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Initiation of swarming behavior and synchronization of mating flights in the leaf-cutting ant *Atta vollenweideri* FOREL, 1893 (Hymenoptera: Formicidae)

Michael STAAB & Christoph J. KLEINEIDAM

Abstract

Leaf-cutting ants of the genus *Atta* build giant nests, inhabited by millions of workers. During a few days in spring, thousands of alates leave their mature home colonies for their mating flights. These flights are synchronized on a large geographical scale, and weather conditions have been reported to play a crucial role in determining when mating flights occur.

Nevertheless, many fundamental aspects of the fascinating swarming behavior in *Atta* are unknown. In this study we describe the three successive phases of the swarming behavior of *A. vollenweideri* FOREL, 1893, the initiation phase, the aggregation phase, and the mating flight. Prior to take-off, alates of both sexes exhibit distinct pre-flight behaviors. *Atta vollenweideri* is a day-flying species, with mating flights occurring in the late afternoon before dusk, and it is the southernmost species of the genus, experiencing strong seasonal climate. In order to identify climatic parameters that induce swarming behavior and elicit a synchronized mating flight, we analyzed 23 swarm events and the corresponding climate data from 2004 - 2010 recorded in northern Argentina. Colonies prepare for mating flights in the spring after a cumulative precipitation of at least 64 mm in the last month before the first mating flight. Only if temperatures rise above 26°C on the days following a major rainfall, alates may leave the nest mound, although they prefer temperatures of about 32°C. When accounted for together, rainfall and a subsequent temperature increase are highly predictive and thus prerequisites for swarming behavior in this species.

We propose that *Atta* species have based on the preferred depth of the founding chamber and local soil conditions species-specific thresholds for cumulative precipitation. In *A. vollenweideri*, the heavy clay soils that are desiccated after the austral winter select for mating flights and subsequent colony founding only after very high precipitation.

Key words: *Atta vollenweideri*, leaf-cutting ants, weather induction, synchronization, Formicidae, mating flight.

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Michael Staab (contact author), Department of Behavioral Physiology and Sociobiology, Biocenter, University of Würzburg, Am Hubland, 97074 Würzburg, Germany; Nature Conservation and Landscape Ecology, Faculty of Environment and Natural Resources, University of Freiburg, Tennenbacher Str. 4, 79106 Freiburg, Germany. E-mail: michael.staab@nature.uni-freiburg.de

Christoph J. Kleineidam, Department of Behavioral Physiology and Sociobiology, Biocenter, University of Würzburg, Am Hubland, 97074 Würzburg, Germany; Behavioral Neurobiology, Department of Biology, University of Konstanz, 78457 Konstanz, Germany.

Introduction

The nests of leaf-cutting ant colonies are conspicuous elements of the Neotropical fauna. Millions of workers inhabit numerous, interconnected nest chambers where they cultivate a symbiotic fungus on harvested plant material (WEBER 1972, HÖLLDOBLER & WILSON 1990, 2008). All the workers are offspring of a single queen, as all *Atta* species are strictly monogynous (BORGMEIER 1959, WEBER 1972, HÖLLDOBLER & WILSON 1990, 2008).

The life cycle of a colony starts with alates leaving their parental nest for the mating flight (MOSER 1967, MOSER & al. 2004). These mating flights, which last only for a few hours, are indispensable for mating. The flights are a decisive life stage for *Atta* queens, as mortality risk during the mating flight, nest foundation, and colony establishment is extremely high. More than 99.95% of *Atta* queens die during their first year, many of them during the few hours between emerging from their home nest and the foundation of a new colony (WEBER 1966, FOWLER & al. 1986). After a colony is established successfully, the queen uses the sperm stored from multiple matings during her mating flight to inseminate eggs during her entire lifetime of up to 15 years (FJERDINGSTAD & BOOMSMA 1998, DEN BOER & al. 2009).

Many aspects of the biology of *Atta* have been extensively studied (reviewed in WEBER 1972, HÖLLDOBLER & WILSON 1990, 2008), for example nest construction and nest ventilation (KLEINEIDAM & al. 2001, BOLLAZZI & al. 2012, COSARINSKY & ROCES 2012, PIELSTRÖM & ROCES 2013), organization of leaf-cutting and foraging (RÖSCHARD & ROCES 2003, 2011), the effects of worker polymorphism on the division of labor (WILSON 1980, KLEINEIDAM & al. 2007b), cultivar fungus genetic diversity (MIKHEYEV & al. 2007, 2010), fungus garden hygiene (BOT & al. 2001, CURRIE 2001, FERNANDEZ-MARIN & al. 2009, YEK & al. 2012), and mating system evolution (VILLESEN & al. 2002, BAER & al. 2009, DEN BOER & al. 2009, EVISON & HUGHES 2011). Nevertheless, little is known about the swarming behavior of leaf-cutting ant colonies.

Only MOSER (1967) performed a comprehensive study revealing the swarming behavior in *Atta texana* (BUCKLEY, 1860), a species that flies at dawn. Before and since then, only anecdotal records, mostly on the day-flying *A. sexdens* (LINNAEUS, 1758), have provided further information (e.g., EIDMANN 1932, GEIJSKES 1953, CHERRETT 1968, MOSER & al. 2004). Mating flights take place during a few days at the beginning of the rainy season, which for subtropical species occurs during the spring. The mating flights are highly synchronized within a colony as well as across different colonies at a large geographical range, thereby promoting outbreeding and the reduction of predatory pressure on alates by predator oversaturation (MOSER 1967, AMANTE 1972, MOSER & al. 2004).

