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Foraging behavior and Preferences for Alternative Supplementary Feeds by the African Weaver Ant, *Oecophylla longinoda* Latreille (Hymenoptera, Formicidae)

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Abstract

Weaver ants, *Oecophylla* spp, are effective predators that control a wide range of insect pests in multiple crops when maintained at high population. Supplementary feeding, particularly during reduced food availability is one of the management practices that maintain and boost weaver ants' populations. Experiments were conducted between September and October 2013, January and February, 2014 to determine the type of food preferred by weaver ants, *O. longinoda*. Twenty colonies of *O. longinoda* were provided with four types of food to determine their feeding preferences. These include anchovy, chicken intestine, fish intestines and earthworms. We examined food preferred by ants based on weight of the food removed and activity of the ants on foods. Furthermore, we examined foraging behavior of ant workers on anchovy food (fresh-ground and dry-ground) in nine *O. longinoda* colonies. Thereafter, small and large particles of dried-ground anchovy were tested. The results showed that *O. longinoda* preferred anchovy to other foods provided. However, the results of Analytical Hierarchy Process showed that earthworm and fish intestine were the most accessible food types by farmers, as determined by availability, affordability and applicability. We observed more ants on fresh-ground as opposed to dry anchovy; similarly, large particles were more easily removed than was the case with small particles. Thus, during reduced food availability, farmers in the study area should use earthworms and fish intestines feeds to supplement *O. longinoda* colonies. Fresh moist anchovy or dry anchovy of large particle sizes can be used where available.

Keywords
African Weaver Ant, citrus, cashew, food preference, anchovy, Tanzania

Introduction

Two species of weaver ants, *Oecophylla longinoda* Latreille and *Oecophylla smaragdina* Fabricius are generalist predators that protect crops against insect pests (Way and Khoo 1991; Peng and Christian 2007; Van Mele 2008; Materu et al. 2014). The use of *Oecophylla* as a biocontrol can lead to increased fruit yield and quality (Barzman et al. 1996; Peng and Christian 2005; Olotu et al. 2013a). The ants prey directly on insect pests and obtain energy from honeydew produced by homopterans or from plant nectaries (Way and Khoo 1992). Crop protection is therefore more successful when there is a high and stable population of weaver ants. For instance, Stathers (1995) revealed that cashew trees colonized by high number of *O. longinoda* (>500 foraging *O. longinoda* workers) recorded lower damage by coreid bugs (*Helopeltis anacardii* Miller and *Pseudotheraptus wayi* Brown) than those with few *O. longinoda* (1-20). Adequate palm protection is realized when two or more nests of *O. longinoda* are found in the palm crown (Way 1953) or when 60–70% of the palms are colonized by *O. longinoda* (Way and Khoo 1992). As Sporleder and Rapp (1998) reported, the population of *P. wayi* sinks to zero after a long and stable occupation of palm trees by *O. longinoda*.

In addition to biocontrol, *Oecophylla* is used as a valuable source of food for humans (Sribandit et al. 2008), contributing directly to food security (Offenberg and Wiwatwitaya 2010) and also serve as a feed for song birds in Indonesia (Césard 2004). Populations of weaver ants in crop fields are, however, not stable, as they can commonly drop to very low levels in the field, resulting in inadequate crop protection. This can be caused by many factors one of which is movement of colonies to non-agricultural fields. Different management practices are being developed in order to maintain, boost, and expand the existing colonies to optimum levels. These management practices include artificial nests, (Offenburg 2014), the use of pesticides that are less harmful to the ants, protection of ants from competitors like black ant *Dolichoderus thoracicus* (Smith), facilitation of colony expansion by using strings and poles to connect trees (Van Mele and Cuc 2000; 2007) and maintenance of ground vegetation to control *Pheidole* spp (Way and Khoo 1992; Seguni et al. 2011). Furthermore, technologies for rearing weaver ants in nurseries are being developed (Peeters and Andersen 1989; Ouagoussounon et al. 2013)

Social insect populations are negatively affected when food is scarce (Dusstour and Sympson 2012). Weaver ant colonies may even move among trees in search of forage (Van Mele and Cuc 2007). The goal of biocontrol is to have large and stable colonies thus food supplementation may help to boost population size.

