Carriers and cutters: size-dependent caste polyethism in the tropical fire ant (*Solenopsis geminata*)

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Abstract

Body size is an important life-history trait in eusocial insects which plays a key role in colony fitness. The division of labour, represented by caste polyethism, correlates with divergent morphological traits. Size polymorphism has been noted in the tropical fire ant, *Solenopsis geminata*; however, little is known regarding the differences in the size distributions of workers performing foraging tasks. In the present study, task partitioning was observed in the foraging activities of *S. geminata*. Two subgroups among foraging workers of *S. geminata* were discovered using the Gaussian mixture model: a large worker group (head width ≥ 0.924 mm) and a small worker group (head width < 0.924 mm). The foraging worker population comprised two distinct groups – 25.64% were large workers and 74.36% were small workers. Larger workers delivered heavier seeds faster than smaller workers, but this difference became less apparent when lighter seeds were being carried. When large prey such as crickets was encountered during foraging, *S. geminata* partitioned their tasks into cutting and transportation. The large workers were observed to cut cricket prey into fragments with their longer mandibles, and the small workers then transported these fragments back to the nest. These results present evidence of task partitioning among tropical fire ants, with different tasks being performed by ants of different castes.

Introduction

The caste systems in social Hymenoptera are marked by three major thresholds: the evolution of eusociality, the evolution of queen–worker dimorphism and the evolution of polymorphic worker castes (Wheeler, 1986). The sterile worker caste has evolved to be the key character of eusocial insects (Nonacs, 2014; Olejarz *et al.*, 2017). The division of labour in an ant colony creates specialized behavioural groups which perform different tasks (task specialization) to ensure the survival of the colony. Specialization of workers is one potential explanation for the origin and/or maintenance of the diversity of the worker size and morphology found within a single colony (Wheeler, 1986; Hölldobler and Wilson, 1990; de Toledo *et al.*, 2016). Some studies have supported the idea that the worker morphology influences task performance (Hasegawa, 1993*a*; Powell and Franks, 2006; Powell, 2009). For instance, the major workers of *Colobopsis nipponicus* have been shown to specialize in nest defence and food storage (Hasegawa, 1993*a*, 1993*b*). In addition, the soldier caste in *Cephalotes* is morphologically specialized for blocking nest entrances (Powell, 2008, 2009). Moreover, variation in morphological traits may have originated from different behaviours (i.e., caste polyethism) (Oster and Wilson, 1978), although variation in the ant worker body size is proximately influenced by larval nutrition (Wheeler, 1991), heritable factors (Hughes *et al.*, 2003; Smith *et al.*, 2008), evolutionary constraints (Trible and Kronauer, 2017) and the physical and enemy environment (Wills *et al.*, 2018).

Body size is a critical life-history trait in ants which affects colony fitness (Wills *et al.*, 2018). In continuously polymorphic ant species, size variation potentially makes the division of labour more flexible through task partitioning. Task partitioning occurs when a specific task is divided among two or more types of workers (Ratnieks and Anderson, 1999). Material transfer by different-sized workers during foraging activities is an example of task partitioning (Jeanne, 1986). This has been supported by theoretical simulations in which the workers' body size influenced foraging efficiency (López, 1987) and by empirical studies of size-dependent caste polyethism in seed-harvester ants (*Pogonomyrmex* spp.) (Kaspari, 1996) and leafcutter ants (*Atta* spp.) (de Toledo *et al.*, 2016).

Size polymorphism has been observed in the workers of the continuously polymorphic tropical fire ant, *Solenopsis geminata*, and some research has suggested that it is continuous but weakly bimodal in its frequency distribution (Wilson, 1978). Moreover, *S. geminata*

workers have been suggested to be more polymorphic than other fire ants (Wilson, 1978), including the closely related species *S. invicta* (Tschinkel, 2013). A small group of much larger workers (head width ≥ 1.31 mm), namely 'major workers' or 'soldiers' (Wilson, 1978; Trager, 1991), are characterized by very large quadrangular heads with strong and highly incurved toothless mandibles. These major workers engage in self-grooming and milling of harvested seeds, which means that they tend to remain inside the nest. By contrast, 'minor workers' (head width ≤ 0.79 mm) are smaller, have tapering mandibles and engage in various tasks such as foraging, brood care and nest excavation. The 'media workers' (head width 0.80–1.30 mm) of *S. geminata* retrieve prey and excavate nest materials (Wilson, 1978). In general, the function of the larger media and major workers is seed grinding (Wilson, 1978, 1979), whereas the minor and small media workers, which comprise the majority of *S. geminata*, engage in different tasks. However, such classifications can be problematic because of the varying size distributions between colonies, where the average worker size and skewness of the size distributions can alter throughout colony growth (Tschinkel, 1988). Although behavioural bioassay has been conducted (Wilson, 1978) to shed light on this topic, the nature of the division of workers remains controversial. Size-dependent caste polyethism is supported by a study that revealed a correlation between workers' head width and the size of harvested seeds (Kaspari, 1996). Wilson (1978) suggested that there were no clear differences in the size distributions or performance of minor workers working outside the colony. In addition, the media workers were more likely to represent the transition between the minor and major workers, with the larger media workers behaving similarly to the major workers whereas the smaller media workers behaving similarly to the minor workers.

