

*Use of termites, Reticulitermes virginicus,
as a springboard in the invasive success
of a predatory ant, Pachycondyla
(=Brachyponera) chinensis*

D. M. Bednar & J. Silverman

Insectes Sociaux

International Journal for the Study of
Social Arthropods

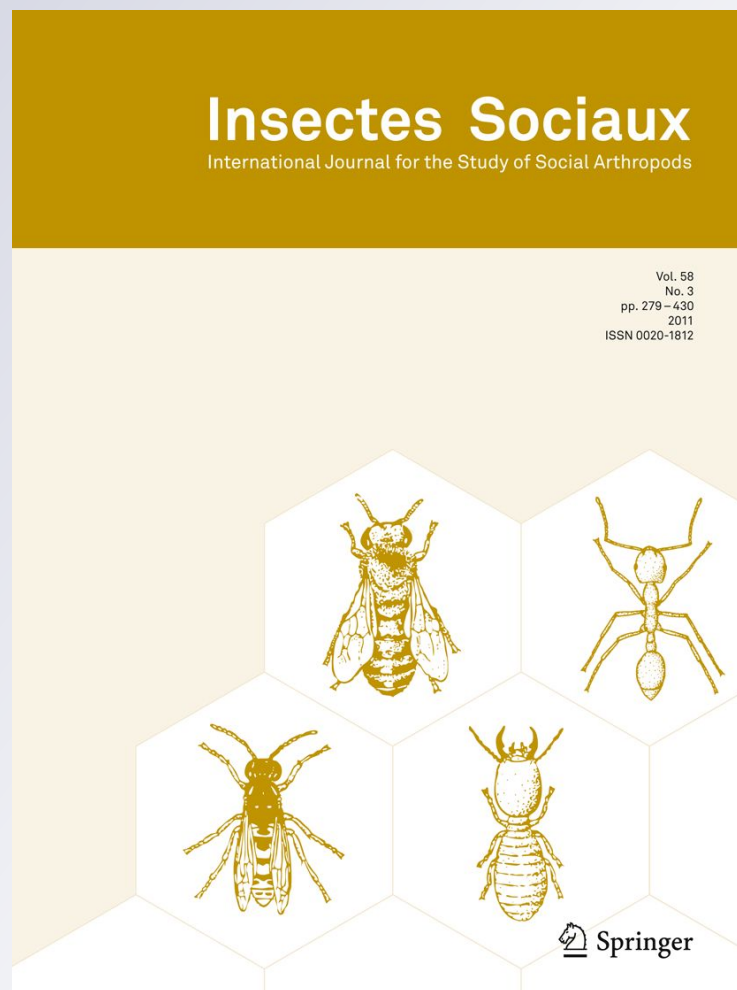
ISSN 0020-1812

Volume 58

Number 4

Insect. Soc. (2011) 58:459-467

DOI 10.1007/s00040-011-0163-0



Your article is protected by copyright and all rights are held exclusively by International Union for the Study of Social Insects (IUSI). This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your work, please use the accepted author's version for posting to your own website or your institution's repository. You may further deposit the accepted author's version on a funder's repository at a funder's request, provided it is not made publicly available until 12 months after publication.

Use of termites, *Reticulitermes virginicus*, as a springboard in the invasive success of a predatory ant, *Pachycondyla* (= *Brachyponera*) *chinensis*

D. M. Bednar · J. Silverman

Received: 9 September 2010 / Revised: 19 February 2011 / Accepted: 12 April 2011 / Published online: 4 May 2011
© International Union for the Study of Social Insects (IUSSI) 2011

Abstract Invasive ant species have general diet and nest requirements, which facilitate their establishment in novel habitats and their dominance over many native ants. The Asian needle ant, *Pachycondyla chinensis*, native throughout Australasia was introduced to the southeastern US where it has become established in woodland habitats, nests in close proximity to and consumes subterranean termites (Rhinotermitidae). *P. chinensis* do not occur in habitats lacking Rhinotermitidae. We suggest that subterranean termites are critical for *P. chinensis* success in new habitats. We demonstrate that *P. chinensis* is a general termite feeder, retrieving *Reticulitermes virginicus* five times more often than other potential prey near *P. chinensis* colonies. Odors produced by *R. virginicus* workers, as well as other potential prey, attract *P. chinensis*. Furthermore, *P. chinensis* occupy *R. virginicus* nests in the lab and field and display behaviors that facilitate capture of *R. virginicus* workers and soldiers. Termites are an abundant, high quality, renewable food supply, in many ways similar to the hemipteran honeydew exploited by most other invasive ant species. We conclude that the behavior of *P. chinensis* in the presence of termites increases their competitive abilities in natural areas where they have been introduced.

Keywords Invasive ants · Predation · *Pachycondyla chinensis* · Asian needle ant · Subterranean termites

Introduction

Recent work suggests that many successful invasive ants rely on a mutualism with Hemiptera for their invasion success (*Linepithema humile*: Grover et al., 2007; Tillberg et al., 2007; Rowles and Silverman, 2009; *Solenopsis invicta*: Helms and Vinson, 2008). Phloem-feeding Hemiptera, such as aphids or scale insects, produce honeydew as a waste product. Honeydew contains sugars, and several amino acids (Mittler, 1958). Ants are attracted to the honeydew and protect the hemipterans from predators forming a mutualism that has been studied extensively. Hemipteran honeydew is abundant and available throughout most of the year (year-round in some climates). This food source allows ants, such as *L. humile*, to grow massive colonies, and expand their territories (Morales, 2000; Helms and Vinson, 2002; Lach, 2005; Grover et al., 2007; Tillberg et al., 2007; Helms and Vinson, 2008; Rowles and Silverman, 2009). It has been suggested that one of the reasons that invasive ants tend to be dietary generalists is that such generalization makes them able to take advantage of hemipteran resources (Buckley and Gullan, 1991; Helms and Vinson, 2002; Lach, 2003). However, a “new” invasive ant, *Pachycondyla chinensis* appears to break this rule of hemipteran dependence.