The feeding preferences of *Oecophylla longinoda* are not well known. In Vietnam, farmers provide fish and chicken intestines to *O. smaragdina* as supplementary foods during scarcity (Van Mele and Cuc 2000). Food supplementation (Van Mele and Cuc 2000; Lim 2007) or feeding (Offenburg and Wiwatwitaya 2010) of weaver ants

is reported to increase weaver ant populations. Furthermore, population increase of *O. longinoda* was reported in fed colonies (Abdula et al. 2015). However, weaver ants consume food in order of preference. *O. smaragdina* prefer mealworm to fish, honey or weaver ant formula (Lim 2007).

The practice of food supplementation for *O. longinoda* is limited by inadequate knowledge on food preference (based on cheap local available feeds), forms or states as well as particle sizes. Therefore, the objective of this study was to determine the type of food preferred by weaver ants in terms of form and size. A good understanding of food preferences by weaver ants and their foraging behavior is essential for effective management of *O. longinoda* during food scarcity.

Materials and methods

We conducted experiments at Naliendele Agricultural Research Institute (NARI), Mtwara Region, in Southern Tanzania (40°09'57.05"E, 10°21'22.49"S, 140 m asl). The region has a unimodal rainfall pattern, starting from November/December to April/May, with a single peak in January. The annual rainfall ranges from 810 to 1090 mm, whereas mean temperature ranges from 23°C in July to 27°C in December. Relative humidity ranges from 79% in October to 87% in March.

The study was approved by the Directorate of Research and Postgraduate Studies (DRPG) of SUA, a body responsible for monitoring and evaluating compliance to ethical conduct of staff and students undertaking research. The research complied with Code of Conduct for Research Ethics of Sokoine University of Agriculture (SUA) available at www.drpgs.suanet.ac.tz. Food preferences were tested for 10 days in orchards colonized by weaver ants. The tests were conducted for two seasons, between September and October 2013 (dry season), and between January and February 2014 (rainy season). Two orchards one of cashew, *Anacardium occidentale* L. and another of orange, *Citrus sinensis* L were selected.

In each orchard we selected trees with at least 40% of branches occupied by ants (as assessed by as per Peng et al. (2008). Each tree had between 5 and 25 nests. Each colony was provided with four types of foods; (i) earthworm (ii) chicken intestine (iii) fish intestine and (iv) anchovy.

Intestines and anchovy were ground by locally made mortar and pestle. Earthworms were dug out of wet soils (close to water ponds and irrigated fields) and chopped into small pieces (approximately 0.5–1 cm in length) by a kitchen knife. About 6 g of each food type were placed in a 0.01×0.1 m bowl, set on a feeding platform. Ants could access the bowls by crawling through a guiding stick. Feed bowls were placed equidistant from the middle of the feeding platform. Feeds and water were provided *ad libitum* throughout the experimental period. All the food types were tested in 10 colonies in each orchard. Preferences were determined by i) counting all foraging workers observed on food station and inserting their mouths into a food type and ii) weighing the amount of each food removed by the ants. Counting started 60 minutes after more

than one forager had discovered each food type. Thereafter, the weight of the remaining food in each bowl was determined. The amount of food type removed by the ants was determined by establishing the difference in weight between the food supplied and the food which remained in the bowl. In each case, the weight loss due to evaporation was deducted. Weight loss due to evaporation was determined in the control food types that were inaccessible by ants.

A sample of each food type was analyzed for nutrient compositions at the University of Dar es Salaam. The total carbohydrate, crude protein, total lipids (Fat) and vitamin A were determined according to the procedures described by Allen (1989). The total flavonoids were determined based on the procedures described by Bonvehi et al. (2001); the moisture content was determined gravimetrically after oven has dried at 105°C for 24 hours.