Swarming behavior is triggered by weather conditions, and can only be observed after substantial rainfalls and the wetting of the upper soil layers (EIDMANN 1932, AMANTE 1972, MOSER 1967, JONKMAN 1980a, MOSER & al. 2004). This is not only true for *Atta* but appears to be a general phenomenon in ground nesting ants across various habitats all over the world (e.g., HÖLLDOBLER 1976, BOOMSMA & LEUSINK 1981, JOHNSON & RISSING 1993, KENNE & DEJEAN 1998, GÓMEZ & ABRIL 2012). On swarm days, aggressive workers gather on the nest surface and exhibit pre-swarming behavior (MOSER 1967, MOSER & al. 2004). Prior to the mating flight, first the males and then the gynes (i.e., virgin queens) accumulate at the nest mound. Likewise, the males take-off shortly before the gynes (MOSER 1967, MOSER & al. 2004). In all *Atta* species, gynes mate with multiple males high in the air (MURAKAMI & al. 2000, VILLESEN & al. 2002, EVISON & HUGHES 2011), but copulation has never been directly observed. Swarming behavior ends with the landing of the mated queens on the ground, where they dig themselves into the soil and build a founding chamber at a depth of about 30 cm (CAMARGO & al. 2011, FRÖHLE & ROCES 2012).

Substantial precipitation is necessary, but not sufficient, to initiate swarming behavior. Even after heavy rain falls, colonies may or may not initiate swarming behavior during the following days. Thus, besides rain, other factors, probably climate parameters, are being taken into account by leaf-cutting and other ants to decide on suited conditions for the timing of mating flights. Swarming days have been reported as warm, sunny, and calm (EIDMANN 1932, MOSER 1967, AMANTE 1972, BOOMSMA & LEUSINK 1981, MOSER & al. 2004, DEPA 2006), and ant alates have been reported to be more likely to fly at less suitable weather conditions towards the end of the mating season (BOOMSMA & LEUSINK 1981).

Of all subtropical leaf-cutting ant species, *Atta vollenweideri* FOREL, 1893 has the most southerly distribution range in the genus *Atta*. As a result, colonies face pronounced changes in weather conditions from cool and dry winters to hot and humid summers with large amounts of precipitation marking the shift from winter to spring. After the first heavy spring rainfalls, weather conditions are highly variable, allowing us to test which weather parameters are important factors in the initiation of mating flights. Hence, *A. vollenweideri* is a prime species to study swarming behavior in the genus *Atta*.

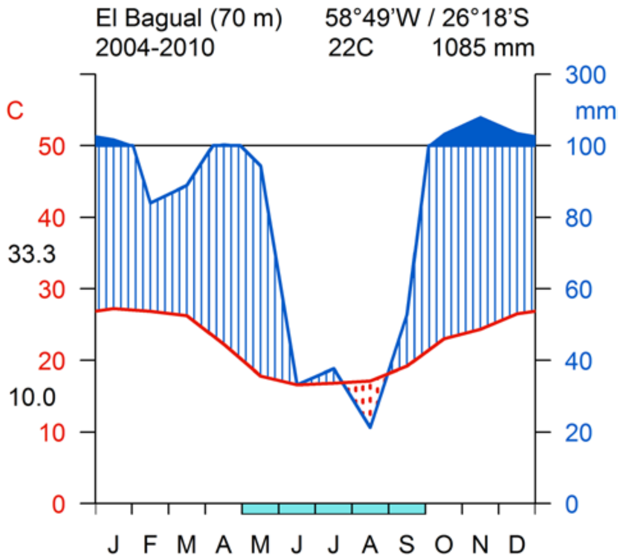

Fig. 1: Walter and Lieth climate diagram of monthly average temperature (red solid line) and precipitation (blue solid line) at Reserva Ecologica El Bagual.

In this study, we first describe the phases of the swarming behavior of *Atta vollenweideri* colonies and the distinct stereotypic behavior of alates prior to the mating flight. Secondly, we correlate climate data with 23 mating flights in order to identify the weather conditions that lead to the initiation of swarming behavior and that trigger mating flights.

Material and methods

Study site: The grass-cutting species *Atta vollenweideri* has its distribution range in the central part of South America from 19° to 31° southern latitude, mostly in the Gran Chaco region (BORGMEIER 1959, JONKMAN 1976, FOWLER & al. 1986). The predominant vegetation of the Gran Chaco is an open *Copernicia alba* MORONG, 1893 palm savanna with abundant patches of Chaco forest and reed-beds (SEIBERT 1997, MATURO & al. 2005).