S. geminata is a globally invasive species that was introduced to Taiwan at least since the 1980s (Nambu and Tano, 1983; Gotzek *et al.*, 2015). It is widely distributed in agricultural fields and weedy habitats (Lai *et al.*, 2009). With small invertebrates and plant seeds as its main food sources, tropical fire ants significantly affect the local ecosystem by reducing the prey population and promoting seed dispersal at the same time (Taber, 2000; Lai *et al.*, 2018). Previously, we have observed divergence of foraging behaviour in *S. geminata* when handling different prey species. Unlike a plant seed, which can be carried by a single worker, invertebrate prey is usually fragmented by cooperative teams of workers before being retrieved. The present study sought evidence on task partitioning during foraging activities of tropical fire ant, *S. geminata*, in Taiwan. The head width and behavioural preference of subgroups of foraging workers were examined using a mixture distribution model and bioassay, respectively. Parameters of each distribution were used to determine the categories to which individual foraging workers collected from the field in Taiwan belonged. The performance and preference of the foraging behaviours in each group were examined by providing plant seeds and invertebrate preys in varying sizes.

Materials and methods

Collection of S. geminata *colonies and morphological examination*

Twenty-eight colonies of *S. geminata* were excavated from eight study areas in central [Taichung (24°15′ N, 120°31′ E), Changhua (24°09′ N, 120°30′ E) and Yunlin (23°44′ N, 120°24′ E)], southern [Chiayi (23°27′ N, 120°19′ E), Tainan (22°55′ N, 120°17′ E), Kaohsiung (22°41′ N, 120°18′ E) and Pingtung (22°40′N, 120°29′E)] and eastern [Hualien (23°51′ N, 121°33′ E)] Taiwan between August 2013 and July 2014. All colonies were transported in plastic containers coated with Fluon (NP115; Northern Products Inc., Woonsocket, RI, USA) to prevent the ants from escaping. The *S. geminata* colonies were kept in soil and maintained at room temperature (27–28°C) and relative humidity (50–60%) in the laboratory.

To determine the size categorization, 600 workers were sampled from each of the 28 colonies collected. The foraging workers on the nest surface were sampled. However, the major workers might be accidently sampled. Six hundred workers from each colony might have contained both foraging workers and major workers, because most of the major workers hide inside the nest during any nest interference (Wilson, 1978), the low number of ants accidentally sampled were not expected to bias the estimation. Worker ants were anaesthetized and preserved in the freezer (−10°C) before examination. The body sizes of the workers were quantified by measuring the head width, which has been proposed as a standard measure (a convenient index) of overall body size in polymorphic ant species (Wilson, 1953; Tschinkel, 2013). The head width (mm) (i.e., length between the eyes) was measured using a standard ocular micrometre calibrated with a stage micrometre under the compound microscope (Trager, 1991; Lin and Wu, 1996).
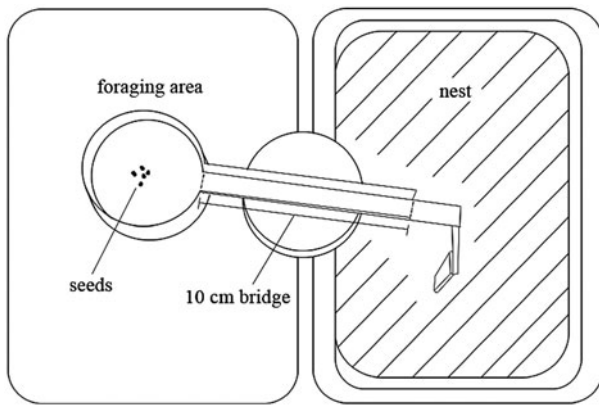
Behavioural experiments

The ants used for the laboratory experiments were collected from Taichung (24°15′ N, 120°31′ E) in 2017–2018. Each colony was excavated and maintained with soil in a $55 \times 39 \times 30$ cm³ plastic container. After acclimation for one night in the laboratory, the colonies were provided with *ad libitum* water and commercial insect jellies (Beetle jelly, Han Shuo Food Co., Ltd, Taiwan). The colonies were maintained in the laboratory prior to use in behavioural experiments for a maximum of 30 days. For standardization, all of the experiments were conducted between 2 and 4 pm in the laboratory.

Speed of seed delivery

Three colonies collected in 2017 were used in this experiment. In the behavioural experiments, *Casuarina equisetifolia* seeds were used on the basis of the previous study which revealed that a great quantity of *C. equisetifolia* was found in the seed caches of *S. geminata* colonies (Lai *et al.*, 2018). The speed of seed delivery was determined by measuring the amount of time a worker ant spent passing through a 10-cm bridge while carrying a seed (fig. 1). The bridge (10×1 cm²) was first spread with ant trail pheromone to connect the nest and foraging area (a 5.5-cm-diameter plastic Petri dish) with 40 seeds of *C. equisetifolia*. After all the seeds were harvested by the worker ants, the connection was removed. For speed measurement, five test seeds were placed in the foraging area with a worker ant, which was attracted from the nest by using *C. equisetifolia* seeds. The amount of time that a worker ant spent passing through the 10-cm bridge was measured using a stopwatch with a resolution of 1/100th of a second. The head widths of the worker ants were measured using ImageJ software after removing the head. Besides, images of heads were captured using a digital camera (Stylus TG-4 Tough, Olympus, Japan).