One possibility is that *P. chinensis* simply succeeds in some other way. Success may hinge on a disruption in trophic interactions, parasitism, vectoring disease, or a combination of several complex mechanisms (Kenis et al., 2009). Another possibility, however, and the one that we explore here, is that *P. chinensis* actually uses termites much in the way that fire ants or Argentine ants use hemipteran exudates. Termites, like Hemiptera, present an excellent candidate as a springboard for invasive predatory ants.

Termite colonies in the southeastern US occur as frequently as 4.42 colonies ha⁻¹ with each colony containing

D. M. Bednar · J. Silverman (✉)
Department of Entomology, North Carolina State University,
Raleigh, NC 27695, USA
e-mail: jules_silverman@ncsu.edu

anywhere from a few thousand individuals to 9.5 million (Howard et al., 1982; Su et al., 1993). In some habitats they are as abundant as 10,000 individuals m^{-2} (Eggleton et al., 1996), are available year-round, extremely nutritive (Sogbesan and Ugwumba, 2008), and create suitable nest space for many ant species (Dejean et al., 1996). Several other *Pachycondyla* spp. including *P. commutata*, *P. laevigata*, and *P. marginata*, prey exclusively upon different termite species (Wheeler, 1936; Mill, 1984; Agbogba, 1992; Leal and Oliveira, 1995; García Pérez et al., 1997; Corbara and Dejean, 2000; Bayliss and Fielding, 2002). These reasons lead us to believe that termites are a better candidate than Hemiptera in providing predatory ants with a springboard to invasive success.

Pachycondyla chinensis, native throughout Australasia (Wheeler, 1930; Smith, 1934; Bolton, 1995), and one of the most common native woodland ants throughout Japan (Kubota et al., 2003), is now regarded as an invasive pest in parts of the eastern US (Guénard and Dunn, 2010). Recent evidence indicates that several native ant species, especially *Aphaenogaster rudis*, in southeastern US deciduous forests are negatively impacted by *P. chinensis* and it has been suggested that one mechanism by which *P. chinensis* influences other species is through competition for nest sites and food (Guénard and Dunn, 2010). Nests of *P. chinensis* occur on the forest floor within decayed logs. Subterranean termites in the genus *Reticulitermes* also frequently occupy these logs. Throughout their introduced range *P. chinensis* workers have been observed nesting with and carrying *Reticulitermes* spp. workers in their mandibles (Zungoli et al., 2005; Nelder et al., 2006). In Japan, where *P. chinensis* occur naturally, they have even been referred to as a “termite-hunter” (Matsuura, 2002). Most evidence concerning the diet of *P. chinensis*, is anecdotal, however, being restricted to a few observations, where they are described as generalists or scavengers (Smith, 1934).

Here, we test the hypothesis that the invasive ant, *P. chinensis*, is a generalized termite predator, which prefers termites as food but will accept other prey items. We test this hypothesis by examining the behavior of *P. chinensis* workers towards *Reticulitermes virginicus* (Isoptera: Rhinotermitidae) workers and other arthropods in decayed logs occupied by *P. chinensis* nests. We also examine the movement of *P. chinensis* workers into nests occupied by colonies of *R. virginicus*.

Methods

Collection of colonies and species determination

While *P. chinensis* were easily identified using morphological characteristics (Smith, 1934), *R. virginicus* and *R.*

flavipes could not be distinguished by site identification alone. Termites captured in the field were identified using molecular techniques described by Szalanski et al. (2003). Briefly, DNA was extracted from workers preserved in alcohol using Chelex-100 (Walsh et al., 1991). A portion of the COII region of the mtDNA was then amplified by PCR. The mtDNA was digested with TaqI polymerase and the fragments were electrophoresed on a 2.5% agarose gel and compared with known *R. flavipes* DNA profiles (Szalanski et al., 2003).

We created lab stock colonies of *P. chinensis* and *R. virginicus* from Lake Johnson State Park (35.45°N, -78.43°W) and from Schenck Memorial Forest (35.49°N, -78.43°W) in May 2008. Schenck Memorial forest is dominated by *Pinus taeda* (10–70 years). Lake Johnson State Park is dominated by oak (*Quercus* sp., ca. 85 years) and pine (*Pinus* sp.).

We separated *P. chinensis* (ca. 2,000) and *R. virginicus* (ca. 50,000) from each site. *Pachycondyla chinensis* stock colonies (ca. 1,000 workers, several queens, and brood) were kept in open plastic containers (76 × 76 × 6.4 cm) with plastic Petri dish nests (140 × 15 mm) containing moistened plaster and provided water and food, 10 *R. virginicus* workers/day plus artificial diet (Bhatkar and Whitcomb, 1970) ad libitum. *Reticulitermes virginicus* stock colonies were held in closed plastic containers (46 × 35 × 12 cm) with a moist cellulose matrix plus fragments of the wood they were extracted from. We maintained all insects at 26 ± 0.7°C and 80% RH with a 12:12 L/D cycle.

Pachycondyla chinensis acceptance of potential prey, including *R. virginicus*

A number of different arthropod species, including *R. virginicus*, were collected from logs with *P. chinensis*. Arthropods were removed from field debris and identified to order, and to family and genus, where possible. Potential prey that were recovered included Curculionidae (Coleoptera) adults, Pyralidae (Lepidoptera) larvae, Mycetophilidae (Diptera) larvae, Diplopoda, Machilidae (Archaeognatha), *Reticulitermes virginicus* (Isoptera) workers, Elateridae (Coleoptera) larvae, Trogossitidae (Coleoptera) adults, Chilopoda, *Parcoblatta* spp. (Dictyoptera) larvae, and Collembola. We excluded the several ant species found, due to the variation in species, size, distribution, and availability.

