Phenology of mating flight in Ecitoninae (Hymenoptera: Formicidae) in a Brazilian Atlantic Forest location

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Abstract. The mating flight of Ecitoninae ants was studied using four light traps inspected daily during the whole year of 2004. A total of 1285 males belonging to 15 species was collected, the species number varying monthly from two to 11. For most species, the beginning of the reproductive period occurred in November or in February. Synchronism of the flight activity among species was observed in the genera Neivamyrmex and Eciton, while segregation occurred in two Labidus species. In the model tested to explain male abundance over the year, including rainfall, temperature and interaction between both factors, only temperature was significant.

Résumé. Phénologie du vol nuptial chez les Ecitoninae (Hymenoptera : Formicidae) dans une localité de la forêt atlantique brésilienne. Le vol nuptial chez les fourmis légionnaires Ecitoninae a été étudié en utilisant quatre pièges lumineux visités chaque jour pendant toute l’année 2004. Un total de 1285 mâles appartenant à 15 espèces a été collecté, le nombre d’espèces capturées variant de deux à 11 par mois. Pour la plupart des espèces, le début de la saison de reproduction se situe en novembre ou en février. La synchronisation de l’activité de vol a été observée entre les différentes espèces des genres Neivamyrmex et Eciton, alors qu’il existe une ségrégation chez les deux espèces de Labidus. Un modèle a été testé pour expliquer l’abondance des mâles au cours de l’année, incluant précipitations, et température ainsi que l’interaction entre ces deux facteurs, et la température seule s’est montrée significative.

Keywords: Reproduction, Neotropical region, rainfall, temperature, seasonality.

The Ecitoninae, as well as the other legionary or army ants (generic name given to the ants of the subfamilies Aenictinae, Dorylinae and Ecitoninae), have a unique biology among ants (Hymenoptera, Formicidae), defined as “syndrome of behavioral and reproductive traits” (Gotwald 1982; Brady 2003). These characteristics are: colonies with a large number of individuals, nomadism, colony fission, collective foraging and the presence of a single and highly morphologically modified queen per colony. The colonies do not form permanent nests and their life cycles are marked by alternating nomad and stationary stages (Schneirla 1971; Gotwald 1995). Females are permanently wingless, so that the winged males represent the only agent of cross-fertilization and gene dispersal in all species. Males are produced in large numbers relative to gynes and usually begin their nuptial flight at night. The fact that Ecitoninae males are well attracted to light traps has been known for a long time (Borgmeier 1955).

The timing of reproduction can influence an organism’s reproductive success. Reproductive phenologies often reflect species adaptations to their abiotic and biotic environments (Rathcke & Lacey 1985; Kaspari et al. 2001b; Frederickson 2006). In temperate regions, ant mating flights are restricted to a single day, a few days, or a few weeks per year, occurring sometimes during spring, but principally in summer, such as in Lasius spp. and Myrmica spp. (Boomsma & Leusink 1981; Seifert 2007; Noordijk et al. 2008). On the contrary, in tropical regions, they can have a high flight frequency during several months of the year (Kaspari et al. 2001ab; Torres et al. 2001).

Two patterns of nuptial flights are known for army ants: i) in Dorylinae, flights occur all year, but with higher intensity during the transition period between dry and rainy seasons (Haddow et al. 1966; Leston 1979); and ii) in Ecitoninae living in temperate regions, these flights are characterized by a well defined seasonality and are highly dependent on climatic factors, mainly temperature and rainfall (Baldridge 1972; Baldridge et al. 1980).

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In the Neotropics, the first extensive investigation of flight periodicity was conducted by Kannowski (1969) on Barro Colorado Island, Panama. The occurrence of males of 15 species of Ecitoninae was recorded; eight of them were trapped in the transition period between the dry and rainy seasons, while two species, Labidus coecus (Latreille) and Eciton burchelli (Westwood), were exclusive to the dry season. However, Kannowski’s observations were not conclusive due to the short time period of observation. The only other reference to nuptial flights of South American Ecitoninae is that of Nascimento et al. (2004), who investigated seasonality of mating flight in the genus Labidus through a large temporal series. It is common that ants of congeneric species perform their mating flights in the same period of the year (Delabie & Reis 2000), because they are phylogenetically close (forming taxoecenes, see Kaspari et al. 2000b) and because they respond to similar environmental factors (McCluskey 1965). Sometimes related species respond in a distinct manner to resources and conditions: segregation of ant mates during the mating flight period is a possible indicator of that situation. This can be evolutionarily significant because ecological differences can act as an important mechanism to avoid interspecific hybridization (Hölldobler & Wilson 1990). Examples of nuptial flight segregation in Ecitoninae are recorded in Neivamyrmex by Kannowski (1969) and Baldridge et al. (1980), as well as in Labidus by Nascimento et al. (2004). This segregation can happen in a short time space (approximately 12 hours), as reported in eight species of the doryline genus Dorylus (Haddow et al. 1966), or in four species of Neivamyrmex (Kannowski 1969).

