoing natural selection and the cost to the colony might differ from the cost paid by the individual (e.g. Scharf et al. 2012). Ant foragers are usually the oldest workers and their life expectancy is short (6.1 days in a congeneric species, Schmid-Hempel and Schmid-Hempel 1984). It is thus likely that the additive cost of injury to the colony, if it does not cause death within the first day or 2, is relatively low. The lab results indicate a negative association between injury and survival (Figure 3). There are numerous mechanistic and trade-off costs of injury potentially leading to the ultimate cost of shortened survival (see Section 1, Introduction). A scoring system of injuries in fighting fig wasps (Murray 1987; Hardy et al. 2013) ranks a leg injury more severely than an antenna injury and our results support this ranking.

Considering our experiments in the 2 arenas and the mazes, none of the significant results is surprising. We expected to detect interactions between arena length, group size, and injury type but instead were only able to demonstrate that ants require more time to reach the food in longer arenas and that larger groups of workers reach the food faster than smaller groups (Figure 2). There is a positive correlation of colony size with the number of foraging workers in this species as well as between habitat complexity or the path length the workers need to traverse to reach the food and the time required to do so (Bega et al. 2019). We did not control for the worker age, as colonies were not kept in the lab for a long time. However, the workers were randomly allocated to treatments, so worker age was probably homogenous across treatments. Using workers of a known age would contribute to reducing the background noise and might make more subtle differences significant. Finally, our failure to detect injury-inflicted effects on foraging or searching success does not indicate that such effects do not exist in the studied species. For example, examining the foraging of injured workers over several successive days might lead to different results and a stronger impact of injuries.

Additional research is required to examine how specific injuries affect survival in different ant species. Ant species/populations that tend to get injured more may tolerate better such injuries. Future studies should also examine the effect of different types of injury on foraging and survival in the field, as it is possible that injured foragers travel over shorter distances to find food or choose a different type of food. It also remains to be tested whether injury, similar to sickness, interferes with various social behaviors workers usually engage in (Bos et al. 2012; Miler et al. 2017).

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Author Contributions

T.G., A.S., and I.S. designed the study together and wrote the manuscript. T.G. and A.D. conducted the study. I.S. and T.G. conducted the statistical analysis.

Conflict of Interest

We declare no conflict of interest.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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