We placed individuals from the same source colony ($n = 5$; 1 queen, 25 workers, and 5 brood) of *P. chinensis* in a plastic fluon-coated tray (25 × 18 × 5 cm). Each tray was provisioned with a nest that consisted of a Petri dish (90 × 10 mm) with moistened cellulose. Ants were fed artificial diet (Bhatkar and Whitcomb, 1970) for 24 h, after which, diet was removed and experimental prey were

introduced. Of the arthropods recovered from logs with *P. chinensis*, we only tested those present year-round during our collections; *Reticulitermes virginicus* (workers), Elateridae (larvae), Trogossitidae (adults), Chilopoda, *Parcoblatta* spp. (larvae), and Collembola.

We performed a no-choice experiment with these putative arthropod prey species. We introduced a different prey type on each of five consecutive days. For example, on day 1, we introduced one *Parcoblatta* larva to each of the five *P. chinensis* colonies, Chilopoda on day 2, etc. Prey not retrieved (brought to the nest) within 10 min were removed. We introduced the next prey item after 40 min. The percent of each prey type retrieved was compared across all species using a χ^2 analysis in JMP (SAS-v7.0 2007).

Pachycondyla chinensis detection of *R. virginicus* and other prey olfactory cues

We evaluated *P. chinensis* worker response to *R. virginicus* worker scent in a Y-tube olfactometer. Three Petri dishes (50 × 9 mm) were connected via a plastic Y-tube (2.2 cm × 3 mm ID, 60° angle). We placed a termite worker in either the “right” or “left” dish ($n = 30$, 15 per side). We then introduced one *P. chinensis* worker to the “center” dish, and covered the dishes with their lids. We recorded the direction of travel (right or left) by the ant from the center dish. We considered that a choice was made when the terminus of the ant’s abdomen passed the intersection of the tube. Thus, we avoided recording “investigating” behavior, where ants placed their head in the intersection and antennated without proceeding. Ants, termites, and Y-tubes were replaced once a choice was recorded. We repeated this procedure using *Parcoblatta* larvae ($n = 20$, 10 per side), and collembolans ($n = 20$, 10 per side) paired with *R. virginicus* workers as well as cellulose powder previously occupied by termites (ca. 1 g dry wt.) paired with fresh autoclaved cellulose (ca. 1 g dry wt.) ($n = 20$, 10 per side). We first determined that there were no differences between right/left orientation using a χ^2 analysis, then pooled these data to compare the various choices made by *P. chinensis* workers with χ^2 in JMP (SAS-v7.0, 2007).

Pachycondyla chinensis nest choice assays

Termite monitoring stations

We used termite-monitoring stations in laboratory and field experiments to determine if *P. chinensis* moved into substrate containing termites. Each station contained a wooden block [southern yellow pine (*Pinus taeda* L.)] in the base, and a cartridge filled with cellulose in the space above the

wooden block. Subterranean termites (Rhinotermitidae) consumed the cellulose matrix and the wooden block, but nested within the wooden block (Rojas and Morales-Ramos, 2001).

Lab assay

Two stations were placed 10 cm apart in a plastic container (46 × 35 × 17 cm) with sandy soil (9,660 cm³). We removed the cellulose matrix from each station, then exposed 10 of these cellulose matrices to 1,000 *R. virginicus* each, for 30 days. The cellulose matrices with the termites were kept in separate containers until 24 h prior to the experiment. *Pachycondyla chinensis* ($n = 100$ workers, 1 queen, and 5 brood) were housed in Petri dishes (95 × 15 mm) containing moist plaster and covered with red cellophane.

Each of 10 replicate containers had one termite-containing cellulose matrix (TCM) and one without termites (CM) in the station, with a divider between the stations to prevent termite movement between cellulose matrices. After 24 h, we placed the dish with the *P. chinensis* colony between the two stations. Preliminary experiments revealed that *P. chinensis* moved out of a plaster nest into the cellulose matrix. Using instantaneous sampling, we recorded the position of worker ants within each container hourly on the first day (10.00–16.00 h) and then once daily (10.00 h) for 5 days. The measure obtained through this type of sampling is the proportion of sample points during which the behavior is occurring; for our experiment it is expressed as:

$\frac{P}{E}$, where P is the number of ants observed in a particular location and E is the number of sampling events. The resultant number has no units, but is a score of frequency for that behavior (Martin and Bateson, 1993). We analyzed these data using a Friedman test where frequency data were scored between locations and the χ^2 test statistic was calculated (Ambrose et al., 2002).

Field assay 1

We identified seven logs containing *P. chinensis* nests at three locations: Research Triangle Park (RTP) (1 log), Walnut Creek (WC) (3 logs), and Schenck Memorial Forest (S) (3 logs). On 1 March 2009, we buried monitoring stations in the soil parallel to each log 30 cm apart for a total of 10 stations per log, 5 stations on each side. Logs at each site were spaced at least 100 m apart, while a minimum of 6.7 km separated the three sites. Stations were inspected weekly for ants and/or termites for 3 months. After which time, the cellulose portions of the stations were returned to the laboratory and thoroughly inspected.

Field assay 2

After inspection of cellulose matrices ($n = 70$) from field assay 1, we found no arthropods. We then moistened the cellulose in all stations with distilled water. One-half of these ($n = 35$) were each exposed to 1,000 *R. virginicus* workers for 30 days. Termite-excavated galleries and termite workers were evident within the cellulose matrix by day 30.

The *R. virginicus*-infested and un-infested cellulose matrices were returned to the field and inserted within the stations as in field assay 1. In contrast to the previous assay, five of the stations on one side of the log contained termites while those on the other side did not. The stations with and without termites were assigned randomly. We recorded the presence or absence, as well as any visible counts of termites and ants in each station daily, for 6 days. The proportion of instances where ants were present in stations containing termites was compared to those without termites and analyzed using a χ^2 analysis in JMP (SAS-v7.0, 2007).