Furthermore, we used anchovy food to test for food forms and particle sizes that can be preferred by *Oecophylla longinoda*. Anchovy was used because it is processed in a standard form. We hypothesized that anchovy type (dry and fresh) affected the foraging behavior of workers. We also hypothesized that the particle size of dry anchovy affects the foraging behavior of workers. The experiments were conducted between May and July 2014. The first experiment involved two different forms of anchovy, dried and fresh. This experiment was conducted for 10 days, with the observation starting around 0900 am each day. The anchovy was sun dried (27–29 °C) for 7 days before grinding. We used fresh-ground anchovy of approximately similar size as the dried one. A Y- shaped feeding arena made up of wood was used as a feeding platform. A feeding bowl was placed on top of a board tied at each end of the Y shaped arena. This gave an equal chance for the workers to access each of the anchovy food types. Nine colonies were used. Each colony occupied at least two citrus trees. One bowl of each anchovy food was supplied per colony. The foraging behavior was assessed by counting workers carrying food particles from the source. The counting was done ten times at an interval of one minute (ten observations) every day per each colony for 10 days consecutively. In the end, we calculated the average number of foraging workers per minute per colony for a given food form.

The second experiment involved dried-ground anchovy of different particle sizes. The particles were measured by using laboratory test sieves (Wagtech International Ltd UK). Two particles sizes were selected; particles ranging from 0.5 to 1 mm in diameter (referred hereinafter as small particles) and; particles of 2 mm d (referred hereinafter as large particles). The experiment was conducted on six weaver ant colonies for 10 days using similar procedures for testing food types (above).

Thereafter, Analytic Hierarchy Process (AHP) (Saaty 1980) was used to determine the food type that would be accessed by the farmers. The set of evaluation criteria consisted of affordability, availability and applicability. The set of alternative options among which the decision was made consisted of four food types. The weights for each evaluation criterion were generated. The score for each criterion was assigned according to the pair wise comparisons of the options (on a scale of 1 – 9). Finally, the criteria weights and the options scores were used to compute the global score for a given option, as a weighted sum of the scores obtained with respect to all the criteria.

Data analysis

The analyses were performed using JMP 10.00 software. A non parametric one way ANOVA was used followed by Multiple Comparison-Wilcoxon Each Pair for counting forage workers and the amount of food removed under food preferences. Mann-Whitney tests were performed to compare the number of forage workers on dried and fresh anchovy; similar comparisons were done for small and large particle sizes.

Results

Nutrients composition of the feeds fed to the ants are presented in Table 1. Crude protein ranged from 47.7% (from fish intestine) to 31.2% (chicken intestine). All tested feeds, except earthworms contained flavonoids. The highest amount of flavonoids was 0.013 mg/g.

The numbers of workers foraging on food types were significantly (p=0.05) different in both citrus and cashew orchards, during both dry and rainy seasons (Tables 2, 3). Significantly, (p=0.05) more workers foraged on anchovy than they did on other food types. However, in citrus during dry season, the numbers of workers foraging on anchovy and chicken intestine were not significantly (p=0.05) different. The preference was the highest for anchovy and fish intestine and the least for earthworm and chicken intestine across both seasons and orchards except in citrus during the dry season (Figure 1a, 1b). The quantities of food types taken by ant workers were significantly (p=0.05) different in citrus but not in cashew orchard during the dry season (Tables 2, 3). In contrast, the quantities of food taken by ant workers in both orchards were statistically different (p=0.05) during the rainy season. Workers took significantly (p=0.05) more anchovy than they did to other food types. In all situations, the preference was the lowest for chicken and fish intestine (Fig. 2a, b). The results show further that more workers foraged on fresh-ground than they did on dried-ground anchovy (Fig. 3a). Similarly, more workers foraged on large particles than they did on small particles of ground-dried anchovy (Fig. 3b). The results of the AHP show that of the three criteria, earthworm ranked the highest followed by fish and chicken intestine.

Table 1. Nutrients composition of the feeds fed to ants.

Parameters	Food type Anchovy	Fish intestine	Chicken intestine	Earthworm
Crude Protein (%)	44.6	47.68	31.2	45.6
Total carbohydrate (g/100g)	0.2	3.1	6.21	0.01
Fat content(g/g)	0.059	0.078	0.087	0.005
Moisture content(%)	89.4	52.7	78.4	92.9
Vitamin A(mg/100g)	4.5	3.4	5.6	0
Flavonoids(mg/g)	0.013	0.0002	0.001	0.00
Energy(kj/g	9.711	11.414	9.553	7.834