Our study was conducted at the Reserva Ecologica El Bagual (58° 49' W / 26° 18' S; 70 m a.s.l.) from 2004 - 2010, and at the National Park Rio Pilcomayo (58° 10' W / 25° 07' S; 68 m a.s.l.) during October and November 2009. Both study sites are located in the Formosa province of northern Argentina and belong to the humid part of the Gran Chaco with a seasonal, subtropical climate. Mean annual temperature is 22.0°C, mean precipitation is 1085 mm, with a dry season from June to September (Fig. 1). During the rainy season, occasional flooding is common. Due to the open vegetation at our study sites, we could easily spot mature nests using satellite images from Google Earth® (compare aerial photographs in JONKMAN 1976) and visual inspection in the field.

Behavioral observations: In 2009, three mature nests with mound diameters of approximately 8 m were selected in the Rio Pilcomayo National Park for observation during the expected swarming period after the first heavy precipitation in the beginning of austral spring (JONKMAN 1980b). Prior to the mating flight, many workers gather at the top of the nest mound, indicating the beginning of swarming (MOSER 1967, FOWLER 1982, MOSER & al. 2004). In order to identify such behavior, nests were inspected in the afternoon around 13:00 and 17:00 from 3 October to 14 November 2009. Another early sign of swarming behavior is alates positioned about 20 cm inside the central tunnels of the nest at night, so nests were inspected again between 20:00 and 23:00.

Once workers started to appear on the nest mound, workers, and later alates were observed continuously until the end of the mating flights. Photographs were taken in order to document distinct behaviors of alates. Whenever we refer to gynes, we address unmated, virgin queens. A total of eight swarming events were observed closely on the three focal nests. At two nests three swarming events each and at one nest two swarming events were monitored.

Sex allocation: In order to assess alate numbers per colony, we used two different methods. Males aggregating on the nest surface before take-off were counted on entire nest overview photographs taken from close-by trees. As gynes do not aggregate densely on the nest surface (see below), we counted them in a given time interval on video recordings taken from the side of the nest during take-off. Observations from all mating flights of a given nest were pooled to estimate total alate numbers. To calculate total alate biomass we collected specimens from all three nests, killed them in a freezer and dried them at 55°C, males (n = 13) for 96 hours and gynes (n = 12) for 192 hours, to account for differences in body size. Individuals were weighted after drying, using a microbalance. Based on estimated mean individual numbers and mean dry weights per sex we calculated the operational sex allocation ratio with the methods described in detail by DIJKSTRA & BOOMSMA (2008). We also calculated the expected worker optimum sex allocation using an effective queen mating frequency of 2.5 based on literature values of other *Atta* species (FJERDINGSTAD & BOOMSMA 1998, 2000, MURAKAMI & al. 2000, HELMKAMPF & al. 2008), as there are no patrilineal data for *A. vollenweideri* available.

Recording and analysis of climate data: Our aim was to correlate different weather parameters with swarming behavior in order to test which of these parameters predict the behavior in a statistical model. Hence, climate data were recorded at both study sites using standard weather stations (Vantage Pro2 at Rio Pilcomayo National Park, MB3-LR Vantage Pro-R at Reserva El Bagual, both Davis Instruments®, Hayward, California, USA). We analyzed the following variables: precipitation, temperature, solar radiation, atmospheric pressure, relative humidity, and wind velocity. On 1 November, sunrise and sunset at our study sites are at 6:04 and 19:08 local time (UTC -3), respectively.

After the first rainfalls in spring, the heavy clay soil soaks up the water, and stays moist for prolonged time due to low soil water conductivity. To address this storage effect, we calculated the cumulative precipitation from the last 30 days before the first mating flight of each year. For all of the other parameters we compared the values on the day of the mating flights (n = 23) with the day before (n = 19). We calculated mean values for a time window of six hours beginning at 12:30, representing the time frame from the first signs of swarming behavior until the departure of the first alates. Thus, this time span represents a biological reasonable period to trigger changes in the behavior of the ants.

As mating flight occurrence and the associated climate data were recorded in different years, we used generalized linear mixed models (glmms, R function glmmPQL) with a binomial error distribution and a logit link function (ZUUR & al. 2009). Year of observation was treated as random factor in order to account for a possible non-independence of climate data in a given year. Observed flight occurrence was used as response variable. Before fitting the initial full model, all climate parameters were analyzed for possible multicollinearity. If two parameters were correlated with Spearman's $\rho > 0.7$, only one parameter was used. Spearman's ρ was taken as a measure of correlation as solar radiation and wind velocity were not normally distributed. Solar radiation was strongly correlated with relative humidity ($\rho = -0.77$) and not used to fit glmms. Hence, the initial full model contained temperature, atmospheric pressure, relative humidity, and wind velocity as explanatory variables. Precipitation was not included in the initial model as it is an obvious pre-requisite for the initiation of swarming behavior. Using the coefficients of the final model, we fitted a logistic regression.

In order to test if the time since reaching the cumulative precipitation threshold and the actual flight day is correlated with the climate parameters at the flight day, we used a glmm with Poisson error distribution and a log link function. The initial full model was fitted with the number of days since reaching the threshold as response variable and the same explanatory variables were used as in the models above. To obtain minimum adequate models, all glmms were reduced by a stepwise backwards selection process to remove non-significant explanatory variables. All statistical analyses were done using R 2.15.1 (R CORE TEAM 2012).

