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Initial behavior in colony fragments of an introduced population of the invasive ant *Wasmannia auropunctata*

Comportement initial des fragments de colonies de populations introduites de la fourmi envahissante Wasmannia auropunctata

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ABSTRACT

We investigated in the laboratory the initial behavior of propagules of the invasive ant *Wasmannia auropunctata* in Cameroon where it has been introduced. Both workers and queens at first feigned death (*thanatosis*), and then the workers slowly moved around the experimental arena; the queens did the same about 10 seconds later. Each queen antennated selected workers that then aggregated together by grasping the hind leg of another ant with their mandibles. When encountering the queen again, the lead worker climbed up the queen's hind leg and onto her back, followed by some other individuals. The remaining workers followed the queen to a location in the experimental arena. When brood was present, the workers transferred it to this location. Orphaned workers did not aggregate, but gathered the brood together and took care of it. By permitting propagules to survive, these behaviors likely contribute to the success of *W. auropunctata* as an invader.

Mots clés :
Agrégation
Feindre la mort
Thanatose
Transport d'ouvrières
Invasion biologique
Petite fourmi de feu

RÉSUMÉ

Nous avons étudié le comportement initial des propagules de la fourmi envahissante *Wasmannia auropunctata* au Cameroun où elle a été introduite. Tout d'abord les ouvrières et les reines font les mortes (*thanatose*), puis les ouvrières bougent lentement dans l'aire expérimentale ; dix secondes plus tard les reines font de même. Chaque reine palpe certaines ouvrières qui forment des agrégats en leur saisissant les pattes postérieures avec leurs mandibules. À la prochaine rencontre avec la reine, la première ouvrière et quelques autres montent sur une patte postérieure de la reine puis sur son dos. Les ouvrières restantes suivent la reine dans un emplacement de l'aire expérimentale où elle s'installe. Les ouvrières transportent le couvain vers la reine. En absence de reine les ouvrières ne s'agrègent pas au début mais regroupent le couvain et en prennent soin. En permettant à la propagule de survivre, ces comportements contribueraient au succès de *W. auropunctata* comme envahisseur.

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1. Introduction

About 150 out of the approximately 12,600 known ant species have been transported and introduced into many parts of the world. Few of these colonies survived, although some of them proliferated quickly to become devastating invaders [1]. Biological invasions can be broken down into three distinct populational processes (i.e., arrival, colonization, and spreading) amongst which the colonization phase represents a critical period during which populations grow, and then expand their area of distribution [2]. To survive in a novel environment, a species must overcome a series of obstacles including the biotic and abiotic resistance of the recipient environment. Factors affecting the successful establishment of a species – as well as those that determine the expansion of their area of distribution and impact – have been well documented [2–9]. Among these aspects, the competitive ability of the introduced species and propagule pressure (i.e., the number of individuals initially released into a region where they are not native, or propagule size, plus the number of separate release events, or propagule number) are emerging as explanations. In ants, propagule size can correspond to the number of workers and/or queens being introduced within a single colony, or to the number of introduced colonies [10–14]. An important step, then, in developing strategies to prevent or mitigate future invasions is to reconstruct the history and routes of introduction of exotic organisms [15,16].

Native to South and Central America, *Wasmannia auropunctata* has been introduced into various island groups in the Caribbean and Pacific Oceans, Australia, Florida and Israel [17,18]. It has also been found in tropical green houses in Great Britain and Canada [19]. In Africa, it was first reported in Gabon [20] and later in Cameroon [21].

Recent observations in Cameroon indicate that this ant is spreading, and has invaded many more locations in the country (Tindo et al. unpub. data). However, probably due to its peculiar clonal reproductive strategy where all of the queens of one lineage are genetically identical [22,23], propagule pressure does not appear to play a role for *W. auropunctata*, and most of the ant's colonization success has been due to the single introduction of a pair of clones [15,23]. Nevertheless, because a queen without workers cannot successfully establish a colony [24], the initial behavior of introduced individuals is decisive in the group's survival since dispersal and disorientation can lead to the failure to colonize a new area. In human-disturbed areas of Brazil, a part of *W. auropunctata* native range, queens are known to transport up to six workers that aggregate in her presence [25].

