UNIWERSYTET JAGIELLOŃSKI
W KRAKOWIE

Faculty of Biology

Institute of Environmental Sciences

Determinants of rescue behaviour in ants (Hymenoptera: Formicidae)

Filip Turza

PhD thesis performed in the Life History Evolution Team,
Institute of Environmental Sciences,
Supervisor: prof. dr hab. Marcin Czarnołęski
Auxiliary supervisor: dr hab. Krzysztof Miler

Kraków 2024

Table of contents

Acknowledgements

I would like to thank my supervisor, Marcin Czarnołęski, for his invaluable support and insightful discussions throughout my PhD journey. I am also deeply grateful to my co-supervisor, Krzysztof Miler, for our long-standing and highly productive collaboration. Finally, I extend my heartfelt thanks to my family for their unwavering faith in me and my passion for ants.

Financial support for my research was provided by the Institute of Environmental Sciences, Faculty of Biology, Doctoral School of Exact and Natural Sciences, and Jagiellonian University, as part of the ID.UJ 2024 competitions: Visibility & Mobility Module and Excellence Module. During my PhD studies (2020–2024), I was also supported by the PRELUDIUM 16 NCN grant (2018/31/N/NZ8/02312) and BEKKER 4 NAWA Programme (BPN/BEK/2021/1/00214).

Summary

This PhD thesis aimed to explore unanswered questions about ants and one of the most intriguing forms of pro-social and altruistic behaviour observed in the animal kingdom, rescue behaviour. This behaviour is manifested as helping relatives in danger. Despite a growing number of studies devoted to rescue behaviour, the sources of variation in its expression remain unclear. The present dissertation consists of a general introduction (**Chapter I**), three main chapters in the form of two published papers (**Chapters II & III**) and one manuscript submitted for publication (**Chapter IV**), and a general discussion (**Chapter V**). In the first two studies, I focused on factors influencing the expression of rescue behaviours in ants on a within-species scale, while in the third study, I examined the conditions under which these behaviours are more likely to be expressed on a between-species scale. My first study (**Chapter II**) shows that body size is an important factor in determining behavioural persistence in ants. Specifically, the rescue persistence of workers that provide help to others and the activity of individuals that require help is higher in smaller than in larger workers. My second study (**Chapter III**) demonstrates that injury in ants leads to a survival cost, decreasing life expectancy. My results show that this handicap affects rescue behaviour toward nestmates but not aggressive behaviour toward alien species. Specifically, injured workers take part in rescue actions more likely toward intact nestmates. My third study (**Chapter IV**) indicates that inter-species variation in ant rescue tendencies can be partly explained by general worker life expectancy. Specifically, ant species differ strongly in how long their workers generally live and rescue proneness is more frequent in species characterised by higher worker life expectancy compared to those with lower life expectancy. The presented results demonstrate a notable variance in rescue behaviour expression both within and between species. Furthermore, they highlight the importance of body size and individual as well as species-characteristic life expectancy for shaping rescue patterns in ants. Importantly, this phenomenon calls for more extensive

research, considering the diverse physiologies, life histories, and ecologies across different species. Overall, the thesis holds significant implications for understanding differences in decision-making processes in social animals and provides potential directions for future studies.

Summary in Polish

Niniejsza rozprawa doktorska miała na celu zgłębienie nierozwiązanych zagadnień dotyczących mrówek oraz jednej z najbardziej intrygujących form prospołecznego i altruistycznego zachowania obserwowanego w królestwie zwierząt, zachowania ratunkowego. Zachowanie to polega na udzielaniu pomocy krewniakom w niebezpieczeństwie. Pomimo rosnącej liczby badań poświęconych zachowaniu ratunkowemu, źródła zmienności w jego ekspresji pozostają niejasne. Rozprawa składa się z ogólnego wprowadzenia (**Rozdział I**), trzech głównych rozdziałów w formie dwóch opublikowanych artykułów (**Rozdziały II & III**) i jednego manuskryptu przesłanego do publikacji (**Rozdział IV**), oraz ogólnej dyskusji (**Rozdział V**). W pierwszych dwóch badaniach skoncentrowałem się na czynnikach wpływających na ekspresję zachowań ratunkowych u mrówek w skali wewnątrzgatunkowej, natomiast w trzecim badaniu przetestowałem warunki, w jakich zachowania te są bardziej prawdopodobne do występowania w skali międzygatunkowej. Moje pierwsze badanie (**Rozdział II**) pokazuje, że wielkość ciała jest istotnym czynnikiem pod względem wytrwałości w zachowaniu mrówek. To znaczy, że wytrwałość ratowniczek oraz aktywność robotnic, które potrzebują pomocy, jest wyższa u małych niż dużych osobników. Moje drugie badanie (**Rozdział III**) wykazuje, że urazy kończyn u mrówek prowadzą do kosztów, skracając ich przeżywalność. Wyniki pokazują, że pogorszenie stanu zdrowia robotnic wpływa na występowanie zachowań ratunkowych wobec współtowarzyszek z kolonii, ale nie na agresję w stosunku do mrówek obcego gatunku. Oznacza to, że ranne robotnice częściej biorą udział

w akcjach ratunkowych wobec nienaruszonych współtowarzyszek. Moje trzecie badanie (**Rozdział IV**) wskazuje, że międzygatunkowe zróżnicowanie w zachowaniu ratunkowym mrówek można częściowo wyjaśnić oczekiwaną długością życia robotnic. Okazuje się, że gatunki mrówek różnią się znacznie pod względem długości życia robotnic, a skłonność do ratowania jest częstsza u gatunków charakteryzujących się wyższą oczekiwaną długością życia w porównaniu z gatunkami o niższej oczekiwanej długości życia. Przedstawione wyniki pokazują znaczną zmienność w ekspresji zachowań ratunkowych zarówno w obrębie gatunku, jak i między gatunkami. Podkreślają one znaczenie wielkości ciała oraz charakterystycznej dla gatunku oczekiwanej długości życia dla kształtowania wzorców zachowań ratunkowych u mrówek. Zjawisko to wymaga bardziej rozległych badań, uwzględniających zróżnicowaną fizjologię, historię życia i ekologię różnych gatunków. Podsumowując, niniejsza praca ma istotne implikacje dla zrozumienia różnic w procesach podejmowania decyzji u zwierząt społecznych, a także dostarcza potencjalnych kierunków dla przyszłych badań.

Chapter I: General introduction

The aims of the thesis

Understanding the proximate and ultimate causes of animal behaviour is fundamental to ecological studies. My PhD thesis aims to address questions arising from the current state of knowledge about rescue behaviour, using ants (Hymenoptera: Formicidae) as the study system. Specifically, I focused on examining factors that affect the engagement of workers in rescue actions within a species. I considered two potential determinants of this phenomenon, namely body size and life expectancy of rescuing individuals and the individuals that require help. Furthermore, I examined rescue behaviour on a broader scale, among different ant species, to elucidate whether short-lived species differ in the level of rescue behaviours from long-lived species. The factors that I studied in the context of rescue behaviour are known to be closely linked to many life-history traits and behavioural parameters in social animals, especially ants. I assessed the relative impact of these factors on rescue behaviour between interacting nestmates using a standard artificial entrapment bioassay (described in detail below). Research problems addressed in this dissertation are presented in the last part of the introduction chapter, but at the beginning, I provide a wider background of my research.

Definition of rescue behaviour

Pro-social behaviour refers to any voluntary action that benefits another individual (Hamilton 1963). It plays a critical role for group-living animals by facilitating their survival and increasing fitness (de Waal 2008). In recent years, specific pro-social behaviours have gained increased attention, becoming one of the most commonly studied aspects of social animals' lives (e.g., Cronin 2012, Marshall-Pescini 2016, Nafcha et al. 2023, Chen et al. 2023). Although numerous field and experimental studies have been conducted, behavioural ecologists

have predominantly focused on one type of pro-sociality: rescue behaviour. In animal studies, rescue behaviour is also known as helpfulness or helping behaviour (e.g., Lafleur 1940, Ueno 2019). It is defined as a type of pro-social behaviour performed by a rescuer intended to help another individual, the so-called victim, to meet its needs or improve its situation (Nowbahari and Hollis 2010). Rescue behaviour is also considered "altruistic" in the biological sense, as it incurs fitness costs to the rescuer while benefiting the individual being rescued (Hamilton 1963). Performing rescue behaviour always involves the use of energy and/or increased exposure to risks (e.g., injury or death) without obtaining a direct benefit by the rescuer. Specifically, four conditions must be met to classify a given pro-social behaviour as rescue: 1) the victim must be in danger and immediate physical risk; 2) the behaviour of the rescuer must be adapted to the circumstance and type of danger encountered; 3) the rescuer must take a risk when carrying out the rescue action; 4) the rescue action must not provide a direct benefit to the rescuer (Nowbahari and Hollis 2010). To date, rescue behaviour has been described in various forms across many taxa, including vertebrates and invertebrates, but rescue proneness is especially commonly studied in ants (reviewed in Miler and Turza 2021).

Studies on rescue behaviour in ants

Eusocial insects, such as ants, dominate the Earth (Wilson 1971). This impressive evolutionary and ecological "success" is mainly due to extreme altruistic tendencies (Hölldobler and Wilson 1990). These tendencies of ants are results of kin selection, so they do not indicate truly altruistic acts in evolutionary sense. For example, by sacrificing their own reproductive opportunities and devoting their energy solely to raising the queen's offspring, ant workers benefit by increasing the number of their gene copies that are propagated by their close relatives (Hamilton 1963). Although this form of "reproductive altruism" may involve partial coercion (Ratnieks and Wenseleers 2007), its persistence is primarily due to the indirect fitness

benefits gained by each participating altruist. Furthermore, ants exhibit ostensibly voluntary altruistic actions. For example, some ants can leave their nests to die in social isolation, thereby reducing the risk of disease spread within the colony (Heinze and Walter 2010), or to close the nest entrance from the outside, sacrificing themselves by facing predators (Shorter and Rueppell 2012). Another example is provided by the rescue behaviours, which I studied here, and which also lacks evidence of coercion.

Rescue behaviour in ants is frequently discussed in the literature as an example of highly advanced cognitive behaviour and thus of high scientific value (Hollis and Nowbahari 2022). Historical references of this phenomenon date back to the 19th century, when British naturalist Thomas Belt (1874) anecdotally mentioned that army ants (*Eciton hamatum* (Fabricius, 1782)) tried to help nestmates stuck under stones. Since then, rescue behaviour has been noted several times by different authors and in various ecological contexts, including backfilling the nest chambers (Markl 1965, Spangler 1968, Hangartner 1969), antlion or spider capture (Czechowski et al. 2002, Uy et al. 2019) and during fights with termites (Frank et al. 2017). Considerable attention has been devoted to artificial ensnarement, a simulation of entrapment under fallen sand or debris and/or predator capture (Nowbahari et al. 2009).

In the artificial entrapment bioassay, the victim is captured and inserted into a nylon loop attached to a piece of filter paper, partially covered with sand particles, and another ant, the potential rescuer, is placed near the victim (see Figure 1). Various rescue components performed by the rescuer can be observed, including digging close to the entrapped victim, removing the covering sand, or more advanced rescue behaviours such as pulling at the victim's body parts and biting the thread that holds it. According to Hollis and Nowbahari (2013), when examining ant rescue behaviour, specific variables should be included: the time delay to the first episode of rescue (latency), the time devoted to any rescue component (total rescue duration), and the duration of each component of rescue (digging duration, pulling duration,

sand transport duration and thread biting duration). All components of rescue should be measured using standard units [s].

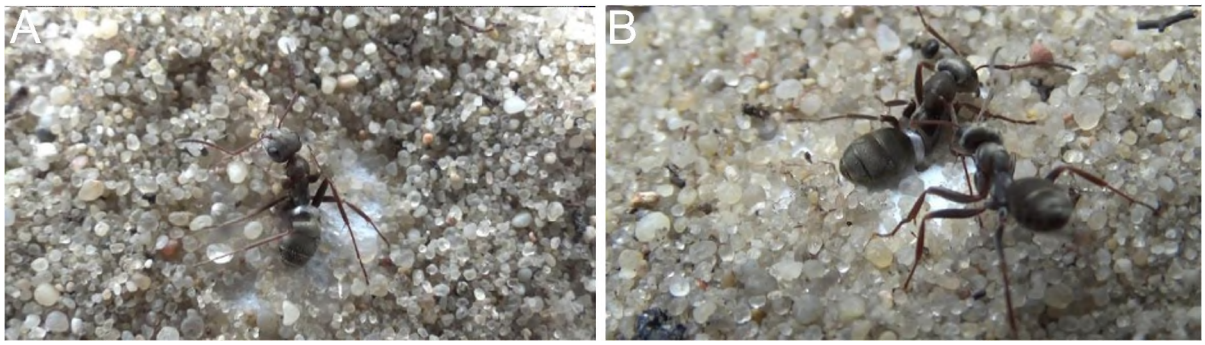


Figure 1. Artificial ensnarement prepared for *Formica cinerea* workers based on the setup proposed by Nowbahari et al. (2009). (A) An entrapped victim ant, with a visible nylon thread ensnaring it to the piece of paper hidden beneath the sand surface. (B) A rescue action, with the rescuer ant pulling at the leg of the victim. Photographs: F. Turza.

Research gaps in the field of ant rescue behaviour

Previous research indicates significant variation in the expression of rescue behaviour both among and within ant species. However, the causes of this variation are often overlooked. Rescue actions bring both benefits and costs, the balance of which surely contributes to this variation. For instance, Frank et al. (2017) provided evidence that without help, 32% of injured termite-eating ants (*Megaponera analis* (Latreille, 1802)) would die, whereas rescue reduced these losses and increased colony size by 28.7%. Similarly, rescue acts toward nestmates stuck in spider webs are recognised as essential to maintaining large ant colonies (Uy et al. 2018). Kwapich and Hölldobler (2019) estimated that the loss of just five workers of harvester ants (*Veromessor pergandei* (Mayr, 1886)) a day, imposed by spider predation, could cost colonies 65,700 seeds annually. Nevertheless, rescue behaviour is also costly. According to Kwapich and Hölldobler (2019), more than 6% of *V. pergandei* foragers attempting to remove spider webs to rescue their nestmates are killed by spiders. Furthermore, another study demonstrated

that rescuers involved in helping ants exposed to the risk of predation by antlions also can become victims of the predators, and rescue actions in such contexts are rarely effective (Turza et al. 2020). These pieces of evidence suggest that many factors simultaneously can affect the expression of rescue behaviour, but, to date, these triggers remain largely unknown.

Some studies indicate the role of body size in ant rescue behaviour, at least in polymorphic species (Kwapich and Hölldobler 2019). Although polymorphic species exhibit distinct phenotypes, such as minor, media, and major workers (Hölldobler and Wilson 1990), even monomorphic species can exhibit extreme body size variation among workers of the same sub-caste (e.g., Herbers and Cunningham 1983, Ishii and Hasgeawa 2013). Differences in body size can lead to significant behavioural variability among workers in both polymorphic and monomorphic species (Herbers and Cunningham 1983, Willott et al. 2000, Ishii and Hasgeawa 2013). For example, the minor workers of polymorphic harvester ants (*Messor bouvieri* (Forel, 1890)) are more efficient at finding seeds, whereas the major workers are more efficient at transporting these seeds to the nest (Willott et al. 2000). Furthermore, smaller workers of the monomorphic ant *Temnothorax longispinosus* (Roger, 1863)) are more likely to engage in social interactions (Herbers and Cunningham 1983), while larger workers of the monomorphic *Myrmica kotokui* (Forel, 1911)) are more likely to be inactive (Ishii and Hasgeawa 2013). Such task engagement is particularly tied to energy expenditure and mortality risks, contributing to the increased fitness of the whole colony (Moroń et al. 2008). Studies suggest that size-dependent differences in behaviour are common, yet we have limited direct evidence for their adaptive consequences.

Worker behavioural variation can also occur regardless of body size differences, particularly in species with strong exploratory activity (Gilad et al. 2022). For example, sand-dwelling ants (*Formica cinerea* (Mayr, 1853)) are highly exposed to predator injuries, leading to a decrease in life expectancy and physiological deterioration (Miler et al. 2017b). Some

studies indicate that experimentally shortening worker life expectancy (e.g., by poisoning with carbon dioxide, physical harm, or parasitic infection) leads to a transition from intranidal tasks to the risky task of foraging outside the nest in ants and honeybees (Moroń et al. 2008, Woyciechowski and Moroń 2009). However, little is known about the effect of shortened life expectancy on worker behaviour in contexts other than the division of labour. For example, it remains unexplored whether similar patterns exist in behaviours that do not require coercion (Ratnieks and Wenseleers 2007). An important question arises as to whether individuals with shorter life expectancies exhibit greater risk-taking tendencies or whether longer-lived individuals are more favourably rescued during life-threatening situations (Miler et al. 2017b, Gilad et al. 2021). Impaired performance, increased susceptibility to predation or infection, and shorter life expectancy may render injured workers less valuable to the colony, serving as the so-called disposable caste (Porter and Jorgensen 1981). This expectation aligns with age-dependent changes in behaviour observed in social insects (Woyciechowski and Kozłowski 1998, Hartmann et al. 2020). Since the primary purpose of rescue behaviour is to benefit the colony (Frank et al. 2017, Kwapich and Hölldobler 2019), saving soon-to-die individuals should be inefficient, but this still awaits testing (Miler et al. 2017b).

Another important gap in understanding rescue behaviour in Formicidae lies in its interspecies variation. Unfortunately, only 23 species out of over 16,000 known species of ants (Bolton 2024) have been studied so far in this context, indicating that our knowledge about rescue actions in ants is limited. Exploring new species is essential for uncovering potential general patterns that may shed light on the occurrence of rescue only in certain species. It is particularly pressing to understand why some species exhibit no such behaviour, even though they seem to encounter rescue-demanding situations in natural conditions (Hollis and Nowbahari 2013, Miler et al. 2017a). One possible cause of this inconsistency may lie in differences in life expectancy between species. To date, we know that such differences can

lead to differences in aggression levels or activity timing (Bos et al. 2012, Hartmann et al. 2020). These effects seem to be related to maximizing Darwinian fitness in ant colonies. Analogically, other behaviours, like the rescue behaviour that I studied, should be promoted more in long-lived species, i.e., species in which individuals will still work for the colony benefits after rescue action, preferably for a long time (Miler et al. 2017b). In other words, ant species that are characterised by short-lived workers may engage in rescue less than those with long-lived workers because such investment – or even investment in social interactions more broadly – does not pay off. However, no one has yet studied the relationship between the general life expectancy of workers and rescue proneness on the between-species scale.

Research questions addressed in the thesis

My first study (**Chapter II**) investigates whether the expression of rescue behaviour depends on body size of a worker that has a chance to engage in rescue actions. To address this question, I measured the rescue tendency and body size of the monomorphic ant *Formica cinerea*. I chose this species due to its large and unexplained behavioural variation in rescue activity (e.g., Miler et al. 2017a, Turza and Miler 2022) and because the workers of the same subcaste, the so-called foragers working outside the nest, are visibly diverse in size (e.g., Seifert 2002, Czechowski et al. 2012). I hypothesised that rescue activity would be higher when performed by small rather than large individuals. This is expected because the colony invested less in the production of smaller individuals (Kay and Rissing 2005). Larger workers are often more valuable due to the substantial investment required for their development and the high energy cost when they are lost (Billick 2002). Consequently, smaller individuals may exhibit a higher propensity for engaging in risky rescue activities. Alternatively, I hypothesised that rescue activity would be higher in large rather than small workers. Some studies indicate the role of body size in ant rescue behaviour, at least in polymorphic species (Kwapich and Hölldobler 2019). This could

be due to the potentially greater efficiency of larger-bodied workers in executing rescue due to their higher metabolic rate and/or greater strength (Hurlbert et al. 2008, Packard 2020). Since there is some evidence that workers of different sizes can differ in their life expectancy (Shik 2010), I measured this parameter in the studied species. Moreover, given the scarcity of data on victim behaviour (Nowbahari and Hollis 2010), I conducted an initial attempt to measure and quantify the behaviour of individuals in the need of rescue. This aimed to explore whether victim activity correlates with victim size and rescue occurrence.

My next study (**Chapter III**) explores whether the injury might affect worker survival and lead to behavioural variation in rescue among individuals that provide help to others and/or those in need of help. To check for context-specificity, I also tested another common and risky behaviour, aggression. This question is addressed with a case study on *Formica cinerea*. This species is characterized by high behavioural variation in rescue activity (Turza and Miler 2022), is vulnerable to attacks of predatory antlions and slave-making ants and so has a high number of injured individuals within their ranks, and is both competitive and aggressive towards aliens (Czechowski et al. 2002). I hypothesised that (1) injured workers would be characterized by lower life expectancy than intact individual, consistent with previous studies involving experimental groups with artificially shortened life expectancy (e.g., Moroń et al. 2012). To increase ecological relevance, I compared the survival rates of naturally injured workers with those of experimentally injured ones. Second, I hypothesised that (2) injured workers would be more responsive in terms of rescue and aggression. Impaired performance, increased susceptibility to predation or infection, and shorter life expectancy may render injured workers less valuable to the colony, serving as the so-called disposable caste (Porter and Jorgensen 1981). This expectation aligns with age-dependent changes in behaviour observed among social insects (Woyciechowski and Kozłowski 1998, Hartmann et al. 2020). Third, I hypothesised that (3) rescue would be directed less toward injured than intact workers.

Since the primary purpose of rescue behaviour is to benefit the colony, saving soon-to-die individuals should be inefficient (Miler et al. 2017b).

My third study (**Chapter IV**) investigates whether the general life expectancy of workers explains variation in rescue behaviour between species regardless of phylogenetic relatedness. To investigate this, I gathered ants belonging to fourteen species originating from different subfamilies and tribes. I estimated the lifespan of workers in each species and their rescue tendency. To standardize the type of individuals tested, I only collected ants found outside the nest, foragers, identified as the most rescue-prone among ant castes (Nowbahari et al. 2012). I hypothesised that rescue is more likely in species that are characterized by longer-lived workers. This is because rescue efforts are expected to occur primarily in species with foragers of high value, namely individuals that will continue to contribute to the colony's productivity after being rescued (foragers with relatively high life expectancy). However, in some species, rescue has no chance of being beneficial. Such a situation may occur in species with workers characterised by generally low life expectancy, with a high turn-over of workers. In other words, I hypothesised that there is no point in helping each other when individuals generally die off quickly. The cost associated with rescuing such individuals is disproportionately high compared to the benefits derived from it (Porter and Jorgensen 1981, Tofilski 2002, 2009).