This study presents, for the first time in the Neotropics, data on the reproductive phenology of 15 species of Ecitoninae, studied during a one year period. Our aims were: i) to determine periodicity and seasonality of mating flights of the genera Eciton, Labidus, Neivamyrmex and Nomamyrmex under tropical conditions; ii) to test mating synchronism in congeneric species; and iii) to test the influence of climatic factors (temperature and rainfall) on the abundance of Ecitoninae males throughout the year.

Materials and Methods

Study area

This study was conducted at the village of Barramares (14°37’04”S; 30°04’07”W) in a “restinga” (typical coastal vegetation of Brazil) area, located at the northern coast of the county of Ilhéus, Bahia, Brazil. The region is located in the Atlantic Forest biome and is covered by several vegetation types, the coastal savanna and mangroves being the predominant fea-

ures (Delabie et al. 1998). According to Köppen’s classification, the climate of the area is tropical, hot and wet (Af), without a defined dry season. Rainfall is above 2000 mm; monthly average precipitation is never lower than 100 mm (Frota 1972). Monthly mean temperature is 20 °C from June to August (winter) and higher temperatures (monthly mean = 26 °C) occur from December to March (summer) (fig. 1b).

Sampling

Males of Ecitoninae were sampled with four light traps of the “Luiz de Queiroz” type (Silveira Neto & Silveira 1969) modified by Ferreira & Martins (1982), placed 2 m above the soil. Light traps were crepuscular/nocturnal, in activity from 18:00 to 6:00. They were set up from January 1st, 2004 to January 1st, 2005 and inspected daily. Two light traps were placed amid the vegetation 100 m from the sea shore in bushy grassland where cashew (Anacardium occidentale) and palm trees (Cocos nucifera) were predominant. A second set of two light traps was placed 800 m from the sea shore, on the margin of a secondary forest.

The ant specimens were preserved in 70% ethanol and latter mounted and labeled at the laboratory for identification. Identification was carried out using taxonomic keys for Ecitoninae (Borgmeier 1955; Watkins 1976). Voucher specimens were deposited in the collection of the Myrmecology Laboratory of the Coca Research Center, Ilhéus, State of Bahia, Brazil. Climatic variables were collected daily in the sampling areas. The average daily temperature was obtained by a thermohyrometer Model FUESS 115 T in a meteorological shelter, with three observations per day (9:00; 15:00 and 21:00 h). The rainfall was measured in a rain cage Model “Ville de Paris”.

Analysis of the data

Mating flight segregation was tested by means of an ANOVA. The genera were analyzed separately. The number of males of each species sampled was used as the response variable while collecting date was used as the explanatory variable. For p values greater than 0.05, the hypothesis of flight segregation was rejected. The model formed by temperature x rainfall and the interaction between these factors to explain Ecitoninae male abundance during the year was also tested by ANOVA. In this analysis, only species represented by more than 20 individuals distributed over the whole study period were considered. Non significant variables were excluded from the model until an adequate model was obtained (Crawley 2002).

The analyses were performed using a General Linear Model (GLM) and were carried out within the R Statistical package, version 1.7.1© 2003 of the R Development Core Team (Ihaka & Gentleman 1996).

Results

Species composition

During the 365 nights of the experiment, 1285 males belonging to 15 species of Ecitoninae were collected. They belonged to the genera Eciton (3 species), Labidus (2 species), Neivamyrmex (9 species) and Nomamyrmex (1 species) (tab. 1). Neivamyrmex had the highest abundance, with 62.7% of all individuals collected (tab. 1). Eciton (22.6%) and Labidus (12.8%)
were the second and third most abundant genera. Two species, *Neivamyrmex detectus* and *N. piraticus*, only represented by a single individual each, are new records for the State of Bahia.

