



## RESEARCH ARTICLE - ANTS

## Mating Behavior of the African Weaver Ant, *Oecophylla longinoda* (Latreille) (Hymenoptera: Formicidae)

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### Abstract

Mating in most species of ants occur during nuptial flights. In the African weaver ant, *Oecophylla longinoda* Latreille, mating has previously been hypothesized to take place within the nest before the nuptial flight but no research data has ever been presented to support this. Understanding the mating strategy of *O. longinoda* is important for its successful application in biological control programs. Here we report on the findings from studies conducted in Tanzania to determine whether mating occur prior to dispersal flight. Winged *O. longinoda* queens collected at four steps; before taking flight, immediately after leaving the nest, up to 12h after leaving the nest and after settling naturally following the dispersal flights were examined. Mating in captivity with varied number of males and queens was also assessed. Results showed that no eggs hatched from any of the 527 winged queens that were collected prior to their dispersal flights and no mating attempts in captivity lead to viable offspring. Only eggs produced by queens collected after settling naturally (N=65) hatched into larvae. High percentages (88.73) of eggs that hatched were laid by queens that shed wings and laid their eggs within 3 days after nuptial flights. Findings from the current study suggest that mating of *O. longinoda* queens take place during a nuptial flight and does not take place within the nest, as previously suggested. Time from nuptial flights to shedding of wings and egg laying translates to hatchability of the eggs.

### Introduction

The African Weaver Ant (AWA), *Oecophylla longinoda* Latreille (Hymenoptera: Formicidae) is a predatory species which controls a wide range of insect pests in multiple crops (Way and Khoo, 1992; Van Mele, 2008). The specie is increasingly being used in biological control programs in tropical plantations (Van Mele, 2008). A stable and high population level is usually required for effective control of insect pests by *O. longinoda* (Sporleder & Rapp, 1998). Introduction of *O. longinoda* into crop fields requires mapping of colonies in natural habitat, subsequent harvesting and transplanting. However, the transplantation of colonies into new plantations is hampered by the difficulties in finding the single maternal queen in colonies comprising up to several hundreds of nests (Ouagoussounon et al., 2013; Rwegasira et al., 2015).

*Oecophylla* spp. queens disperse from their natal colonies at the onset and/or during the rainy season (Vanderplank, 1960; Offenberg & Wiwatwitaya, 2010; Peng et al., 2013). The copulation of unrelated sexual forms during nuptial flights (Depa, 2006), provides an opportunity for genetic mixing and plays an important role in the life cycle of many ant species (Dhami & Booth, 2008). There are mainly two mating systems which have been reported in ants namely; male aggregation and female calling. Male aggregation (MA) is a system that winged males and females meet during nuptial flights. Females enter a swarm, which is dominated by males and subsequently mate with one or more of these males and return from the flight as fertile queens (Kaspariet al., 2001). Female calling (FC) is a system that winged females position themselves near their natal nests and emit pheromones to attract winged males with whom they mate, before they disperse or re-enter their



natal colony as fertile queens (Kaspari et al., 2001). In rare cases, mating occur within the nest, for instance in *Formica aquilonia* (Yarrow) (Forterius, 2005) and *Linepithema humile* (Myr) (Aron, 2001). In independent founding species, including *Oecophylla* spp., fertilized queens search for nesting places and shed their wings after the mating (Hölldobler & Wilson, 1978; Dhama & Booth, 2008). Queens then rear their first brood by using food reserves stored in the fat body and their wing musculature (Keller & Passera, 1989).

Mating strategy of *O. longinoda* has never been established. Understanding the mating strategy of *O. longinoda* is an important prerequisite for their successful use in integrated pest management (IPM), particularly in collection of mated queens to stock ant nurseries (Ouagoussounon et al., 2013; Peng et al., 2013). Way (1954) collected alate and dealated queens of *O. longinoda* in the field, during the ants' mating seasons in Tanzania which laid eggs that subsequently hatched into viable offspring. However, it was not known how and when mating occurred. Vanderplank (1960) hypothesized that mating by *O. longinoda* takes place within the nest, before dispersal of the queens. In a latter case, males liberated from their natal nests, disperse and enter into the nests of other colonies, where mating with alate queens takes place. Reported facts about sexuals of the closely related Asian green tree ant, *Oecophylla smaragdina*, unveiled that mating occurs during nuptial flights (Peng et al., 2013). Similar case would be true to *O. longinoda* but evidence based on data was required to disprove Vanderplank's hypothesis.