Chapter II: Small workers are more persistent when providing and requiring help in a monomorphic ant

scientific reports

Check for updates

OPEN

Small workers are more persistent when providing and requiring help in a monomorphic ant

Filip Turza[1,2] & Krzysztof Miler[3]

The common sand-dwelling *Formica cinerea* ants possess monomorphic workers, yet with considerable and easily identified size variation. Considering the importance of body size in polymorphic ants and other animals, we test whether size-dependent differences in behaviour occur in this species. We focus on the behaviour of large and small foragers in the context of rescue occurring between nestmates when one of them is entrapped and requires help. We show that workers of different sizes are characterized by a similar frequency of rescue activity and time delay to the first act of rescue. However, small workers rescue for longer than large workers. These results indicate that, although there is no size-related rescue specialization in *F. cinerea* foragers, small rescuers behave differently than large ones in terms of rescue persistence. Additionally, we show that small workers are more active when trapped. We suggest that variation in behavioural persistence of differently-sized workers may increase the efficiency of rescue actions. This study is the first to find a connection between body size and rescue behaviour in ants and the first to quantify and analyze the behaviour of individuals in need of rescue. These findings add substantially to our understanding of social insects and, more generally, highlight the need to study among-individual behavioural variation in social animals, including those in which body size is judged minute and irrelevant.

Rescue is a situation in which one individual provides help to another in danger[1]. In many animals including, e.g., bottlenose dolphins[2], elephants[3], rats[4], some birds[5], humpback whales[6], mice[7], wild boars[8], and many species of primates[9–12], rescue behaviour draws high scientific interest. It is especially well-researched and common in ants (reviewed in[13,14]). Ants are considered to be organisms with a widespread rescue tendency, which might contribute to their ecological and evolutionary success[15,16]. Belt[17] first described army ants (*Eciton hamatum*) which tried to help their nestmates stuck under stones. Then, more detailed studies provided descriptions of rescue in ants trapped under the soil[18–20], captured by predators such as antlions and spiders[1,21], or in confrontation with termites[22]. Rescue behaviour in ants can be studied both in the laboratory[23] and in the natural setting[24] using the same test that simulates natural rescue contexts in which one ant, the victim, is artificially trapped—tied by a thread to a piece of paper—and partially buried in the sand. When the victim is exposed to free nestmates, the latter often attempt to release the victim by pulling at its body, digging around it, excavating the sand, or even biting the thread entrapping it[23]. Although what the rescuers do in such situations is well quantified, the behaviour of the victims is largely neglected. These trapped individuals are considered passive during rescue attempts and dependent on their rescuers[25].

There is a considerable, albeit largely unexplained variation in rescue behaviour expression between workers of the same colony[16,26]. On the one hand, rescue behaviour increases colony fitness and so one might expect its high occurrence. For example, in harvester ants (*Veromesor pergandei*), the loss of 5 workers a day by spider predation translates to the loss of 65,700 seeds per year, which is a cost avoidable if nestmates show rescue behaviour towards endangered individuals[27]. In termite-eating ants (*Megaponera analis*), 32% of the injured ants die after confronting termites, but rescue behaviour minimizes these losses, allowing for up to 28.7% higher size of the colony and so mitigating the costs of loss of individuals[22]. On the other hand, rescue behaviour is considered risky for rescuing individuals[25,28], which might lower its occurrence. Therefore, some regulating processes connected to rescue behaviour expression are plausible. In general, little is known about these regulating processes[13].

Body size is one of the most essential attributes of any living organism. It is tightly linked to many life-history traits and behavioural parameters[29]. It is also an organismal feature that is evolutionarily flexible and is one of the

[1]Doctoral School of Exact and Natural Sciences, Jagiellonian University, Prof. S. Łojasiewicza 11, 30-348 Kraków, Poland. [2]Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland. [3]Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, 31-016 Kraków, Poland. email: filip.turza@uj.edu.pl; miler@isez.pan.krakow.pl

first to respond to natural selection[30,31]. Research devoted to insect body size takes advantage of the fact that the exoskeleton is easier to measure than the soft bodies of other animals[32,33]. Studying insects in this context greatly expands our knowledge about the importance of body size. In social insects, body size is indicated as a driver of the non-reproductive division of labour in workers of eusocial wasps [34], termites[35], bumblebees[36] and other bees[37], as well as ants[38,39]. Moreover, as shown by Trible and Kronauer[40], accounting for body size is necessary to understand caste evolution and development among Formicidae.

In the so-called polymorphic ants with workers characterized by different sizes and shapes[41], the relationship between morphology and task allocation is quite well studied[42–52]. Among ants, however, a majority of species are monomorphic[53], i.e., without broad size or shape variation between workers[54]. These workers typically follow strong age polyethism and progress from performing one type of task to others as they age[41,53], but they might show some specialization or personality (consistent behavioural variation) towards different tasks in relation to their body size[55,56]. These tendencies can be a result of factors influencing the development of larvae, such as nutrition or temperature (reviewed in[57]). Similarly, the relationship between body size and behaviour in monomorphic species is thought to increase the ergonomics of labour[58–64]. Factors such as the efficiency of energy expenditure or a decrease in mortality risks might shape task engagement in workers according to their body size[50,51].

Although size-dependent differences in behaviour seem to be common[43–47] we have limited knowledge of their true adaptive significance, particularly within monomorphic ants[65]. We investigate, therefore, rescue behaviour variation among size-diversified workers that perform tasks outside the nest, the foragers. We use the monomorphic ant *Formica cinerea* as a model species because the evidence for inter-individual variation in the rescue behaviour of this species is well documented[66–72] and because foragers of *F. cinerea* are visibly diverse in size[73,74]. Our primary aim is to check whether foragers characterized by different body sizes differ in rescue behaviour directed towards nestmates. We propose a hypothesis that rescue activity would be higher when performed by small rather than large individuals. This is reasonable to expect in terms of risk management within the colony[75]. Specifically, larger workers can be more valuable than smaller workers because of different costs that need to be invested by the colony for the development of such workers in terms of biomass, which is difficult to compensate for when lost[76]. If so, small individuals should engage in risky rescue more than large individuals. Our alternative hypothesis is the reverse, i.e., that rescue activity would be higher in large rather than small workers. Rescue may be more efficient when performed by workers characterized by larger body sizes because of their higher rates of metabolism[77,78] or higher strength[79]. Considering that in workers of at least some polymorphic ants, larger workers live longer than smaller ones[80], we measured this parameter among our foragers to exclude the possibility that our results would be related to life expectancy[68]. Indeed, life expectancy can play a role in rescue actions[66,68,72]. For instance, the ants with lower life expectancies seem to better discriminate which nestmates are worth rescuing[72]. Additionally, due to the general scarcity of data related to the behaviour of the victims, we present the first attempt at measuring and quantifying the behaviour of individuals in need of rescue. We explored whether the victim activity would depend on the size of the victim and rescue occurrence.

Methods

The study was conducted in the field near Klucze (Błędowska Desert, Poland, 50°21′22″N 19°31′03″E) and the laboratory in Kraków (Institute of Environmental Sciences, Jagiellonian University, Poland). We used four independent colonies of *F. cinerea,* at least 500 m apart from each other. All studied colonies were polycalic, i.e., within which workers moved freely without aggression over various nests[74]. The colonies have been previously used in studies devoted to rescue behaviour[67,70–72].

Behavioural experiment in the field

We performed four types of dyadic rescue behaviour tests, each with the victim classed as large (L) or small (S) and the potential rescuer also classed as large (L) or small (S).

The testing procedure was similar to that used in earlier studies[15,24,71]. For each test, a plastic ring (7 cm diameter × 7 cm high) was placed in an ant-free area near the nest entrance. The ring wall was coated with fluon (Sigma–Aldrich, Germany) to prevent nearby workers from entering the test area. Then, using clean forceps, a forager belonging to one of the size classes (L or S) was captured within a nearby area and inserted into a nylon loop attached to a piece of filter paper. Such a victim was placed inside the test area and partly covered with the surrounding sand. Immediately after placing a victim, a potential rescuer of a certain body size (L or S) was captured within a nearby area and placed in the test area. The ants were classified as large or small by eye in the field. Each ant, nylon thread, and filter paper were used only once. Each test lasted 5 min, started immediately after the placement of the victim and potential rescuer inside the ring, and was recorded using Sony HDR-CX625 cameras. After each test, forceps were sterilized in 98% ethanol to avoid direct transfer of cuticular hydrocarbons via the tested ants. All ants used in the tests were placed in plastic containers (separate for individuals categorized as large or small) and transported to the laboratory after the tests on the given day finished. The procedure was repeated 30 times for each dyad type (i.e., LL, LS, SL, SS) and colony (a total of 480 tests). We collected the data during an active foraging period of *F. cinerea*, i.e., between 9 AM and 6 PM[71,81]. The order of the tests was counterbalanced by dyad type and colony, i.e. every day we performed tests for all four types of dyads for each of the four colonies. The order of the tested colonies and tests was changing daily (we performed 20 of all types of dyads per colony per day, i.e., 80 tests in total per day). The temperature during the six days over which the behavioural experiments in the field were performed ranged from 24–27 °C and humidity was between 52 and 65%. After each test day, all tested individuals were frozen and preserved in 98% ethanol until measured.

Analysis of the recordings from the behavioural experiment

Each recording was coded (blinded) to prevent any potential observer bias and then analysed using the Behavioural Observation Research Interactive Software (BORIS;[82]). We noted whether in a given test any rescue behaviour such as digging around the entrapped individual, pulling at its body parts, transporting the sand covering it, and/or biting the thread entrapping it occurred at all, as well as the time to the first act of rescue and the total duration of rescue. This categorization of rescue behaviour was proposed by Nowbahari et al.[23] and then subsequently used by multiple authors with similar dependent variables measured in each case, including the latency and total rescue duration[26,67,71,83]. In addition, we analysed the behaviour of the ants in need of rescue. We measured the total duration of the victim's activity including opening mandibles, kicking, and/or sand throwing. All behaviours were measured in seconds.

Survival experiment in the laboratory

400 *F. cinerea* foragers were handpicked near the nest entrance from the four focal colonies (100 workers per colony) and transported to the laboratory. Ants during handpicking were divided into two classes: large (L) and small (S) workers (50 individuals per group, respectively). Similarly to e.g., Miler[66], the ants were kept in plastic boxes (18 × 15 × 7 cm) separate for each colony and class, with fluon coating the inner walls (Sigma–Aldrich, Germany) to prevent escape, at a constant temperature of 24 °C, 40–60% relative humidity and a 12:12 day/night cycle. Ants were provided with water and 10% sucrose solution ad libitum. Ant mortality was checked daily from the moment of collection in the field until the end of the experiment, i.e., 200 days. Each day dead individuals were frozen and preserved in 98% ethanol until measured. The same was done with ants that remained alive after 200 days when the experiment was terminated.

Body size measurements

To investigate the general distribution of size among foragers and confirm its unimodality in *F. cinerea*, we collected 400 active foragers (100 from each of the four focal colonies) and measured their body size. We also measured the body size of all frozen individuals that originated from the behavioural experiment in the field and survival experiment in the laboratory, in which the ants were categorized as large or small (1360 individuals in total, 960 workers from the behavioural experiment in the field and 400 workers from the survival experiment in the laboratory). We chose the maximum head width as a proxy of body size (a standard measurement of body size in ants, including *Formica* ants, e.g., [84–86]) which was measured to the nearest 0.001 mm, using a digital microscope Delta Optical Smart 5MP PRO under 65 × magnification and Delta Optical Smart Analysis Pro software.

Statistical analysis

All analyzes were performed using R[87]. To compare rescue occurrence (1—rescue, 0—no rescue), we used a generalized linear mixed model (lme4 package;[88]) with a binomial residual distribution, logit link function and included a random factor "colony" and a fixed factor "rescuer size" (L vs. S). Then, for further analysis, we used a subset of data that included only tests in which any rescue behaviour was observed. First, to compare the latency to the first rescue attempt, we used a generalized linear mixed model (lme4 package;[88]) with a gamma residual distribution, log link function and included a random factor "colony" and fixed factor "rescuer size" (L vs. S). Second, to compare the duration of rescue, we used a mixed-effects Cox proportional hazards model fit by maximum likelihood (coxme package;[89]) with a random factor "colony" and a fixed factor "rescuer size" (L vs. S). Data for tests during which rescue behaviour was interrupted at the end of the recording (i.e., 5 min) was censored to indicate that the behaviour of interest occurred beyond the ant's observation time (as in[72]). This was done to avoid biased outcomes within any group of workers due to some individuals that were still engaged in rescue after a recording period. Among a total number of 201 tests with any rescue, 81 were censored.

Furthermore, to compare the duration of rescue categories, we also used mixed-effects Cox proportional hazards models fit by maximum likelihood (coxme package;[89]) with a random factor "colony" and a fixed factor "rescuer size" (L vs. S). Data for tests during which a given rescue category was interrupted at the end of the recording (i.e., 5 min) was censored. Analogically as described above, 81 tests were censored for the same reasons as in the case of the total duration of rescue.

To compare the duration of activity in the victims, we used a mixed-effects Cox proportional hazards model fit by maximum likelihood (coxme package;[89]) with a random factor "colony" and the fixed factors of "victim size" (L vs. S) and "rescue occurrence" (0 vs. 1). We used a subset of data that included only tests in which any activity in the victim was observed. Similarly to the rescue duration analyses, data for tests during which victim activity was interrupted at the end of the recording (i.e., 5 min) was censored. Among a total number of 417 tests with any victim activity, 118 were censored.

To analyze mortality in large and small workers, we used a mixed-effects Cox proportional hazards model (coxme package;[89]) with a random factor "colony" and a fixed factor "group" (L vs. S). Data for ants that remained alive at the end of the survival experiment (i.e., after 200 days) was censored. Among a total number of 400 ants, 181 workers were censored.

To analyze body size distribution in each focal colony, we used a Shapiro–Wilk test of normality. To compare body size between workers classed as large and small in our two experiments, we used a linear mixed model (lme4 package;[88]) and two explanatory variables, a random factor "colony" and a fixed factor "group" (L vs. S).

Ethical approval

No approval of research ethics committees was required to accomplish the goals of this study, because experimental work was conducted with an unregulated invertebrate species.

Results

Rescue occurrence was similar between the two groups of workers (rescuer size: z = 1.58, $p = 0.11$; mean ± SE for small workers = 0.45 ± 0.03, mean ± SE for large workers = 0.38 ± 0.03). The latency to the first episode of rescue did not differ between tests (rescuer size: $t_{1,197} = 0.77$, $p = 0.44$; mean ± SE for small workers = 98.6 ± 8.09, mean ± SE for large workers = 110.6 ± 9.27; Fig. 1a). In terms of the duration of rescue behaviour, however, we found a significant effect of the rescuer size (z = − 2.36, $p = 0.02$; Fig. 1b), with small workers rescuing longer than large workers (mean ± SE for small workers = 70.40 ± 6.57, mean ± SE for large workers = 60.08 ± 7.58). In the analysis of rescue categories, differences were significant for the duration of digging (z = -3.26, p = 0.001) and sand transport (z = − 2.36, $p = 0.02$) but not pulling (z = − 1.89, $p = 0.06$) and thread biting (z = − 1.56, $p = 0.12$). In other words, small rescuers performed more digging and sand transport than large rescuers, which resulted in higher total durations of rescue in the former.

Small victims were characterized by higher activity than large victims (z = − 2.22, $p = 0.03$; Fig. 2a; mean ± SE for small workers = 103.59 ± 5.23, mean ± SD for large workers = 87.53 ± 4.67). In terms of tests with and without rescue behaviour, victim activity was lower in the former type of tests (z = 2.64, $p = 0.01$; Fig. 2b; mean ± SD for tests with rescue = 90.10 ± 5.05, mean ± SD for tests without rescue = 100.77 ± 4.94).

Mortality did not differ between workers assigned to the two groups of workers: large vs. small (z = 0.48, $p = 0.63$, Fig. 3).

In *F. cinerea*, forager body size had a unimodal distribution (Fig. 4a). Shapiro–Wilk test of normality for each of the four colonies utilized in the study showed non-significant results and indicated that distributions did not deviate from normality (colony 1: W = 0.99, $p = 0.58$, colony 2: W = 0.99, $p = 0.57$, colony 3: W = 0.99, $p = 0.48$, colony 4: W = 0.98, $p = 0.24$). In turn, the results for the ants tested in our two experiments showed that there were significant size differences between workers classed as large or small ($F_{1,1355} = 3354.40$, $p < 0.001$; Fig. 4b). Although there was some overlap in size between classes, which indicated that our classification was not error-free, the vast majority of individuals used in the study were clearly larger or smaller and appropriately classed as such.

Discussion

The present study offers the first investigation into the effect of the body size in a monomorphic (or any other) ant species in the context of rescue behaviour. Here we show a considerable size diversity in foragers in *F. cinerea*. As demonstrated by the results, these differently-sized individuals show no differences in longevity when measured in laboratory conditions. Furthermore, neither of our hypotheses regarding the effect of body size on rescue activity is confirmed. The decision to rescue a nestmate is not related to the body size of the rescuer, and this is also the case in terms of the time delay to the start of rescue activity. Small workers perform rescue for longer than large ones, but it seems likely that it is simply evidence of higher behavioural persistence. This difference between

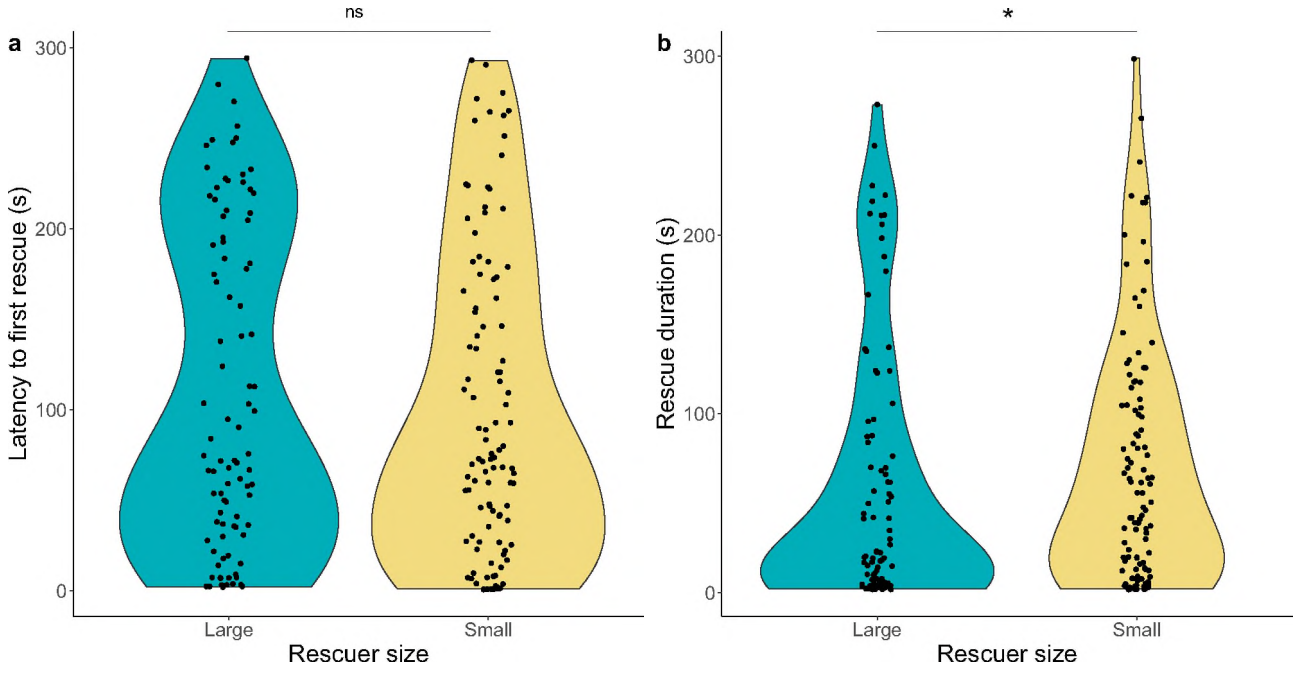


Figure 1. The latency to the first episode of rescue (**a**) and the duration of rescue (**b**) in differently-sized workers of *F. cinerea*. Dots represent each test with rescue occurrence. The violin plot outlines illustrate kernel probability density. Above plots, the "ns" indicates not significant ($p > 0.05$), whereas the asterisk indicates significant difference ($p < 0.05$) between groups. The latency to initiate the rescue episode showed no differences between tests. In terms of the duration of rescue behaviour, small workers demonstrated a longer time spent on rescue compared to large workers.

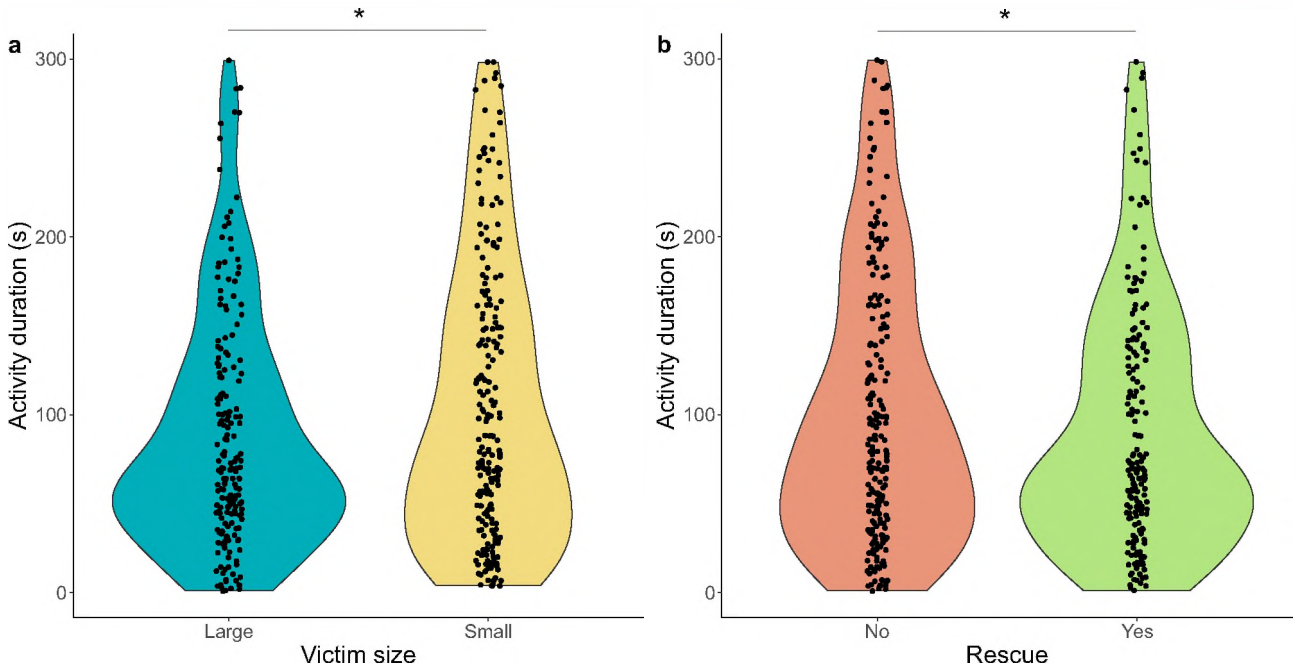


Figure 2. The duration of activity of the differently-sized victims of *F. cinerea* (**a**) and the duration of victims' activity during rescue behaviour tests in which rescue occurred or not (**b**). Dots represent each test with activity occurrence. The violin plot outlines illustrate kernel probability density. Above plots, the asterisk indicates significant difference ($p < 0.05$) between groups. Small victims spent significantly more time being active than large victims. In terms of tests with and without rescue behaviour, victim activity was significantly lower in tests with rescue.