Because seed weight had the potential to influence the delivery speed, test seeds with three different weights were manufactured

Figure 1. Diagram of the behavioural experiments. For the speed measurement, five test seeds of *C. equisetifolia* were placed in the foraging area (5.5-cm diameter plastic Petri dish), which was connected to the nest. The speed of seed delivery was determined by measuring the amount of time that a foraging worker ant spent crossing the bridge (10×1 cm²) while carrying a seed. See Supplementary Movie S3 (a).

from the *C. equisetifolia* seeds collected from the field. The three different test seeds used were as follows: (1) cut one seed into four equal pieces ($0.25 \times = \sim 0.3$ mg); (2) used one whole seed ($1 \times = \sim 1.3$ mg) and (3) stuck all three seeds together using white glue ($3 \times = \sim 3.9$ mg). In each experiment, five seeds with the same weight were provided as the test seeds. Each ant colony was tested three times using the three different test seeds.

Behavioural preferences of different-sized worker ants

Behavioural preferences were examined by comparing the sizes of the worker ants engaging in different foraging behaviours. Three experiments were conducted by providing different foods in the foraging area which connected to the nest, including *C. equisetifolia* seeds of different weights, the whole body of a cricket, and cricket fragments that had been shredded into pieces that ranged from 0.3 to 0.4 cm in size.

The *C. equisetifolia* seeds were manufactured according to the aforementioned methods. The seeds were cut or stuck together to create five different weights: $0.25 \times$, $1 \times$, $3 \times$, $6 \times$ (~ 7.6 mg) and $12 \times$ (~ 15.6 mg). Three seeds of each weight (15 seeds in total) were placed in the foraging area. The worker ants that lifted a seed and moved toward the nest were collected. The seed weight was recorded (AJ-620E, Vibra, Shinko Denshi Co., Ltd, Japan) and returned to the foraging area. The head width was recorded by cutting the ant head off, photographing it, and then followed by measurement using ImageJ software.

The crickets used in the present experiment were frozen adults of *Gryllus bimaculatus*. We categorized worker behaviour into two groups: 'cutters' and 'carriers'. Cutters were defined as *S. geminata* workers that cut the prey into pieces when handling larger prey. Carriers were defined as workers that transported the fragments of the prey back to the nest. Since larger prey is unable to be carried alone, *S. geminata* workers cut the prey into smaller pieces. The cutter continually masticates the prey until a fragment is dismantled from the body, and then the carrier raises the fragment and moves toward the nest. During the experiments, both the cutter and carrier were observed handling the whole cricket body, whereas the only carrier was observed when the cricket fragment was provided. After determined the behaviours, the worker ants were collected and subjected to head width measurement as previously described.

In the behavioural experiments, each of the behaviours was examined with three to seven colonies selected from the total of 13 colonies (Supplementary Material S2). In addition, because of the obvious behavioural preference in handling whole crickets (see fig. 5), we display that data independently. In fig. 6, we included the data from additional six pre-examined colonies. The colonies had been maintained in the laboratory for few months which make the nest condition inconsistent. They are not included in the previous analyses to reduce the artificial error, but independently showed in fig. 6 since the worker displayed a similar behavioural pattern regardless of the nest condition.

Statistical analyses

The head widths of worker ants from the 28 colonies (600 individuals per colony) were analysed using the Gaussian mixture model. The mixture distribution model assumes that subgroups within an observed frequency distribution have previously been applied in order to estimate the number of larval instars of gypsy moth according to measurements of the head capsule width (McClellan and Logan, 1994). Here we used the Gaussian mixture model to determine the number of subgroups categorized according to the ant-head width, the model was tested using different subgroup numbers (1 to 4), and criteria were selected on the basis of the highest-modified Akaike information criterion (Benaglia *et al.*, 2009), which considers the number of components as a penalty (Young, 2007). The boundaries of each normal distribution were determined by crossing each probability density function (McClellan and Logan, 1994). A general boundary was set by including all 16,800 individuals to characterize worker ants in the behavioural experiment. On the basis of the boundary, the worker ants were categorized into the subgroup of 'large worker' (head width ≥ 0.924 mm) and subgroup 'small worker' (head width < 0.924 mm) groups (fig. 2) for the behavioural experiments.

A linear mixed-effect model was applied to test the correlation between the head width and delivery speed (i.e., amount of time spent passing through the 10-cm bridge), with the colonies as a random effect. The significance of the fixed effect was tested by removing the effect term using the likelihood ratio test. After separating the ant workers into two groups, the data from the three colonies were pooled and compared using pairwise Wilcoxon rank sum tests adjusted by Bonferroni correction.

The proportions of large worker ants were compared among foraging behaviours using generalized linear mixed models with binomial errors. The proportions of the large worker ants were considered response variables, the eight foraging behaviours [delivery of seeds with different weights ($0.25 \times$, $1 \times$, $3 \times$, $6 \times$ and $12 \times$), delivery of previously shredded cricket fragments, carrier and cutter] were considered fixed effects, and the colony was considered a random factor. The significance of the fixed effects was tested by comparing the full model with all fixed effects and the model with subset combinations of the fixed effect terms using the likelihood ratio test. *Post hoc* multiple comparisons of the eight behavioural categories were conducted using Tukey's all-pairwise comparisons. To further confirm the different compositions of worker ants in the cutter and carrier categories, the proportion of large workers in each colony was calculated.