In order to support the assumption that alates wait readily in their home nest for substantial rainfalls and these rainfalls alone can initiate swarming behavior if weather conditions after rain are suitable, we performed an additional watering experiment with one nest at Rio Pilcomayo National Park. During a longer dry period on 13 October 2009, the nest mound was uniformly sprinkled with around 2,200 l of water over a period of 30 minutes. The water quantity simulates approximately 40 mm of precipitation, which is comparable to heavy natural thunderstorms during the mating season.

Results

Behavior: Swarming behavior can always be described as having three consecutive and clearly distinguishable phases: initiation phase, aggregation phase, and mating flight.

We use the term "swarming behavior" when referring to the entire behavioral sequence. The term "mating flight" is used for the phase of the swarming behavior when alates take off from the nest. The day on which the mating flight takes place is referred to as "flight day".

Initiation phase: Each swarming behavior begins with the initiation phase. After rainfalls, workers enlarge the big, central nest entrances and establish additional entrances in the nest periphery. During the nights before a flight day, some alates can already be observed in the central tunnels.

Fig. 2: Close up photograph of a mature *Atta vollenweideri* nest during aggregation phase. The nest surface is covered with males shortly before the mating flight starts.

We observed alates to be highly sensitive to disturbance and strictly photonegative but did not quantify these behaviors. On flight days, large workers and soldiers run quickly and excitedly over the nest surface and through the nearby vegetation. The ants are very aggressive and attack anything, living or not, found on the nest. The workers' pre-swarming behavior starts at around noon and peaks at the start of the mating flight, though the workers remain aggressive until the end of the mating flight. Only during swarming behavior large numbers of workers can be seen on top of the nest mound and this never happens at any other time of the year, except after massive disturbance of the nest mound.

Aggregation phase: The initiation phase ends when the males begin to appear on the nest mound at around 16:00. At approximately 17:30, males begin to spread all over the nest surface, moving rapidly (Fig. 2) with frequent stops to lick their front legs with their mouthparts (Fig. 3, Appendix S1, as digital supplementary material to this article, at the journal's web pages). At the beginning of the aggregation phase, males only appear from the central tunnels, and later they crawl out from over 90% of the nest openings. Shortly before the mating flight, the entire nest is so densely covered with males that they sometimes create several layers of ants (Appendix S2).

Mating flight: Finally, at around 18:30 the first males take off, ending the aggregation phase and starting the mating flight. In contrast to the males, gynes can only be observed in the large, central tunnels at around the time when males begin to leave. Around 10 - 15 minutes after the males started to depart, the gynes appear but never spread densely on the nest's surface and take off soon after the males (Fig. 4, Appendix S3). While standing on their middle and hind legs, the gynes lift their heads and shake them from side to side. At the same time, they flap their wings and paddle with their front legs (Fig. 5, Appendix S4). Aside from unavoidable collisions, workers and alates have never been observed interacting during fully developed swarming behavior. However, when swarming behavior is terminated soon after it starts, workers can prevent alates of both sexes from emerging onto the nest mound (see discussion).

Although not quantified in detail, the number of males leaving the nest after the beginning of mating flight appears to increase exponentially, culminating in a mass flight before sunset at about 18:40 - 19:00. After this peak, the vast majority of males have already left so the take-off rate drops rapidly. The last single males depart at sunset, between 19:10 - 19:20. The first gynes depart during the males' mass flight. Gyne take-off peaks at 18:50 - 19:10. As in the males' case, take-off frequency drops quickly afterwards. The last gynes fly at approximately 19:15 - 19:25. Gynes are slow, clumsy flyers, and usually need several attempts to get air-borne (Appendix S3). Alates fly straight up into the air, quickly reaching high altitudes, and most head in a similar direction. We never observed mating leks or copulation as it takes place far away from the nest, presumably high in the air.

Throughout aggregation phase and mating flight, many bird species prey on the alates, most of them selectively picking gynes (Appendix S5, S6). After a mating flight ended, we frequently observed queens with shed wings, digging founding tunnels into the wet soil. Occasionally, queens were killed by conspecific workers as well as by

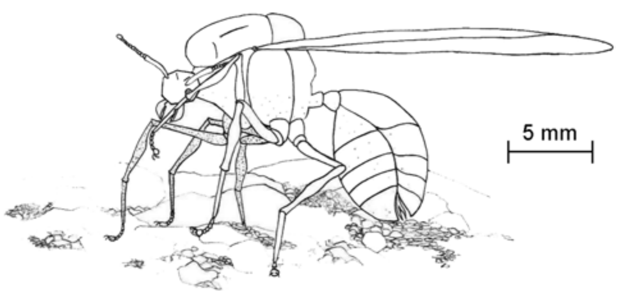

Fig. 3: Pre-flight behavior of males: All males lick their front legs quickly alternating with the mouthparts while standing still on the nest surface. Drawing by Ceara Elhardt.

Fig. 4: Gynes leaving the nest out of central entrances and showing pre-flight behavior during early mating flight.