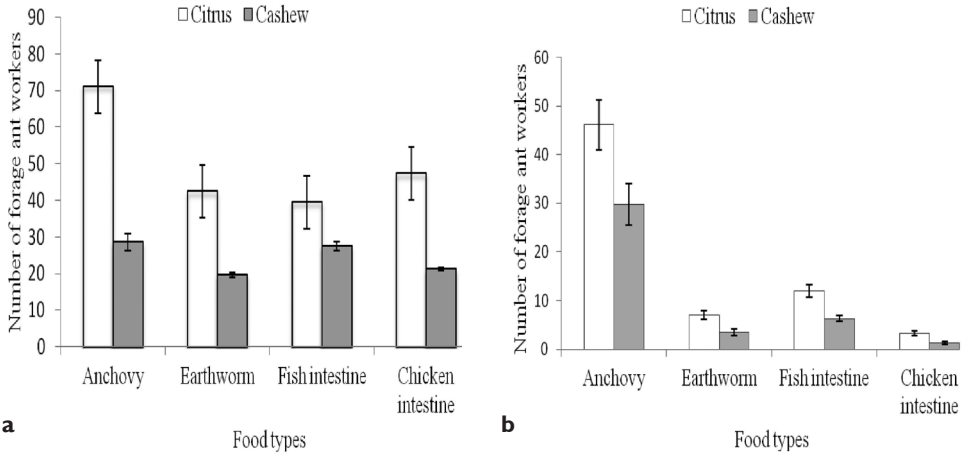

Figure 1. Number of Weaver Ant workers at food sources after 60 minutes foods supplied daily for 10 days between (**a**) September-October 2013 (dry season), and (**b**) January-February 2014 (rainy season) at Naliendele Citrus and Cashew orchard, Tanzania.

Table 2. The p-values for weaver ant counting and amount of food removed (g)/hour in 20 days between September and October 2013 and January and February 2014 in citrus orchard, Naliendele, Tanzania. (Kruskal-Wallis Multiple Comparison-Wilcoxon Each Pair test).

Food types	Citrus dry season		Citrus rainy season	
Pairwise comparison	Weaver ant counting (P-values)	Amount of food removed (g) (P-values)	Weaver ant counting (P-values)	Amount of food removed (g) (P-values)
Anchovy versus Chicken intestine	0.054	0.042	0.0002	0.0011
Anchovy versus Earthworm	0.021	0.108	0.0002	0.0011
Anchovy versus Fish intestine	0.014	0.0018	0.0003	0.0011
Chicken intestine versus Fish intestine	0.121	0.0095	0.0002	0.0011
Chicken intestine versus Earthworm	0.68	0.77	0.004	0.0011
Earthworm versus Fish intestine	0.33	0.014	0.0155	0.0011

Table3. The p-values for weaver ant counting and amount of food removed (g)/hour in 20 days between September and October 2013 and January and February 2014 in cashew orchard, Naliendele, Tanzania. (Kruskal-Wallis Multiple Comparison-Wilcoxon Each Pair test).

Food types	Cashew dry season		Cashew rainy season	
Pairwise comparison	Weaver ant counting (P-values)	Amount of food removed (g) (P-values)	Weaver ant counting (P-values)	Amount of food removed (g) (P-values)
Anchovy versus Chicken intestine	0.0013	0.51	0.0002	0.0002
Anchovy versus Earthworm	0.0006	0.817	0.0002	0.0002
Anchovy versus Fish intestine	1.00	0.86	0.0002	0.0002
Chicken intestine versus Fish intestine	0.0008	0.76	0.0002	0.0002
Chicken intestine versus Earthworm	0.068	0.84	0.023	0.0002
Earthworm versus Fish intestine	0.0003	0.92	0.005	0.0002