Pachycondyla chinensis worker foraging in the presence and absence of termites

We developed a laboratory assay allowing for direct observation of *P. chinensis* worker responses to *R. virginicus* workers and soldiers within their nest. Two hundred *R. virginicus* (198 workers plus 2 soldiers) were housed in Petri dish nests (60×15 mm) containing moist pine sawdust. These nests were connected to an empty Petri dish (50×9 mm) by plastic tubing (10×2 mm ID). *Pachycondyla chinensis* (100 workers, 1 queen, and 10 brood) were housed in a moist plaster-filled Petri dish (100×15 mm), which was attached to the above empty dish (60×15 mm) on one end and another empty dish distal to the termite nest. The empty dish on the end was provisioned with 25% sucrose solution and freshly killed cockroaches, *Blattella germanica*, ad libitum. Access of *P. chinensis* workers to the sawdust nest was prevented for 24 h prior to the observation period by blocking the tube entering the dishes with and without termites.

We recorded the behavior of *P. chinensis* workers at the entrance to the sawdust-filled nests with ($n = 5$) and without ($n = 5$) *R. virginicus*. Observations of *P. chinensis* contact with or entering the termite (or empty) nest entrance were recorded for 4 min every hour for 24 h. Each experimental set-up was replicated five times simultaneously. We considered that *P. chinensis* 'entered' the nest when the entire length of the body was within the nest. If an ant contacted the nest entrance and immediately entered the nest we only recorded this as an entering event. Data for the contact events were tested using repeated measures analysis of variance with termite-occupied and sawdust-only nests as

between-subjects variable and time the within-subjects variable. Data for the entering events were analyzed similarly. Other between-subjects variables tested were contact versus entering in the termite occupied treatment only with time as the within-subjects variable. We used the JMP (SAS-v7.0 2007) program to analyze these data.

Results

Pachycondyla chinensis acceptance of potential prey, including *R. virginicus*

Pachycondyla chinensis workers retrieved all (100%) of the *R. virginicus* presented. Significantly fewer ($\chi^2 = 95.8$, $P < 0.0001$) Collembola (20%), *Parcoblatta* spp. (8%), Chilopoda (16%), Trogossitidae (0%), and Elateridae (0%) were retrieved. It appeared that the hard exoskeleton of the Elateridae and Trogossitidae made penetration by the *P. chinensis* workers sting ineffectual, and these prey were typically ignored (64 and 40% of encounters, respectively). The size of the Collembola (ca. <1 mm) made them difficult for the ants to discover but easy to capture; in contrast *Parcoblatta* nymphs were large (ca. 15 mm) and fast making them difficult to catch, sting, and retrieve.

Pachycondyla chinensis detection of *R. virginicus* olfactory cues

Pachycondyla chinensis chose the direction towards the dish containing *R. virginicus* 80% of the time regardless of left/right orientation of termite placement ($\chi^2 = 30$, $df = 1$, $P < 0.0001$), revealing that *P. chinensis* detected *R. virginicus* from a distance of at least 7 cm. This confirmed our observations during the prey acceptance study, where *P. chinensis* foragers appeared to 'track' *R. virginicus* workers. However, when presented with a choice between *R. virginicus* workers and *Parcoblatta* nymphs ($\chi^2 = 1.8$, $df = 1$, $P = 0.17$) or collembolans ($\chi^2 = 0.05$, $df = 1$, $P = 0.81$) *P. chinensis* did not choose the direction of the termites more frequently. Similar results were obtained when *P. chinensis* workers were exposed to cellulose powder fed on by termites compared to that which had not been ($\chi^2 = 0.8$, $df = 1$, $P = 0.37$).

Pachycondyla chinensis nest choice assays

Lab assay

Most *P. chinensis* workers vacated the plaster nest and moved into the cellulose matrix containing *R. virginicus* (TCM) within the first 5 h of introduction to the arena. We continued recording their location during the next 5 days to

see if the ants would continue inhabiting the termite nest. *Pachycondyla chinensis* were recorded in the TCM during the entire 5-day period. We were able to reject the null hypothesis that the frequency of *P. chinensis* workers would be found at equal rates between the three locations ($\chi^2 = 10.95$, $df = 2$, $P < 0.05$). We observed *P. chinensis* workers in the TCM ($n = 80$, avg. freq. = 0.46) almost two and one-half times more often than the CM ($n = 80$, avg. freq. = 0.20). We also, observed worker *P. chinensis* carrying sister workers in their mandibles from their original nest into the cellulose matrix occupied by *R. virginicus* as well as into the unoccupied cellulose matrix. Preliminary results indicate that these recruited workers dig tunnels in new nest space, and/or transport dead termites.

Field assay 1

Bait stations that were neither inoculated with *R. virginicus* nor moistened never contained *P. chinensis* or *R. virginicus* during the 3-month pretrial period.

Field assay 2

Seventy-five percent of the stations inoculated with *R. virginicus* were occupied by *P. chinensis* workers, whereas zero percent of stations lacking termites contained *P. chinensis* ($\chi^2 = 41.31$, $P < 0.0001$). After 6 days, 49% of the stations that had been inoculated with termites no longer contained termites, leading to a 57% decrease in stations occupied by *P. chinensis* between day 3 and 6.

Pachycondyla chinensis worker foraging in the presence and absence of termites

The first *P. chinensis* worker that encountered the termite nest entrance behaved in an excited manner while circling the entrance several times. When a termite worker came to the entrance, presumably to extend the tunnel, it was seized and stung. The termite was released after it became immobilized, whereupon the ant again circled the nest. After ca. 2 min the worker collected the dead termite and returned it to the *P. chinensis* nest. Once inside the ant nest many workers pulled at the termite with their mandibles and began feeding upon it. Approximately five workers then rapidly evacuated the nest. The first worker that brought back the termite did not lead or carry any workers back to the termite nest. *Pachycondyla chinensis* workers that stung and killed *R. virginicus* at the nest entrance did not carry them back to the nest immediately. Frequently, other *P. chinensis* carried the dead termites back to the ant nest. After ca. 25 min ca. 10 workers were at the *R. virginicus* nest entrance, removing termites as they appeared.