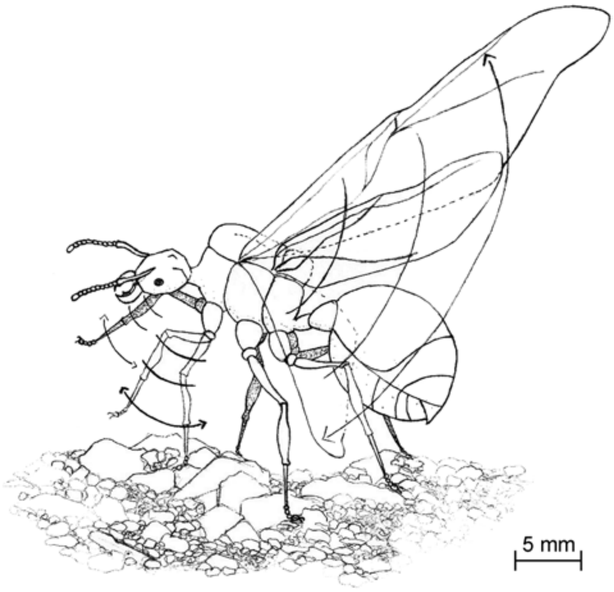

Fig. 5: Pre-flight behavior of gynes: All gynes shake their heads and paddle with their front legs while moving their wings. Characteristic movements are indicated with arrows. Drawing by Ceara Elhardt.

other ant species. Scavenging ants were often seen carrying dead or dying males.

Sex allocation: Based on photographs and video recordings of the three focal nests in Rio Pilcomayo National Park during aggregation phases and mating flights, we estimate that a mature colony produces 30,000 - 40,000 males and 4,000 - 5,000 gynes yearly. Thus, the ratio of gynes to males is approximately 1:8 - 1:10. Gynes have a dry weight of 391.9 ± 32.1 mg (n = 12), while males have a mass of only 22.5 ± 2.1 mg (n = 13). Based on these numbers, each mature colony produces 1.5 - 2 kg of gynes and 0.7 - 0.9 kg of males, giving a total weight of 2 - 3 kg of alates per year. The operational sex allocation ratio in *Atta vollenweideri* is 0.49 and the expected worker optimum sex allocation 0.64.

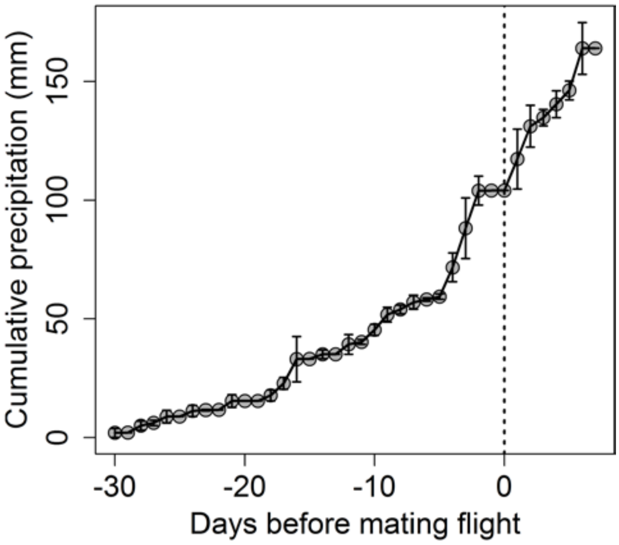

Fig. 6: Cumulative precipitation for the 30 days before and the seven days after the first mating flight of a year. During 30 days prior to a mating flight, precipitation was 102.6 ± 17.0 mm. There was no precipitation on flight days. Rainfall patterns after the first mating flight are highly variable. The day of mating flight is indicated by the dashed line. Shown are mean ± SEM (n = 7).

General timing of swarm days: In the period from 2004 to 2010, a total of 23 flight days were recorded at the Reserva Ecologica El Bagual. The number of mating flights per year varied from two (2004, 2008, 2009) to six (2005), with a median of three flight days per year. Mating flights always occurred in austral spring, the earliest flight day was on 6 October in 2006, the latest was on 8 December in 2010. The median flight day was 24 October. Mating flights in a given year were separated by a median of four days. However, mating flights also took place on consecutive days (four times), and can be separated by up to 44 days (2010).

Climate data: Substantial precipitation of 102.6 ± 17.0 mm (mean ± SEM, n = 7) was recorded during the 30 days preceding the first flight day of a given year (Fig. 6). The least precipitation measured before the first flight day was 64.2 mm in 2010. Between the first flight day and subsequent flight days, substantial precipitation often occurred with up to 88.6 mm per day. In seven cases, no precipitation was recorded between consecutive flight days. The flight days were always without precipitation (Fig. 6), except for three cases of minor drizzle (≤ 0.5 mm / day) in the early morning hours. The last rain day before a mating flight was at a median of two days prior to the flight day (range one to five days). The last rain event before a flight day had at least 9.9 mm precipitation, but the mean value was much higher (40.0 ± 6.3, n = 16). When mating flights took place on two consecutive days, precipitation in the preceding rain event was high (57.1 ± 9.8 mm, n = 4), although not significantly higher than before separated flight days (34.3 ± 7.2 mm, n = 12, $p = 0.10$, W = 10, Mann-Whitney U-test).