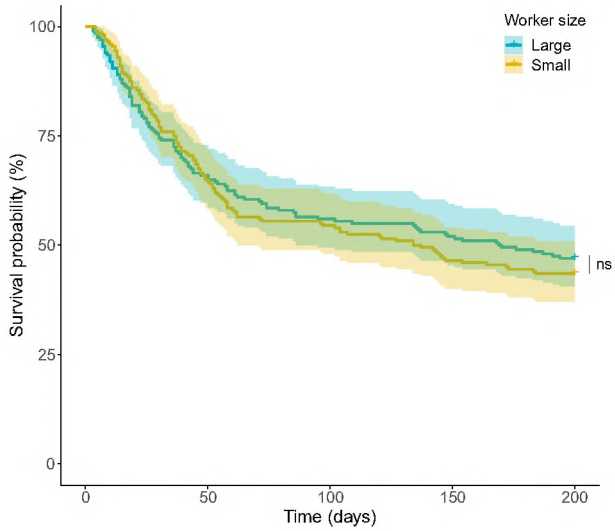


Figure 3. Survival curves for large and small foragers in *F. cinerea*. Shading indicates confidence intervals. There is no statistically significant difference between the groups (indicated by the "ns").

small and large workers stems from higher engagement in digging and sand transport in the former. It is also visible in the behaviour of workers during entrapment. Specifically, the activity of entrapped individuals is higher in small than large workers, which suggests higher persistence in attempts to self-release in the former. A recent study reported similar results in the highly polymorphic *Atta* leaf-cutting ants, in which smaller individuals are more persistent fighters, although the general probability of attack does not differ between workers of different sizes[90]. The authors suggest that smaller individuals might stimulate other individuals, including larger ones, to show aggression during colony defence. Somewhat increased rescue engagement which we describe here in small individuals may analogically function to facilitate nearby nestmates to join rescue action. Although this has not been explicitly studied, Nowbahari et al. already suggested that nestmates might facilitate rescue engagement[23].

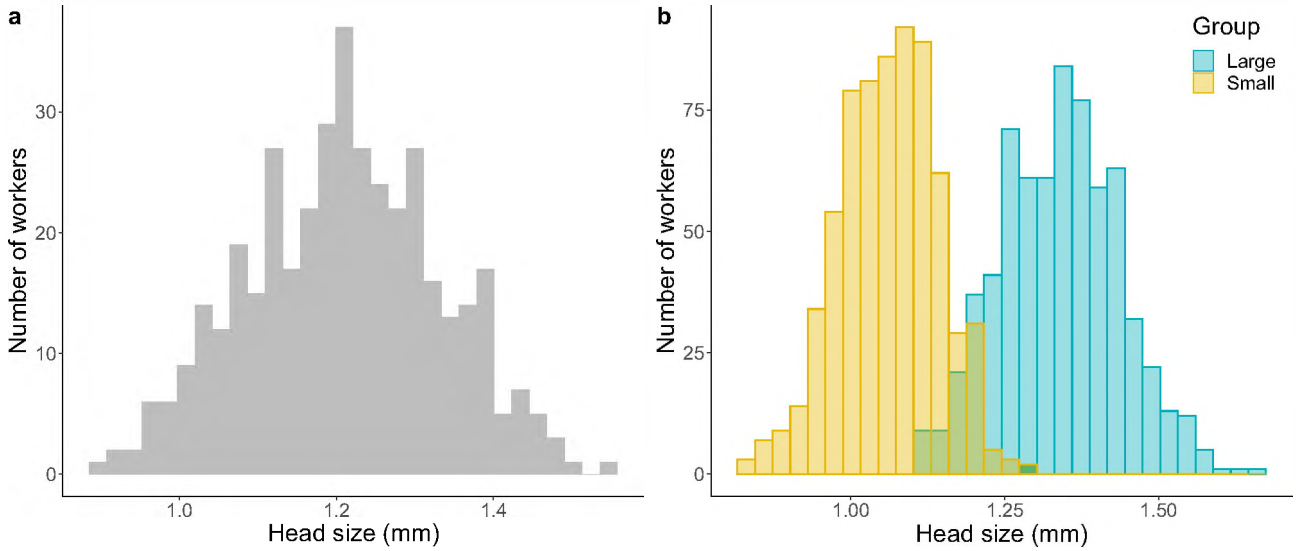


Figure 4. The unimodal distribution of head sizes among foragers (n = 400) of *F. cinerea* (**a**) and the distribution of head sizes among foragers targeted for our experiments and classed as large (n = 680) or small (n = 680) (**b**). The two distributions have significantly different means (small workers = 1.061 mm, large workers = 1.339 mm).

Differently sized workers engage in risky rescue acts with similar frequencies to those reported in previous research utilizing the same model species[66–71]. Of note, however, this is the first study to also measure, quantify, and report results concerning the activity of the victims. Their activity might indicate self-release attempts, as it causes physical disturbances which can remove whatever is entrapping them, and/or attract the attention of nestmates that might provide help. Activity is higher when the entrapped individual is small, which is reasonable considering that such individuals need more effort to remove whatever is ensnaring them and/or trigger rescue action. Nowbahari et al.[83] reported that the use of motionless victims, anesthetized by chilling at − 4 °C for 2 min, triggers no rescue action compared to untreated active individuals. Thus, the activity of victims might be potentially used as an indicator of visual, chemical, and/or vibroacoustic cues to attract the attention of nestmates[1, 22, 23]. Curiously, in our study, victim activity decreases significantly in tests with rescue behaviour. This counterintuitive result, in our opinion, likely indicates that rescue is a cue for the victim to cease activity and wait. Future research needs to explore rescue from the perspective of the victims[13]. Indeed, the victim's behaviour may explain the rescuer's response and contribute to how well the rescue is performed, similarly to the social carrying behaviour in which the transported ant freezes to facilitate transport by another ant[38, 41, 91, 92].

Regarding the behaviour of differently sized workers, there are some noteworthy results already reported in the literature in contexts other than rescue. For example, Herbers and Cunningham[55] demonstrated that small workers in a monomorphic *Temnothorax longispinosus* engage in social interactions more frequently than large ones. Beshers and Traniello[59] suggested that the most costly to produce and valuable (i.e., relatively large) individuals should be the least active. This is evident, for example, in the monomorphic *Myrmica kotokui*[93], in which the largest individuals are the laziest ones. Large individuals of the monomorphic *Leptothorax acervorum* are also less aggressive than small ones[56]. How general such patterns of activity are in size-diversified workers of the monomorphic ant species remains to be determined. However, this requires a comprehensive approach that includes several measures of the behaviour, as in the current study and previous research devoted to polymorphic species[49–51].

The workers in our study are arbitrarily classed into two size groups (large and small), the extremes of an unimodal distribution. Body size measurements and analysis confirmed that this method is accurate, however, it has some limitations. Such categorization is more appropriate for polymorphic ants, in which the discrete morphological castes arise from different development pathways (reviewed in[39]). Grouping monomorphic workers similarly to polymorphic workers might lead to misinterpretation of the results. Thus we suggest that in the case of monomorphic ants, treating the worker size as a continuous variable might be more appropriate. This, however, requires a different strategy of data collection than employed here in this study. In any case, in contrast to earlier findings, which mostly focus on size differences in workers performing different tasks[58–64], our results show that in the monomorphic ant *F. cinerea,* body size diversity of workers may be observed even among the same task group, i.e., foragers. This highlights the importance of this body parameter, even in species with narrow and seemingly irrelevant worker size variation.

Conclusions

Taken together, our data support a growing number of studies showing size-specific behavioural variation in animals[91, 94]. More specifically, we show that behavioural persistence in the context of rescue is higher in smaller sized workers of a monomorphic ant species. This research also introduces the measurement and analysis of the activity of ant workers in need of help. We suggest that future research on rescue behaviour should consider the poorly understood behaviour of entrapped individuals and its potential effect on rescuers.

Data availability

The datasets generated and analysed during the current study are available in the RODBUK repository, [https://doi.org/10.57903/UJ/RSAJWP].

Received: 27 June 2023; Accepted: 2 December 2023
Published online: 07 December 2023

References

1. Czechowski, W., Godzińska, E. J. & Kozłowski, M. W. Rescue behavior shown by workers of *Formica sanguinea* Latr., *F. fusca* L. and *F. cinerea* Mayr (Hymenoptera: Formicidae) in response to their nestmates caught by an ant lion larva. *Ann. Zool.* **52**, 423–431 (2002).
2. Siebenaler, J. B. & Caldwell, D. K. Cooperation among adult dolphins. *J. Mammal.* **37**, 126–128 (1956).
3. Bates, L. *et al.* Do elephants show empathy?. *J. Conscious Stud.* **15**, 204–225 (2008).
4. Bartal, I. B. A., Decety, J. & Mason, P. Empathy and pro-social behavior in rats. *Science* **334**, 1427–1430 (2011).
5. Hammers, M. & Brouwer, L. Rescue behaviour in a social bird: Removal of sticky 'bird catcher tree' seeds by group members. *Behaviour* **154**, 403–411 (2017).
6. Pitman, R. L. *et al.* Humpback whales interfering when mammal-eating killer whales attack other species: Mobbing behavior and interspecific altruism?. *Mar. Mamm. Sci.* **33**, 7–58 (2017).
7. Ueno, H. *et al.* Helping-like behaviour in mice towards conspecifics constrained inside tubes. *Sci. Rep.* **9**, 5817 (2019).
8. Masilkova, M. *et al.* Observation of rescue behaviour in wild boar (*Sus scrofa*). *Sci. Rep.* **11**, 16217. https://doi.org/10.1038/s41598-021-95682-4 (2021).
9. Rood, J. P. Banded mongoose rescues pack member from eagle. *Anim. Behav.* **31**, 1261–1262 (1983).
10. Vogel, E. R. & Fuentes-Jiménez, A. Rescue behavior in white-faced capuchin monkeys during an intergroup attack: Support for the infanticide avoidance hypothesis. *Am. J. Primatol.* **68**, 1012–1016 (2006).
11. Gardner, C. J., Radolalaina, P., Rajerison, M. & Greene, H. W. Cooperative rescue and predator fatality involving a group-living strepsirrhine, Coquerel's sifaka (*Propithecus coquereli*), and a Madagascar ground boa (*Acrantophis madagascariensis*). *Primates* **56**, 127–129 (2015).
12. Huang, J. *et al.* Having infants in the family group promotes altruistic behavior of marmoset monkeys. *Curr. Biol.* **30**, 4047–4055 (2020).
13. Miler, K. & Turza, F. "O sister, where art thou?"—A review on rescue of imperiled individuals in ants. *Biology* **10**, 1079. https://doi.org/10.3390/biology10111079 (2021).
14. Hollis, K. L. & Nowbahari, E. Cause development, function, and evolution: Toward a behavioral ecology of rescue behavior in ants. *Learn. Behav.* **50**, 329–338 (2022).
15. Taylor, K., Visvader, A., Nowbahari, E. & Hollis, K. L. Precision rescue behavior in north American ants. *Evol. Psychol.* **11**, 665–677 (2013).
16. Andras, J. P., Hollis, K. L., Carter, K. A., Couldwell, G. & Nowbahari, E. Analysis of ants' rescue behavior reveals heritable specialization for first responders. *J. Exp. Biol.* **223**, jeb212530 (2020).
17. Belt, T. *The Naturalist in Nicaragua* (John Murray, 1874).
18. Markl, H. Stridulation in leaf-cutting ants. *Science* **149**, 1392–1393 (1965).
19. Spangler, H. W. Stimuli releasing digging behavior in the Western harvester ant. *J. Kan. Entomol. Soc.* **41**, 318–323 (1968).
20. Hangartner, W. Carbon dioxide, a releaser for digging behavior in *Solenopsis geminata* (Hymenoptera: Formicidae). *Psyche* **76**, 58–67 (1969).
21. Uy, F. M. K., Adcock, J. D., Jeffries, S. F. & Pepere, E. Intercolony distance predicts the decision to rescue or attack conspecifics in weaver ants. *Insectes Soc.* **66**, 185–192 (2018).
22. Frank, E. T. *et al.* Saving the injured: Rescue behavior in the termite-hunting ant *Megaponera analis*. *Sci. Adv.* **3**, e160218. https://doi.org/10.1126/sciadv.1602187 (2017).
23. Nowbahari, E., Scohier, A., Durand, J. & Hollis, K. L. Ants, *Cataglyphis cursor*, use precisely directed rescue behavior to free entrapped relatives. *PLoS One* **4**, e6573. https://doi.org/10.1371/journal.pone.0006573 (2009).
24. Hollis, K. L. & Nowbahari, E. A comparative analysis of precision rescue behavior in sand-dwelling ants. *Anim. Behav.* **85**, 537–544 (2013).
25. Nowbahari, E. & Hollis, K. L. Rescue behavior: Distinguishing between rescue, cooperation, and other forms of altruistic behavior. *Commun. Integr. Biol.* **3**, le3. https://doi.org/10.4161/cib.3.2.10018 (2010).
26. Nowbahari, E., Hollis, K. L., Bey, M., Demora, L. & Durand, J. L. Rescue specialists in *Cataglyphis piliscapa* ants: The nature and development of ant first responders. *Learn. Behav.* **50**, 71–81 (2022).
27. Kwapich, C. L. & Hölldobler, B. Destruction of spiderwebs and rescue of ensnared nestmates by a granivorous desert ant (*Veromessor pergandei*). *Am. Nat.* **194**, 395–404 (2019).
28. Duhoo, T., Durand, J. L., Hollis, K. L. & Nowbahari, E. Organization of rescue behaviour sequences in ants, *Cataglyphis cursor*, reflects goal-directedness, plasticity and memory. *Behav. Processes.* **139**, 12–18 (2017).
29. Schmidt-Nielsen, K. *Scaling: Why is Animal Size so Important?* (Cambridge University Press, 1984).
30. Anderson, A. M. *et al.* Consistent declines in wing lengths of *Calidridine sandpipers* suggest a rapid morphometric response to environmental change. *PLoS ONE.* **14**, e0213930. https://doi.org/10.1371/journal.pone.0213930 (2019).
31. Esquerré, D. *et al.* Phylogenomics, biogeography, and morphometrics reveal rapid phenotypic evolution in pythons after crossing wallace's line. *Syst. Biol.* **69**, 1039–1051 (2020).
32. Daly, H. V. Insect morphometrics. *Annu. Rev. Entomol.* **30**, 415–438 (1985).
33. Csősz, S. *et al.* Insect morphometry is reproducible under average investigation standards. *Ecol. Evol.* **11**, 547–559 (2020).
34. O'Donnell, S. & Jeanne, R. L. The roles of body size and dominance in division of labor among workers of the eusocial wasp *Polybia occidentalis* (Olivier) (Hymenoptera: Vespidae). *J. Kans. Entomol. Soc.* **68**, 43–50 (1995).
35. Crosland, M. W. J., Lok, C. M., Wong, T. C., Shakarad, M. & Traniello, J. F. A. Division of labour in a lower termite: The majority of tasks are performed by older workers. *Anim. Behav.* **54**, 999–1012 (1997).
36. Jandt, J. M. & Dornhaus, A. Spatial organization and division of labour in the bumblebee *Bombus impatiens*. *Anim. Behav.* **77**, 641–651 (2009).
37. Gruter, C., Menezes, C., Imperatriz-Fonseca, V. L. & Ratnieks, F. L. W. A morphologically specialized soldier caste improves colony defense in a neotropical eusocial bee. *Proc. Natl. Acad. Sci. USA* **109**, 1182–1186 (2012).
38. Wilson, E. O. *The Insect Societies* (Harvard University Press, 1971).
39. Wills, B. D., Powell, S., Rivera, M. D. & Suarez, A. V. Correlates and consequences of worker polymorphism in ants. *Annu. Rev. Entomol.* **63**, 575–598 (2018).
40. Trible, W. & Kronauer, D. J. C. Ant caste evo-devo: Size predicts caste (almost) perfectly. *Trends Ecol. Evol.* **8**, 671–673 (2021).
41. Hölldobler, B. & Wilson, E. O. *The Ants* (Harvard University Press, 1990).
42. Wilson, E. O. The Ergonomics of caste in the social insects. *Am. Nat.* **102**, 41–66 (1968).

43. Burd, M. Body size effects on locomotion and load carriage in the highly polymorphic leaf-cutting ants *Atta colombica* and *Atta cephalotes*. *Beh. Ecol.* **11**, 125–131 (2000).
44. Hart, A. & Ratnieks, F. Task partitioning, division of labour and nest compartmentalisation collectively isolate hazardous waste in the leafcutting ant *Atta cephalotes*. *Behav. Ecol. Sociobiol.* **49**, 387–392 (2001).
45. Medan, V. & Josens, R. B. Nectar foraging behaviour is affected by ant body size in *Camponotus mus*. *J. Insect. Physiol.* **51**, 853–860 (2005).
46. Schwander, T., Rosset, H. & Chapuisat, M. Division of labour and worker size polymorphism in ant colonies: The impact of social and genetic factors. *Behav. Ecol. Sociobiol.* **59**, 215–221 (2005).
47. Arnan, X., Ferrandiz-Rovira, M., Pladevall, C. & Rodrigo, A. Worker size-related task partitioning in the foraging strategy of a seed-harvesting ant species. *Behav. Ecol. Sociobiol.* **65**, 1881–1890 (2011).
48. Theraulaz, G., Bonabeau, E. & Deneubourg, J. L. The origin of nest complexity in social insects. *Complexity* **3**, 15–25 (1998).
49. Beshers, S. N. & Fewell, J. H. Models of division of labor in social insects. *Annu. Rev. Entomol.* **46**, 413–440 (2001).
50. Detrain, C. & Pasteels, J. M. Caste polyethism and collective defense in the ant, *Pheidole pallidula*: The outcome of quantitative differences in recruitment. *Behav. Ecol. Sociobiol.* **29**, 405–412 (1992).
51. Detrain, C. & Pasteels, J. M. Caste differences in behavioral thresholds as a basis for polyethism during food recruitment in the ant, *Pheidole pallidula* (Nyl.) (Hymenoptera: Myrmicinae). *J. Insect. Behav.* **4**, 157–176 (1991).
52. Gordon, D. M. The organization of work in social insect colonies. *Nature.* **380**, 121–124 (1996).
53. Oster, G. F. & Wilson, E. O. *Caste and Ecology in the Social Insects* (Princeton, 1978).
54. Waser, N. M. Task-matching and short-term size shifts in foragers of the harvester ant, *Messor pergandei* (Hymenoptera: Formicidae). *J. Insect. Behav.* **11**, 451–462 (1998).
55. Herbers, J. M. & Cunningham, M. Social organization in *Lepthotorax longispinosus* Mayr. *Anim. Behav.* **31**, 759–771 (1983).
56. Kühbandner, S., Modlmeier, A. P. & Foitzik, S. Age and ovarian development are related to worker personality and task allocation in the ant *Leptothorax acervorum*. *Curr. Zool.* **60**, 392–400 (2014).
57. Trible, W. & Kronauer, D. J. C. Caste development and evolution in ants: It's all about size. *J. Exp. Biol.* **220**, 53–62 (2017).
58. Burton, J. L. & Franks, N. R. The foraging ecology of the army ant *Eciton rapax*: An ergonomic enigma?. *Ecol. Entomol.* **10**, 131–141 (1985).
59. Beshers, S. N. & Traniello, J. F. A. Polyethism and the adaptiveness of worker size variation in the attine ant *Trachymyrmex septentrionalis*. *J. Insect. Behav.* **9**, 61–83 (1996).
60. Fournier, D., Battaille, G., Timmermans, I. & Aron, S. Genetic diversity, worker size polymorphism and division of labour in the polyandrous ant *Cataglyphis cursor*. *Anim. Behav.* **75**, 151–158 (2008).
61. Westling, J. N., Harrington, K., Bengston, S. & Dornhaus, A. Morphological differences between extranidal and intranidal workers in the ant *Temnothorax rugatulus*, but no effect of body size on foraging distance. *Insectes. Soc.* **61**, 367–369 (2014).
62. Grześ, I. M., Okrutniak, M. & Grzegorzek, J. The size-dependent division of labour in monomorphic ant *Lasius niger*. *Eur. J. Soil. Biol.* **77**, 1–3 (2016).
63. Vele, A. & Modlinger, R. Body size of wood ant workers affects their work division. *Sociobiology* **66**, 614–618 (2019).
64. Okrutniak, M., Rom, B., Turza, F. & Grześ, I. M. Body size differences between foraging and intranidal workers of the monomorphic ant *Lasius niger*. *Insects* **11**, 433. https://doi.org/10.3390/insects11070433 (2020).
65. Okrutniak, M. *et al.* The ant *Lasius niger* shows no relationship between task efficiency and body size variation among workers. *Entomol. Sci.* **26**, e12549. https://doi.org/10.1111/ens.12549 (2023).
66. Miler, K. Moribund ants do not call for help. *PLoS One* **11**, e0151925. https://doi.org/10.1371/journal.pone.0151925 (2016).
67. Miler, K. & Kuszewska, K. Secretions of mandibular glands are not involved in the elicitation of rescue behaviour in *Formica cinerea* ants. *Insectes Soc.* **64**, 303–305 (2017).
68. Miler, K., Symonowicz, B. & Godzińska, E. J. Increased risk proneness or social withdrawal? The effects of shortened life expectancy on the expression of rescue behavior in workers of the ant *Formica cinerea* (Hymenoptera: Formicidae). *J. Insect. Behav.* **30**, 632–644 (2017).
69. Miler, K., Yahya, B. E. & Czarnoleski, M. Pro-social behaviour of ants depends on their ecological niche: Rescue actions in species from tropical and temperate regions. *Behav. Proc.* **144**, 1–4 (2017).
70. Turza, F. *et al.* Ants co-occurring with predatory antlions show unsuccessful rescue behavior towards captured nestmates. *J. Insect Behav.* **33**, 1–6 (2020).
71. Turza, F. & Miler, K. Comparative analysis of experimental testing procedures for the elicitation of rescue actions in ants. *Curr. Zool.* **68**, 159–168 (2022).
72. Turza, F. & Miler, K. Injury shortens life expectancy in ants and affects some risk-related decisions of workers. *Anim. Cogn.* **26**, 1643–1647 (2023).
73. Seifert, B. A taxonomic revision of the *Formica cinerea* group (Hymenoptera: Formicidae). *Abh. Naturforsch. Ges. Gorlitz.* **74**, 245–272 (2002).
74. Czechowski, W., Radchenko, A. & Czechowska, W. *The ants (Hymenoptera, Formicidae) of Poland* (Museum and Institute of Zoology PAS, 2012).
75. Kay, A. & Rissing, S. W. Division of foraging labor in ants can mediate demands for food and safety. *Behav. Ecol. Sociobiol.* **58**, 165–174 (2005).
76. Billick, I. The relationship between the distribution of worker sizes and new worker production in the ant *Formica neorufibarbis*. *Oecologia* **132**, 244–249 (2002).
77. Hurlbert, A. H., Ballantyne, F. & Powell, S. Shaking a leg and hot to trot: The effects of body size and temperature on running speed in ants. *Ecol. Entomol.* **33**, 144–154 (2008).
78. Packard, G. C. Rethinking the metabolic allometry of ants. *Evol. Ecol.* **34**, 149–161 (2020).
79. Paul, J. Mandible movements in ants. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **131**, 7–20 (2001).
80. Shik, J. The metabolic costs of building ant colonies from variably sized subunits. *Behav. Ecol. Sociobiol.* **64**, 1981–1990 (2010).
81. Markó, B. & Czechowski, W. *Lasius psammophilus* Seifert and *Formica cinerea* Mayr (Hymenoptera: Formicidae) on sand dunes: Conflicts and coexistence. *Ann. Zool. Fenn.* **54**, 365–378 (2004).
82. Friard, O. & Gamba, M. BORIS: A free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol. Evol.* **7**, 1325–1330 (2016).
83. Nowbahari, E., Hollis, K. L. & Durand, J. L. Division of labor regulates precision rescue behavior in sand-dwelling *Cataglyphis cursor* ants: To give is to receive. *PLoS One.* **7**, e48516. https://doi.org/10.1371/journal.pone.0048516 (2012).
84. Deslippe, R. J. & Savolainen, R. Role of food supply in structuring a population of *Formica* ants. *J. Anim. Ecol.* **63**, 756–764 (1994).
85. Sorvari, J. & Hakkarainen, H. Forest clear-cutting causes small workers in the polydomous wood ant *Formica aquilonia*. *Ann. Zool. Fenn.* **46**, 431–438 (2009).
86. Haatanen, M. K. & Sorvari, J. Similarity of body size in queens of the wood ant *Formica aquilonia* from optimal and sub-optimal habitats indicates a strong heritable component. *J. Insect. Sci.* **13**, 115 (2013).
87. R Core Team. R: A language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. https://www.R-project.org (2023).
88. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48 (2015).
89. Therneau, T. M. Coxme: Mixed effects cox models. R package version 2.2-16. https://CRAN.R-project.org/package=coxme (2020).