The main objective of the present study was to document if aggregation and worker transportation exist in an introduced population of *W. auropunctata*. Indeed, this behavior permits the individuals in colony fragments to reorganize themselves and establish new nests, and so might facilitate the success of this ant as an invader, as suggested by Feitosa [25].

2. Material and methods

Thirty nest fragments containing workers, brood, and queens were collected from the field in Yaoundé, Cameroon (03°51'N, 11°27'E). Individuals were sorted, transferred to Petri dishes (Ø = 5.5 cm; Height = 1 cm) and observed through a stereomicroscope (Wild M3Z) in the zoology laboratory at the university of Yaoundé I. The interactions between workers and queens were recorded in the following combinations:

- young and old workers plus a queen;
- old workers and a queen;
- young workers and a queen;
- workers (young and old) without a queen;
- workers (young and old) plus brood without a queen;
- workers (young and old) plus brood and a queen.

When brood was present, a piece of moist paper was introduced into the experimental arena to serve as a refuge. An experimental unit consisted of 20 workers and two queens and was observed for 15 minutes. Thirty replicates were conducted for each combination. The duration of significant acts was recorded, and the means compiled and compared using Student's *t*-test (XLSTAT 2010 software).

3. Results

Data on the interactions between workers and queens are summarized in Table 1. In general, after being introduced into the experimental area, both the workers and queens at first immobilized themselves and curled up into a nymphal position, feigning death. The queens spent significantly more time motionless than the workers (5.50 ± 0.86 seconds; $n = 30$ versus 2.96 ± 0.90 seconds, $n = 120$; $t = 13.83$, df = 148, $P < 0.0001$). The workers then started moving slowly around the experimental arena. About 10 seconds later, the queen also began moving and antennated certain workers. The antennated workers then formed aggregates with no particular shape (2 to 6 per replicate, Mean ± SD = 3.63 ± 1.13, n =3 0; Fig. 1A). In these aggregates, individuals generally hold each other by a hind leg. The next time the lead worker encountered the queen, it seized her hind leg (Fig. 1B) and then progressively climbed onto the queen's back followed by other workers from the aggregate (Fig. 1C). In general, only antennated workers climbed onto the queen's back. Other workers followed this formation to a location in the experimental arena where the queen was finally covered by workers (Fig. 1D).

The queens spent significantly more time antennating young workers than she did antennating old workers (3.20 ± 0.91 seconds; $n = 97$ versus 1.34 ± 0.58 seconds; $n = 52$; $t = 13.36$, df = 147, $P < 0.0001$). In contrast to old workers that aggregate after being antennated by the queen, young workers did not aggregate and, consequently, did not climb onto the queen's back, but they finally joined her at a location in the experimental arena (Table 1).

In the absence of a queen, no particular formation by workers was observed. Both young and old workers moved slowly around the experimental arena, mutually

Table 1
Interactions between *Wasmannia auropunctata* workers and queens recorded in six different combinations. Observations were conducted using a stereomicroscope (Wild M3Z) in the zoology laboratory at the university of Yaoundé I in Cameroon. Each combination was repeated 30 times.