Despite no active recruitment, there was a dramatic increase in foragers at the termite nest entrance within the first hour. *Pachycondyla chinensis* workers were three times more likely to contact the termite nest entrance than the unoccupied nest during the 24 h of observation ($F = 6.73$, $df = 1,8$, $P = 0.0319$, Fig. 1a). When other workers contacted the termite nest entrance their behavior was similar to that of the initial worker, including excited circling, and the occasional removal of foraging termites. Interestingly, *P. chinensis* workers did not enter termite-occupied versus unoccupied nest nests more often ($F = 1.04$, $df = 1,8$, $P = 0.3379$, Fig. 1b). We demonstrated that, in the presence of termites, ants were six times more likely to contact the nest entrance rather than enter it over a 24-h period ($F = 10.5$, $df = 1,8$, $P = 0.0118$).

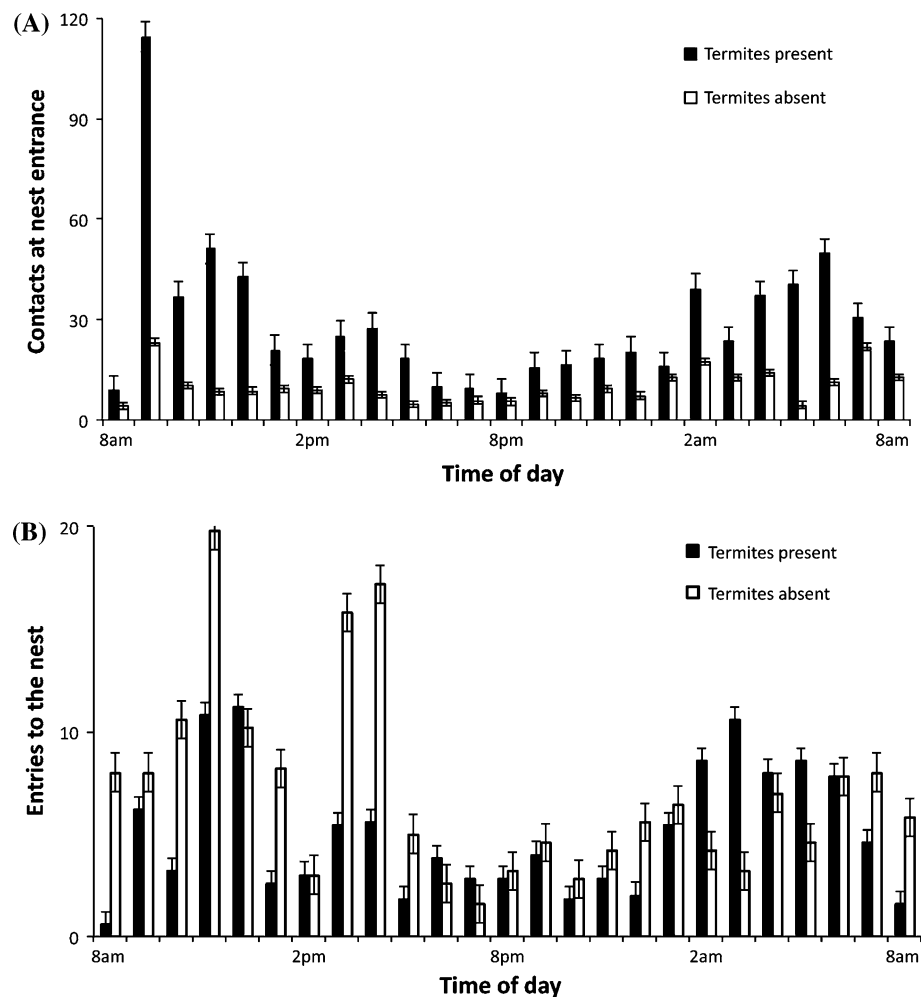
Discussion

We provide four lines of evidence that subterranean termites are an important resource for *P. chinensis* and thus may aid in their successful establishment and spread throughout new habitats. First, of the potential prey near *P. chinensis* colonies, *R. virginicus* workers were preferred. Second, *P. chinensis* oriented towards *R. virginicus* from a distance in an olfactometer, suggesting the ability to detect termite odors as well as other prey items. Third, *P. chinensis* workers occupied *R. virginicus* nests in the lab and field. Finally, *P. chinensis* workers elicit behaviors enabling them to capture *R. virginicus* individuals.

In our laboratory assay, *P. chinensis* largely ignored many different arthropods considered as potential prey occurring in and around logs. It is possible that during our field surveys we missed collecting some important arthropod prey; however, if these prey were indeed an important part of the diet of a large invasive ant colony they should be fairly conspicuous. We showed that *P. chinensis* workers exhibit plasticity in their feeding habits, attacking several types of prey introduced, but were only able to retrieve termites consistently. *Parcoblatta* only exist at moderate densities (60 cockroaches m^{-2}) (Horn and Hanula, 2002); and Collembolans occur at lower densities (82 collembolans m^{-2}) (Hansen et al., 2001), are very small in size, and appear difficult for *P. chinensis* to detect. Neither *Parcoblatta* nor Collembola are as abundant as termites (ca. 10,000 m^{-2} ; Eggleton et al., 1996). For these reasons we conclude that termites make the most suitable and accepted prey items for *P. chinensis* colonies establishing in new habitats.

Many invasive ant species decimate local arthropod fauna (Holway et al., 2002) and subsequently switch their diet to that largely comprised of hemipteran honeydew (Abbott and Green, 2007; Tillberg et al., 2007); an

Fig. 1 Mean observations ($n = 5$) every 4 min where *P. chinensis* workers contacted (a) and entered (b) the sawdust nest entrance in the presence (solid bars) and absence (open bars) of *R. virginicus* during a 24-h period



abundant, high quality, renewable food supply. We found no evidence that *P. chinensis* workers tended honeydew-excreting hemipterans at or above the soil surface. Ponerine ants in general do not tend hemipterans because they do not possess a crop (Hölldobler and Wilson, 1990). The crop allows ant species to share liquid resources between nest mates through trophallaxis (Paul and Roces, 2003). It is unlikely that hemipteran honeydew (comprised mostly of liquid simple carbohydrates) is a significant dietary component.