90. Valadares, L., Nascimento, F. S. & Chaline, N. Small workers are more persistent fighters than soldiers in the highly polymorphic *Atta* leaf-cutting ants. *Anim. Behav.* **189**, 15–21 (2022).
91. Abraham, M. & Pasteels, J. M. Social behaviour during nest-moving in the ant *Myrmica rubra* L. (Hym. Form.). *Insectes Soc.* **27**, 127–147 (1980).
92. Cerdá, X. & Retana, J. A behavioural study of transporter workers in "*Cataglyphis iberica*" ant colonies (Hymenoptera Formicidae). *Ethol. Ecol. Evol.* **4**, 359–374 (1992).
93. Ishii, Y. & Hasgeawa, E. The mechanism underlying the regulation of work-related behaviors in the monomorphic ant, *Myrmica kotokui*. *J. Ethol.* **31**, 61–69 (2013).
94. Briffa, M. & Archer, R. Size specific boldness associated with differences in resource requirements and habitat use: A cross-sectional study in hermit crabs. *Curr. Zool.* **3**, 360–366 (2023).

Author contributions

Both authors contributed to the design and implementation of the research, to the analysis of the results, to the writing of the manuscript, and approved the final version.

Funding

Financial support for this research was provided by the National Science Centre, Poland (grant PRELUDIUM 16, number 2018/31/N/NZ8/02312) to FT.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to F.T. or K.M.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Chapter III: Injury shortens life expectancy in ants and affects some risk-related decisions of workers

Animal Cognition (2023) 26:1643–1647
https://doi.org/10.1007/s10071-023-01810-0

ORIGINAL PAPER

Injury shortens life expectancy in ants and affects some risk-related decisions of workers

Filip Turza[1,2] · Krzysztof Miler[3]

Received: 27 February 2023 / Revised: 7 July 2023 / Accepted: 9 July 2023 / Published online: 14 July 2023

Abstract

The *Formica cinerea* ants are known to be highly territorial and aggressively defend their nest and foraging areas against other ants. During the foraging, workers engage in large-scale battles with other colonies of ants and injuries often occur in the process. Such injuries open the body up to pathologies and can lead to costs expressed in lower survival. Here, we addressed the significance of injury in dictating decisions related to engagement in risky behavior in ants (i.e., rescue and aggression). We manipulated the life expectancies of *F. cinerea* workers by injury and found that the survival of injured workers was shorter compared to the intact individuals. Furthermore, we found that injured workers discriminated between the intact and injured nestmates and showed more rescue behavior toward intact individuals. These rescue actions were expressed as digging around the trapped ant in need of rescue, pulling at its body parts, transporting the sand covering it, and biting the thread entrapping it. In turn, intact and injured workers showed similar and high levels of aggression toward heterospecifics. Our findings highlight the role of behavioral context in the studies devoted to the decision-making processes among social insects and the importance of life expectancy in their behavioral patterns.

Keywords Aggression · Ants · *Formica cinerea* · Rescue behavior · Survival

Introduction

Injuries result from various factors, such as fights over resources or failed predation. Ants are commonly characterized by non-lethal injuries (Gilad et al. 2022). For instance, slave-making ants (*Formica sanguinea*) inflict wounds on their hosts, such as the removal of one or more legs or antennae (Hölldobler and Wilson 1990). Confrontation with termites leads to similar injuries in termite-eating ants (*Megaponera analis*) (Frank et al. 2017). These injuries might have various costs and create a risk of higher mortality by opening the body up to infection, desiccation, function impairment, and/or other pathologies.

Not only in ants but also in many other species of social insects, task engagement is dictated by life expectancy (Woyciechowski and Kozłowski 1998). Indeed, young and fit workers choose tasks inside the nest and switch to foraging outside when they get older (Hölldobler and Wilson 1990). This is accompanied by many physiological changes, e.g., altered protein and hormone levels (Hartmann et al. 2020). Behavioral variation can be observed even within groups of workers belonging to the same sub-caste, as in termites, in which older soldiers are more risk-prone than younger soldiers (Yanagihara et al. 2018, see also Moroń et al. 2008). Here, we decided to test the importance of life expectancy in the context of risky rescue behavior, in which individuals act to free a nestmate from danger after it signals distress (Czechowski et al. 2002; Miler and Kuszewska 2017). The literature refers to various contexts where ants show this common behavior (Miler and Turza 2021). However, there is a considerable unexplained variation in rescue occurrence on both between-species and between-individuals levels (Miler et al. 2017a; Andras et al. 2020). In our study, we examined

Filip Turza
filip.turza@uj.edu.pl

Krzysztof Miler
miler@isez.pan.krakow.pl

1 Doctoral School of Exact and Natural Sciences, Jagiellonian University, Prof. S. Łojasiewicza 11, 30-348 Kraków, Poland

2 Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland

3 Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, 31-016 Kraków, Poland

how an injury might affect survival and the decision-making process in terms of rescue engagement in *Formica cinerea* ants. To assess context-specificity, we checked also another risky behavior, aggression. As our model species is vulnerable to attack from predators such as antlions, and other ants, it has a high number of injured individuals and shows marked levels of both rescue and aggression (Czechowski et al. 2002; Miler 2016).

We hypothesize that (1) injured workers would be characterized by lower life expectancy than intact individuals. This would be in line with studies that utilized experimental groups in which life expectancy was artificially shortened (e.g., Moroń et al. 2012). To add ecological relevance, we compare the survival of naturally and experimentally injured workers. Second, we hypothesize that (2) injured workers would be more responsive in terms of rescue and aggression. Injured workers could be less valuable to the colony due to their impaired performance, susceptibility to predation/infection, and shorter life expectancy, so they might show a lower threshold for engaging in more risky tasks than intact workers (Tofilski 2009). This is reasonable to expect based on the age-dependent changes in behavior among social insects (Woyciechowski and Kozłowski 1998; Hartmann et al. 2020). Third, we hypothesize that (3) rescue would be directed less toward injured than intact workers. Since rescue occurs to benefit the colony, saving soon-to-die individuals should be counterproductive (Miler et al. 2017b).

Materials and methods

Ant foragers were collected near Klucze (Poland, 50°21′22″N 19°31′03″E) from three colonies, with a week-long break between colonies, in the summer of 2022. Ants were transported to the laboratory, kept in containers (40 × 30 × 10 cm) at a constant temperature of 24 °C, 40–60% RH, and 12:12 day/night cycle with ad libitum access to water and 10% sucrose solution.

In the survival experiment, three groups of workers per colony, counting 30 individuals each, were created: a control group (C), a naturally injured group (N), and an experimentally injured group (E). The control group contained only intact individuals. The naturally injured group comprised individuals with at least a part of some extremity (antenna or leg) missing. In the experimentally injured group, each individual had the left or right hind leg removed at the femur using microscissors. All groups of ants were kept in the experimental boxes (18 × 15 × 7 cm) separate for each group and colony for the standard survival rate observation (Miler 2016).

In the behavioral experiment, the analogical control group of intact individuals (C) and experimentally injured group of workers (E) was placed in a shared container and tested for their rescue behavior and aggression toward nestmates and heterospecifics, respectively. Rescue behavior was tested using four standard dyadic rescue behavior tests (CC vs. CE vs. EC vs. EE) (Nowbahari et al. 2009), where a forager from group C or E was captured and placed in the middle of a sand-filled box, partly covered with sand, and a potential rescuer from the same or opposite group was placed in the arena. Aggression behavior was tested using two types of dyadic aggression tests (HC vs. HE), where a heterospecific individual (*Lasius niger*) was placed as the victim, and the procedure was the same as for rescue behavior tests. All behaviors were analyzed using standard rescue and aggression behavioral categories (Nowbahari et al. 2009) (see Appendix A) in terms of occurrence (1/0) and duration (s).

Statistical analyses were performed using R (R Core Team 2023). To analyze survival, we used a mixed-effects Cox proportional hazards model (coxme package; Therneau 2020) with a random factor "colony" and a fixed factor "mortality group" (C vs. N vs. E). Data for ants that remained alive after 50 days were censored. To compare rescue occurrence, we used a generalized linear mixed model (lme4 package; Bates et al. 2015) with a binomial residual distribution, logit link function and included a random factor "colony" and fixed factors "victim type" (C vs. E) and "rescuer type" (C vs. E), and their interaction. To compare the duration of rescue, we used a mixed-effects Cox proportional hazards model (coxme package; Therneau 2020) with the same factors. To compare aggression occurrence, we used a binomial generalized linear mixed model (lme4 package; Bates et al. 2015) with a random factor "colony" and a fixed factor "aggressive group" (C vs. E). To compare the duration of aggression, we used the mixed-effects Cox model (coxme package; Therneau 2020), with the same factors. In the Cox models, the data for tests during which rescue and aggression were interrupted at the end of the recording (i.e., 5 min) was censored.

Results and discussion

In agreement with our first hypothesis, we observed lower survival of injured foragers (as shown by the comparison of the survival in groups C and E, $z = 5.490$, $p < 0.001$; Fig. 1). Although this result might seem intuitive, it is crucial to demonstrate. At times, surprising patterns related to life expectancy are revealed (e.g., some parasitised ants show prolonged lifespans, see Beros et al. 2021). Notably, we also observed a significant difference in the survival of the experimentally treated and naturally injured group ($z = 2.240$, $p < 0.001$; Fig. 1) (see Appendix B for full results). This result might stem from differences between conditions in

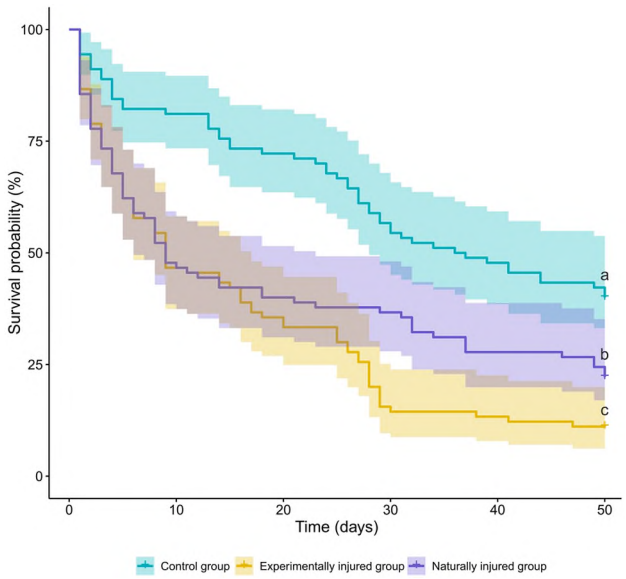


Fig. 1 Survival curves for different groups of foragers in *F. cinerea*. Shading indicates 95% confidence intervals. Small letters above crosses indicate significance yielded by between group comparisons (with $p < 0.001$)

which the ants were injured. Indeed, a laboratory setting with abnormal colony interactions, e.g., without wound grooming by healthy individuals inside the nest (Frank et al. 2018), was likely affecting the mortality rate. In any case, we confirm treatment by injury is effective in reducing life expectancy. However, further research should focus on the possibility of creating more natural conditions in the laboratory, e.g., by keeping experimentally treated ants with access to the full colony, and so ensuring the presence of natural interactions between individuals of different castes and/or health status.

That said, our second and third hypotheses were only partially confirmed. Rescue probability differed between tests (victim type: $\chi^2 = 0.027$, $p = 0.871$; rescuer type: $\chi^2 = 2.256$, $p = 0.133$; interaction: $\chi^2 = 6.177$, $p = 0.013$; Fig. 2A). Specifically, injured workers took part in rescue actions more likely toward intact nestmates. Regarding the aggression directed toward heterospecific ants, there was no difference between the intact and injured groups ($\chi^2 = 0.437$, $p = 0.500$; Fig. 2B). In both rescue and aggression, the total duration of these behaviors was similar in all tests (see Appendix B for full results). A higher probability of providing help to individuals with higher life expectancies rather than soon-to-die individuals illustrates the adaptive significance of rescue (Miler 2016). However, why only injured workers discriminated between intact and injured individuals remains unclear. Although the latter likely differ in cuticular hydrocarbon profiles and/or volatile pheromones from normal individuals (Csata et al. 2023), a lack of discrimination between nestmates that differ in life expectancies is already reported in the literature (Leclerc and Detrain 2016). Regarding aggression, previous research suggests that some ants with shortened life expectancies increase their level of aggression toward heterospecific ants (Bos et al. 2012). Why is the decision to attack not related to life expectancy in our species unclear, but possibly, *F. cinerea* is too territorial and aggressive to show dependence on life expectancy in this context (Czechowski et al. 2002). It can be worthwhile to

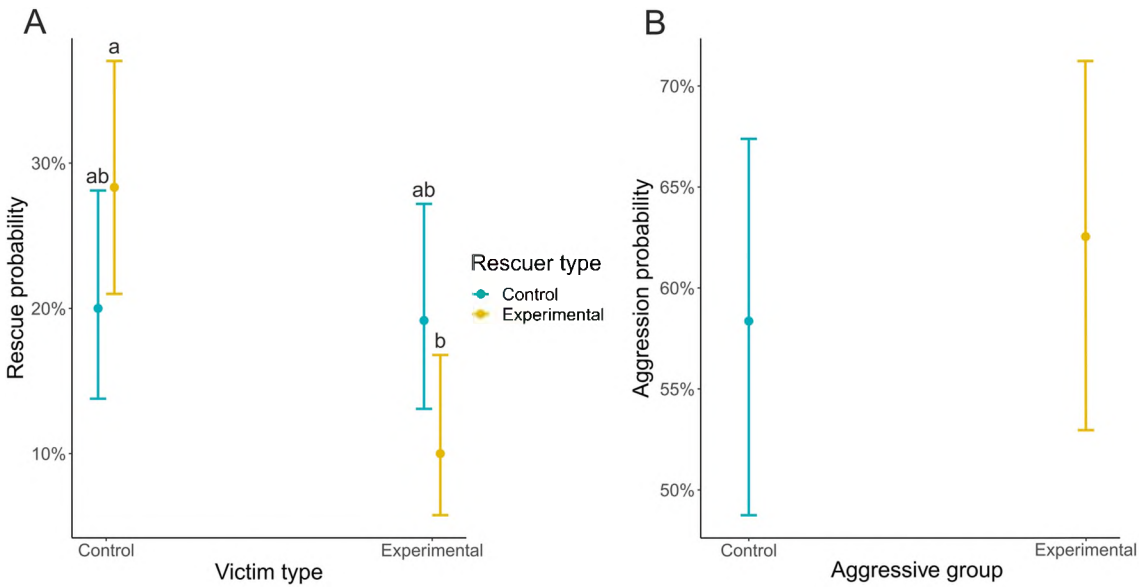


Fig. 2 Probability of rescue (**A**) and aggression (**B**) in *F. cinerea* foragers. Dots represent model predictions and whiskers indicate 95% confidence intervals. Small letters above upper whiskers indicate significance yielded by post hoc Tukey comparisons

compare aggressive tendencies in normal and injured workers of several species differing in their competitiveness.

Our results show that injury in *F. cinerea* foragers leads to a survival cost, and this cost affects rescue but not aggressive behavior. Indeed, injured workers were more likely to perform risky rescue actions toward intact individuals, while this type of discrimination between nestmates did not occur in intact ants. At the same time, aggression toward heterospecifics was similar in both injured and intact workers. This supports a growing number of studies that indicate that ants are highly sensitive to the behavioral context (Duhoo et al. 2017; Turza and Miler 2022). In the case of our study, we demonstrate diverging responses of ants to decreased life expectancy in the context of rescue and aggression. So far, little research attention was placed on the continued role of injured individuals in the colony (Tofilski et al. 2008; Gilad et al. 2022) and we encourage further studies on the role of decreased life expectancy on behavior of social insects. From a larger perspective, to fully describe the effect of life expectancy on behavior, experiments need to include this and other factors, such as aspects of ecology, body size, and/or personality, together (Hollis and Nowbahari 2013; Okrutniak et al. 2020; Maák et al. 2021).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10071-023-01810-0.

Author contributions Both authors contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

Funding Financial support for this research was provided by the National Science Centre, Poland (grant PRELUDIUM 16, number 2018/31/N/NZ8/02312).

Availability of data and materials All data generated and analyzed during the current study are available in the RODBUK repository, [https://doi.org/10.57903/UJ/OAUSU5].

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

References

Andras JP, Hollis KL, Carter KA, Couldwell G, Nowbahari E (2020) Analysis of ants' rescue behavior reveals heritable specialization for first responders. J Exp Biol 223:jeb212530

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48

Beros S, Lenhart A, Scharf I, Negroni MA, Menzel F, Foitzik S (2021) Extreme lifespan extension in tapeworm-infected ant workers. R Soc Open Sci 8:202118

Bos N, Lefèvre T, Jensen AB, d'Ettorre P (2012) Sick ants become unsociable. J Evol Biol 25:342–351

Csata E, Casacci LP, Ruther J, Bernadou A, Heinze J, Markó B (2023) Non-lethal fungal infection could reduce aggression towards strangers in ants. Comm Biol 6:183

Czechowski W, Godzińska EJ, Kozłowski MW (2002) Rescue behavior shown by workers of *Formica sanguinea* Latr., *F. fusca* L. and *F. cinerea* Mayr (Hymenoptera: Formicidae) in response to their nestmates caught by an ant lion larva. Ann Zool 52:423–431

Duhoo T, Durand J-L, Hollis KL, Nowbahari E (2017) Organization of rescue behaviour sequences in ants, *Cataglyphis cursor*, reflects goal-directedness, plasticity and memory. Behav Processes 139:12–18

Frank ET, Schmitt T, Hovestadt T, Mitesser O, Stiegler J, Linsenmair KE (2017) Saving the injured: Rescue behavior in the termite-hunting ant *Megaponera analis*. Sci Adv 3:e160218

Frank ET, Wehrhahn M, Linsenmair KE (2018) Wound treatment and selective help in a termite-hunting ant. Proc R Soc B 285:20172457

Gilad T, Dorfman A, Subach A, Scharf I (2022) Leg or antenna injury in *Cataglyphis* ants impairs survival but does not hinder searching for food. Curr Zool 68:441–450

Hartmann C, Haschlar J, Heinze J, Bernadou A (2020) Activity patterns and age-dependent changes in behavior in the clonal ant *Platythyrea punctata*. J Insect Behav 33:149–157

Hölldobler B, Wilson EO (1990) The ants. Harvard University Press, Cambridge

Hollis KL, Nowbahari E (2013) A comparative analysis of precision rescue behaviour in sand-dwelling ants. Anim Behav 85:537–544

Leclerc J-B, Detrain C (2016) Ants detect but do not discriminate diseased workers within their nest. Sci Nat 103:70

Maák I, Trigos-Peral G, Ślipiński P, Grześ IM, Horváth G, Witek M (2021) Habitat features and colony characteristics influencing ant personality and its fitness consequences. Behav Ecol 32:124–137

Miler K (2016) Moribund ants do not call for help. PLoS One 11:e0151925

Miler K, Kuszewska K (2017) Secretions of mandibular glands are not involved in the elicitation of rescue behaviour in *Formica cinerea* ants. Insectes Soc 64:303–305

Miler K, Turza F (2021) "O sister, where art thou?"—a review on rescue of imperiled individuals in ants. Biology 10:1079

Miler K, Yahya BE, Czarnoleski M (2017a) Pro-social behaviour of ants depends on their ecological niche—Rescue actions in species from tropical and temperate regions. Behav Proc 144:1–4

Miler K, Symonowicz B, Godzińska EJ (2017b) Increased risk proneness or social withdrawal? The effects of shortened life expectancy on the expression of rescue behavior in workers of the ant *Formica cinerea* (Hymenoptera: Formicidae). J Insect Behav 30:632–644

Moroń D, Witek M, Woyciechowski M (2008) Division of labour among workers with different life expectancy in the ant *Myrmica scabrinodis*. Anim Behav 75:345–350

Moroń D, Lenda M, Skórka P, Woyciechowski M (2012) Short-lived ants take greater risks during food collection. Am Nat 180:744–750

Nowbahari E, Scohier A, Durand J, Hollis KL (2009) Ants, *Cataglyphis cursor*, use precisely directed rescue behavior to free entrapped relatives. PLoS One 4:e6573

Okrutniak M, Rom B, Turza F, Grześ IM (2020) Body size differences between foraging and intranidal workers of the monomorphic ant *Lasius niger*. Insects 11:433

R Core Team (2023) R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. https://www.R-project.org. Accessed 19 June 2023

Therneau TM (2020) coxme: mixed effects Cox models. R package version 2.2–16. https://CRAN.R-project.org/package=coxme. Accessed 19 June 2023

Tofilski A (2009) Shorter-lived workers start foraging earlier. Insectes Soc 56:359–366

Tofilski A, Couvillon MJ, Evison SE, Helanterä H, Robinson EJ, Ratnieks FL (2008) Preemptive defensive self-sacrifice by ant workers. Am Nat 172:E239–E243

Turza F, Miler K (2022) Comparative analysis of experimental testing procedures for the elicitation of rescue actions in ants. Curr Zool 68:159–168

Woyciechowski M, Kozłowski J (1998) Division of labor by division of risk according to worker life expectancy in the honey bee (*Apis mellifera* L.). Apidologie 29:191–205

Yanagihara S, Suehiro W, Mitaka Y, Matsuura K (2018) Age-based soldier polyethism: old termite soldiers take more risks than young soldiers. Biol Lett 14:20180025

Supplementary Materials

Appendix A. Operational definitions of the behavioural categories of rescue and aggression.