**Phenology**

Males of Ecitoninae were collected during the whole year, with the number of species per month varying from two to eleven. Most of them were yet flying between November and February (fig. 1a, where this period appears divided in January/February and November/December, due to the distribution of our observations along one year). The increase in species number is mainly because *Neivamyrmex* species begin mating activity in November (fig. 1). On the contrary, in March, males of *Neivamyrmex* were completely absent from the traps and the number of Ecitoninae species strongly decreased. The analyses do not include *N. piraticus* and *N. detectus*, recorded respectively in January and February, as they were represented by singletons.

During the year, all species exhibited bimodal curves, with the majority having maximum activities during the summer, although activity peaks may occur for at least six months (fig. 2). *Eciton burchelli* males had a flight activity approximately throughout the year, even though only 10% of the males were collected out of the flight activity peak that occurs from December and February (fig. 2). No segregation was observed between *E. burchelli* and *Eciton mexicanum* on the day of flight (*p* = 0.512, *F* = 0.428, df = 125, fig. 2). All *Neivamyrmex* species concentrated their flight activity from November to February and no segregation was observed between species on the day of the flight (*p* = 0.5283, *F* = 0.397, df = 212, fig. 2). In contrast to other Ecitoninae species, males of *Labidus coecus* started their flight activity during the coldest months of the year (July to September). They showed a clear segregation of flight activity from those of *L. praedator* (*p* < 0.001, *F* = 7.078, df = 70) that flew from January to May (fig. 3).

**Climatic variables**

When male Ecitoninae abundance was analyzed with rainfall, temperature and the interaction between the two factors, only temperature was found to be significant (tab. 2A). Therefore, the simplest model to explain male abundance includes only temperature (tab. 2B). The number of Ecitoninae males is positively, although weakly, correlated with temperature (*r* = 0.20; *p* = 0.0045) (fig. 4).

**Discussion**

The high quantity of males reported in this study only gives a partial idea of the reproductive activity among Ecitoninae species at Ilhéus. The main reason is

<table>
<thead>
<tr>
<th>Species</th>
<th>Nb individuals</th>
<th>% individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eciton burchelli</em> (Westwood 1842)</td>
<td>248</td>
<td>19.26</td>
</tr>
<tr>
<td><em>Eciton mexicanum</em> Roger 1863</td>
<td>32</td>
<td>2.48</td>
</tr>
<tr>
<td><em>Eciton vagans</em> (Olivier 1791)</td>
<td>12</td>
<td>0.93</td>
</tr>
<tr>
<td><em>Labidus coecus</em> (Latreille 1802)</td>
<td>139</td>
<td>10.80</td>
</tr>
<tr>
<td><em>Labidus praedator</em> (Fr. Smith 1858)</td>
<td>27</td>
<td>2.09</td>
</tr>
<tr>
<td><em>Neivamyrmex clavifemur</em> Borgmeier 1953</td>
<td>20</td>
<td>1.53</td>
</tr>
<tr>
<td><em>Neivamyrmex detectus</em> Borgmeier 1953</td>
<td>1</td>
<td>0.07</td>
</tr>
<tr>
<td><em>Neivamyrmex guerini</em> (Shuckard 1840)</td>
<td>169</td>
<td>13.13</td>
</tr>
<tr>
<td><em>Neivamyrmex leptognathus</em> (Emery 1900)</td>
<td>67</td>
<td>5.20</td>
</tr>
<tr>
<td><em>Neivamyrmex pilosus</em> (Fr. Smith 1858)</td>
<td>23</td>
<td>1.78</td>
</tr>
<tr>
<td><em>Neivamyrmex piraticus</em> Borgmeier 1953</td>
<td>1</td>
<td>0.07</td>
</tr>
<tr>
<td><em>Neivamyrmex spinolai</em> (Westwood 1842)</td>
<td>12</td>
<td>0.93</td>
</tr>
<tr>
<td><em>Neivamyrmex sp. prox. spinolai</em></td>
<td>17</td>
<td>1.32</td>
</tr>
<tr>
<td><em>Neivamyrmex swainsoni</em> (Shuckard 1840)</td>
<td>498</td>
<td>38.69</td>
</tr>
<tr>
<td><em>Nomamyrmex esenbeckii</em> (Westwood 1842)</td>
<td>19</td>
<td>1.47</td>
</tr>
<tr>
<td>Total</td>
<td>1285</td>
<td>100</td>
</tr>
</tbody>
</table>
that it is estimated that there is 3000 males produced per year against only six apterous females in a single colony of *Eciton hamatum* (Fabricius) (Passera & Aron 2005); this ratio can be similar in the other Ecitoninae, but no data is available for the other species of the group.