Data analyses and visualization were implemented using R software (version 3.5.2, R Core Team, 2018) with the following packages: mixtools, for building the Gaussian mixture model;
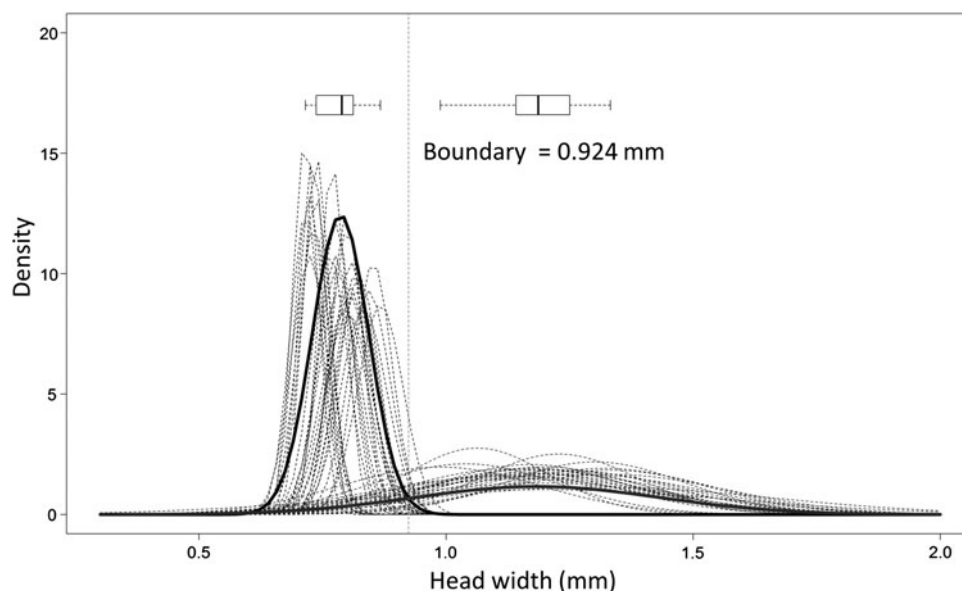
Figure 2. Head-width distribution of *S. geminata*, estimated using the Gaussian mixture model. The dashed line represents the distribution of each of the 28 colonies, and the solid line represents the distribution from the pooled data. The boundary indicates the head width at the intersection of the two distributions. The boxplots indicate the estimated mean value of the head width in each of the 28 colonies.

lme4, for building the linear mixed-effects model and generalized linear mixed model and multcomp, for the post hoc multiple comparisons.

Results

Morphological examination

In each of the 28 colonies examined, two subgroups were identified among the foraging workers (fig. 2, Supplementary S1) on the basis of the head width. The average head width was 0.79 mm (0.72–0.87 mm) for the small workers and 1.23 mm (0.99–1.33 mm) for the large workers (Table 1). The boundary between the two distributions was 0.924 mm (fig. 2). After characterizing small workers and large workers according to the head size, the small workers constituted 74.36% (52.43–84.83%) of the foraging worker population whereas the large workers occupied 25.64% (15.17–47.57%) of the population (Table 1).

Behavioural experiments

Speed of seed delivery

From 381 workers, the average time a worker spent passing through the bridge was 9.20 s (3.12–33.13 s) [Supplementary Movie S3 (a)]. There was a significant positive correlation between the speed and size of worker ants in the delivery of heavier seeds (1× and 3×) but not in the delivery of lighter seeds (0.25×) (fig. 3, Table 2). The large workers (head width ≥ 0.924 mm) were faster at delivering heavier seeds (1× and 3×) than the small workers (head width < 0.924 mm) (fig. 4).

Behavioural preferences of different-sized worker ants

The proportion of large worker ants differed in each foraging behaviour (fig. 5). The proportion of large worker ants was higher in the cutter behaviour category than in any of the other categories. The delivery of crickets and larger seeds (3×, 6× and 12×) was performed with a similar composition of workers. The large workers

engaged in the delivery of small seeds (0.25× and 1×) in the smallest proportion. The same pattern was generally observed in all of the colonies examined (fig. 6). Despite the varying proportions of large worker ants in cutter and carrier tasks among different colonies, the large workers were often the ones tended to fragment the cricket prey [Supplementary Movie S3 (b)].

Discussion

Variation in the worker body size within a colony can be influenced by internal and external factors, including evolutionary constraints, genetic factors, the social environment, larval nutrition, the physical environment and competition (Wills *et al.*, 2018). There is also evidence that size variation improves efficiency in nest tasks (Reyes and Fernández Haeger, 1999; Arnan *et al.*, 2011*a*; de Toledo *et al.*, 2016). In the present study, we provide evidence of size division among foraging workers of the tropical fire ant *S. geminata* and the relevant caste polyethism in handling food items. Two distinct groups of foraging workers were identified among each colony of *S. geminata* in Taiwan. Overall, the small workers and large workers comprised 74.36 and 25.64% of the total foraging worker population, respectively. These two size-based groups of workers displayed divergent foraging behaviours: small workers preferred delivering food items, whereas large workers preferred fragmenting prey and exhibited higher efficiency in delivering heavier food items.