The current study aimed at examining the mating strategies of *O. longinoda* by testing the fertility of queens at different dispersal stages (pre-dispersing, dispersing, post-dispersing and natural settling).

## Materials and Methods

### *The study area*

Field Experiments were conducted from 2012 to 2014 at Naliendele Agricultural Research Institute (NARI) in Mtwara region, southern Tanzania (40°09' 57.05" E, 10° 21' 22.49" S). The region has a unimodal rainfall lasting from November/December to April/May and the mean annual rainfall ranges from 810 to 1090 mm. The mean maximum and minimum temperatures are 27 °C and 23 °C respectively.

### *Collection of queens at different stages of dispersal*

Winged queens were caught during different stages of their dispersal from their natal nests. Four different stages of nuptial flights were considered; i) pre-dispersal, ii) dispersal, iii) post-dispersal and iv) after naturally settling. In the first stage, alate queens were caught before dispersal, either from surface of the nests or from vegetation in the immediate neighborhood of nests. In the second stage, queens were

collected immediately after they took off from their nests. The queens were trapped with mosquito nets that covered cashew trees in the field as well as plastic sheets that covered potted mango and cashew seedlings. In the third stage, queens that attempted to disperse from four colonies established on cashew seedlings were collected from the roof of the screen house, twelve hours after each dispersal flight. In the fourth stage, queens were collected by using artificial nests, after settling naturally as described by Peng et al. (2013). In this technique, a total of 1000 artificial nests were constructed on 10 citrus trees.

### *Testing for fertility of queens collected at different stages of dispersal*

Each collected queen was kept in a match box (53x36x17mm) placed on tables in a screen house and protected from other ants as described by Way (1954). Queens were provided with distilled water soaked in a cotton wool every day. A 20% sucrose solution was also provided after the first workers emerged. Rearing continued for 60 days after the queens were collected. Number of queens that shed wings, numbers of queens that laid eggs, and queens whose eggs hatched and developed into larvae, pupa and imago were recorded. The mortality of queens after 60 days of development was calculated and compared between the tested groups.

### *Testing for fertility of queens in captivity*

Queens and males were collected from nest surfaces and nearby vegetation just before mating. A queen was paired with 0, 1, 2, 3, 4, or 5 males in a matchbox during the first mating season (December 2012 to April 2013). The same combinations of males were paired with two queens in a matchbox, except in the second season (December 2013 to April 2014). Each combination was made of two different kinship relations; i.e with queens and males originating from the same or different colonies and replicated three times. Queens and males were maintained for 60 days as in the queen fertility tests at varied stages of dispersal described above. The number of queens that shed their wings, numbers of queens that laid eggs, and queens whose eggs hatched and developed into larvae, pupa, imago workers or workers were recorded.

### *Developmental times of initial stages of weaver ants*

Queens were collected using artificial nests placed on citrus trees at Moma (040°18' 25.1" E, 10° 39' 71.7" S, 34 m asl) and Sogea (040°14' 71.4" E, 10° 37' 79.1" S, 142 m asl) sites in Mtwara from January to April 2015. Founding queens were reared in matchboxes placed on cages in the shade house. Number of queens that shed wings (n=103) as well as number of queens that laid eggs (n=103) within 3, 6 and above six days from nuptial flights were recorded. Also recording

was done on number of queens with hatched eggs from 10<sup>th</sup> to 14<sup>th</sup> and beyond 14<sup>th</sup> days after nuptial flights (n=69). The temperature during rearing period ranged between 28 and 29 °C. Queens were provided with distilled water *ad libitum*.

*Data analysis*

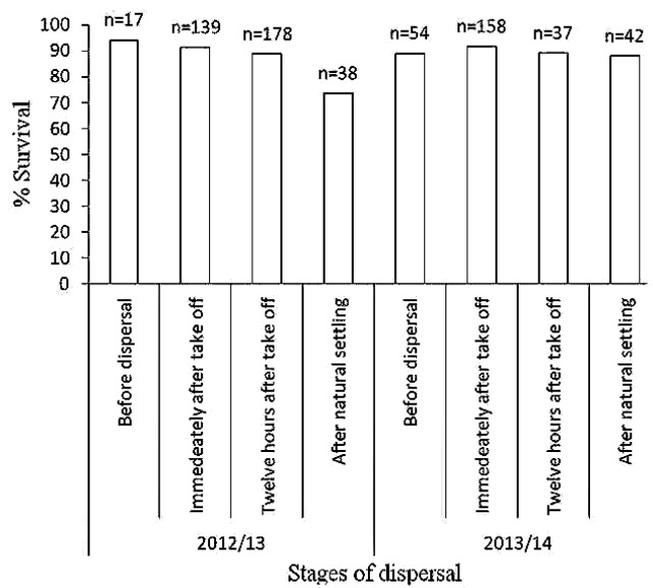
Collected data were analysed using JMP 10.0 software (SAS, 1995). Chi Square was used to test dependency of wing shedding, egg laying, egg hatching and queens’ survival, on different stage of queen dispersal. The dependency of wing shedding, egg laying and egg hatching, on days from nuptial flights were also analysed by Chi Square test.