Yet, *R. virginicus* and other termites may provide benefits to *P. chinensis* that are similar to the value of hemipteran honeydew for other invasive ants: they are abundant (Wood and Sands, 1978), of high nutrient value (Sogbesan and Ugwumba, 2008), easily processed (possessing thin cuticle), difficult to overexploit [refuge in narrow galleries and have a defensive soldier caste (Prestwich, 1984; Jaffe et al., 1995; Matsuura, 2002; Buczkowski and Bennett, 2008)] and renewable (numerous progeny produced year-round) (Matsuura et al., 2009). However, it is likely that termites increase the ability of

P. chinensis to produce more workers and gynes as in other specialists (for example, *Plectrotena minor*: Suzzoni et al., 2000), thus providing a springboard for their success. The access to a high quality food source year round allows invasive ants, like *P. chinensis*, to exploit dietary nitrogen. Native ant species may also be reliant on termites as a source of nitrogen (Davidson, 1997; Blüthgen et al., 2003; Palmer, 2003; Dejean et al., 2007).

Pachycondyla chinensis workers oriented to *R. virginicus* workers prior to physical contact, suggesting a role for olfactory cues in prey detection. However, the response to prey items is most likely general, i.e. to CO₂. We determined, in our olfactometer experiment, that *P. chinensis* workers detect odorants emitted by all prey items separate from its nesting material. Such a general response likely places *P. chinensis* in close proximity to most any prey item with the most abundant prey, e.g. termites, located most frequently. While we determined that the ants moved into nests containing termites, it was interesting that *P. chinensis* workers did not respond differently to substrate previously occupied by termites.

We determined that *P. chinensis* workers moved into *R. virginicus* nests. Nests, both occupied and abandoned by termites, provide shelter for invertebrates (Dejean et al., 1996) including 75 different species of ants, as well as non-native termites (Dejean and Féneron, 1999). Upon inspecting the stations occupied by *P. chinensis* workers in the lab and field, we found ants had excavated the cellulose matrix. Modification of termite tunnels by *P. chinensis* workers as they prey upon termites suggests a certain level of termitolesty.

Eurhopalothrix heliscata (Wilson and Brown, 1984), *Pachycondyla cafferaria* (Agbogba, 1992), and *Centromyrmex bequaerti* (Dejean and Féneron, 1999), exhibit varying degrees of termitolesty, where the ants live with and prey upon termites. Wilson and Brown (1984) suggested that by living close to their food source, *E. heliscata* might have a competitive advantage over other ant species. By occupying termite galleries, *P. chinensis* workers possess a competitive advantage over other ground-nesting ants that prey on termites, such as *Aphaenogaster rudis* s.s. (Buczowski and Bennett, 2007).

The strategy employed by *P. chinensis* coincides with that of *E. heliscata* and may, in part, explain the displacement of native ants by *P. chinensis*. We recorded a threefold increase in *P. chinensis* workers foraging at the entrance to *R. virginicus* nests compared with unoccupied nests. This behavior had not been previously recorded for other *Pachycondyla* species that mass raid when hunting for termites. Following discovery of a termite nest, a scout *Pachycondyla analis* deposits a pheromone trail while returning to its nest and then recruits a raiding party, which it leads to the termite nest. Ant workers enter the nest bringing termites back to the outside nest surface (Wheeler, 1936; Corbara and Dejean, 2000; Bayliss and Fielding, 2002). We did not observe mass nest raiding in *P. chinensis* workers. Instead, *P. chinensis* foragers amassed at the termite nest entrance and grabbed *R. virginicus* individuals as they appeared, rather than enter the nest to raid it. Many *P. chinensis* workers at the termite nest entrance may interfere with native ant species ability to prey upon termites.

Subterranean termites are widespread and abundant in the southeastern US (Watt et al., 1997) and thus may constitute a critical food source for *P. chinensis*, facilitating its establishment and spread in deciduous and coniferous forests. While subterranean termites may have a positive near-term impact on *P. chinensis*, invasive *P. chinensis* may deplete native subterranean termite populations, with cascading effects at other trophic levels. Subterranean termites burrow through soil and wood, consuming cellulose, and thus contribute to litter decomposition, soil aeration, and carbon recycling (Wood and Sands, 1978; Dejean et al., 2007). Other invasive ant species have been shown to reduce the number of termite colonies (Dejean et al., 2007).

Therefore, this ant may negatively impact long-term ecosystem processes.

Future work on the impact of *P. chinensis* on native termite populations should involve studies quantifying the effect of termite populations on *P. chinensis* colony establishment. A temporal sampling of termite colonies in areas where *P. chinensis* colonies are excluded compared to areas where they have been recently established over a 10–15 year period would give an indication as to what level of impact *P. chinensis* colonies have on long-term ecosystem processes. In contrast, population studies of *P. chinensis* colony establishment in areas with and without termites would be beneficial in knowing what habitat requirements are most beneficial to its persistence in new habitats.

Acknowledgments We thank R. Dunn and E. Vargo for providing improvements to the manuscript. J. Brightwell, M. Green, B. Guénard, P. Labadie E. Spicer and D. Tarcy provided technical assistance and C. Arellano provided statistical advice. This study was supported by the Blanton J. Whitmire Endowment at North Carolina State University.