In all of the 28 examined colonies, the number of small workers was higher than that of large workers; however, the ratios varied. Colony age may have an effect on the distribution of worker sizes; a similar phenomenon was observed in *S. invicta* colonies, which typically produce larger workers as the colony increased in age and size (Tschinkel, 1988, 1993). Additionally, nutrition is one possible factor affecting worker size. The provision of carbohydrate in the ants' food increases the number and average body size of the workers, whereas the body size of workers was not affected by the provision of extra dietary amino acids to ants (Wills *et al.*, 2015). This further implicates the importance of

Table 1. Head widths (distance between the eyes) and percent of the 600 randomly sampled foraging workers in each of the 28 examined colonies of *S. geminata*

Locality	Small worker		Large worker	
	Head width (mm)	Percent (%)	Head width (mm)	Percent (%)
Changhua	0.82 ± 0.04	84.67	1.18 ± 0.21	15.33
Changhua	0.83 ± 0.06	71.79	1.28 ± 0.24	28.21
Changhua	0.80 ± 0.05	67.78	1.20 ± 0.22	32.22
Chiayi	0.72 ± 0.03	79.30	1.19 ± 0.39	20.70
Chiayi	0.74 ± 0.03	71.45	1.10 ± 0.25	28.55
Chiayi	0.72 ± 0.03	74.62	0.99 ± 0.20	25.38
Chiayi	0.81 ± 0.04	70.52	1.33 ± 0.22	29.48
Hualien	0.80 ± 0.04	76.00	1.18 ± 0.24	24.00
Hualien	0.84 ± 0.04	84.83	1.26 ± 0.22	15.17
Hualien	0.80 ± 0.04	69.06	1.17 ± 0.19	30.94
Kaohsiung	0.78 ± 0.04	80.63	1.12 ± 0.21	19.37
Kaohsiung	0.80 ± 0.03	83.00	1.19 ± 0.31	17.00
Kaohsiung	0.82 ± 0.04	81.00	1.13 ± 0.25	19.00
Pingtung	0.77 ± 0.03	67.33	1.24 ± 0.22	32.67
Pingtung	0.78 ± 0.03	80.13	1.23 ± 0.24	19.87
Pingtung	0.78 ± 0.04	84.64	1.31 ± 0.35	15.36
Taichung	0.82 ± 0.04	68.79	1.23 ± 0.16	31.21
Taichung	0.80 ± 0.03	64.21	1.17 ± 0.21	35.79
Taichung	0.85 ± 0.04	77.67	1.32 ± 0.21	22.33
Taichung	0.74 ± 0.03	65.78	1.17 ± 0.25	34.22
Taichung	0.72 ± 0.04	52.43	1.12 ± 0.24	47.57
Taichung	0.78 ± 0.04	65.33	1.30 ± 0.18	34.67
Tainan	0.72 ± 0.03	73.67	1.20 ± 0.20	26.33
Tainan	0.73 ± 0.03	80.13	1.06 ± 0.14	19.87
Tainan	0.87 ± 0.05	69.17	1.33 ± 0.24	30.83
Yunlin	0.76 ± 0.04	76.92	1.16 ± 0.23	23.08
Yunlin	0.73 ± 0.03	84.64	1.04 ± 0.19	15.36
Yunlin	0.79 ± 0.05	76.46	1.15 ± 0.27	23.54
Pooled	**0.79 ± 0.06**	**74.36**	**1.23 ± 0.22**	**25.64**

The bold values in the last row are calculated by pooling the data from the 28 examined colonies.

plant-based resources in the growth of the colony (Wills *et al.*, 2015). In our previous study, seeds of Gramineae plants comprise more than 50% of all seeds in the seed caches of *S. geminata* nests (Lai *et al.*, 2018). Vegetation that is mainly composed of Gramineae plants provides a vast quantity of plant seeds that are rich in carbohydrates and lipids, which support colony development. The requirement for seed harvesting is in accordance with our behavioural experiments, showing that the small worker tends to act as the carrier, hauling the plant seeds and prey fragments. By contrast, the large worker acts as the cutter, fragmenting the invertebrate prey's body, which would be too heavy to be carried by a single worker alone. Retana and Cerdá (1994) suggest that the size of harvested seeds depends on the forager size (the concept of size matching). Despite no clear evidence, a rising in the proportion of large workers along with the colony growth

might be beneficial since the colony can utilize the wider size range of food items and subsequently increase its foraging efficiency (Retana and Cerdá, 1994; Arnan *et al.*, 2011*b*).