**Results**

*Fertility status and survival of collected queens*

All collected queens produced eggs, regardless of stage of collection. Egg production was not dependent on stage of collection in both the first ( $X^2=7.418$ ,  $p=0.06$ ) and second ( $X^2=5.88$ ,  $p=0.12$ ) season. Only queens that settled naturally after dispersal flights produced viable eggs (Fig 1). The proportion of queens that produced eggs was higher than the proportion that shed their wings (Fig 1). The proportion of queens that shed their wings varied between 60.6 and 100%. Shedding of wings was significantly dependent on stage of queen collection in both first ( $\chi^2=24.83$ ;  $p<0.0001$ ) and second season ( $\chi^2= 28.60$ ;  $p<0.0001$ ). The highest proportion of queens that shed wings were those collected after settling naturally.

Results on survival of queens collected at the different stages of dispersal are presented in Fig 2. In all cases, survival was above 88 %, except for queens that settled naturally after dispersal (73.7% to 94.1). Survival of queens was significantly dependent on stage of collection in the first season ( $\chi^2=8.2$ ,  $p=0.043$ ) but not in the second season ( $\chi^2=0.80$ ,  $p=0.85$ ). Queens collected pre-dispersal to 12 hours after dispersal



**Fig 2.** Survival rates of queens caught at different stages of dispersal from their natal nests in 60 days of rearing.

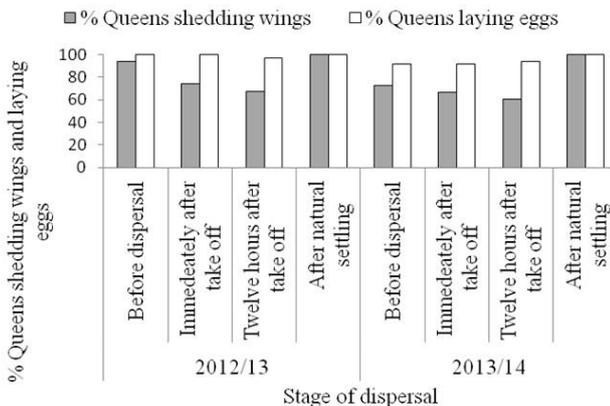
had higher survival rates while those collected after settling naturally had lowest survival rate.

*Mating in the captivity*

None of the males that were introduced to alate queens in match boxes survived, regardless of the combination. Males died within approximately two days after they were placed in the boxes of alate queens. Dead males were removed from the rearing chamber (match boxes). Many of the test queens (N=83) kept in captivity shed off their wings and initiated egg laying but none of them (N=90) produced viable eggs.

*Developmental times of eggs and wing shedding*

All the collected queens (experiment conducted from January to April 2015) laid eggs and shed their wings. However, only eggs from 71 queens were hatched out of 103, making eggs hatching successful by 68. 93 %. Wing shedding was significantly dependent on time from nuptial flights (Table 1). More queens shed their wings within 3 days after nuptial flights. Similarly, egg laying was significantly dependent on time from nuptial flights (Table 1). More queens (68.93%) laid eggs within 3 days after flights. A high percentage of eggs that hatched (88.73) were laid by queens that shed their wings and laid eggs within 3 days after nuptial flights ( $\chi^2=21$ ,  $p<0.0001$ ,  $n=71$ ). Only 11.27% of eggs that hatched were laid by queens between 4<sup>th</sup> and 6 days after nuptial flights. Egg hatching was also dependent on days from nuptial flights (Table 2). A significantly high number of queens had their eggs hatched from day 11 to 13 after nuptial flights. No hatching was recorded in eggs laid 6 days after nuptial flights.