References

- Abbott K.L. and Green P.T. 2007. Collapse of an ant-scale mutualism in a rainforest on Christmas Island. *Oikos* **116**: 1238–1246
- Agbogba C. 1992. Settlement in the prey termite nest by the ponerine ant *Pachycondyla cafferaria* (Smith), and tandem running signal analysis for the following ant. *J. Ethol.* **10**: 133–137
- Ambrose H., Ambrose K., Emlen D. and Bright K. 2002. *A Handbook of Biological Investigation*. Hunter Textbooks Inc. Winston-Salem, NC. 188 pp
- Bayliss J. and Fielding A. 2002. Termitophagous foraging by *Pachycondyla analis* (Formicidae, Ponerinae) in a Tanzanian coastal dry forest. *Sociobiology* **39**: 103–122
- Bhatkar A. and Whitcomb W.H. 1970. Artificial diet for rearing various species of ants. *Fla. Entomol.* **53**: 229–232
- Blüthgen N., Gebauer G. and Fiedler K. 2003. Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. *Oecologia* **137**: 426–435
- Bolton B. 1995. A taxonomic and zoogeographical census for the extant ant taxa (Hymenoptera, Formicidae). *J. Nat. Hist.* **29**: 1037–1056
- Buckley R. and Gullan P. 1991. More aggressive ant species (Hymenoptera: Formicidae) provide better protection for soft scales and mealybugs (Homoptera: Coccidae, Pseudococcidae). *Biotropica* **23**: 282–286
- Buczowski G. and Bennett G. 2007. Protein marking reveals predation on termites by the woodland ant, *Aphaenogaster rudis*. *Insect. Soc.* **54**: 219–224
- Buczowski G. and Bennett G. 2008. Behavioral interactions between *Aphaenogaster rudis* (Hymenoptera: Formicidae) and *Reticulitermes flavipes* (Isoptera: Rhinotermitidae): the importance of physical barriers. *J. Insect Behav.* **21**: 296–305
- Corbara B. and Dejean A. 2000. Adaptive behavioral flexibility of the ant *Pachycondyla analis* (= *Megaponera foetens*) (Formicidae: Ponerinae) during prey capture. *Sociobiology* **36**: 465–483
- Davidson DW. 1997. The role of resource imbalances in the evolutionary ecology of tropical arboreal ants. *Biol. J. Linn. Soc.* **61**: 153–181