Behavioral acts	Young and old workers plus a queen (%)	Young workers and a queen (%)	Old workers and a queen (%)	Workers (young and old) without a queen (%)	Workers (young and old) plus brood without a queen (%)	Workers (young and old) plus brood and a queen (%)
Feigned death	100	100	100	100	100	100
Detection by contact	100	100	100	100	100	100
Short antennation	73.3		100			75
Prolonged antennation	26.7	100				25
Mutual antennation				100	100	
Self-grooming				100	100	
Care of queen		100				83.3
Aggregation	73.3		100			65.5
Climb onto the queen's back	13.3		16.7			12.5
Run after the queen	60		83.3			50
Brood transportation					100	16.7
Queen coverage	100		100			100

antennating one another and self-grooming, and moving the brood into the humid refuge provided in all cases (Table 1). In contrast, in the presence of the queen, the workers preferred taking care of the queen rather than the brood (Table 1). The brood was later transferred to the same location as the queen (83.3% of cases, $n = 30$), and only rarely did the queen later move toward the brood (16.7% of cases, $n = 30$).

4. Discussion

Feigning death is a form of self-defense used against predators by a wide range of taxa, including ants [3,26]; for example, young fire ants increased their probability of survival fourfold when they feigned death in the presence of aggressive neighbours [27]. In Brazil, *W. auropunctata* queens and workers reacted to a very strong disturbance (these individuals were gathered using Winkler extractors) by staying motionless during a few seconds prior to organizing themselves [25]. In contrast, in their introduced range, they feigned death (*thanatosis*), although, comparatively, we had disturbed them only slightly.

This study shows that the aggregation and worker transportation behavior known in *W. auropunctata* in its native range [25] also exists in an introduced range. This behavior, which is unique amongst ant species, may represent an organizational strategy designed to minimize the loss of individuals to predation or disorientation. It might allow the individuals in colony fragments carried long distances to reorganize themselves and find favorable sites for new nests, with an adequate number of workers present to lead the initial tasks in colony foundation. As suggested by Feitosa [25], the aggregation behavior is likely triggered by the emission of a volatile pheromone (i.e., an alkylpyrazine emitted from the mandibular gland; [28]). In our study it appears that, although they have no specific shape, after being formed, the aggregates are maintained through a contact pheromone while the aggregates around the queen may be maintained through a kind of queen pheromone.

Our results indicate that the presence of a queen is crucial to the initial organization of the propagule since in her absence no "elaborate" reorganization was observed. Workers that had not been antennated by the queen did not participate in the formation of the aggregate. However, despite the fact that the queen spent more time antennating young workers than she did antennating old workers, certain young workers did not participate in the formation of the aggregate indicating that they did not react to the queen's signal. Therefore, old workers with more experience are more numerous than young workers in the formation of the propagule.

It is known that *W. auropunctata* workers alone are incapable of founding a new colony and that independent colony founding is highly unlikely [5,24]. By rearing colonies of the Argentine ant that differed both in worker and queen number, Hee et al. [29] showed that queens need workers to survive and to successfully establish colonies, and that small propagules (i.e., queens accompanied by as few as 10 workers) have high survivorship rates and can grow quickly. Our study demonstrates that the presence of the queen is more decisive than that of the brood in the establishment of a new colony of *W. auropunctata*. Nevertheless, we also observed that workers from orphaned propagules gathered the brood together and took care of it, but we could not establish if this strategy permits the production of new queens and males as reported for other invasive species like the Argentine ant [30,31] and the pharaoh's ant [32,33]. Indeed, this strategy can increase the probability of small, isolated propagules successfully establishing themselves in new areas. As a follow-up study, it would be interesting to monitor in the laboratory the development of propagules made up of various combinations (e.g., present, absent, different quantities) of brood, workers and queens to evaluate their success and the production or lack of production of new queens and males in orphaned propagules.

In conclusion, feigning death and forming aggregates that include queens that transport workers likely contribute to the success of *W. auropunctata* as an invader.