The flight activity of males of at least two species of Ecitoninae per month is reported for the first time in the subfamily. Besides the possibility of reproduction over a longer period in the year, there is evidence that the flight season is longer in tropical areas than in temperate zones. For example, in Ilheus, *Neivamyrmex swainsoni* males flew for approximately 90 days and *L. coecus* for about 100 days, whereas Baldridge *et al.* (1980) reported a flight season of 79 and 71 days for these same species in Texas, respectively. The data of our study add to those summarized by Kaspari *et al.* (2001a) and support the hypothesis of the existence of a latitudinal gradient in the duration of the reproductive period in ants.

Although less intense during the Austral winter, from March to September, out of the main reproductive peak period, males of *E. burchelli* has a flight activity during almost the whole year, that can be due to the
multiple mating activity already recorded for this ant (Denny et al. 2004). The occurrence of male flight at least several months of the year indicates that the colonies are producing an additional sexual brood per season, since emerged males remain a maximum of two weeks in their original colonies (Schneirla 1971). This result was not expected, since in Eciton, as well as in other genera of legionary ants, mass production of males with a strong seasonality is generally observed (Schneirla 1971; Gotwald 1995). The small number of males sampled out of the peak period suggests that fission of colonies could happen outside the ideal reproductive period. However, our results suggest more likely that, at least in E. burchelli, local climatic factors are not limiting factors of mate production, as suggested for other Ecitoninae (Schneirla 1971). A second possibility is that Eciton males trapped out of the peak period are produced by workers. Although Eciton workers cannot mate, some of them have functional ovaries and should be able to produce unfertilized eggs that could potentially develop into males (Whelden 1963). This fact has already been reported for E. burchelli by Kronauer et al. (2007).

A similar pattern of male production in Dorylus army ants (Dorylinae) during the whole year, with peaks distributed during three or four months, was reported by Haddow et al. (1966) and Leston (1979) in two African sites. According to Schneirla (1957), Eciton and Dorylus are homologous in their reproductive behavior, suggesting the monophyly of these ants. Brady (2003) showed that these ants belong to a monophyletic group that implies a behavioral inheritance and reproductive adaptation. This may partially explain the similarity between the reproductive phenology in Eciton and Dorylus, but more information is required for further clarification. The remaining species of Ecitoninae follow the unimodal pattern of activity curves (Baldridge et al. 1980; Nascimento et al. 2004).

Figure 3
Phenology of flight activity of males of Labidus coecus (n = 139) and L. praedator (n = 27) sampled monthly by light traps at Ilhéus, Bahia, Brazil, from January to December, 2004.

Figure 4
Relation between temperature and the number of Ecitoninae males sampled by light traps at Ilhéus, Bahia, Brazil, from January to December, 2004. The upper limit of male flight activity was drawn as an exponential curve.
The species of *Neivamyrmex* studied here exhibited a similar pattern in their phenologies. Activity curves are almost identical in some pairs of species, such as *N. clavifemur* x *N. swainsoni* and *N. pilosus* x *N. leptognathus* (fig. 2). The hypothesis of flight segregation with males released on different days, as a factor of reproductive isolation, was not verified. However, one cannot exclude the possibility of flight segregation on a smaller time scale (12 or 24 hours), as reported by Kannowski (1969) for *Neivamyrmex* and by Haddow *et al.* (1966) for *Dorylus*. This could not be detected in this experiment as the data were collected over continuous 12 hour periods. Unlike species of *Neivamyrmex*, *L. coecus* and *L. praedator* have completely distinct flight phenologies over the year. Their pattern reproduces exactly the same found for these two ants in another locality about 1000 km south (Nascimento *et al.* 2004). The non-overlapping flight seasons of *L. coecus* and *L. praedator* might have evolved to avoid hybridization. It can also be speculated that similar segregation occurs in *Eciton vagans* when compared with *E. mexicanum* (fig. 2). However, the little number of males collected did not allow any statistical analysis. Flight segregation has also been suggested for other species of ants of temperate regions (Noordijk *et al.* 2008), as well as North American Ecitoninae (Baldridge *et al.* 1980; Hölldobler & Wilson 1990) and African Dorylinae (Haddow *et al.* 1966). However, this is not evident in most of species assemblies of tropical regions (Kaspari *et al.* 2001b).