**Fig 1.** Reproductive success of queens collected at different dispersal stages.

**Table 1.** Time taken for initiation of wing shedding and egg laying in founding *O. longinoda* queens.

Duration (days)	1-3 days	4-6 days	>6 days	Statistic	N
% Shedding wings	78.64 (81)	8.74 (9)	12.62 (13)	$\chi^2=52.12$ , df, 2, $p<0.0001$	103
% Laying eggs	68.93 (71)	13.59 (14)	17.48 (18)	$\chi^2=67.79$ , df, 2, $p<0.0001$	103

## Discussion

In this study only the queens that dispersed and subsequently settled naturally were able to produce viable offspring. These queens had an opportunity to fly to heights beyond 10 meters (the size of a shade house). This observation suggests that mating took place after their dispersal from the nest and that a certain flying height was needed before mating occurred. This correlates well with the Australian species, *O. smaragdina* that mate at some greater heights in the air and not inside their nests (M.G. Nielsen et al., unpublished data). Mating at great heights is not a unique character to *Oecophylla* species. Other ant species are known to fly high before mating. For instance, *Solenopsis invicta* has been reported to reach at height of 60-100 m during their mating flights (Dhamb & Booth, 2008).

The current study has shed light on the myth that surrounded the collected alate *Oecophylla* queens that produced viable offspring (Way, 1954; Lokkers, 1990) without clear understanding of the dispersal stage at which the queens were collected. It also brings to an end the speculation (Vanderplank, 1960) that mating by *O. longinoda* on Zanzibar took place inside their nests before dispersal. Since Vanderplank's alate queens were reportedly collected from the nest surface and that the queens subsequently produced viable offsprings with imago workers. Queens were collected after nuptial flights, and were most probably founding queens. Centrally to that, the difference between Vanderplank's and the present study might be hard to explain unless behavioral variation among populations exists. If the behavior observed by Vanderplank (1960) holds true, it would be a rare strategy only used by few species and not even by the sister species, *O. smaragdina* (Peeters & Molet, 2010).

**Table 2:** Time taken to hatching of eggs into larvae.

Eggs hatch day	Number of queens hatched	% queens hatched	Statistics
Day 10	2	2.9	
Day 11	11	15.94	
Day 12	16	23.18	$\chi^2=60.07$ , df, 5, $p<0.0001$
Day 13	32	46.38	
Day 14	4	5.8	
Above 14	4	5.8	
Total	69	100	

In the present study, many of the infertile queens shed their wings and laid eggs. Thus, wing shedding and egg-laying cannot be used as indicators of fertility or viability among *O. longinoda* queens. It is surprising that queens shed their wings while still unmated as they would need them in subsequent attempts to locate potential mating partners. Further studies would be needed to unveil triggers for wing shedding in queens which occurred regardless of mating or fertilization of the queen's eggs.

Queens that were allowed to settle naturally had lower survival rates exhaustion due to higher energy expenditure. As these queens had been on a mating flight and subsequently fed their developing larvae with food derived from their body reserves. They used more energy than queens that did not participate in mating flights. This higher energy consumption led to a slightly lower survival (King'ori, 2012).

All the founding queens laid eggs and shed their wings but only eggs from 71 queens (68.93%) were hatched. Thus, mating for viable offsprings may sometimes be unsuccessful. We hypothesized that hatching of the laid eggs is affected by the environmental conditions that prevailed in rearing units particularly the temperature. Further research might be needed to confirm this supposition. Time from nuptial flights to egg laying and shedding wing was associated with eggs hatching. Early laid eggs hatched successfully unlike late laid ones. No eggs hatched from any queen that started laying eggs beyond 6<sup>th</sup> day after the flight. High percentage (88.73%) of queens with hatched eggs laid eggs within three days following nuptial flights. This means that, eggs hatching can be predicted by more than 88 % within three days rearing.

Variation in time taken to egg hatching was also noted. More egg hatches were observed in the 11<sup>th</sup>, 12 and 13<sup>th</sup> days, with the highest on the 13<sup>th</sup> day. These results contrast with Vanderplank (1960) who reported that, time from nuptial flights to egg hatching is the same for all new queens reared at the same temperature. He reported that eggs from 24 queens reared at 28 °C took 6 and half days to hatch where as at 33 °C took only 4 days.

We therefore conclude that mating is most likely to take place after queens disperse. This is further supported by the fact that attempts to mate queens in artificial nests was unsuccessful, and that several hundred attempts to mate Australian and Thai *O. smaragdina* sexuals in artificial enclosures have been, equally, unsuccessful (Morgens G. Nielsen, Personal communication).

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