We induced swarming by artificial watering. After sprinkling a nest mound at the Rio Pilcomayo National Park on 13 October 2009, the colony elicited mating flights on 17,

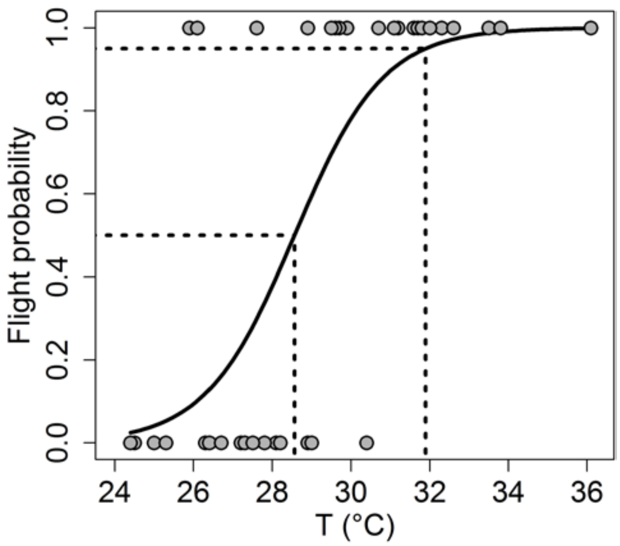

Fig. 7: Logistic regression of flight probability as a function of temperature. Dashed lines mark 50% (28.6°C) and 95% (31.9°C) flight probability. $n_{(preFD)} = 19$; $n_{(onFD)} = 23$.

Tab. 1: Climate parameters for the day preceding a flight day (preFD) and the flight day (onFD). Values are presented as mean ± SEM. p-values are from the binomial glmm. $n_{(preFD)} = 19$; $n_{(onFD)} = 23$. Abbreviations: T (temperature), p (atmospheric pressure), RH (relative humidity), Wind (wind velocity).

Parameter	preFD	onFD	p
T (°C)	27.0 ± 0.4	30.9 ± 0.5	0.001
p (hPa)	1001.3 ± 0.7	998.6 ± 0.9	ns
RH (%)	59.5 ± 2.5	50.2 ± 1.7	ns
Wind (m / s)	1.3 ± 0.2	1.6 ± 0.1	ns

18, and 20 October although none of the un-manipulated nests showed any indication of swarming behavior.

In order to identify weather parameters that may enhance the chances of a mating flight, we compared weather conditions before a flight day with the conditions on a flight day using binomial glmms. In the initial full model on the occurrence of mating flights, we tested for the effect of the four climate parameters: temperature, atmospheric pressure, relative humidity, and wind velocity. The final minimum adequate model contained only temperature as significant explanatory variable (estimate = 0.89, se = 0.25, DF = 34, p = 0.001) (Tab. 1). The high AUC value of 0.91 confirms the reliability of the final model.

The lowest temperature recorded on a flight day was 25.9°C and the maximum was 36.1°C (mean 30.9 ± 0.5°C). Flight probability can be illustrated with a logistic regression, revealing 50% and 95% flight probability at 28.6°C and 31.9°C, respectively (Fig. 7).

Relative humidity and atmospheric pressure were lower on flight days compared to the day before, although these differences were not statistically significant (Tab. 1). However, we experienced flight days to be sunny and not humid. Calm wind conditions have been suggested as favorable for mating flights, but we did not find significant differences in wind conditions between flight days and the days before. We did also not find any correlation between the number of days since reaching the cumulative precipitation and the climate parameters.

During our observations in the Rio Pilcomayo National Park in 2009, mating flights took place on the same days as in the Reserva Ecologica El Bagual, on 23 October, 8 and 9 November with temperatures of 33.4°C, 26.4°C, and 32.0°C, respectively.

Discussion

Behavior: Sequences of behavior described here were highly consistent in all observed swarms. There was no difference in the swarming behavior of swarms induced by watering and of those which occurred due to natural rainfall. Swarming behavior can be divided into three consecutive phases: initiation, aggregation, and mating flight. The initiation phase begins after sufficient precipitation in springtime. Colonies remain in initiation phase up to five days after the last rain, in order to wait for weather conditions favoring a mating flight. At this time, further nest entries are constructed, probably to enable efficient mass aggregation of alates on the nest surface, and alates of both sexes appear in the tunnels near the nest entrances during the night. It seems that the colony prepares for aggregation phase and we speculate that workers play an important role in the sequence of swarming behavior.

Initiation phase starts on the flight day with early pre-swarming, the appearance of aggressive workers on the nest surface. Workers behave very aggressive, attacking everything foreign in their proximity. Pre-swarming is a conclusive sign that a mating flight may soon occur, and most likely evolved to banish or kill potential predators of alates (MOSER 1967, FOWLER 1982). Worker behavior seems to be initiated by males. Previous experiments showed that the male mandibular gland secretion, largely an equal mixture of 4-Methyl-3-Heptanone and 4-Methyl-3-Heptanol (DONASCIMENTO & al. 1993, HERNANDEZ & al. 1999), induces pre-swarming in *Atta* (FOWLER 1982, BENTO & al. 2007). As part of the aggregation phase, both sexes show specific stereotyped behavioral sequences that have not been described before and can only be observed in that phase of swarming behavior. Hence, we suspect that the behaviors play a role in the maintenance of pre-copulatory communication between males and gynes or between alates and workers. Most likely, mandibular gland pheromones are important communication signals during this phase among members of a single colony, which is further supported by changes in the mandibular gland content during pre-swarming (DONASCIMENTO & al. 1993, HERNANDEZ & al. 1999). However, the exact role of these pheromones is still not fully understood.