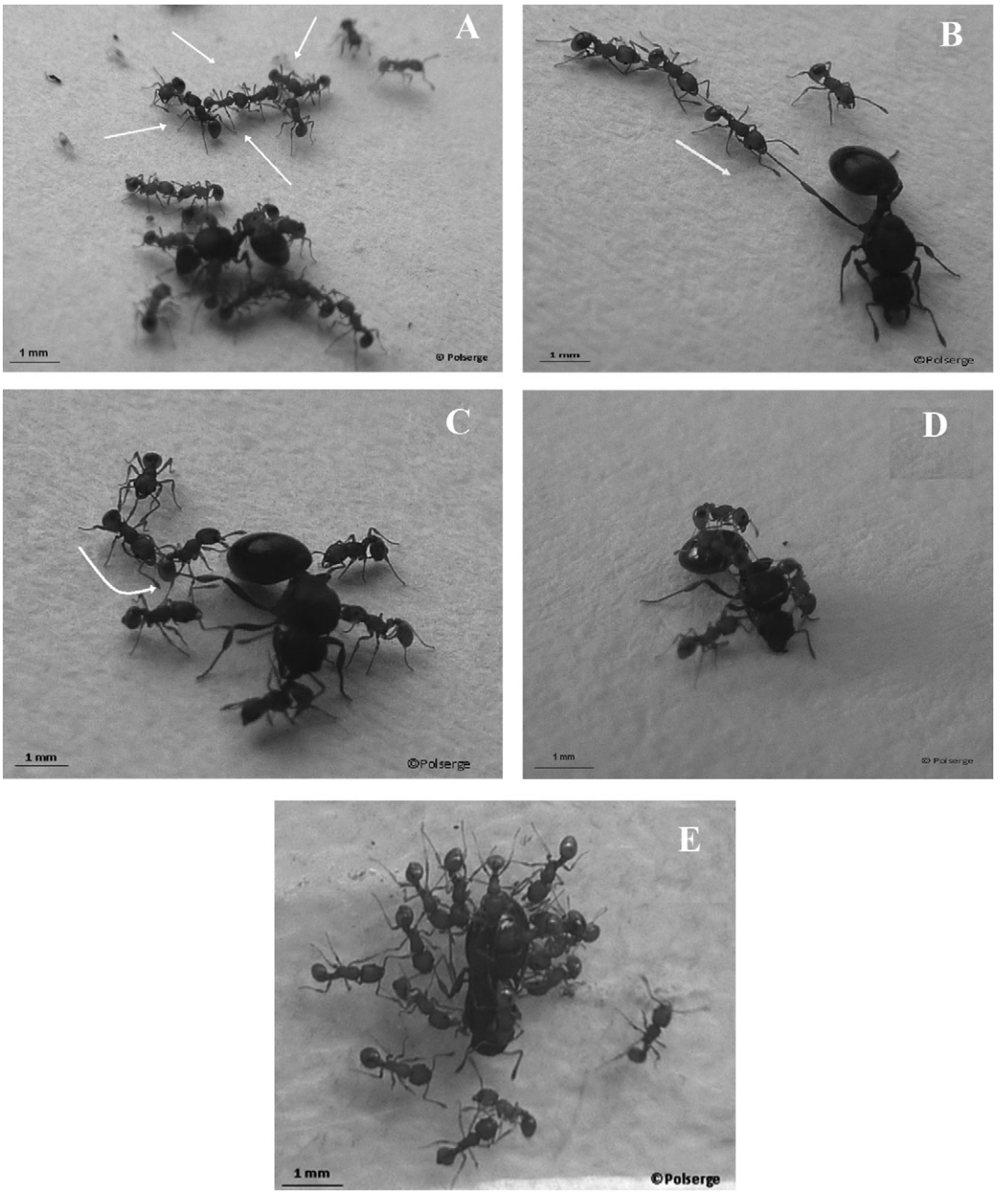

Fig. 1. Initial behavior in colony fragments of *W. auropunctata* in an introduced range. A. Aggregation of workers after antennation by the queen. B. The leading worker followed by other workers holding the queen's hind leg. C. The leading worker followed by other workers climbing onto the queen's back. D. Queen carrying three workers on her back. E. Aggregation of workers around the queen.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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