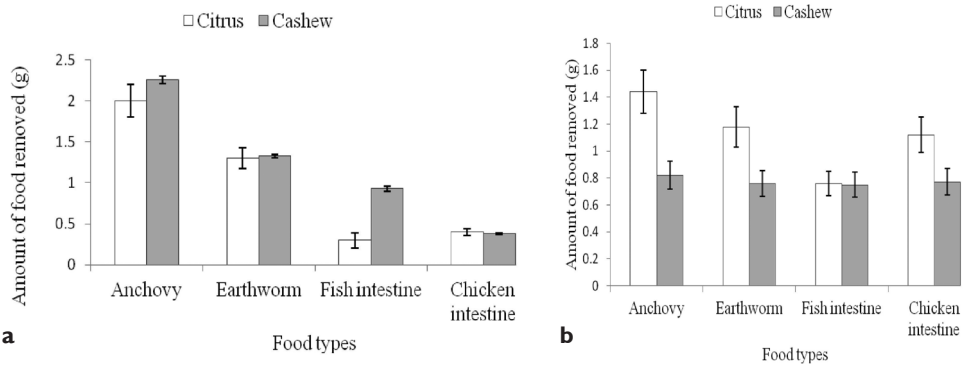

Figure 2. The average amount of food removed by the forage workers after 60 minutes foods supplied/day for 10 days between (**a**) September-October 2013 (dry season), and (**b**) January-February 2014 (rainy season) at Naliendele Citrus and Cashew orchard, Tanzania.

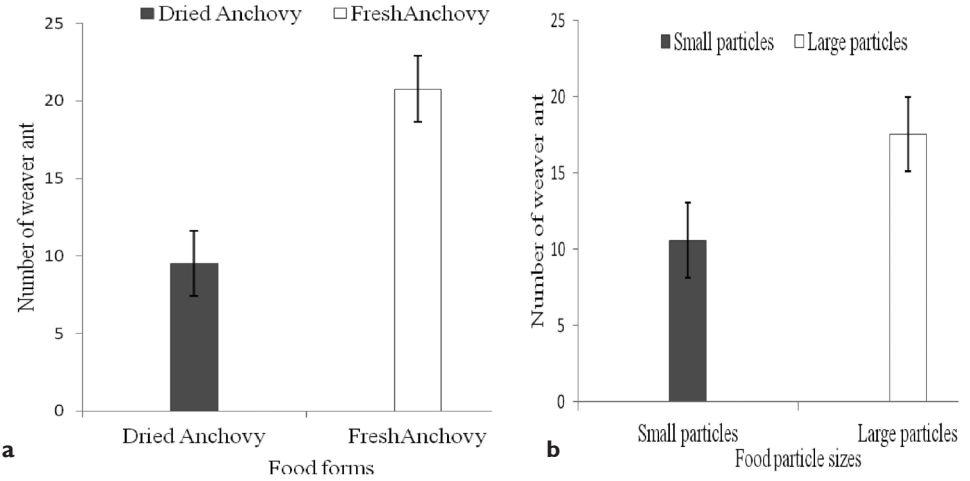

Figure 3. Food forms (**a**): Wilcoxon test; N=90; Prob.<0.0001, Food particle sizes (**b**) Wilcoxon test; N=60; Prob.<0.0001. Number of Weaver Ant counting /10 minutes for 10 days between May and July, 2014 for the given anchovy food forms and different particle sizes at Naliendele Citrus orchard, Tanzania, 2014. Large particle sizes refer to all particles that do not pass on a sieve of less or equal to 1mm, where as small particle sizes are those particle passes on 0.5 or 1 mm sieves.

Table 4. Analytical Hierarchy Process results on farmers access to food types for weaver ants.

Alternatives	Weights (Eigen Vector)*			Global score
	Affordability (0.539)	Availability (0.0845)	Applicability (0.126)	
Earthworm	0.59	0.63	0.41	0.42
Fish intestine	0.25	0.19	0.27	0.18
Chicken intestine	0.13	0.28	0.22	0.12
Anchovy	0.04	0.054	0.11	0.04

*Maximum Eigen Value = 0.24, CI = -1.09, CR = -0.27

Discussion

This study revealed that food preferences and foraging behavior by the *Oecophylla longinoda* can be influenced by food type, form, as well as particle size. Anchovy was highly preferred by *O. longinoda* in both citrus and cashew orchards during both dry and rainy seasons. The reason for forage workers' preference on anchovy is however not clear and could not be confirmed by this study. High preference for anchovy could be due to nutritional composition, particularly flavonoids or proteins. The foraging rate of *Pheidole megacephala* (Fabricius) depends on the type of protein (Cornelius and Grace 1997). It has also been reported that the velvety tree ant, *Liometopum occidentale* Emery prefers anchovy to earthworm (Hoey-Chamberlain and Rust 2014).

Anchovy was the highest preferred food across orchards and seasons but the order of preference for other food types in both orchards varied between seasons.