Once the aggregation phase is initiated, colonies complete mating flights on the same day. However, if weather conditions abruptly change during aggregation phase, the entire sequence of swarming behavior is aborted (GÖTSCH 1938). On one occasion, workers were observed grabbing and pulling alates, mostly males, back into the nest. Similar behavior has been reported in *Camponotus herculeanus* (LINNAEUS, 1758) (HÖLLDOBLER & MASCHWITZ 1965). The swarming behavior in that year (2011) was not studied in detail but it seems that high wind velocity (> 3 m / s) prevented the colonies from developing a full mating flight.

Only a few males and gynes left the nest mound for a mating flight on that day (S. Koch & L. Kling, unpubl.). We hypothesize that, despite there is no difference in wind velocity in our statistical analyzes, a sudden rise in wind velocity can influence and abort swarming behavior on rare occasions.

During mating flight, gynes appear only shortly before taking-off, in order to expose themselves to predation for as short as possible. All species of the genus *Atta* show a male aggregation syndrome (BOOMSMA & al. 2005) and males leave the nest in a synchronized mass swarm shortly before gynes. Both sexes quickly reach high altitudes, and the ants probably mate in large, male-dominated mating leks high in the sky (AMANTE 1972). As there are no direct observations of copulation in *A.* the actual mating behavior can only be speculated. MOSER & al. (2004) classified *A.* species into day- and night-flyers, based on eye morphology and previously published literature. *Atta vollenweideri* was classified as a night-flyer with day-adapted vision. Based on our observations, we can solve this inconsistency by identifying *A. vollenweideri* as a day-flyer.

Evolutionary drivers for synchronization: The large numbers of males and gynes leaving a single nest are impressive. The question arises as to how much a mature colony invests per year in alates. Here we report that *Atta vollenweideri* produces several thousand males and gynes per year with a strongly male dominated sex-ratio. FOWLER & al. (1986) showed that numerical sex-ratios in *A.* species are at least slightly dominated by males but can have huge intra and interspecific variation. However, it is unclear what factors cause these differences. In eusocial Hymenoptera, as a consequence of the haplo-diploid sex determination system, a conflict between workers and queens over the sex allocation ratio of a colony's offspring has been shown (e.g., BOOMSMA & al. 1995, MEHDIABADI & al. 2003, DIJKSTRA & BOOMSMA 2008). In theory, if under worker control, the sex allocation ratio should be biased towards gynes, while under queen control this ratio should be near parity. Recently, this sex allocation conflict has been investigated in fungus-growing ants others than *Atta* (ICHINOSE & al. 2007, DIJKSTRA & BOOMSMA 2008). Depending on the species, support for both, either for worker or for queen control has been found. To our knowledge, our study on *A. vollenweideri* presents the first, very tentative estimation of operational sex allocation in *Atta*. In contrast to other fungus-growing ants, the operational sex allocation in *A. vollenweideri* appears to be under queen control as the ratio of 0.49 is almost identical to the expected queen optimum of 0.50, and is quite different from the expected worker optimum of 0.64. However, our calculations are based on limited data and more detailed studies are necessary for validation.

Individual gynes are extraordinarily heavy for an ant (FJERDINGSTAD & BOOMSMA 1997, DIJKSTRA & BOOMSMA 2008, SEAL 2009). The mean biomass of an *Atta* colony is estimated to be approximately 9 kg (FOWLER & al. 1986). Our estimate of 3 kg of alates per year illustrates that this investment is a substantial part of the entire colony biomass. In particular, gynes are especially resource demanding and valuable due to their high proportion of body fat (SEAL 2009, FUJIHARA & al. 2012).

As in all ants with male aggregation there are likely two main selective pressures that lead to a large-scale synchronization of swarming behavior, and especially of mating flights: high predation pressure and enhanced genetic outbreeding with multiple males. Ant queens face numerous natural enemies during mating flight and colony establishment (WARTER & al. 1962, WEBER 1966, 1972, FOWLER & al. 1986, KENNE & DEJEAN 1998, LEVIN & al. 2009). We observed many bird species selectively picking the nutritious gynes even in the early phase of the mating flight when males are much more numerous. Similarly, most birds preying on airborne alates preferably catch gynes. Losses due to predation can be very high (WEBER 1966, FOWLER & al. 1986). Albeit, if all colonies in a given area swarm at the same time, predators get over-saturated with prey and a few gynes have a good chance to survive the mating flight and eventually found a new nest.

Only less than 0.05% of the gynes leaving the nest survive the first year, but life expectancy of a colony whose queen withstands an initial period is more than 15 years during which the queen can lay 150 - 200 million eggs (HÖLLDOBLER & WILSON 2008). In order to achieve high amounts of sperm that directly prolongs colony life span, queens of all *Atta* species mate multiple times during their single mating flight (MURAKAMI & al. 2000, VILLESEN & al. 2002, EVISON & HUGHES 2011). Multiple mating leads to outbreeding with males of various natal nests and a genetically variable worker force. Genetically heterogeneous ant colonies benefit in pathogen resistance, which may be critical for colony development, division of labor and fungus cultivation (HUGHES & BOOMSMA 2004, OLDROYD & FEWELL 2007).