Rescue	**Operational definition**
Digging	The ant stands in front of the victim and flicks sand backward, using legs
Pulling	The ant grabs any part of the body of the victim and drags it backward, using mandibles
Sand transport	The ant picks up one or more sand particles, covering the filter paper or the victim, and moves it away, using mandibles
Thread biting	The ant bites and tugs on the nylon thread holding the victim, using mandibles
Aggression	**Operational definition**
Biting	The ant bites any part of the body of the victim, using mandibles and flexes its gaster underneath its body
Formic acid projection	The ant flexes its gaster underneath its body and projects formic acid in the direction of the victim
Threatening	The ant opens mandibles to their maximally widest position in the direction of the victim

Table 1. Definitions of rescue and aggression categories used in the study.

Appendix B. Full results of the Cox mixed-effects models conducted in the study.

	exp(coef)	se(coef)	z	P-value
C vs. E	2.692	0.181	5.490	< 0.001
C vs. N	1.849	0.182	3.390	< 0.001
E vs. N	0.687	0.167	2.240	< 0.001

Table 1. Full results of the survival analysis conducted using the Cox mixed-effects model by maximum likelihood for the 3 treatments: a control group of intact individuals (C), a group comprised of individuals with at least a part of some extremity (antenna or leg) missing (N), and a group treated experimentally in which intact individuals had one of their hindlegs removed at the femur (E).

	χ^2	P-value
Victim type	0.376	0.540
Rescuer type	0.050	0.823
Victim type × rescuer type	1.009	0.315

Table 2. Full results of the rescue duration analysis conducted using the Cox mixed-effects model by maximum likelihood with a random factor "colony" and fixed factors "victim type" and "rescuer type" and their interaction. Data for tests during which rescue behaviour was interrupted at the end of the recording (i.e., 5 minutes) was censored.

	χ^2	P-value
Aggressive group	2.374	0.123

Table 3. Full results of the aggression duration analysis using the Cox mixed-effects model by maximum likelihood with a random factor "colony" and a fixed factor "aggressive group". Data for tests during which aggression behaviour was interrupted at the end of the recording (i.e., 5 minutes) was censored.

Chapter IV: Life expectancy in ants explains variation in helpfulness regardless of phylogenetic relatedness

Life expectancy in ants explains variation in helpfulness regardless of phylogenetic relatedness

Filip Turza[1,2], Daniel Stec[3], Diego Fontaneto[4,5], Krzysztof Miler[3]

[1] Doctoral School of Exact and Natural Sciences, Jagiellonian University, prof. S. Łojasiewicza 11, 30-348 Kraków, Poland

[2] Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland

[3] Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, 31-016 Kraków, Poland

[4] Molecular Ecology Group (MEG), National Research Council of Italy, Water Research Institute (CNR-IRSA), Largo Tonolli 50, Verbania Pallanza, Italy

5 National Biodiversity Future Center (NBFC), Piazza Marina 61, Palermo, Italy

Abstract

Rescue behaviour aims at removing an individual from harm. Ants are particularly known for such helpfulness and, perhaps not coincidentally, also show the highest level of social organization in the animal kingdom, i.e., eusociality. However, even among social species such as ants, there is a huge variation in rescue proneness and little is understood about the underlying causes of this variation. In this study, we explore the relationship between helpfulness in the form of rescue and life expectancy, focusing on 14 ant species with diverse phylogenetic affiliations. We posit that species with longer worker life expectancies are more prone to engaging in rescue actions. To test this, we assessed worker lifespan in each species and conducted behavioural tests simulating entrapment scenarios involving a nestmate ensnared by an artificial obstacle. Observed behaviours involved contact with the nestmate, digging around it, pulling at its body parts, and biting the entrapping obstacle. Our findings reveal that species with longer worker life expectancies exhibit higher proneness to rescue endangered nestmates, irrespective of phylogenetic relatedness. Furthermore, we found no trace of a phylogenetic

signal in the life expectancies or helpfulness of workers belonging to different species. The results underscore the significance of life expectancy as a key factor influencing the likelihood of rescue behaviour in ants. This phenomenon warrants further investigation, given the varied physiologies, life histories, and ecologies observed among species. Nevertheless, the impact of life expectancy on behavioural patterns in social insects suggests that this parameter is likely significant across diverse taxa.

Keywords: ants, altruism, Formicidae, life expectancy, pro-social behaviour, rescue behaviour

1. Introduction

Pro-sociality involves individuals voluntarily benefiting a group through their actions (Hamilton, 1963). These actions impose unavoidable costs on pro-social individuals (Decety and Svetlova, 2012; Decety et al., 2016). Given the role of pro-sociality in promoting group survival (de Waal, 2008), it is prevalent in social organisms (Pennisi, 2005). Our need for understanding pro-social behaviour in non-human species is strongly marked in the scientific literature (e.g., Cronin, 2012; Marshall-Pescini, 2016; Nafcha et al., 2023; Chen et al., 2023). A type of pro-sociality that has garnered special attention is rescue. During rescue, one individual (the rescuer) provides help to another individual (the victim) in a dangerous situation (Nowbahari and Hollis, 2010). This altruistic act carries the potential risk of injury or even death for the rescuer (Vasconcelos et al., 2012) at no immediate advantage to that particular individual (Nowbahari et al., 2016). Rescue actions seem widespread across species. They have been observed in various taxa, including birds (Hammers and Brouwer, 2017; Crampton et al., 2022), elephants (Bates et al., 2008), primates (e.g., Vogel and Fuentes-Jiménez, 2006; Huang et al., 2020), rodents (Bartal et al., 2011; Ueno et al., 2019), whales (Siebenaler and Caldwell, 1956, Pitman et al., 2017), wild boars (Masilkova et al., 2021) and pigs (Moscovice et al., 2023).

Rescue has been documented not only in vertebrates but also in ants (reviewed in Miler and Turza, 2021; Hollis and Nowbahari, 2022), making it a general topic in research devoted to social interactions across various organisms, including both vertebrates and invertebrates (Nowbahari et al., 2016).

Ants (Hymenoptera: Formicidae) are characterized by eusociality, the highest level of social organization in the animal kingdom (Hölldobler and Wilson, 1990). Rescue likely contributes to ants' ecological and evolutionary success (Andras et al., 2020). The earliest recorded instance of army ants (*Eciton hamatum* (Fabricius, 1782)) attempting to help nestmates trapped beneath stones dates back to the 19th century (Belt, 1874). Subsequent studies have provided evidence of ant rescue actions in other situations, such as entrapment under the soil (Markl, 1965; Spangler, 1968; Hangartner, 1969), capture by predatory antlions and spiders (Czechowski et al., 2002; Uy et al., 2019), or after injuries inflicted by termites (Frank et al., 2017). Rescue offers significant fitness advantages to ants by enabling them to respond to threats and save fellow group members. For example, Frank et al. (2017) found that without assistance, 32% of injured termite-eating ants (*Megaponera analis* (Latreille, 1802)) would perish, but rescue mitigated these losses, resulting in a 28.7% increase in colony size. Kwapich and Hölldobler (2019) observed that harvester ants (*Veromessor pergandei* (Mayr, 1886)), after aiding nestmates entangled in spider silk, removed the remaining webs, reducing the risk of other colony members getting ensnared. This is vital for these ants, as losing just five foragers per day would equate to a loss of more than 65,500 seeds annually (Kwapich and Hölldobler, 2019). These studies highlight the broader benefits of rescue actions for the entire ant colony. However, rescue is considered costly and risky (Nowbahari and Hollis, 2010; Duhoo et al., 2017). For example, Kwapich and Hölldobler (2019) estimated that 6.3% of *V. pergandei* foragers engaged in web removal are killed by spiders. There is significant variability in rescue occurrence among ant species (e.g., Czechowski et al., 2002; Hollis and Nowbahari,

78 2013; Miler et al., 2017a; Andras et al., 2020) and our understanding of the proximate and
79 ultimate causes of differences in helpfulness between species remains limited (Hollis and
80 Nowbahari, 2022).

81 Ageing is the most widespread and inevitable phenomenon in biology (Cohen et al.,
82 2022). How the ageing process impacts the life-history traits and behavioural characteristics of
83 living organisms is a problem long-present in the scientific literature (e.g., Medvedev, 1990;
84 Viña et al., 2007; Siracusa et al., 2022). Investigating this problem is difficult because in many
85 cases, age is a worse measure of longevity than is life expectancy. Although age strongly
86 correlates with life expectancy (i.e., increasing age decreases life expectancy), the latter may
87 be shaped by factors other than age. For example, it can decrease in organisms exposed to higher
88 risks of diseases, parasite infestation, and predation, or in those more fecund (e.g., Zwaan, 1999;
89 Stearns et al., 2000; Chapuisat and Keller, 2002). The issue is particularly complex in social
90 insects (Korb and Heinze, 2021; Heinze and Giehr, 2021). The evolution of eusociality is
91 associated with a 100-fold increase in life expectancy (Keller and Genoud, 1997), suggesting a
92 co-evolution between insect sociality and extended longevity (Carey, 2001). Indeed, it is
93 thought that more advanced social behaviours provide insurance-based survival advantages,
94 becoming one of the main drivers of extended life expectancy. Nevertheless, although social
95 species may live longer lives than their non-social relatives, life expectancies of the former still
96 strongly differ for many reasons (Hölldobler and Wilson, 1990).

97 Here we integrate the issues of pro-social helpfulness towards group members and life
98 expectancies in different species of ants. We test how ants with varying worker lifespans differ
99 in their behavioural traits, particularly rescue behaviour. A recent study has shown that ant
100 species with different mortality rates might vary in such traits as aggression levels or activity
101 timing (Kwon et al., 2022). Yet, the potential for an across-species pattern in the pro-sociality
102 of ants depending on their life expectancies remains unexplored. Different species of ants are

characterized by different rescue proneness (Hollis and Nowbahari, 2013; Miler et al., 2017a) and ant species exhibit significant variation in life expectancy even among closely related species (Hölldobler and Wilson, 1990; Keller and Genoud, 1997; Kramer and Schaible, 2013). While there have been suggestions that life expectancy may influence rescue actions within species (e.g., Miler, 2016; Miler et al. 2017b; Frank et al., 2018; Turza and Miler, 2023), this has not been explored on a larger, cross-species scale. In this study, we explore whether worker life expectancy among 14 ant species representing diverse phylogenetic affiliations is related to their pro-social behaviour in the form of rescue. Our primary hypothesis is that rescue is more likely in species that are characterized by longer-lived workers because helpfulness in groups with high mortality of individuals would probably be less productive.

2. Materials and methods

2.1 Field site and ant species

We gathered ants belonging to 14 species and from colonies situated in Lesser Poland, spanning a latitude range of 49°03′ to 51°29′ and a longitude range of 18°12′ to 23°24′ (Table 1). We picked five distinct colonies of each species to gather relatively reliable species-level data. To ensure uniformity in the individuals tested, we only collected ants found outside the nest, the so-called foragers, also indicated as most rescue-prone among ant castes (Nowbahari et al., 2012). Furthermore, we timed our collections to coincide with the peak of colony development and the highest activity among individuals, typically occurring during the period of nuptial flights, which spans from April to September, depending on the species (Table 1). Approximately 200 foragers from each colony were collected and placed in plastic containers along with some nest material. Subsequently, the ants from each colony were transported to the laboratory. Species identification was carried out following Czechowski et al. (2012).

Subfamily	Tribe	Species	Nuptial flights
Dolichoderinae	Dolichoderini	*Dolichoderus quadripunctatus* (Linnaeus, 1771)	July
Myrmicinae	Myrmicini	*Myrmica rubra* (Linnaeus, 1758)	August – September
		Myrmica rugulosa (Nylander, 1849)	August – October
		Manica rubida (Latreille, 1802)	April & August – September
	Crematogastrini	*Tetramorium caespitum* (Linnaeus, 1758)	June - August
Formicinae	Formicini	*Formica fusca* (Linnaeus, 1758)	July – August
		Formica cinerea (Mayr, 1853)	July – August
		Formica cunicularia (Latreille, 1798)	August
		Formica sanguinea (Latreille, 1798)	July – August
	Lassini	*Lasius niger* (Linnaeus, 1758)	July – August
		Lasius emarginatus (Olivier, 1792)	July – August
		Lasius brunneus (Latreille, 1798)	June – July
		Lasius fuliginosus (Latreille, 1798)	May – October
		Lasius umbratus (Nylander, 1846)	July – September

Table 1. Ant species within the Formicidae family used in the study, with specified subfamilies, tribes and dates of nuptial flights, which indicates the points of highest colony development and the time of worker collection in the current study (based on Czechowski et al., 2012).

2.2 Rearing conditions

Ants obtained from each colony were separately housed in a plastic container measuring 40 × 30 × 10 cm. The inner walls of each container were covered with fluon (Sigma-Aldrich, Germany) to prevent escape attempts. The laboratory conditions were maintained at a constant temperature of 24 °C, relative humidity ranging from 40% to 60%, and a 12-hour day and night cycle. The ants were provided with an *ad libitum* supply of water and a Bhatkar diet rich in protein, carbohydrates, vitamins, and minerals, as recommended for many ant species by Czechowski and Pisarski (1992). The ants were acclimated to the laboratory conditions for two days before any subsequent procedures. This acclimatization period aligns with established protocols (Miler and Kuszewska, 2017; Turza and Miler, 2022).

2.3 Survival experiment

After acclimation, we took 50 individuals from each colony to create a separate survival group for the life expectancy analysis. These ants were housed in plastic containers measuring 18 ×

15 × 7 cm. The interior walls were treated with fluon (Sigma-Aldrich, Germany) to prevent the escape of animals. The laboratory conditions provided mirrored those described above. The ants had access to *ad libitum* water and the abovementioned diet throughout the experiment. We monitored mortality daily until day 250 since the establishment of each survival group. We analyzed the mortality of a total of 3500 individuals (14 species × 5 colonies × 50 individuals = 3500 individuals).

2.4 Behavioural experiment

The individuals remaining after the establishment of groups for the survival experiment were designated for the behavioural experiment. We performed standard laboratory simulations of entrapment (Nowbahari et al., 2009), the most widely used method for studying rescue behaviour in ants. In each trial, an individual ant, designated as the victim, was tied using nylon thread passed over the petiole to a piece of filter paper and placed inside the circular arena (diameter: 7 cm, height: 8 cm), partially filled with dry sand. This arrangement ensured that the victim's body remained visible while the filter paper was concealed beneath a thin layer of sand. Following this, a second ant, designated as the potential rescuer, was introduced into the testing arena. The test was immediately initiated, with each lasting 5 minutes. All tests were camera recorded using Sony HDR-CX625 cameras positioned directly above the testing arenas. The tests were between 8 AM – 6 PM on a single day for each colony, always in the abovementioned laboratory conditions. Until tests, the ants had constant access to *ad libitum* water and food in their containers. We performed 50 tests for each colony, amounting to 3500 dyadic tests conducted (14 species × 5 colonies × 50 tests = 3500 tests).

2.5 Analysis of the recordings

The tests amounted to a total of 292 hours of recordings. To quantify behaviours exhibited, we measured their duration (in seconds). The analysis was performed using the BORIS software v. 7.9.19 (Friard and Gamba, 2016). We adhered to a standardized ethogram across species (Table

169 2). For rescue to occur, contact between the victim and the rescuer is necessary and often quite
170 prolonged. Thus, the measured behaviours included contact, digging around the victim, pulling
171 the victim's body parts, transporting the sand covering the victim, and biting the nylon thread.
172 Due to the very low frequency of occurrence, we excluded sand transport as a category before
173 proceeding to formal data analysis.

Behaviour	Operational definition
Contact	The ant makes physical contact with the victim's body, using its antennae
Digging	The ant positions itself close to the victim and pushes sand backwards using its legs
Pulling	The ant grabs the victim's body and moves it in a backward direction, using its mandibles
Sand transport	The ant grabs a nearby pebble and transports it away from the victim, using its mandibles
Thread biting	The ant bites the nylon thread that ensnares the victim, using its mandibles

174 **Table 2**. An ethogram of behaviours that were assessed in the study (based on Hollis and
175 Nowbahari, 2013). Sand transport was excluded from analysis in the current study due to very
176 low occurrence.

177 *2.6 Phylogenetic analysis*

178 To allow a reliable comparison of the effect of life expectancy on rescue between species, we
179 used phylogenetic comparative methods to account for the confounding factor of potential
180 phylogenetic autocorrelation between the two analyzed traits. Phylogenetic relationships were
181 thus obtained as a backbone for the phylogenetic comparative models using DNA from the
182 same animals used for the behavioural analyzes. Two individuals from each species were taken
183 for DNA sequencing. Genomic DNA was extracted from whole individuals using Syngen DNA
184 Mini Kit (Syngen Biotech, Poland) following the manufacturer protocol. Using PCR reaction,
185 we amplified two DNA fragments, one nuclear (28S rRNA gene) and one mitochondrial (COI).
186 For COI amplification we used the following primers: LCO1490-JJ (5′-
187 CHACWAAYCATAAAGATATYGG-3′), HCO2198-JJ (5′-
188 AWACTTCVGGRTGVCCAAARAATCA-3′) (Astrin and Stüben, 2008). To amplify 28S

189 rRNA we designed primers *de novo* based on several ant 28S rRNA gene sequences from
190 GenBank. The primers are as follow: 28S_ant_F1 (5′-CAAGACGGGTCCTAAGAGTACC-
191 3′), 28S_ant_R1 (5′- CAAGACGGGTCCTAAGAGTACC-3′). PCR cocktails, profiles,
192 sequencing reactions, and all respective product cleanings follow the pipeline provided by Stec
193 et al. (2020). Sequencing products were read with the ABI 3130xl sequencer (Genomed,
194 Poland). Sequences were processed in BioEdit v. 7.2.5 (Hall, 1999), checked in BLAST
195 (Altschul et al., 1990) to confirm species IDs, and submitted to GenBank (Supplementary
196 Material 1). *Paraponera clavata* (Fabricius, 1775) served as an outgroup.
197 All obtained sequences represented a single haplotype for each species thus, for
198 phylogenetic analysis, we used only sequences of one specimen per species. The sequences
199 were aligned using the default settings (in the case of COI) and the Q-INS-I method (in the case
200 of the 28S rRNA gene) of MAFFT v. 7 (Katoh et al., 2002; Katoh and Toh, 2008) and manually
201 checked against nonconservative alignments in BioEdit. Then, the aligned sequences were
202 trimmed to 685 bp for the 28S rRNA gene and 658 bp for the COI gene. All COI sequences
203 were translated into protein sequences in MEGA11 (Tamura et al., 2021) to check against
204 pseudogenes. The sequences were then concatenated using SequenceMatrix (Vaidya et al.,
205 2011) and appropriate models of sequence evolution, as well as the best partitioning scheme,
206 were chosen using PartitionFinder v. 2.1.1 (Lanfear et al., 2016) under the Akaike Information
207 Criterion (AIC). The ultrametric trees were calculated in BEAST v. 2.6 (Bouckaert et al., 2019).
208 Parameters in BEAST were set as default, except for appropriate models from PartionFinder2,
209 relaxed clock log normal, 100,000,000 generations with tree samples in each 10,000 generation.
210 Three independent BEAST analyzes were run through the CIPRES online portal (Miller et al.,
211 2010). The program Tracer v. 1.6 (Rambaut et al., 2014) was then used to ensure Markov chains
212 had reached stationarity and to determine the correct 'burn-in' for the analysis which was the
213 first 10% of generations. The ESS values were greater than 200 and the consensus tree was

obtained after summarising the resulting topologies and discarding the 'burn-in'. Trees from all three runs were combined into one file using LogCombiner while the consensus tree (Maximum Clade Credibility Tree) was built using TreeAnnotator (both applications distributed with BEAST).

2.7 Index calculation and statistics

For each species, we obtained phylogenetic data alongside survivorship and behavioural data from the experiments. All analyzes were performed using R v. 4.2.3 (R Core Team, 2023).

To characterize the lifespans of workers within each species, we constructed a Kaplan-Meier plot, identified the maximum time for which all species had a non-zero survival probability, and calculated this probability beyond that time for each species ('survival' and 'survminer' packages; Therneau, 2020; Kassambara et al., 2021). This survivorship index was used in subsequent analysis.

To derive an informative summary variable capturing the behaviour of each species during the tests, we conducted a Principal Component Analysis (PCA) using the data from four measured behaviours: contact, digging around the victim, pulling the victim's body parts, and biting the nylon thread. We generated 95 % confidence ellipses around centroids (means) from the PCA (PC1 and PC2) for the species. These means were then extracted as the helpfulness index and employed in subsequent analysis. We utilized the 'ggcorrplot', 'ggbiplot', 'ggfortify', and 'factoextra' packages to conduct the PCA (Vu, 2011; Horikoshi and Tang, 2016; Kassambara and Mundt, 2020; Kassambara, 2023).

To analyze the relationship between helpfulness and survivorship, taking into account the non-independence of data due to phylogenetic relatedness between the species, we used the phylogenetic generalised least squares model (PGLS) (Symonds and Blomberg, 2014; Ravell and Harmon, 2022). The model computed how the helpfulness index depended on the survivorship index and included the phylogenetic relationships in the variance-covariance

matrix. K and λ were estimated to investigate the presence of phylogenetic signals in the two analyzed traits, the survivorship and helpfulness indices. We utilized the 'caper' package for PGLS (Orme et al., 2023).

3. Results

The Kaplan-Meier plot visualizing mortality across species revealed that the maximum time for which all species had a non-zero survival probability was 80 days (Supplementary Material 2). Thus, we calculated the survival probability of workers at 80 days in each species and retained it as the survivorship index.

The first and second dimensions of the PCA for the measured behaviours accounted for nearly 80% of the variance. We extracted the helpfulness index for each species (Supplementary Material 2).

The PGLS model demonstrated that the helpfulness index depended on the survivorship index ($F_{1,12} = 10.50$, $p = 0.007$, $R^2 = 0.467$), with species characterized by higher worker life expectancies being more helpful towards nestmates (Fig. 1). There was no phylogenetic signal in either the survivorship index ($K = 0.210$, $p = 0.098$; $\lambda = 0.502$, $p = 0.649$) or the helpfulness index ($K = 0.053$, $p = 0.791$; $\lambda < 0.001$, $p = 1$).