The relatively high number of large workers in *S. geminata* colonies might also contribute to seed harvest due to increased efficiency in delivering heavier seeds. The positive correlation between worker size and carrying efficiency was first predicted by López (1987) and was empirically supported in a study on *S. geminata* (Kaspari, 1996). Furthermore, Arnan *et al.* (2011*a*) also discovered that the small workers of *Messor bouvieri* were faster at finding seeds and large workers were better at transporting seeds. However, the efficient delivery of prey of various weights might be one of the less likely (or at least not the only) factors responsible for promoting the size division in *S. geminata*. Despite the higher efficiency in delivering larger seeds, the
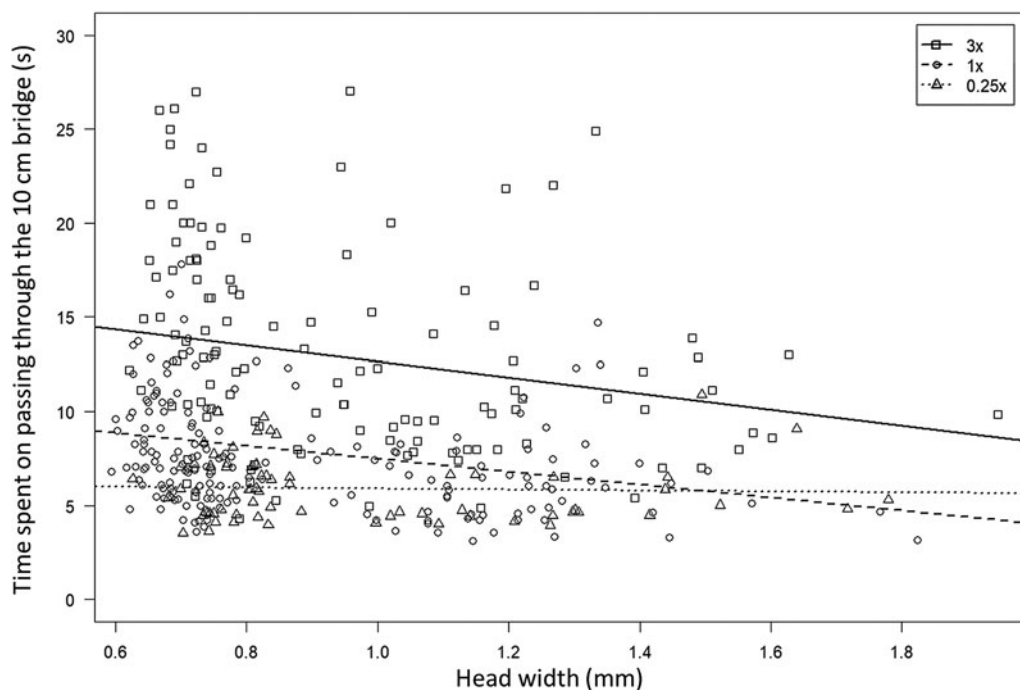
Figure 3. Speed of *S. geminata* workers delivering different weights of *C. equisetifolia* seed. The lines represent the correlations between the size and delivery speed of worker ants according to a linear regression model. The seed weights are presented as factors of one *C. equisetifolia* seed; 3× the seed weight averaged 3.9 mg, 1× was 1.3 mg and 0.25× was 0.3 mg. Levels of significance are calculated from the likelihood ratio test for the linear mixed models (the details are shown in Table 2).

**Table 2.** Fixed effects of the seed weight on the seed delivery speed

Seed weight	Sloop	χ^2	P value	n
0.25×	−0.23	0.1347	0.7136	69
1×	−3.44	14.543	**0.0001**	187
3×	−4.26	5.8029	**0.016**	125

The bold values indicate the significant correlations between the size and delivery speed of worker ants.

speed of delivering smaller seeds (0.25 × weight of a *C. equisetifolia* seed) was not significantly affected by worker size, and this is the common seed weight seen in the seed caches of *S. geminata* in Taiwan (Lai *et al.*, 2018). In addition, the proportion of large workers delivering the *C. equisetifolia* seeds did not linearly increase with the seed weight but instead reached a plateau when three seeds which glued together were offered to the colonies. A lower proportion of large workers involved when carrying the extremely small seeds might not be due to the seed weight but instead caused by the volume of seed, i.e. tiny seeds might be too small to be held by mandibles of major workers (Wilson, 1978). This could explain the positive correlation between seed volume and worker size in the study by Kaspari (1996).

Among fire ant species, *S. invicta* exhibit weak polymorphism, whereas *S. geminata* have greater body-size variations between the minor and major worker castes (Wilson, 1978). Body size differences are reflected in the division of labour. *S. geminata* have more behavioural specializations than *S. invicta* (Hölldobler and Wilson, 1990). Therefore, the behavioural experiments in this study were conducted in order to determine whether differences