**Climatic factors: temperature x rainfall**

Two abiotic factors (temperature and rainfall) are probably key components for understanding reproductive phenology in ants (Kaspari *et al.* 2001a). Ant mating flights seem to be regulated mostly by rainfall, in regions where there is low significant variations in temperature. The dependence of mating flights on rainfall has been reported for several species of ants (Boomsma & Leusink 1981; Kaspari *et al.* 2001a). On the contrary, in our study, male flight activity during the year cannot be explained by rainfall in any of the studied Ecitoninae species. In sites with marked seasonality, the transition between dry and rainy seasons indicates the beginning of the mating period for many ant species, as observed by Kannowski (1969) and Baldridge *et al.* (1980) in Ecitoninae. At Ilhéus, monthly average rainfall is never lower than 100 mm (Frota 1972), with nearly 200 mm per month in 2004. For that reason, rainfall is certainly not a limiting factor for mate production (or at least, not the only one), since there is little rainfall seasonality.

Another non-exclusive possibility to explain rainfall dependence of ant mating flights is the necessity of a wet soil allowing new fertilized haplometrotic queens to excavate their nests (Hölldobler & Wilson 1990). In the particular case of Ecitoninae, new colonies are exclusively formed by fission, where gyne are followed by workers (pleometrotic foundation) and there is no permanent nest. Thus, rainfall dependence to perform mating flights does not appear as important in these ants.

In general, males had maximum flight activity during the warmest months. Only *L. coecus* had its flight activity during the coldest months (average of 22 °C). Maximum flight occurrence of *L. coecus* during cold periods was also reported in Barro Colorado Island, Panama (Kannowski 1969); Texas (Baldridge 1972; Baldridge *et al.* 1980), and in Minas Gerais, Brazil (Nascimento *et al.* 2004).

In addition to the factors that allow male release, another important factor, although poorly understood, is the mechanism that promotes immature sexual production in Ecitoninae. Schneirla (1971) has

| Table 2. Analysis of variance of Ecitoninae male abundance and two climatic variables: rainfall and temperature. A) Complete model; B) Simplified model. |
|------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| A)                                       |                 |                 |                 |                 |                 |                 |
| Coefficient of variation                | df   | Sums of Squares | Mean Squares | F    | P    |                 |
| Complete                                | 365  | 13175.53       | 365.5         | 1.721 | 0.1903 |                 |
| Rainfall                                | 1    | 59.5           | 59.5          | 1.721 | 0.1903 |                 |
| Temperature                              | 1    | 590.5          | 590.5         | 17.077 | <0.001* |                 |
| Rainfall x Temperature                   | 1    | 3.4            | 3.4           | 0.099 | 0.7529 |                 |
| Residual                                | 362  | 12521.8        | 34.59         |       |       |                 |
| B)                                       |                 |                 |                 |                 |                 |                 |
| Variation coefficient                    | df   | Sums of Squares | Mean Squares | F    | P    |                 |
| Complete                                | 365  | 13175.53       | 365.5         | 1.721 | 0.1903 |                 |
| Temperature                              | 1    | 613.1          | 613.1         | 17.766 | <0.001* |                 |
| Residual                                | 364  | 12562.2        | 34.51         |       |       |                 |
proposed that the effect of environmental changes, especially drought periods, can act directly or indirectly on the queen physogastrity determining the occurrence of immature sexual forms. On the other hand, this again may not apply to the conditions in the Ilhéus region due to the lack of a marked dry season.

Although our data encompasses only one year of study, the lack of variation in Ecitoninae reproductive phenology is expected to be similar from one year to the other, as already observed by Nascimento et al. (2004) studying a large Labidus data series taken over 18 years. This kind of information is important in investigating the dynamics of Ecitoninae ants and in the maintenance of biodiversity in tropical ecosystems. Furthermore, it helps in understanding possible mechanisms of speciation that act on the reproductive behavior of these organisms.

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