4. Discussion

Our results reinforce the idea that pro-social tendencies among Formicidae are associated with workers' life expectancies. Specifically, longer-lived ant species are more helpful and more likely take part in rescue actions toward nestmates than shorter-lived ant species. This result extends far beyond previous studies devoted to the effect of life expectancy on rescue behaviour proneness within a species (Miler, 2016; Miler et al., 2017b; Frank et al., 2018). Miler (2016) demonstrated that *Formica cinerea* Mayr, 1853 ants with shortened life expectancy elicited

lower levels of rescue behaviour than healthy individuals. Another study showed that shortened life expectancy increased social withdrawal in this species (Miler et al., 2017b). Also, Frank et al. (2018) found that heavily injured *Megaponera analis* (Latreille, 1802) ants (i.e., individuals with low life expectancy) did not receive help and were not treated after being placed in the nest in comparison to uninjured or less severely injured individuals. Heavily injured ants also showed social withdrawal, ignored their nestmates and resisted being picked up and transported to the nest. Life expectancy is one of the most important factors driving the behaviour of social insects, particularly ants, on a within-species scale (Woyciechowski and Kozłowski, 1998; Tofilski, 2002). As we show here, this is the case as well on a between-species scale, at least in terms of pro-social tendencies. Future research must investigate whether species with varying helpfulness, particularly rescue proneness, differ in fitness outcomes during ecologically relevant situations, such as nest collapse. This could provide novel, direct evidence for an ant colony as a higher-order unit of selection compared to the individual (Queller and Strassmann, 1998; Helanterä, 2009).

In species of ants with relatively low worker life expectancy rescue actions were undetected or infrequent. In turn, species with relatively high life expectancy in workers demonstrated increased proneness for helpfulness. Importantly, neither life expectancies nor helpfulness of workers showed phylogenetic constraints. A plausible explanation for this pattern is that the two traits coevolved independently many times, with a direct effect of one on the other (Carey, 2001). Specifically, more pro-social behaviour can help in maintaining health and thus increase life expectancy in different species. Additionally, this pattern might also reflect a high colony-level cost to saving members of the colony with relatively low life expectancies. In ant colonies, resources and energy are allocated to ensure the survival and high productivity of the entire colony (Hölldobler and Wilson, 1990). Investing resources in interacting with and saving individuals that die soon anyway might be unproductive, especially

289 if the energy spent on such risky and costly behaviour could be directed toward tasks that
290 contribute more significantly to the overall success of the colony, e.g., foraging (Bar et al.,
291 2023). Indeed, Frank et al. (2017) found that *M. analis* ants rescued 9–15 workers per day and
292 most of those saved (around 95%) participated in future colony activities, mitigating the need
293 to replace them with new workers. Further exploration of the trade-offs and costs associated
294 with rescue is essential to fully understand this phenomenon.

295 Our study offers an investigation into the life expectancy parameter under controlled
296 conditions across species representing diverse phylogenetic affiliations. It shows significant
297 differences in the life expectancy of foragers among various ant species. Kwon et al. (2022)
298 found that the forager mortality rate due to interspecific competition in the field is higher in
299 small-bodied, submissive and subordinate species than in large-bodied, aggressive and
300 dominant species. An interesting question for future research is how environments of varying
301 riskiness shape worker life expectancy among ants. Although such an investigation is beyond
302 the scope of the current study, our results suggest that life expectancy might be interrelated to
303 other factors, such as body size or dominance. Specifically, species that lived the longest were
304 bigger and more dominant than those that lived for the shortest periods (Hölldobler and Wilson,
305 1990; Czechowski et al., 2012). Additionally, this similarity between our results and those of
306 Kwon et al. (2022) adds ecological relevance to our measurements of survivorship which, even
307 without extrinsic causes of mortality, revealed similar mortality patterns as in the field (de Vries
308 et al., 2023).

309 Rescue behaviour has been observed in several distinct subfamilies, including
310 Dolichoderinae, Formicinae, Myrmicinae, and Ponerinae (e.g., Czechowski et al., 2002, Hollis
311 and Nowbahari 2013; Miler, 2016; Miler et al., 2017a; Frank et al., 2017; Uy et al., 2019). Thus,
312 it is clear that rescue occurs in distantly related ant species, which makes it plausible that this
313 behaviour evolved many times (Miler and Turza, 2021). Indeed, we did not find a strong

314 phylogenetic signal for this trait, indicating that it is phylogenetically labile and not constrained
315 in its evolution. However, only 23 species have been studied for such behaviour out of over
316 16,000 known species of ants so far (Bolton World Catalog, 2023). It is worth pointing out that
317 in our study, 11 out of 14 species were tested for the first time in the context of rescue behaviour.
318 Our data indicate that rescue behaviour of the tested species varies from pronounced to weak.
319 The results are directly in line with previous findings suggesting that different species of ants
320 represent different levels of rescue (Hollis and Nowbahari, 2013; Miler et al., 2017a). This
321 represents a substantial contribution to our understanding of pro-sociality within this group of
322 insects. Future research should further develop this comprehensive approach. To date, most
323 research questions regarding rescue behaviour were addressed only in two species of
324 ants, *Cataglyphis piliscapa* (Forel, 1901) (formerly *C. cursor* (Fonscolombe, 1846)) and *F.*
325 *cinerea* (e.g., Nowbahari et al., 2009; Duhoo et al., 2017; Miler, 2016; Turza et al., 2020). It is
326 unclear how generalizable are the results obtained solely for these species. Already for these
327 two species, we can find conflicting evidence in the available literature. For example, in the
328 case of *C. piliscapa*, the most common type of rescue behaviour is digging around the victim
329 (Duhoo et al., 2017), while in *F. cinerea* ants it is pulling the victim (Turza and Miler, 2023).
330 The differences in the rescue actions of different ant species may suggest that these actions
331 evolved in various contexts, depending on the selective pressure of the environment and
332 ecology specific to a given species (Hollis and Nowbahari, 2013). This underlines the necessity
333 of investigating this phenomenon in species that have not been explored so far.

334 Some studies suggest that rescue behaviour can depend on the ecology of the species in
335 terms of nesting habitat (Hangartner, 1969; Uy et al., 2019). For example, more frequent rescue
336 behaviour was observed in sand-dwelling ants (*Cataglyphis floricola* Tinaut, 1993 and *C.*
337 *piliscapa*) than in those living in more compact soils, like *Messor barbarus* (Linnaeus, 1767)
338 and *M. marocanus* Santschi, 1927 ants (Hollis and Nowbahari, 2013). In our study, ant species

339 characteristic for sandy and sunny habitats (i.e., *F. cinerea*, *Manica rubida* (Latreille, 1802)
340 and *Tetramorium caespitum* (Linnaeus, 1758)) were indeed characterized by a high level of
341 helpfulness. Expanding the number of studied species is crucial for definitive results in terms
342 of the effect of species ecology (Miler and Turza, 2021).
343
344 **5. Conclusions**
345 In summary, deciphering how proximate and ultimate factors modulate pro-sociality is one of
346 the central questions in animal behaviour research (e.g., Cronin, 2012; Marshall-Pescini, 2016;
347 Nafcha et al., 2023; Chen et al., 2023). Here, using ants, we showed that their inter-species
348 variation in pro-social tendencies, indicated by helpfulness, can be in part explained by life
349 expectancy. Specifically, ant species characterized by higher worker life expectancies are more
350 helpful towards nestmates. This pattern is similar to that observed in mammals. The effect of
351 age on pro-social behaviour has been demonstrated in wild primates (Lange et al., 2023). Also,
352 group-living species live longer than solitary species, supporting the correlated evolution of
353 social organization and longevity in the animal kingdom (Zhu et al., 2023). We postulate that
354 life expectancy should be considered more frequently as part of various behavioural patterns
355 among group-living animals. The incorporation of this factor can provide a deeper
356 understanding of the mechanisms by which all societies operate, from ants to primates.
357
358 **6. Acknowledgements**
359 Financial support for this research was provided by the National Science Centre, Poland (grant
360 PRELUDIUM 16, number 2018/31/N/NZ8/02312).
361
362 **7. Conflict of Interest**
363 The authors declare no conflict of interest.

8. Author contributions

Filip Turza designed the study, collected, analysed, and interpreted the data, and drafted the manuscript; Daniel Stec and Diego Fontaneto analysed the data and drafted the manuscript; Krzysztof Miler conceived and designed the study, analysed and interpreted the data, and drafted the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

9. Data availability statement

Data will be made available on the Jagiellonian University repository upon article acceptance for publication.

10. References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol. 215: 403–410.

Andras JP, Hollis KL, Carter KA, Couldwell G,Nowbahari E. 2020. Analysis of ants' rescue behavior reveals heritable specialization for first responders. J Exp Biol. 223:jeb212530.

Astrin JJ, Stüben PE. 2008. Phylogeny in cryptic weevils: molecules, morphology and new genera of western Palaearctic Cryptorhynchinae (Coleoptera: Curculionidae). Invertebr. Syst. 22:503–522.

Bar A, Gilad T, Massad D, Ferber A, Ben-Ezra D, Segal D, Foitzik S, Scharf I. 2023. Foraging is prioritized over nestmate rescue in desert ants and pupae are rescued more than adults. Behav Ecol. 34:1087–1096.

Bartal IB-A, Decety J, Mason P. 2011. Empathy and pro-social behavior in rats. Science. 334:1427–1430.

Bates L, Lee, P, Njiraini, N, Poole J, Sayialel K, Sayialel S, Moss C, Byrne R. 2008. Do elephants show empathy? J Conscious Stud. 15:204–225.

Belt T. 1874. The Naturalist in Nicaragua. John Murray.

Bolton World Catalog. https://www.antweb.org/page.do?name=world (accessed on 20 June 2024).

Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, Heled J, Jones G, Kühnert D, De Maio N, Matschiner M, Mendes FK, Müller NF, Ogilvie HA, du Plessis L, Popinga A, Rambaut A, Rasmussen D, Siveroni I, …, Drummond AJ. 2019. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLoS Comput Biol. 15:e1006650.

Carey JR. 2001. Demographic mechanisms for the evolution of long life in social insects. Exp Gerontol. 36:713–22.

Chapuisat M, Keller L. 2002. Division of labour influences the rate of ageing in weaver ant workers. Proc R Soc B. 269:909–913.

Chen YQ, Han S, Yin B. 2023. Why help others? Insights from rodent to human early childhood research. Front Behav Neurosci. 17:1058352.

Cohen AA, Ferrucci L, Fülöp T, Gravel D, Hao N, Kriete A, Levine ME, Lipsitz LA, Olde Rikkert, MGM, Rutenberg A, Stroustrup N, Varadhan R. 2022. A complex systems approach to aging biology. Nat Aging. 2:580–591.

Crampton J, Frère CH, Potvin DA. 2022. Australian magpies *Gymnorhina tibicen* cooperate to remove tracking devices. Aust Field Ornithol. 39:7–11.

Cronin KA. 2012. Prosocial behaviour in animals: the influence of social relationships, communication and rewards. Anim Behav. 84: 1085–1093.

Czechowski W, Godzińska EJ, Kozłowski MW. 2002. Rescue behavior shown by workers of *Formica sanguinea* Latr., *F. fusca* L. and *F. cinerea* Mayr (Hymenoptera: Formicidae) in response to their nestmates caught by an ant lion larva. Ann Zool. 52:423–431.

Czechowski W, Pisarski B. 1992. Laboratory methods for rearing ants (Hymenoptera, Formicoidea). Mem Zoologi. 45:1–32.

Czechowski W, Radchenko A, Czechowska W. 2012. The Ants (Hymenoptera, Formicidae) of Poland. Museum and Institute of Zoology PAS.

Decety J, Bartal IB-A, Uzefovsky F, Knafo-Noam A. 2016. Empathy as a driver of prosocial behaviour: highly conserved neurobehavioural mechanisms across species. Phil Trans R Soc B. 371:20150077.

Decety J, Svetlova M. 2012. Putting together phylogenetic and ontogenetic perspectives on empathy. Dev Cogn Neurosci. 2:1–24.

de Vries D, Galipaud M, Kokko H. 2023. Extrinsic mortality and senescence: a guide for the perplexed. Peer Community. 3:e29.

Duhoo T, Durand JL, Hollis KL, Nowbahari E. 2017. Organization of rescue behaviour sequences in ants, *Cataglyphis cursor*, reflects goal-directedness, plasticity and memory. Behav Processes. 139:12–18.

de Waal FB. 2008. Putting the altruism back into altruism: the evolution of empathy. Annu Rev Psychol. 59:279–300.

Friard O, Gamba M. 2016. BORIS: A free, versatile open-source event-logging software forvideo/audio coding and live observations. Methods Ecol Evol. 7:1325–1330.

Frank ET, Schmitt T, Hovestadt T, Mitesser O, Stiegler J, Linsenmair KE. 2017. Saving the injured: Rescue behavior in the termite-hunting ant *Megaponera analis*. Sci Adv. 3:e1602187.

Frank ET, Wehrhahn M, Linsenmair KE. 2018. Wound treatment and selective help in a termite-hunting ant. Proc R Soc B: Biol Sci. 285:20172457.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 41:95–98.

Hammers M, Brouwer L. 2017. Rescue behaviour in a social bird: removal of sticky 'bird catcher tree' seeds by group members. Behaviour. 154:403–411.

Hamilton WD. 1963. The evolution of altruistic behavior. Am Nat. 97: 354–356.

Hangartner W. 1969. Carbon dioxide, a releaser for digging behavior in *Solenopsis geminata* (Hymenoptera: Formicidae). Psyche. 76:58–67.

Heinze J, Giehr J. 2021. The plasticity of lifespan in social insects. Philos Trans R Soc Lond B Biol Sci. 376:20190734.

446 Helanterä H, Strassmann JE, Carrillo J, Queller DC. 2009. Unicolonial ants: Where do
447 they come from, what are they and where are they going? Trends Ecol Evol. 24:341–349.

448 Hölldobler B, Wilson EO. 1990. The ants. Harvard University Press, Cambridge.

449 Hollis KL, Nowbahari E. 2013. A comparative analysis of precision rescue behaviour in
450 sand-dwelling ants. Anim Behav. 85:537–544.

451 Hollis KL, Nowbahari E. 2022. Cause, development, function, and evolution: Toward a
452 behavioral ecology of rescue behavior in ants. Learn Behav. 50:329–338.

453 Horikoshi M, Tang Y. 2016. ggfortify: Data visualization tools for statistical analysis
454 results. https://CRAN.R-project.org/package=ggfortify (accessed on 20 June 2024).

455 Huang J, Cheng X, Zhang S, Chang L, Li X, Liang Z, Gong N. 2020. Having infants in
456 the family group promotes altruistic behavior of marmoset monkeys. Curr Biol. 30:4047–4055.

457 Kassambara A. 2023. ggcorrplot: Visualization of a correlation matrix using 'ggplot2'.
458 https://CRAN.R-project.org/package=ggcorrplot (accessed on 20 June 2024).

459 Kassambara A, Mundt F. 2020. factoextra: Extract and visualize the results of
460 multivariate data analyses. https://CRAN.R-project.org/package=factoextra (accessed on 20
461 June 2024).

462 Kassambara A, Kosinski M, Biecek P, Scheipl F. 2021. survminer: Drawing Survival
463 Curves using 'ggplot2'. https://CRAN.R-project.org/package=survminer (accessed on 20 June
464 2024).

465 Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid
466 multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30:3059–66.

467 Katoh K, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment
468 program. Brief. Bioinform. 9:286–298.

469 Keller L, Genoud M. 1997. Extraordinary lifespans in ants: a test of evolutionary theories
470 of ageing. Nature 389: 958–960.

Korb J, Heinze J. 2021. Ageing and sociality: why, when and how does sociality change ageing patterns? Philos Trans R Soc Lond B Biol Sci. 376:20190727.

Kramer BH, Schaible R. 2013. Life span evolution in eusocial workers--a theoretical approach to understanding the effects of extrinsic mortality in a hierarchical system. PLoS One. 8:e61813.

Kwapich CL, Hölldobler B. 2019. Destruction of spiderwebs and rescue of ensnared nestmates by a granivorous desert ant (*Veromessor pergandei*). Am Nat. 194:395–404.

Kwon T-S, Lee D-S, Park Y-S. 2022. Ant mortality with food competition in forests along a temperature gradient. Insects, 13:392.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol Biol Evol. 34:772–773.

Lange EC, Zeng S, Campos FA, Li F, Tung J, Archie EA, Alberts SC. 2023. Early life adversity and adult social relationships have independent effects on survival in a wild primate. Sci Adv. 9:eade7172.

Markl H. 1965. Stridulation in leaf-cutting ants. Science. 149:1392–1393.

Marshall-Pescini S, Dale R, Quervel-Chaumette M, Range F. 2016. Critical issues in experimental studies of prosociality in non-human species. Anim Cogn. 19:679–705.

Masilkova M, Ježek M, Silovský V, Faltusová M, Rohla J, Kušta T, Burda H. 2021. Observation of rescue behavior in wild boar (*Sus scrofa*). Sci Rep. 11:16217.

Medvedev ZA. 1990. An attempt at a rational classification of theories of ageing. Biol Rev Cambridge Philos. Soc. 65:375–398.

Miler K. 2016. Moribund ants do not call for help. PLoS One. 11:e0151925.

Miler K, Kuszewska K. 2017. Secretions of mandibular glands are not involved in the elicitation of rescue behavior in Formica cinerea ants. Insectes Soc. 64:303–305.

Miler K, Yahya BE, Czarnoleski M. 2017a. Pro-social behaviour of ants depends on their ecological niche: Rescue actions in species from tropical and temperate regions. Behav Processes. 144:1–4.

Miler K, Symonowicz B, Godzińska EJ. 2017b. Increased risk proneness or social withdrawal? The effects of shortened life expectancy on the expression of rescue behavior in workers of the ant *Formica cinerea* (Hymenoptera: Formicidae). J Insect Behav. 30:632–644.

Miler K, Turza F. 2021. "O Sister, where art thou?"—A review on rescue of imperiled individuals in ants. Biology. 10:1079.

Miller M, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES science Gateway for inference of large phylogenetic trees. GCE Workshop 2010, 1–8.

Moscovice LR, Eggert A, Manteuffel C, Rault J-L. 2023. Spontaneous helping in pigs is mediated by helper's social attention and distress signals of individuals in need. Proc R Soc B: Biol Sci. 290:20230665.

Nafcha O, Vilker D, Shamay-Tsoory S, Gabay S. 2023. Prosocial behavior in competitive fish: the case of the archerfish. Commun Biol. 6:822.

Nowbahari E, Scohier A, Durand J-L, Hollis KL. 2009. Ants, *Cataglyphis cursor*, use precisely directed rescue behavior to free entrapped relatives. PLoS ONE. 4:e6573.

Nowbahari E, Hollis KL. 2010. Rescue behavior: Distinguishing between rescue, cooperation and other forms of altruistic behavior. Commun and Integr Biol. 3:77–9.

Nowbahari E, Hollis KL, Durand J-L. 2012. Division of labor regulates precision rescue behavior in sand-dwelling *Cataglyphis cursor* ants: To give is to receive. PLoS ONE. 7:e4851.

Nowbahari E, Lenoir A, Hollis KL. 2016. Prosocial behavior and interindividual recognition in ant. From aggressive colony defense to rescue behavior. In. J. A. Sommerville and J. Decety (Eds.), Social cognition. Development across the life span. Routledge.

Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2023. caper: Comparative analyses of phylogenetics and evolution in R. https://CRAN.R-project.org/package=caper (accessed on 20 June 2024).

Pennisi E. 2005. How did cooperative behavior evolve? Science. 309:93.

Pitman RL, Deecke VB, Gabriele CM, Srinivasan M, Black N, Denkinger J, Durban JW, Mathews EA, Matkin DR, Neilson JL, Schulman-Janiger A, Shearwater D, Stap P, Ternullo R. 2017. Humpback whales interfering when mammal-eating killer whales attack other species: Mobbing behavior and interspecific altruism? Mar Mammal Sci. 33:7–58.

528 Queller DC, Strassmann JE. 1998. Kin selection and social insects. Bioscience. 48:165–
529 175.

530 Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. Tracer v.1.6.
531 https://beast.bio.ed.ac.uk/Tracer (accessed on 20 June 2024).

532 R Core Team 2023. R: A language and environment for statistical computing. Vienna
533 (Austria): R Foundation for Statistical Computing. https://www.R-project.org (accessed on 20
534 June 2024).

535 Ravell LJ, Harmon LJ. 2022. Phylogenetic comparative methods in R, chapter 3
536 phylogenetic generalized least squares, Princeton University Press.

537 Siebenaler JB, Caldwell DK. 1956. Cooperation among adult dolphins. J Mammal.
538 37:126–128.

539 Siracusa ER, Higham JP, Snyder-Mackler N,and Brent LJN. 2022. Social ageing:
540 exploring the drivers of late-life changes in social behavior in mammals. Biol Lett.
541 18:20210643.

542 Spangler HG. 1968. Stimuli releasing digging behavior in the western harvester ant
543 (Hymenoptera: Formicidae). J Kans Entomol Soc. 41:318–323.

544 Stearns SC, Ackermann M, Doebeli M, Kaiser M. 2000. Experimental evolution of aging,
545 growth, and reproduction in fruitflies. PNAS. 97:3309–3313.

546 Stec D, Kristensen RM, Michalczyk Ł. 2020. An integrative description of *Minibiotus*
547 *ioculator* sp. nov. from the Republic of South Africa with notes on *Minibiotus pentannulatus*
548 Londoño et al., 2017 (Tardigrada: Macrobiotidae). Zool Anz. 286:117–134.

549 Symonds MRE, Blomberg SP. 2014. A Primer on phylogenetic generalised least squares.
550 In: L. Garamszegi . (Eds) Modern phylogenetic comparative methods and their application in
551 evolutionary biology. Springer Berlin Heidelberg.

552 Tamura K, Stecher G,and Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics
553 Analysis Version 11. Mol Biol Evol. 38:3022–3027.

554 Therneau TM. 2020. survival: Survival Analysis. https://CRAN.R-
555 project.org/package=survival (accessed on 20 June 2024).

556 Tofilski A. 2002. Influence of age polyethism on longevity of workers in social insects.
557 Behav Ecol Sociobiol. 51:234–237.

558 Turza F, Zuber G, Bzoma M, Prus M, Filipiak M, Miler K. 2020. Ants co-occurring with
559 predatory antlions show unsuccessful rescue behavior towards captured nestmates. J Insect
560 Behav. 33:1–6.

561 Turza F, Miler K. 2022. Comparative analysis of experimental testing procedures for the
562 elicitation of rescue actions in ants. Curr Zool. 68:159–168.

563 Turza F, Miler K. 2023. Injury shortens life expectancy in ants and affects some risk-
564 related decisions of workers. Anim Cogn. 26:1643–1647.

565 Vaidya G, Lohman DJ, Meier R. 2011. SequenceMatrix: concatenation software for the
566 fast assembly of multi-gene datasets with character set and codon information. Cladistics.
567 27:171–180.

568 Vasconcelos M, Hollis K, Nowbahari E, Kacelnik A. 2012. Pro-sociality without
569 empathy. Biol Lett. 8:910–912.

570 Viña J, Borrás C, Miquel J. 2007. Theories of ageing. IUBMB Life. 59:249–254.

571 Vogel ER, Fuentes-Jiménez A. 2006. Rescue behavior in white-faced capuchin monkeys
572 during an intergroup attack: Support for the infanticide avoidance hypothesis. J Primatol.
573 68:1012–1016.