Climate: Substantial springtime rainfalls induce swarming behavior in *Atta* on a circa-annual rhythm. However, alates are produced many months before the first sign of swarming behavior (EIDMANN 1936, GEIJSKES 1953, MOSER 1967). They wait readily in the parental nest for rainfalls to start the initiation phase. On a broad scale, ground nesting ant species in seasonal habitats have mating flights preferably after rainfalls (e.g., HÖLLDOBLER 1976, BOOMSMA & LEUSINK 1981, JOHNSON & RISSING 1993, KENNE & DEJEAN 1998, GÓMEZ & ABRIL 2012). While heavy precipitation is a prerequisite, it alone is not sufficient to induce a mating flight.

We identified temperature to be the only climate parameter significantly differing between flight days when compared to the preceding day. Thus, we conclude that temperature plays an important role in the initiation of the aggregation phase, by triggering the transition from initiation to aggregation phase up to five days after the last rainfall. Albeit *A. vollenweideri* can fly at temperatures down to 26°C, optimal flight conditions are on days with temperatures above 32°C where the flight probability is above 95%. The validity of our model is supported by the fact that 85% of the days between the last rain day and the flight day had temperatures below the 50% flight probability. In contrast to previous observations on Central European ants (BOOMSMA & LEUSINK 1981), *A. vollenweideri* has constant preferences for climate parameters during the entire mating season and does not fly at less suitable temperature and wind conditions if alates have been waiting in the home colony for a long time.

A rise in temperature from the preceding day to the flight day has also been observed in *Manica rubida* (LATREILLE, 1802) in temperate Poland (DEPA 2006). Rising temperature

Tab. 2: Climatic conditions during the start of mating flights. Data for *Atta vollenweideri* (n = 23) from the present study, for *A. sexdens* (n = 6) from MOSER & al. (2004) and for *A. texana* (n > 50) from MOSER (1967). Abbreviations as in Table 1.

Climate parameter	*A. vollenweideri*	*A. sexdens*	*A. texana*
Minimum rainfall (mm)	64.2	19.4	7.0
Minimum T (°C)	24.2	20.1	16.0
Maximum T (°C)	34.8	29.4	25.5
Wind (m / s)	0 - 2.7	0.9 - 2.7	absent to little
Time of take-off	~ 18:30	11:00 - 15:00	~ 4:00

might indicate a better chance for stable and fair weather conditions in the near future. Besides providing information about suitable conditions for mating flights, ants use thermal information in various other contexts, for example in organizing foraging, for controlling thermal homeostasis in the nest, and for fine-tuned brood care (e.g., HÖLLDOBLER & WILSON 1990, ROCES & NUNEZ 1995, BOLLAZZI & ROCES 2002, AZCARATE & al. 2007, WEIDENMÜLLER & al. 2009). Information about the ambient temperature is provided by thermo-sensitive sensilla on the ants' antennae (DUMPERT 1972, KLEINEIDAM & al. 2007a, RUCHTY & al. 2009, 2010a, 2010b). Recent neurophysiological work has shown that *Atta vollenweideri* workers can detect minute temperature changes and even use thermal cues for orientation (KLEINEIDAM & al. 2007a, RUCHTY & al. 2009, 2010b).

Comparison to other *Atta* species: Suitable precipitation and temperature for mating flights are higher in *Atta vollenweideri* than in *A. sexdens* and in *A. texana* (Tab. 2) (MOSER 1967, MOSER & al. 2004). In the Gran Chaco region, the main distribution range of *A. vollenweideri*, soils are heavily loamy (SEIBERT 1997) and almost impossible to penetrate right after the dry season. Precipitation that moistens at least the top 30 cm of the soil is necessary to allow mated queens to dig founding tunnels and to construct nest chambers (FRÖHLE & ROCES 2012). This is less critical for other *Atta* species which live in habitats with sandy soils that are generally easier to dig in. In Panama, the depth of the founding chamber seems to be species specific in various *Atta* species, as well as in other leaf-cutting ants, which might be related to the drought resistance of the respective species (J.J. Boomsma, unpubl.). We propose that, based on soil conditions and the depth of the founding chamber, different *Atta* species have species-specific thresholds for cumulative precipitation, which need to be exceeded before swarming behavior is initiated. In case of insufficient precipitation, *A. vollenweideri* is able to postpone mating flights until as late as December. A similar shifting of mating flights has also been reported for the harvester ant *Pogonomyrmex rugosus* EMERY, 1895, which inhabits dry regions in the southwestern United States (HELMS & HELMS CAHAN 2010).

Conclusion

The present study is the first since MOSER'S (1967) work, adding important information to the mating biology of a leaf-cutting ant species in a broader context. Nevertheless, several major questions have not been addressed so far, and further experiments are required. For example, more detailed information is needed about the chemical communication signals between workers and alates on the nest mound, as well as between alates during their mating flight high in the sky. This will allow experimental manipulation of swarming behavior and ultimately lead to a better understanding of the fascinating mating biology of leaf-cutting ants.

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