Chicken intestine was the least preferred during the rainy season. This is probably due to the fact that individual ant workers more easily remove fish intestine and earthworm than chicken intestine. Chicken intestine became stickier and bound to the food bowl during the rainy season. Thus, the removal of chicken intestine by foragers was difficult. Foragers spent time trying to take sticky-bound food items but they often failed. A temporal change in foraging activities was observed when the food became sticky and bound to the feeding bowl. At 15-30 minutes after food introduction, many ants were recruited and foraging activities increased with more ants observed on chicken intestine. However, as the food became stickier and bound to the feeding bowl, workers shifted to other food sources. It can be concluded that the nature of food at a particular time determines the foraging behavior of *Oecophylla longinoda* workers, and thereby influences preferences. Previous studies have shown that a large number of nest mates are recruited when ants are facing a non-transportable food items such as shrimps (Cerdá et al. 2009), but, foraging shifts were observed when other food sources were present. According to Lim (2007), ants choose food types which are easier to transport, that is, requiring less energy to remove and transport.

More anchovy was removed by ants as opposed to other feeds across seasons and orchards. The probable reason for this could be the form that anchovy assumes after being ground. Anchovy in the field became moist and grainy and could be removed without difficulty unlike the other food types. According to Hoey-Chamberlain and Rust (2014), the ease with which foragers are able to carry a particular type of food influences the amount of food to be consumed apart from food quality. However, inconsistency was observed for the rest of foods across seasons or orchards. For instance, similar amounts of earthworm and chicken intestine were removed in citrus during the dry season. On the other hand, ants removed more chicken intestine than fish intestine in citrus orchard during the rainy season. Furthermore, similar amounts of food types were removed during the dry season in the cashew orchard. A possible cause of the observed differences in food preferences across seasons could be colony needs at a particular time (Rust et al. 2000; Dussutour and Simpson 2008).

The quantities of food taken did correspond with the number of foraging workers, except for fish intestine in citrus orchard. We recorded more foraging workers on fish intestine than on chicken intestine and earthworm but the amount of food removed was smaller. Sometimes more ants visit particular feed but remove less (Neff et al. 2011).

In this study, we observed higher foraging activities on ground-fresh than on dry anchovy. These results support previous studies whereby three ants, *Linepithema humile* Mayr, *Anoplolepis custodiens* F. Smith and *Crematogaster peringueyi* Emery foraged more on liquid or moist food bait than on dry food bait (Nyamukondiwa and Addison 2014). Similarly, more activities for the ant, *L. lumile* were recorded on a 25% sugar solutions or honey than was the case with solid based protein foods such as tuna (Baker et al. 1985). It can be argued that, *Oecophylla longinoda* prefer fresh, moist foods than dried solid particles.

However, fresh-ground foods become sticky after some time, making it difficult for ant workers to remove them. Therefore, fresh-ground anchovy should be replenished to avoid stickiness; otherwise dried-ground anchovy should be used.

Forage workers easily collected and took large particles back to their nests in their mouthparts. However, they faced difficulties in collecting small particles and spent more time at the food bowl. A similar finding was reported for the fire ant *Solenopsis invicta* Buren (Neff et al. 2011).

Flavonoids have phytochemical properties against fungal, virus, and bacteria (Cushnie and Lamb 2005). They possess pharmacological activities such as antioxidant, anticancer and inhibition of tumor growth in mice (Shama 2006). They are considered to be an integral part of human diet (Arrabi et al. 2004). Therefore, flavonoid rich food types such as anchovy can be regarded as the best food to feed weaver ants. However, results of the AHP indicate that earthworm ranked the highest. This means farmers are more inclined to adopt the food type that is affordable and readily available. Earthworm is a cheap and widely available source of protein in the study area.

Conclusion

The results showed that all four tested feeds were removed by *Oecophylla longinoda* workers but anchovy was the most preferred. Considering the availability and affordability of the tested food sources, earthworms and fish intestine would be recommended as supplements during scarcity to boost weaver ants colonies on understanding that farmers preferred the less costly and sustainable option. All in all, fresh or dried-ground anchovy with particle sizes greater than 1 mm remains the best choice if availability and affordability are not subjects of concern.

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