in worker size were associated with behavioural specializations during foraging activities in *S. geminata*. Our results regarding the different behaviours among the workers imply that size-dependent caste polyethism occurs in *S. geminata*, with findings suggesting that large workers performed with higher efficiency and preferred to deliver heavier seeds. Task partitioning during foraging can occur in the collection and transportation of seeds (Reyes and Fernández Haeger, 1999). Workers cooperate by exhibiting chain transfer behaviour when they encounter heavier seeds. The proportions of large workers were similar in food item delivery (including larger seeds and fragmented crickets), but significantly lower than that of the ants fragmented the crickets. In other words, task partitioning of *S. geminata* workers during foraging results in the division of labour for transportation (carriers) and cutting prey (cutters) when encountering larger prey (such as crickets). This means that large workers can help the colony to forage more efficiently by retrieving large prey items (Evison and Ratnieks, 2007). Similar task partition was observed in leafcutter ants (*Atta* spp.), with the division of labour between major workers that fragmenting fallen fruit and small workers that involving in transport tasks (Hart *et al.*, 2002; Evison and Ratnieks, 2007). In the present study, two groups of workers performed the cutting behaviour in a colony: The major workers gnawed seeds (miller caste) inside the nest, and the large foraging workers fragmented prey outside the nest. In theory, the increasing population of large foraging workers is positively correlated with the protein intake from animal prey, which is beneficial to the development of ant larvae (Hölldobler and Wilson, 1990; Martinez and Wheeler, 1994). When worker replacements are required, the preference for a protein-based diet has been observed in the invasive fire ant (Cook *et al.*, 2016) along with an increase in the size of worker ants in well-
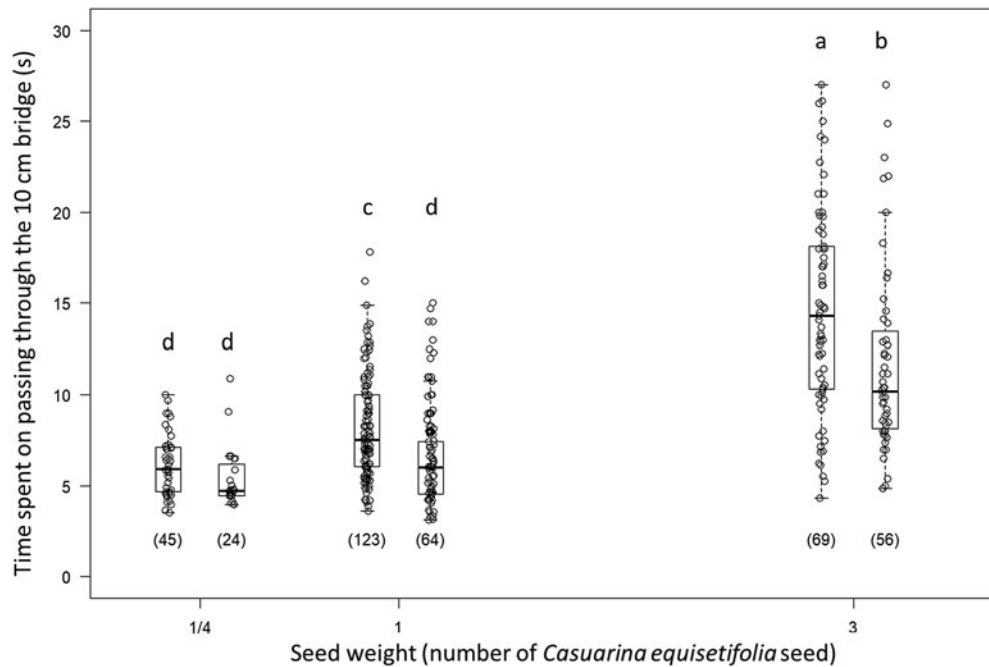
Figure 4. Speed of *S. geminata* workers delivering different weights of *C. equisetifolia* seeds. The workers were categorized into large (right) and small (left) groups on the basis of the head width boundary of 0.924 mm, which was estimated using the Gaussian mixture model. Numbers in parentheses indicate the total number of workers sampled. The letters (a, b, c and d) represent the statistical significance based on pairwise Wilcoxon rank sum tests, adjusted by Bonferroni correction. The seed weights are presented as factors of one *C. equisetifolia* seed; 3× the seed weight averaged 3.9 mg, 1× was 1.3 mg and 0.25× was 0.3 mg.
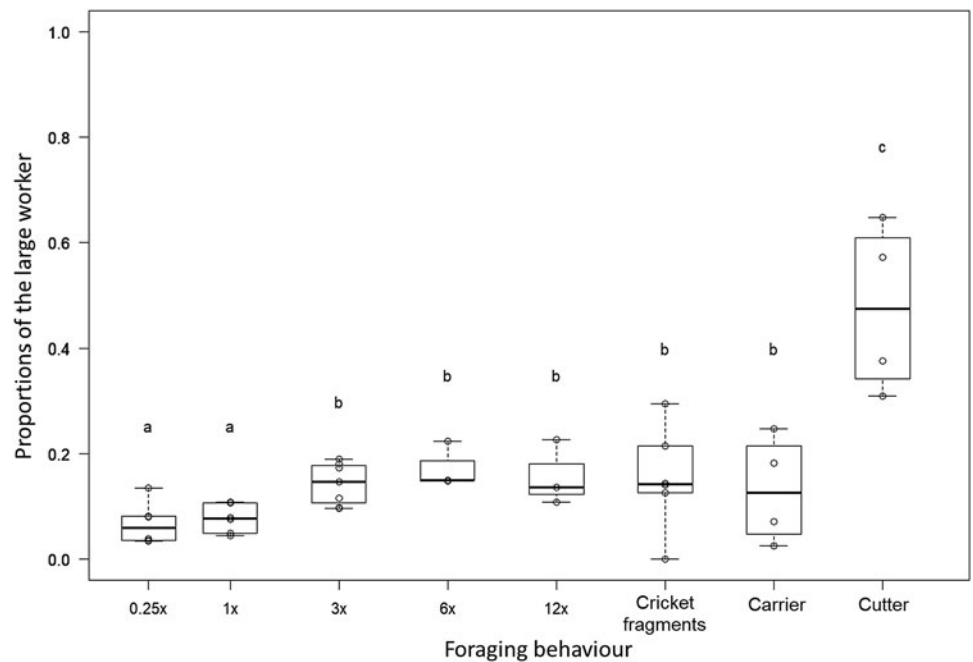
Figure 5. Proportions of large workers among the foraging workers that delivered different weights of *C. equisetifolia* seeds and 'cricket fragments' and fragmented the whole body of crickets. The seed weights were represented by fold multiplication of one *C. equisetifolia* seed; 12 × the seed weight averaged 15.6 mg, 6× was 7.6 mg, 3× was 3.9 mg, 1× was 1.3 mg, and 0.25× was 0.3 mg. 'Cricket fragments' indicates workers delivering cricket parts that had been artificially fragmented beforehand, whereas 'Carrier' indicates workers delivering fragments cut by other workers. 'Cutter' indicates workers fragmenting the cricket body. Level of significance is calculated from the likelihood ratio test for the generalized linear mixed models ($\chi^2 = 297.75$, $df = 7$, $P < 0.001$) with the letters (a, b, c) represent the statistical significance based on pairwise differences (multiple comparisons conducted using a Tukey's honestly significant difference test).