574 Vu VQ. 2011. ggbiplot: A ggplot2 based biplot. http://github.com/vqv/ggbiplot (accessed
575 on 20 June 2024).

576 Woyciechowski M, Kozłowski J. 1998. Division of labor by division of risk according to
577 worker life expectancy in the honey bee (*Apis mellifera* L.). Apidologie. 29:191–205.

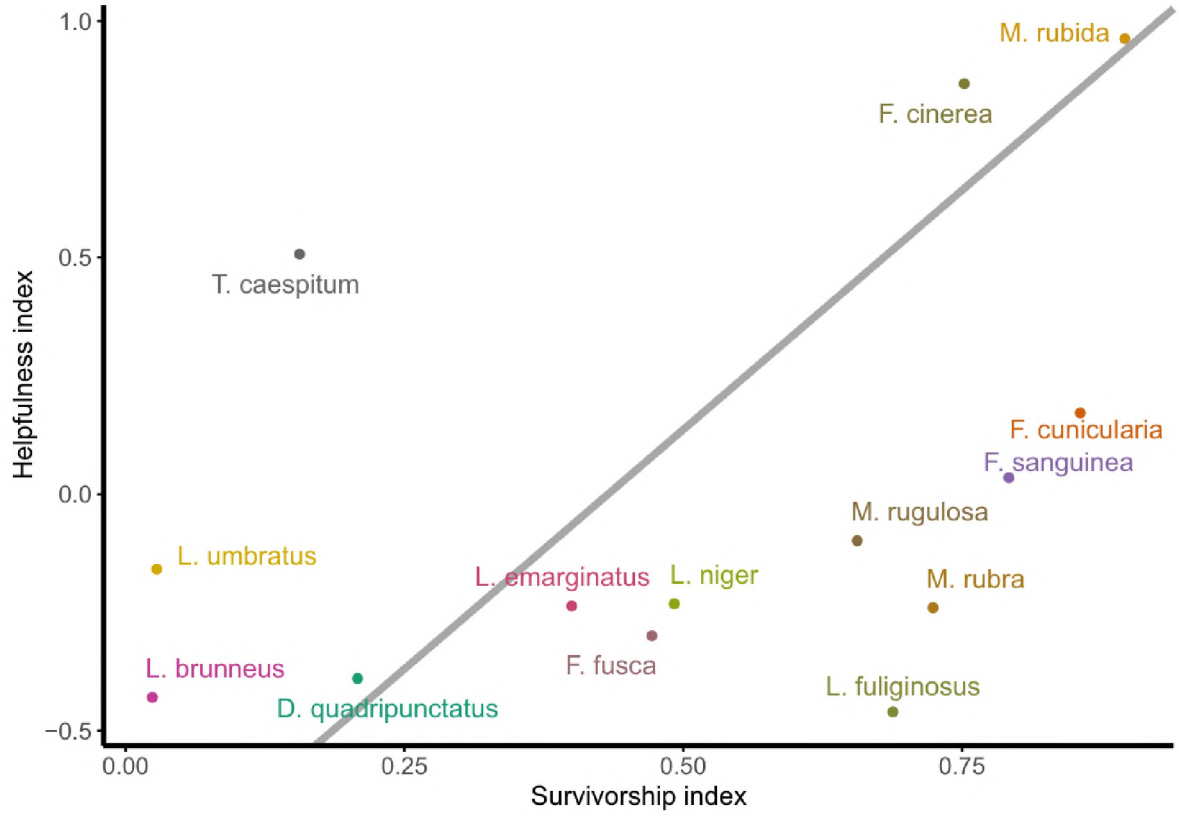
578 Ueno H, Suemitsu S, Murakami S, Kitamura N, Wani K, Matsumoto Y, Okamoto M,
579 Ishihara T. 2019. Helping-like behavior in mice towards conspecifics constrained cnside tubes.
580 Sci Rep. 9:5817.

581 Uy FMK, Adcock JD, Jeffries SF, Pepere E. 2019. Intercolony distance predicts the
582 decision to rescue or attack conspecifics in weaver ants. Insectes Soc. 66:185–192.

583 Zhu P, Liu W, Zhang X, Li M, Liu G, Yu Y, Li Z, Li X, Du J, Wang X, Grueter CC, Li
584 M, Zhou X. 2023. Correlated evolution of social organization and lifespan in mammals. Nat
585 Commun. 14:372.

586 Zwaan BJ. 1999. The evolutionary genetics of ageing and longevity. Heredity. 82:589–
587 97.

588 **Figures**



589

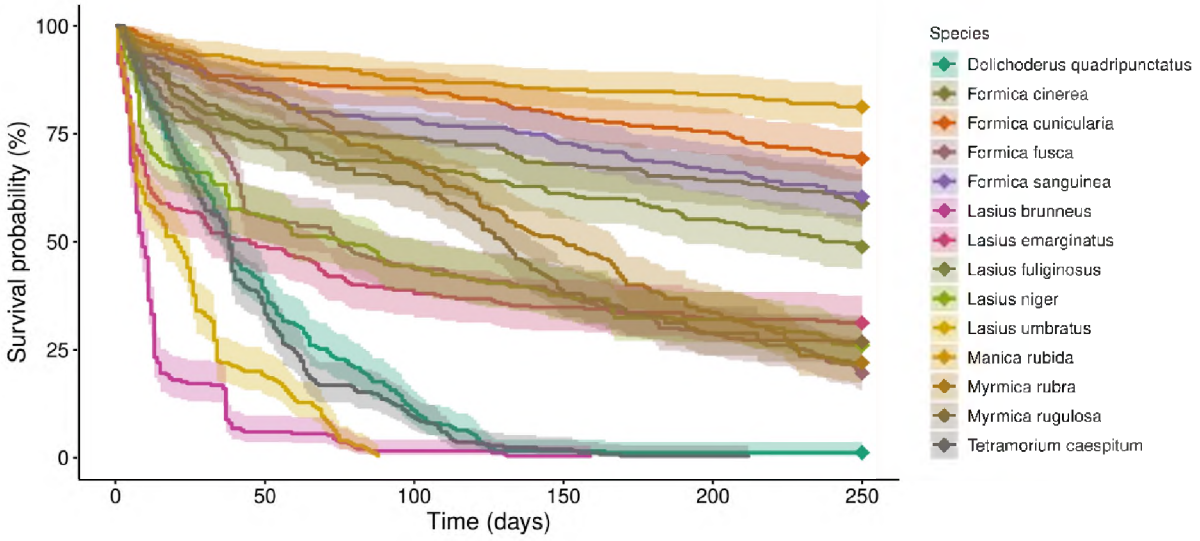
590 **Figure 1.** Helpfulness as a function of survival in the examined species. The gray solid line

591 indicates the regression line estimated by the PGLS model.

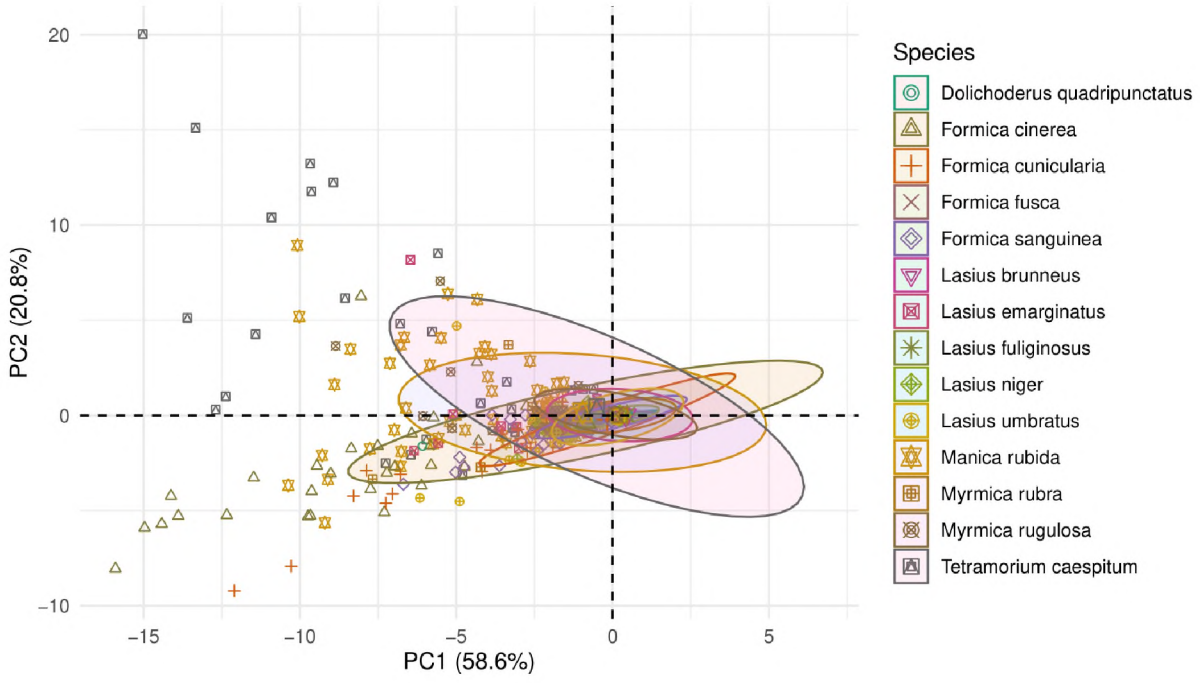
592 **Supplementary Materials**

	Species	28S rRNA	COI
	Dolichoderus quadripunctatus	OR856564	OR856592
	Dolichoderus quadripunctatus	OR856565	OR856593
	Myrmica rubra	OR856586	OR856614
	Myrmica rubra	OR856587	OR856615
	Myrmica rugulosa	OR856588	OR856616
	Myrmica rugulosa	OR856589	OR856617
	Manica rubida	OR856584	OR856612
	Manica rubida	OR856585	OR856613
	Tetramorium caespitum	OR856590	OR856618
	Tetramorium caespitum	OR856591	OR856619
	Formica fusca	OR856570	OR856598
	Formica fusca	OR856571	OR856599
	Formica cinerea	OR856566	OR856594
	Formica cinerea	OR856567	OR856595
	Formica cunicularia	OR856568	OR856596
	Formica cunicularia	OR856569	OR856597
	Formica sanguinea	OR856572	OR856600
	Formica sanguinea	OR856573	OR856601
	Lasius niger	OR856580	OR856608
	Lasius niger	OR856581	OR856609
	Lasius emarginatus	OR856576	OR856604
	Lasius emarginatus	OR856577	OR856605
	Lasius brunneus	OR856574	OR856602
	Lasius brunneus	OR856575	OR856603
	Lasius fuliginosus	OR856578	OR856606
	Lasius fuliginosus	OR856579	OR856607
	Lasius umbratus	OR856582	OR856610
	Lasius umbratus	OR856583	OR856611
OUTGROUP	*Paraponera clavata*	DQ353641	DQ353276

593 **Supplementary Material 1**. Sequence accession numbers submitted to GenBank.



Supplementary Figure 1. Kaplan-Meier survival plot for the species utilized in the study. Shadings indicate confidence intervals. Mortality was assessed for 5 colonies per species and 50 individuals from each colony over 250 days. The probability of worker survival beyond 80 days was used as the survivorship index and retained for further analyses.



Supplementary Figure 2. Principal Component Analysis score plot for the duration of contact with the victim, digging around it, pulling at its body parts, and biting the entrapping thread

602 during behavioural rescue tests in each of the studied species. Shapes and colours differentiate
603 species. Behaviour was assessed for 5 colonies per species and 50 tests from each colony were
604 performed. Ellipses indicate 95 % confidence intervals around means for the species and these
605 means were used as the helpfulness index and retained for further analyses.

Chapter V: General discussion

Results summary

The results of the first study (**Chapter II**) showed that there is a significant size diversity among foragers in *Formica cinerea* ants. Despite this size diversity, these differently-sized individuals exhibited no differences in life expectancy when measured under laboratory conditions. Furthermore, the effect of body size on rescue activity was found. The decision to rescue a nestmate was found to be unrelated to the body size of the rescuer, and this was also observed in the time delay to the first act of rescue. Interestingly, in terms of the duration of rescue behaviour, there was a significant effect of the rescuer's size. Small workers carried out rescue activities for longer periods compared to larger ones. These observations support the assumption that body size can lead to behavioural variation between differently sized workers in terms of rescue persistence (Kwapich and Hölldobler 2019). A similar pattern can be observed in the activity of the victims. Specifically, the activity level of entrapped individuals was higher in small workers compared to large workers, indicating greater persistence in attempts to self-release among the former. Valadares et al. (2022) reported similar findings in the highly polymorphic *Atta* leaf-cutting ants, where smaller individuals exhibited higher persistence in fighting, although the overall probability of attack did not differ between workers of different sizes. The authors proposed that smaller individuals might stimulate aggression in other colony members, including larger ones, during colony defence. Analogously, the increased engagement in rescue activities observed in small individuals in our study may serve to facilitate the involvement of nearby nestmates in rescue actions. While this mechanism has not been explicitly investigated, a previous study also suggested that nestmates may facilitate engagement in rescue actions (Nowbahari et al. 2009).

The second study of this dissertation (**Chapter III**) revealed that, as hypothesised, injured workers showed a lower life expectancy compared to intact individuals. While this finding may seem intuitive, it is crucial to empirically demonstrate, as unexpected patterns related to life expectancy can arise (e.g., some parasitised ants exhibit prolonged life expectancy, see Beros et al. 2021). However, the second and third hypotheses were only partially confirmed. Specifically, injured workers showed a greater tendency to rescue intact nestmates, but no significant differences in aggression towards alien ants were observed between intact and injured individuals. This highlights the adaptive significance of rescue (Frank et al. 2017). However, it remains unclear how ants discriminate between intact and injured individuals. Although differences in cuticular hydrocarbon profiles and/or volatile pheromones may contribute, the lack of discrimination between nestmates of differing life expectancies is noted in the literature (see, for example, Leclerc and Detrain 2016). The lack of heightened propensity in the decision to attack aliens between injured and intact workers may perhaps be explained by the fact that *F. cinerea* is a highly aggressive and territorial species (Czechowski et al. 2002). In other words, aggression may be very high in workers of this species regardless of the context.

Consistent with my hypothesis, the results of the third study (**Chapter IV**) showed that species with relatively higher general life expectancies are more likely to engage in rescue actions than species characterised by relatively shorter lifespans. Notably, my results showed that both worker life expectancies and their helpfulness displayed no phylogenetic constraints. This pattern suggests that these two traits may have evolved independently multiple times. Indeed, a coevolution of social behaviour with life expectancy has been suggested multiple times (e.g., Keller and Genoud 1997, Carey 2001, Zhu et al. 2023). Increased pro-social behaviour may contribute to maintaining health, thereby enhancing life expectancy, and this pattern might be the same across different species. Furthermore, this trend may also reflect the

substantial colony-level costs associated with rescuing members with shorter life expectancies (Miler et al. 2017b). Ant colonies allocate resources and energy to ensure the survival and productivity of the entire colony (Hölldobler & Wilson 1990), making investments in interacting with and providing help to short-lived individuals potentially counterproductive (Miler et al. 2017b). Redirecting energy towards tasks that contribute more significantly to colony success, such as foraging, may offer greater benefits (Bar et al. 2023). In fact, previous research has shown that rescued ants in certain species actively participate in future colony activities, reducing the need for replacement workers (Frank et al. 2017). Undoubtedly, more research should look into the trade-offs and costs related to rescue behaviour to fully understand the evolution of this phenomenon.

Significance of the study

Ant colonies exhibit remarkable organisational complexity similar to that of an organism's body (Hölldobler and Wilson 2008). However, unlike the cells in a body, individual insects retain a significant degree of autonomy and their decisions can strongly influence the collective (Hölldobler and Wilson 1990, Frank et al. 2017, Kwapich and Hölldobler 2019). Therefore, identifying factors that impact individual behaviour is crucial for developing a comprehensive understanding of colony dynamics as a whole (Gordon 2019). This thesis addresses important gaps in our knowledge of ant decision-making and rescue behaviour towards nestmates. Specifically, one of the main goals was to investigate potential triggers of rescue behaviour and address the issues of unexplained behavioural variation in rescue proneness in Formicidae.

The first study (**Chapter II**) provides initial empirical evidence demonstrating the influence of body size on rescue behaviour in monomorphic ants. This finding strongly corroborates previous hypotheses suggesting the significant impact of body size on ant behaviour across various behavioural contexts (see, e.g., Kay and Rissin 2005, Hurlbert et al.

2008, Okrutniak et al. 2020, Valadares et al. 2022), including those related to rescue behaviour (Kwapich and *Hölldobler* 2019). Moreover, the research presented in this dissertation demonstrated behavioural differences in a specific group of ants characterized by monomorphism. This is particularly important because, due to the lack of distinct worker phenotypes, behavioural individual differences in monomorphic ants have been typically overlooked by the mainstream studies (Okrutniak et al. 2020). My evidence also represents the first quantification of the behaviour of ant workers in need of rescue, with indications that the activity of victims can be a proxy of the emission of signal(s) responsible for rescue behaviour expression.

The next study in my thesis (**Chapter III**) indicates clearly that the injury in *F. cinerea* foragers incurs a survival cost, which influences rescue but not aggressive behaviour. These findings support previous suggestions that ants are highly sensitive to behavioural context (Duhoo et al. 2017, Turza and Miler 2022). It is important to note that, to date, there has been limited research on the role of injured individuals within the colony (Tofilski et al. 2008, Gilad et al. 2022) and this thesis offers an interesting take on how physical harm leads to decreased life expectancy, with effects on the behaviour of social insects.

The third study (**Chapter IV**) indicates a link between worker lifespans and rescue behaviour, controlling for phylogenetic distance between species. Specifically, species with longer worker life expectancies show a higher propensity to rescue endangered nestmates. This clearly shows that general worker life expectancy should be more frequently considered in studies of various behavioural patterns among group-living animals. Comparative studies of rescue behaviour in ants are very rare (Hollis and Nowbahari 2013, Miler et al. 2017a). Thus, my study represents one of the first examinations of several species of ants belonging to different taxa. Importantly, out of the fourteen studied ant species, eleven species were tested for the first time regarding rescue occurrence. Thus, in addition to its values for understanding

important natural processes, the implementation of this research has also a high methodological value for the further development of the study of rescue behaviour among Formicidae. Throughout this dissertation, different aspects of rescue behaviour were investigated, and the findings provide a more detailed insight into factors that influence ants' decision-making. They also reveal promising directions for future studies, as outlined below.

Future directions

Despite my research here, providing new knowledge about the eco-evolutionary determinants of rescue occurrence in ants, many fundamental questions remain unanswered. Given the number of unexplored species with respect to this behaviour, it is still unclear how general across Formicidae are the patterns revealed by this study. The detected effects of body size on rescue behaviour in *Formica cinerea* (**Chapter II**) also lead the way for future research on the ultimate causes of rescue behaviour. It would be valuable to check whether the tendency to rescue depends on the general body size of workers in different species. An important question to consider is whether larger or smaller ant species are more likely to have evolved rescue behaviour. Size could play a crucial role in the development of such altruistic behaviours due to differences in colony dynamics, resource allocation and individual value within the colony (Billick 2002, Kay and Rissing 2005). Larger ant species may have more robust physical abilities, potentially allowing them to rescue trapped nestmates more effectively. Additionally, species with larger workers often form smaller colonies than species with smaller workers (Hölldobler and Wilson 1990), where each worker could be more valuable, thereby increasing the likelihood of evolved rescue behaviour as a means of preserving colony fitness. On the other hand, smaller ant species may rely on numbers rather than individual size for survival. With more numerous colonies, rescue behaviour could have evolved as a cooperative strategy to mitigate losses, especially when each worker plays a specific

and essential role in colony function. Undoubtedly, investigating the relationship between body size, colony size and the occurrence of rescue behaviour could provide key insights into the evolutionary pressures that shape altruistic tendencies in social insects. Future studies should also consider the combined effects of life expectancy, ecology, body size, and personality (consistent behavioural variation) (Hollis and Nowbahari 2013, Okrutniak et al. 2020, Maák et al. 2021). The latter is especially increasing scientific interest among Formicidae (e.g., Kolay et al. 2020, Reznikova 2021). Thus, it would be reasonable to investigate whether the tendency to rescue is correlated with other behavioural traits, including altruistic personality of ants.

Similarly to my second study (**Chapter III**), a direction for future work would be to manipulate the perceived value of individuals. This can shed light on how colonies prioritize rescue efforts and how this affects their fitness, especially in nature. In general, as ant behaviour is often context-dependent and may be altered in laboratory observations, more studies should be conducted in the field to verify the results of laboratory experiments (Turza and Miler 2022). Moreover, behavioural ecologists continuing to study similar topics should more often consider the adaptive value of rescue behaviour for different species. This would help understand the ecological and evolutionary aspects of altruism and related phenomena, e.g. the origin of rescue behaviour patterns and/or their high variability in nature. Although methodologically challenging, it would be very useful to conduct some whole-colony perspective studies which sought to explore whether colonies that differ in their average rescue activity also differ in fitness outcomes when facing ecologically relevant situations that trigger rescue behaviour, such as nest collapse (Miler and Turza 2021). Ants exhibit a wide range in terms of the number of workers per colony, which may affect their individual value (Wilson 1971). This depends on the structure and age of the colony, as well as access to food resources (Wilson and Hölldobler 1990). In-depth exploration of how these factors could influence rescue

activity from the perspective of the entire colony might provide evidence supporting the idea that, in ants, a colony is a higher-order unit of selection than the individual (Queller 1998, Helanterä et al. 2009). Furthermore, future studies should explore the genetic basis of rescue behaviour. Rescue behaviour may be more likely to occur among closely related individuals, as predicted by kin selection theory (Hamilton 1963, 1964). Investigating whether genetic relatedness within or between colonies influences rescue efforts could offer insights into the evolutionary drivers of this behaviour. Moreover, by identifying specific genes linked to rescue behaviour, researchers could deepen our understanding of altruism in social animals (Andras et al. 2020, Nowbahari et al. 2022).

Previous studies have been limited to the behaviour of the rescuer, but there is also another key component of rescue, the ant in need of help and its behaviour. My study stressed the importance of including the behaviour of the victim during entrapment (**Chapter II**). Rescue behaviour may also be influenced by the relationship between certain traits (e.g., body size and life expectancy) of both the rescuer and the victim. This perspective extends beyond examining the individual characteristics of either the rescuer or the victim and highlights the significance of the relative value of these traits in determining rescue action. Another important question relates to whether victims modulate rescue actions. The mechanisms underlying rescue behaviour among Formicidae are not well understood. Different types of signals, such as chemical, auditory, or visual cues, need further investigation in the context of rescue occurrence (Markl 1965, Spangler 1968, Hangartner 1969). To date, more than forty exocrine glands have been identified among social insects. These glands produce pheromones, volatile chemical substances that induce behavioural responses in conspecifics (Billen and Morgan 1998). Czechowski et al. (2002) suggest that the association of rescue behaviour in *Formica cinerea* may involve the mandibular, anal, or venom glands. The lack of phylogenetic relatedness effect on rescue behaviour in my study (**Chapter IV**)

suggests that rescue behaviour has evolved multiple times independently in ants. This in turn suggests plausible differences between species in the types of signals that trigger their rescue behaviour.