- Dejean A., Durand J.L., and Bolton B. 1996. Ants inhabiting *Cubitermes* termitaries in African rain forests. *Biotropica* **28**: 701–713
- Dejean A. and Fénelon R. 1999. Predatory behaviour in the ponerine ant, *Centromyrmex bequaerti*: a case of termitolesty. *Behav. Process.* **47**: 125–133
- Dejean A., Kenne M., and Moreau C.S. 2007. Predatory abilities favour the success of the invasive ant *Pheidole megacephala* in an introduced area. *J. Appl. Entomol.* **131**: 625–629
- Eggleton P., Bignell D.E., Sands W.A., Mawdsley N.A., Lawton, Wood T.G., and Bignell N.C. 1996. The diversity, abundance and biomass of termites under differing levels of disturbance in the Mbalmayo Forest Reserve, southern Cameroon. *Phil. Trans. R. Soc. Lond. B.* **351**: 51–68
- García Pérez J.A., Blanco Pinon A., Mercado Hernández R. and Badii M. 1997. Predation of *Pachycondyla harpax* Fabr. (Hymenoptera: Ponerinae) on *Gnathamitermes tubiformans* Buckley (Isoptera: Termitidae) under conditions of captivity. *Southwest. Entomol.* **22**: 345–353
- Grover C.D., Kay A.D., Monson J.A., Marsh T.C., and Holway D.A. 2007. Linking nutrition and behavioural dominance: carbohydrate scarcity limits aggression and activity in Argentine ants. *Proc. R. Soc. B.* **274**: 2951–2957
- Guénard B. and Dunn R.R. 2010. A new (old), invasive ant in the hardwood forests of Eastern North America and its potentially widespread impacts. *PLoS ONE* **5**: e11614. doi:10.1371/journal.pone.0011614
- Hansen R.A., Williams R.S., Degenhardt D.C. and Lincoln D.E. 2001. Non-litter effects of elevated CO₂ on forest floor microarthropod abundances. *Plant Soil* **236**: 139–144
- Helms K.R. and Vinson S.B. 2002. Widespread association of the invasive ant *Solenopsis invicta* with an invasive mealybug. *Ecology* **83**: 2425–2438
- Helms K.R. and Vinson S.B. 2008. Plant resources and colony growth in an invasive ant: The importance of honeydew-producing Hemiptera in carbohydrate transfer across trophic levels. *Environ. Entomol.* **37**: 487–493
- Hölldobler B. and Wilson E.O. 1990. *The Ants*. Belknap Press, Cambridge, Massachusetts, 732 pp
- Holway D.A., Lach L., Suarez A.V., Tsutsui N.D. and Case T.J. 2002. The causes and consequences of ant invasions. *Annu. Rev. Ecol. Syst.* **33**: 181–233
- Horn S. and Hanula J.L. 2002. Life history and habitat associations of the broad wood cockroach, *Parcoblatta lata* (Blattaria: Blattellidae) and other native cockroaches in the coastal plain of South Carolina. *Ann. Entomol. Soc. Am.* **95**: 665–671
- Howard R.W., Jones S.C., Mauldin J.K. and Beal R.H. 1982. Abundance, distribution, and colony size estimates for *Reticulitermes* spp (Isoptera, Rhinotermitidae) in Southern Mississippi. *Environ. Entomol.* **11**: 1290–1293
- Jaffe K., Ramos C. and Issa S. 1995. Trophic interactions between ants and termites that share common nests. *Ann. Entomol. Soc. Am.* **88**: 328–333
- Kenis M., Auger-Rozenberg M.A., Roques A., Timms L., Péré C., Cock M., Settele J., Augustin S. and López-Vaamonde C. 2009. Ecological effects of invasive alien insects. *Biol. Invasions* **11**: 21–45
- Kubota M., Imai H.T., Kondo M., Onoyama K., Ogata K., Terayama M. and Yoshimura M. 2003. *Japanese Ant Image Database*. Japanese Ant Database Group (JADG).
- Lach L. 2003. Invasive ants: unwanted partners in ant-plant interactions? *Ann. Miss. Bot. Gard.* **90**: 91–108
- Lach L. 2005. Interference and exploitation competition of three nectar-thieving invasive ant species. *Insect. Soc.* **52**: 257–262
- Leal I.R. and Oliveira P.S. 1995. Behavioral ecology of the neotropical termite hunting ant *Pachycondyla (=Termitopone) marginata* - colony founding, group-raiding and migratory patterns. *Behav. Ecol. Sociobiol.* **37**: 373–383
- Martin P. and Bateson P. 1993. *Measuring Behavior – An Introductory Guide*. Cambridge University Press. 222 pp
- Matsuura K. 2002. Colony-level stabilization of soldier head width for head-plug defense in the termite *Reticulitermes speratus* (Isoptera : Rhinotermitidae). *Behav. Ecol. Sociobiol.* **51**: 172–179
- Matsuura K., Vargo E., Kawatsu K., Labadie P., Nakano H., Yashiro T. and Tsuji K. 2009. Queen succession through asexual reproduction in termites. *Science* **323**: 1687–1687
- Mill A.E. 1984. Predation by the ponerine ant *Pachycondyla commutata* on termites of the genus *Syntermes* in Amazonian rain-forest. *J. Nat. Hist.* **18**: 405–410
- Mittler T.E. 1958. Studies on the feeding and nutrition of *Tuberolachnus salignus* (Gmelin) (Homoptera, Aphididae) II. The nitrogen and sugar composition of ingested phloem sap and excreted honeydew. *J. Exp. Biol.* **35**: 74–84
- Morales M.A. 2000. Mechanisms and density dependence of benefit in an ant-membracid mutualism. *Ecology* **81**: 482–489
- Nelder M., Paysen E., Zungoli P. and Benson E. 2006. Emergence of the introduced ant *Pachycondyla chinensis* (Formicidae: Ponerinae) as a public health threat in the southeastern United States. *J. Med. Entomol.* **43**: 1094–1098
- Palmer T.M. 2003. Spatial habitat heterogeneity influences competition and coexistence in an African acacia ant guild. *Ecology* **84**: 2843–2855
- Paul J. and Roces F. 2003. Fluid intake rates in ants correlate with their feeding habits. *J. Insect Physiol.* **49**: 347–357
- Prestwich G.D. 1984. Defense mechanisms of termites. *Annu. Rev. Entomol.* **29**: 201–232
- Rojas M. and Morales-Ramos J. 2001. Bait matrix for delivery of chitin synthesis inhibitors to the formosan subterranean termite (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* **94**: 506–510
- Rowles A.D. and Silverman J. 2009. Carbohydrate supply limits invasion of natural communities by Argentine ants. *Oecologia* **161**: 161–171
- SAS Institute. 2007. *JMP*, Version 7. SAS Institute Inc., Cary, NC
- Smith M.R. 1934. Ponerine ants of the genus *Euponera* in the United States. *Ann. Entomol. Soc. Am.* **27**: 557–564
- Sogbesan A.O. and Ugwumba A.A.A. 2008. Nutritional values of some non-conventional animal protein feedstuffs used as fishmeal supplement in aquaculture practices in Nigeria. *Turk. J. Fish. Aqua. Sci.* **8**: 159–164
- Su N.-Y., Ban P.M. and Scheffrahn R.H. 1993. Foraging populations and territories of the eastern subterranean termite (Isoptera: Rhinotermitidae) in southeastern Florida. *Environ. Entomol.* **22**: 1113–1117
- Suzzoni J.P., Schatz B. and Dejean A. 2000. Essential and alternative prey in a ponerine ant: variations according to the colony life cycle. *C.R. Acad. Sci. III* **323**: 1003–1008
- Szalanski A.L., Austin J.W. and Ovens C.B. 2003. Identification of *Reticulitermes* spp. (Isoptera: Reticulitermatidae) from south central United States by PCR-RFLP. *J. Econ. Entomol.* **96**: 1514–1519
- Tillberg C.V., Holway D.A., LeBrun E.G. and Suarez A.V. 2007. Trophic ecology of invasive Argentine ants in their native and introduced ranges. *Proc. Natl. Acad. Sci. U.S.A.* **104**: 20856–20861
- Walsh P.S., Metzger D.A. and Higuchi R. 1991. Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* **10**: 506–513
- Watt A.D., Stork N.E., Eggleton P., Srivastava D., Bolton B., Larsen T.B., Brendell M.J. and Bignell D.E. 1997. Impact of forest loss and regeneration on insect abundance and diversity. In: *Forests and Insects* (Watt A.D., Stork N.E. and Hunter M.D., Eds), Chapman & Hall, London, U.K. pp 273–286

- Wheeler W.M. 1930. A list of the known Chinese ants. *Peking Nat. Hist. Bull.* **5**: 53–81
- Wheeler W.M. 1936. Ecological relations of Ponerine and other ants to termites. *Proc. Am. Acad. Arts Sci.* **71**: 159–242
- Wilson E.O. and Brown W.L. 1984. Behavior of the cryptobiotic predaceous ant *Eurhopalothrix heliscata*, n. sp. (Hymenoptera: Formicidae: Basicerotini). *Insect. Soc.* **31**: 408–428
- Wood T.G. and Sands W.A. 1978. The role of termites in ecosystems. In: *Production Ecology of Ants and Termites* (Brian M.V., Ed), Cambridge University Press, Cambridge, U.K. pp 245–292
- Zungoli P., Paysen E., Benson E. and Nauman J. 2005. Colony and habitat characteristics of *Pachycondyla chinensis* (Hymenoptera: Formicidae). In: *Proc. 5th Int. Conf. Urban Pests* (Young-Lee C. and Robinson W.H., Eds), Perniagaan Ph'ng @ P&Y Design Network, Malaysia. p 571