developed ant colonies (Tschinkel, 2013). With the increased need for protein resources, the growth of worker size in well-developed fire ant colonies, which has been observed in *S. invicta* (Tschinkel, 1988), might be an adaptive response realized by an increased efficiency in dealing with invertebrate prey. The behavioural divergence of foraging workers might provide a possible adaptive link between size distribution and nutritional requirements, based on current understanding.

Approximately 15% of all ant genera (45/297) exhibit worker polymorphism (Hölldobler and Wilson, 1990). In the present study, we demonstrated two size-dependent groups of foraging workers and their different behavioural preferences for delivering and fragmenting prey. The physiological mechanisms underlying the size of the foraging workers are worth noting because the distinct groups might suggest two different developmental trajectories. Moreover, if the categorization is only based on the head
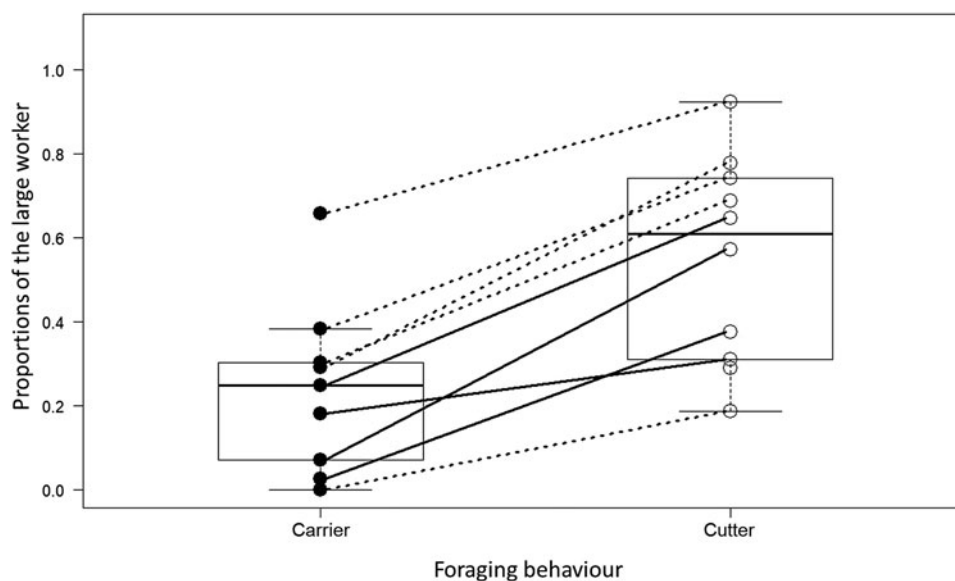
Figure 6. Proportion of large workers involved in delivering cricket fragments (carriers) and fragmenting the whole body of the cricket (cutters), respectively, among the 10 examined colonies. Data from the same colonies are linked by a line while the dotted lines indicate the data from five pre-examined colonies and the solid line indicated the four colonies whose data are also included in the analysis in fig. 5. One pre-examined colonies whose worker did not display the delivering behaviour was only showed the proportion of the cutters. Level of significance is calculated from the likelihood ratio test for the generalized linear mixed models ($\chi^2 = 161.94$, $df = 1$, $P < 0.001$).

width, the two distinct groups might not truly represent the labour division of tropical fire ants, while more factors (e.g. age polyethism) should be taken into consideration when investigating task partitioning inside a colony (Wilson, 1978). The majority of workers in fire ant species are small workers, consisting of various castes engaged in numerous tasks, including brood care, foraging and nest construction. The large foraging workers of *S. geminata* in the present study were observed cutting cricket prey into fragments during foraging. The small workers then transported these fragments back to the nest. Our results present an additional example of task partitioning during foraging in tropical fire ants with separate tasks conducted by different castes of ants.

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