Conclusions

To sum up, my PhD thesis consists of three complementary studies that explore the importance of factors such as body size and life expectancy for the expression of rescue behaviour both within and among different species of ants. My major results demonstrate that (1) smaller workers are more persistent than larger workers in terms of providing and requiring help (**Chapter II**), (2) injury decreases worker survival and leads to behavioural variation among rescuing individuals that provide help to others (**Chapter III**), (3) the expression of rescue behaviour is more frequent in ant species expressing longer general life expectancies of workers (**Chapter IV**). These findings help unravel the links between key life history traits and behaviour, enhancing our understanding of behavioural variation in decision-making among social animals. While there is still much research needed to fully understand the processes structuring rescue behaviour, this dissertation addresses key issues and will hopefully inspire and develop further studies devoted to this phenomenon.

References

- Andras JP, Hollis KL, Carter KA, Couldwell G, Nowbahari E. 2020. Analysis of ants' rescue behavior reveals heritable specialization for first responders. Journal of Experimental Biology 223: jeb212530.
- Bar A, Gilad T, Massad D, Ferber A, Ben-Ezra D, Segal D, Foitzik S, Scharf I. 2023. Foraging is prioritized over nestmate rescue in desert ants and pupae are rescued more than adults. Behavioral Ecology 34: 1087–1096.
- Belt, T. 1874. The Naturalist in Nicaragua. John Murray.
- Beros S, Lenhart A, Scharf I, Negroni MA, Menzel F, Foitzik S. 2021. Extreme lifespan extension in tapeworm-infected ant workers. Royal Society Open Science 8: 202118.
- Billen J, Morgan ED. 1998. Pheromone communication in social insects: sources and secretion, CCR Press.
- Billick I. 2002. The relationship between the distribution of worker sizes and new worker production in the ant *Formica neorufibarbis*. Oecologia 132: 244–249.
- Bolton World Catalog. https://www.antweb.org/page.do?name=world (accessed on 5 June 2024).
- Bos N, Lefèvre T, Jensen AB, d'Ettorre P. 2012. Sick ants become unsociable. Journal of Evolutionary Biology 25: 342–351.
- Carey JR. 2001. Demographic mechanisms for the evolution of long life in social insects. Experimental Gerontology 36: 713–22.
- Chen YQ, Han S, Yin B. 2023. Why help others? Insights from rodent to human early childhood research. Frontiers in Behavioral Neuroscience 17: 1058352.
- Cronin KA. 2012. Prosocial behaviour in animals: the influence of social relationships, communication and rewards. Animal Behaviour 84: 085–1093.
- Czechowski W, Godzińska EJ, Kozłowski MW. 2002. Rescue behavior shown by workers of *Formica sanguinea* Latr., *F. fusca* L. and *F. cinerea* Mayr (Hymenoptera: Formicidae) in response to their nestmates caught by an ant lion larva. Annales Zoologici 52: 423–431.

- Czechowski W, Radchenko A, Czechowska W. 2012. The ants (Hymenoptera, Formicidae) of Poland. Museum and Institute of Zoology PAS.
- de Waal FB. 2008. Putting the altruism back into altruism: the evolution of empathy. Annual Review of Psychology 59: 279–300.
- Duhoo T, Durand JL, Hollis KL, Nowbahari E. 2017. Organization of rescue behaviour sequences in ants, *Cataglyphis cursor*, reflects goal-directedness, plasticity and memory. Behavioural Processes 139: 12–18.
- Frank ET, Schmitt T, Hovestadt T, Mitesser O, Stiegler J, Linsenmair KE. 2017. Saving the injured: Rescue behavior in the termite-hunting ant *Megaponera analis*. Science Advances, 3: e1602187.
- Gilad T, Dorfman A, Subach A, Scharf I. 2022. Leg or antenna injury in *Cataglyphis* ants impairs survival but does not hinder searching for food. Current Zoology 68: 441–450.
- Gordon DM. 2019. The Ecology of collective behavior in ants. Annual Review of Entomology 64: 35–50.
- Hamilton WD. 1963. The evolution of altruistic behavior. American Naturalist 97: 354–356.
- Hangartner W. 1969. Carbon dioxide, a releaser for digging behavior in *Solenopsis geminata* (Hymenoptera: Formicidae). Psyche 76: 58–67.
- Hartmann C, Haschlar J, Heinze J, Bernadou A. 2020. Activity patterns and age-dependent changes in behavior in the clonal ant *Platythyrea punctata*. Journal of Insect Behavior 33: 149–157.
- Heinze J, Walter B. 2010. Moribund ants leave their nests to die in social isolation. Current Biology 20: 249–250.
- Helanterä H, Strassmann JE, Carrillo J, Queller DC. 2009. Unicolonial ants: Where do they come from, what are they and where are they going? 24: 341–349.
- Herbers JM, Cunningham M. 1983. Social organization in *Lepthotorax longispinosus* Mayr. Animal Behavior 31: 759–771.
- Hölldobler B, Wilson EO. 1990. The ants. Harvard University Press, Cambridge.

- Hölldobler B, Wilson EO. 2008. The superorganism: The beauty, elegance, and strangeness of insect societies. W. W. Norton & Company, New York.
- Hollis KL, Nowbahari E. 2013. A comparative analysis of precision rescue behaviour in sand-dwelling ants. Animal Behaviour 85: 537–544.
- Hollis KL, Nowbahari E. 2022. Cause, development, function, and evolution: Toward a behavioral ecology of rescue behavior in ants. Learning Behavior 50: 329–338.
- Hurlbert AH, Ballantyne F, Powell S. 2008. Shaking a leg and hot to trot: The effects of body size and temperature on running speed in ants. Ecological Entomology 33: 144–154.
- Ishii Y, Hasgeawa E. 2013. The mechanism underlying the regulation of work-related behaviors in the monomorphic ant, *Myrmica kotokui*. Journal of Ethology 31, 61–69.
- Kay A, Rissing SW. 2005. Division of foraging labor in ants can mediate demands for food and safety. Behavioral Ecology and Sociobiology 58: 165–174.
- Keller L, Genoud M. 1997. Extraordinary lifespans in ants: a test of evolutionary theories of ageing. Nature 389: 958–960.
- Kolay S, Boulay R, d'Ettorre P. 2020. Regulation of Ant Foraging: A Review of the Role of Information Use and Personality. Frontiers in Psychology 11: 734.
- Kwapich CL, Hölldobler B. 2019. Destruction of spiderwebs and rescue of ensnared nestmates by a granivorous desert ant (*Veromessor pergandei*). The American Naturalist 194: 395–404.
- Lafleur LJ. 1940. Helpfulness in ants. Journal of Comparative Psychology, 30: 23–29.
- Leclerc J-B, Detrain C. 2016. Ants detect but do not discriminate diseased workers within their nest. The Science of Nature 103: 70.
- Maák I, Trigos-Peral G, Ślipiński P, Grześ IM, Horváth G, Witek M. 2021. Habitat features and colony characteristics influencing ant personality and its fitness consequences. Behavioral Ecology 32: 124–137.
- Markl H. 1965. Stridulation in leaf-cutting ants. Science 149: 1392–1393.

- Marshall-Pescini S, Dale R, Quervel-Chaumette M, Range F. 2016. Critical issues in experimental studies of prosociality in non-human species. Animal Cognition 19: 679–705.
- Miler K, Turza F. 2021. "O Sister, where art thou?"—A review on rescue of imperiled individuals in ants. Biology 10: 1079.
- Miler K, Yahya BE, Czarnoleski M. 2017a. Pro-social behaviour of ants depends on their ecological niche: Rescue actions in species from tropical and temperate regions. Behavioural Processes 144: 1–4.
- Miler K, Symonowicz B, Godzińska EJ. 2017b. Increased risk proneness or social withdrawal? The effects of shortened life expectancy on the expression of rescue behavior in workers of the ant *Formica cinerea* (Hymenoptera: Formicidae). Journal of Insect Behavior 30: 632–644.
- Moroń D, Lenda M, Skórka P, Woyciechowski M. 2012. Short-lived ants take greater risks during food collection. The American Naturalist 180: 744–750.
- Moroń D, Witek M, Woyciechowski M. 2008. Division of labour among workers with different life expectancy in the ant *Myrmica scabrinodis*. Animal Behavior 75: 345–350.
- Nafcha O, Vilker D, Shamay-Tsoory S, Gabay S. 2023. Prosocial behavior in competitive fish: the case of the archerfish. Communication Biology 6: 822.
- Nowbahari E, Scohier A, Durand J-L, Hollis KL. 2009. Ants, *Cataglyphis cursor*, use precisely directed rescue behavior to free entrapped relatives. PLOS ONE 4: e6573.
- Nowbahari E, Hollis KL, Durand JL. 2012. Division of labor regulates precision rescue behavior in sand-dwelling *Cataglyphis cursor* ants: to give is to receive. PLOSE ONE 7: e48516.
- Nowbahari E, Hollis KL. 2010. Rescue behavior: Distinguishing between rescue, cooperation and other forms of altruistic behavior. Communicative & Integrative Biology 3: 77–9.
- Nowbahari E, Hollis KL, Bey M, Demora L, Durand JL. 2022. Rescue specialists in *Cataglyphis piliscapa* ants: The nature and development of ant first responders. Learning Behavior. 50: 71–81.

- Okrutniak M, Rom B, Turza F, Grześ IM. 2020. Body size differences between foraging and intranidal workers of the monomorphic ant *Lasius niger*. Insects 11: 433.
- Queller DC, Strassmann J.E. 1998. Kin selection and social insects. Bioscience 48: 165–175.
- Packard GC. 2020. Rethinking the metabolic allometry of ants. Evolutionary Ecology 34: 149–161.
- Porter SD, Jorgensen CD. 1981. Foragers of the harvester ant, *Pogonomyrmex owyheei*: a disposable caste? Behavioral Ecology and Sociobiology 9: 247–256.
- Ratnieks FLW, Wenseleers T. 2007. Altruism in insect societies and beyond: Voluntary or enforced? 23:45–52.
- Reznikova Z. 2021. Ants' personality and its dependence on foraging styles: research perspectives. Frontiers in Ecology and Evolution 9: 661066.
- Seifert B. A taxonomic revision of the *Formica cinerea* group (Hymenoptera: Formicidae). 2002. Abhandlungen der Naturforschenden Gesellschaft zu Görlitz 74: 245–272.
- Shik J. 2010. The metabolic costs of building ant colonies from variably sized subunits. Behavioral Ecology and Sociobiology 64: 1981–1990.
- Shorter JR, Rueppell O. 2012. A review of self-destructive defense behaviors in social insects. Insectes Sociaux 59: 1–10.
- Spangler HW. 1968. Stimuli releasing digging behavior in the Western harvester ant. Journal of the Kansas Entomological Society 41: 318–323.
- Tofilski A. 2002. Influence of age polyethism on longevity of workers in social insects. Behavioral Ecology and Sociobiology 51: 234–237.
- Tofilski A. 2009. Shorter-lived workers start foraging earlier. Insectes Sociaux 56: 359–366.
- Tofilski A, Couvillon MJ, Evison SE, Helanterä H, Robinson EJ, Ratnieks FL. 2008. Preemptive defensive self-sacrifice by ant workers. The American Naturalist 172: E239–E243.

- Turza F, Miler K. 2022. Comparative analysis of experimental testing procedures for the elicitation of rescue actions in ants. Current Zoology 68: 159–168.
- Turza F, Zuber G, Bzoma M, Prus M, Filipiak M, Miler K. 2020. Ants co-occurring with predatory antlions show unsuccessful rescue behavior towards captured nestmates. Journal of Insect Behavior 33: 1–6.
- Valadares L, Nascimento FS, Chaline N. 2022. Small workers are more persistent fighters than soldiers in the highly polymorphic *Atta* leaf-cutting ants. Animal Behavior 189: 15–21.
- Ueno H, Suemitsu S, Murakami S, Kitamura N, Wani K, Matsumoto Y, Okamoto M, Ishihara T. 2019. Helping-like behaviour in mice towards conspecifics constrained inside tubes. Scientific Reports 9: 5817.
- Uy FMK, Adcock JD, Jeffries SF, Pepere E. 2019. Intercolony distance predicts the decision to rescue or attack conspecifics in weaver ants. Insectes Sociaux 66: 185–192.
- Willott SJ, Compton SG, Incoll LD. 2000. Foraging, food selection and worker size in the seed harvesting ant *Messor bouvieri*. Oecologia 125: 35–44.
- Wilson EO. 1971. The insect societies. Harvard University Press, Cambridge.
- Woyciechowski M, Kozłowski J. 1998. Division of labor by division of risk according to worker life expectancy in the honey bee (*Apis mellifera* L.). Apidologie 29: 191–205.
- Woyciechowski M, Moroń D. 2009. Life expectancy and onset of foraging in the honeybee (*Apis mellifera*). Insectes Sociaux 56: 193–201.
- Zhu P, Liu W, Zhang X, Li M, Liu G, Yu Y, Li Z, Li X, Du J, Wang X, Grueter CC, Li M, Zhou X. 2023. Correlated evolution of social organization and lifespan in mammals. Nature Communication 14: 372.

Author contribution statements

Filip Turza
Imię i nazwisko Kandydata
Name and Surname of Candidate

Kraków, 17.09.2024
Miejscowość i data
Place and date

Oświadczenie / Statement

Ja niżej podpisany jako współautor artykułu pt. "Small workers are more persistent when providing and requiring help in a monomorphic ant" oświadczam, że mój wkład w przygotowaniu publikacji polegał na udziale w częściach dotyczących:

I, undersigned as a co-author of the article entitled entitled "Small workers are more persistent when providing and requiring help in a monomorphic ant" I declare that my contribution to the preparation of the publication consisted of participation in the following parts:

- Opracowania koncepcji badań
  Development of research concepts
- Opracowania metodyki badań
  Development of research methodology
- Zbieraniu danych
  Data collection
- Analizie i interpretacji wyników
  Analysis and interpretation of results
- Przygotowaniu manuskryptu
  Manuscript preparation
- Redagowaniu manuskryptu
  Manuscript editing
- Przeprowadzenia procesu recenzji
  Manuscript revision

a mój indywidualny procentowy wkład w powstanie artykułu wynosi 70 %
and my individual percentage contribution to the article is 70 %

Filip Turza
.....
Czytelny podpis Kandydata/
Legible Candidate/

Krzysztof Miler
Imię i nazwisko Współautora
Name and Surname of Co-Author

Kraków, 17.09.2024
Miejscowość i data
Place and date

Oświadczenie / Statement

Ja niżej podpisany jako współautor artykułu pt. "Small workers are more persistent when providing and requiring help in a monomorphic ant" oświadczam, że wkład doktoranta w przygotowaniu publikacji polegał na udziale w częściach dotyczących:

I, undersigned as a co-author of the article entitled "Small workers are more persistent when providing and requiring help in a monomorphic ant" I declare that the PhD student contribution to the preparation of the publication consisted of participation in the following parts:

- Opracowania koncepcji badań
  Development of research concepts
- Opracowania metodyki badań
  Development of research methodology
- Zbieraniu danych
  Data collection
- Analizie i interpretacji wyników
  Analysis and interpretation of results
- Przygotowaniu manuskryptu
  Manuscript preparation
- Redagowaniu manuskryptu
  Manuscript editing
- Przeprowadzenia procesu recenzji
  Manuscript revision

a jego indywidualny procentowy wkład w powstanie artykułu wynosi 70 %
and his individual percentage contribution to the article is 70 %

Czytelny podpis Współautora
Legible Co-author signature

Filip Turza
Imię i nazwisko Kandydata
Name and Surname of Candidate

Kraków, 17.09.2024
Miejscowość i data
Place and date

Oświadczenie / Statement

Ja niżej podpisany jako współautor artykułu pt. "Injury shortens life expectancy in ants and affects some risk-related decisions of workers" oświadczam, że mój wkład w przygotowaniu publikacji polegał na udziale w częściach dotyczących:

I, undersigned as a co-author of the article entitled entitled "Injury shortens life expectancy in ants and affects some risk-related decisions of workers" I declare that my contribution to the preparation of the publication consisted of participation in the following parts:

- Opracowania koncepcji badań
  Development of research concepts
- Opracowania metodyki badań
  Development of research methodology
- Zbieraniu danych
  Data collection
- Analizie i interpretacji wyników
  Analysis and interpretation of results
- Przygotowaniu manuskryptu
  Manuscript preparation
- Redagowaniu manuskryptu
  Manuscript editing
- Przeprowadzenia procesu recenzji
  Manuscript revision

a mój indywidualny procentowy wkład w powstanie artykułu wynosi 70 %
and my individual percentage contribution to the article is 70 %

Filip Turza
.....
Czytelny podpis Kandydata/
Legible Candidate/

Krzysztof Miler
Imię i nazwisko Współautora
Name and Surname of Co-Author

Kraków, 17.09.2024
Miejscowość i data
Place and date

Oświadczenie / Statement

Ja niżej podpisany jako współautor artykułu pt. "Injury shortens life expectancy in ants and affects some risk-related decisions of workers" oświadczam, że wkład doktoranta w przygotowaniu publikacji polegał na udziale w częściach dotyczących:

I, undersigned as a co-author of the article entitled "Injury shortens life expectancy in ants and affects some risk-related decisions of workers" I declare that the PhD student contribution to the preparation of the publication consisted of participation in the following parts:

- Opracowania koncepcji badań
  Development of research concepts
- Opracowania metodyki badań
  Development of research methodology
- Zbieraniu danych
  Data collection
- Analizie i interpretacji wyników
  Analysis and interpretation of results
- Przygotowaniu manuskryptu
  Manuscript preparation
- Redagowaniu manuskryptu
  Manuscript editing
- Przeprowadzenia procesu recenzji
  Manuscript revision

a jego indywidualny procentowy wkład w powstanie artykułu wynosi 70 %
and his individual percentage contribution to the article is 70 %

Czytelny podpis Współautora
Legible Co-author signature

Filip Turza
Imię i nazwisko Kandydata
Name and Surname of Candidate

Kraków, 17.09.2024
Miejscowość i data
Place and date

Oświadczenie / Statement

Ja niżej podpisany jako współautor artykułu pt. "Life expectancy explains variation in helpfulness across several ant species" oświadczam, że mój wkład w przygotowaniu publikacji polegał na udziale w częściach dotyczących:

I, undersigned as a co-author of the article entitled entitled "Life expectancy explains variation in helpfulness across several ant species" I declare that my contribution to the preparation of the publication consisted of participation in the following parts:

- Opracowania koncepcji badań
  Development of research concepts
- Opracowania metodyki badań
  Development of research methodology
- Zbieraniu danych
  Data collection
- Analizie i interpretacji wyników
  Analysis and interpretation of results
- Przygotowaniu manuskryptu
  Manuscript preparation
- Redagowaniu manuskryptu
  Manuscript editing
- Przeprowadzenia procesu recenzji
  Manuscript revision

a mój indywidualny procentowy wkład w powstanie artykułu wynosi 60 %
and my individual percentage contribution to the article is 60 %

Filip Turza
.....
Czytelny podpis Kandydata/
Legible Candidate/

Daniel Stec
Imię i nazwisko Współautora
Name and Surname of Co-Author

Kraków, 17.09.2024
Miejscowość i data
Place and date

Oświadczenie / Statement

Ja niżej podpisany jako współautor manuskryptu pt. "Life expectancy explains variation in helpfulness across several ant species" oświadczam, że wkład doktoranta w przygotowaniu publikacji polegał na udziale w częściach dotyczących:

I, undersigned as a co-author of the manuscript entitled "Life expectancy explains variation in helpfulness across several ant species" I declare that the PhD student contribution to the preparation of the publication consisted of participation in the following parts:

- Opracowania koncepcji badań
  Development of research concepts
- Opracowania metodyki badań
  Development of research methodology
- Zbieraniu danych
  Data collection
- Analizie i interpretacji wyników
  Analysis and interpretation of results
- Przygotowaniu manuskryptu
  Manuscript preparation
- Redagowaniu manuskryptu
  Manuscript editing
- Przeprowadzenia procesu recenzji
  Manuscript revision

a jego indywidualny procentowy wkład w powstanie artykułu wynosi 60 %
and his individual percentage contribution to the article is 60 %

Daniel Stec
.....
Czytelny podpis Współautora
Legible Co-author signature

Diego Fontaneto
Imię i nazwisko Współautora
Name and Surname of Co-Author

Verbania Pallanza, 17.09.2024
Miejscowość i data
Place and date

Oświadczenie / Statement

Ja niżej podpisany jako współautor manuskryptu pt. "Life expectancy explains variation in helpfulness across several ant species " oświadczam, że wkład doktoranta w przygotowaniu publikacji polegał na udziale w częściach dotyczących:

I, undersigned as a co-author of the manuscript entitled "Life expectancy explains variation in helpfulness across several ant species" I declare that the PhD student contribution to the preparation of the publication consisted of participation in the following parts:

- Opracowania koncepcji badań
  Development of research concepts
- Opracowania metodyki badań
  Development of research methodology
- Zbieraniu danych
  Data collection
- Analizie i interpretacji wyników
  Analysis and interpretation of results
- Przygotowaniu manuskryptu
  Manuscript preparation
- Redagowaniu manuskryptu
  Manuscript editing
- Przeprowadzenia procesu recenzji
  Manuscript revision

a jego indywidualny procentowy wkład w powstanie artykułu wynosi 60 %
and his individual percentage contribution to the article is 60 %

Czytelny podpis Współautora
Legible Co-author signature

Krzysztof Miler
Imię i nazwisko Współautora
Name and Surname of Co-Author

Kraków, 17.09.2024
Miejscowość i data
Place and date

Oświadczenie / Statement

Ja niżej podpisany jako współautor manuskryptu pt. "Life expectancy explains variation in helpfulness across several ant species " oświadczam, że wkład doktoranta w przygotowaniu publikacji polegał na udziale w częściach dotyczących:

I, undersigned as a co-author of the manuscript entitled "Life expectancy explains variation in helpfulness across several ant species" I declare that the PhD student contribution to the preparation of the publication consisted of participation in the following parts:

- Opracowania koncepcji badań
  Development of research concepts
- Opracowania metodyki badań
  Development of research methodology
- Zbieraniu danych
  Data collection
- Analizie i interpretacji wyników
  Analysis and interpretation of results
- Przygotowaniu manuskryptu
  Manuscript preparation
- Redagowaniu manuskryptu
  Manuscript editing
- Przeprowadzenia procesu recenzji
  Manuscript revision

a jego indywidualny procentowy wkład w powstanie artykułu wynosi 60 %
and his individual percentage contribution to the article is 60 %

Krzysztof Miler

Czytelny podpis Współautora
Legible Co-author signature