

Behavioural assays testing the appeasement allomone of *Polyergus rufescens* queens during host-colony usurpation

A. MORI¹, R. VISICCHIO¹, M.F. SLEDGE², D.A. GRASSO¹, F. LE MOLI^{1,4}, S. TURILLAZZI², S. SPENCER³ and G.R. JONES³

¹ Dipartimento di Biologia Evolutiva e Funzionale, Università degli Studi di Parma, Viale delle Scienze, I-43100, Parma, Italy

² Dipartimento di Biologia Animale e Genetica, Università degli Studi di Firenze, Via Romana 17, I-50125, Firenze, Italy

³ Chemical Ecology Group, School of Chemistry and Physics, Keele University, Staffordshire, ST5 5BG, England

Received 18 January 2000, accepted 5 June 2000

The role of decyl butyrate (the main component of the secretion of Dufour's gland in newly-mated queens) during host-colony usurpation was investigated in the European amazon ant *Polyergus rufescens*. The effect of this ester on the behaviour of workers of the common host species *Formica cunicularia* was analysed. We used a laboratory aggression test during which workers of the species *Camponotus ligniperda*, masked with synthetic decyl butyrate, were introduced into colony fragments of *F. cunicularia*. The results showed that this compound drastically lowers the degree of overt aggression against the introduced ants, and we suggest that it acts as an appeasement allomone. Moreover, pieces of filter-paper soaked with decyl butyrate were more attractive than pieces of paper treated with solvent. The results are discussed in relation to a chemical strategy that likely allows an easier invasion and usurpation of host colonies by queens of this obligatory slave-making ant.

KEY WORDS: Formicinae, slave-making ants, *Polyergus rufescens*, colony usurpation, Dufour's gland, decyl butyrate, bioassay, appeasement allomone.

INTRODUCTION

In ants, newly-mated queens generally display two main strategies of colony founding. After insemination, they either withdraw into a brood chamber and start a new colony without the help of workers (i.e. independent foundation), or they are supported from the beginning of colony formation by their nestmates or by workers from another colony (i.e. dependent foundation) (cf. HÖLLDOBLER & WILSON 1990,

⁴ Corresponding author.

KELLER 1991, BOURKE & FRANKS 1995). Socially parasitic ants normally exhibit a dependent mode of colony foundation. In particular, queens of inquilines (permanent parasites which generally have lost their worker caste) gain acceptance into complete host colonies, coexisting with the resident queen/s and producing only sexual offspring, which is reared by the host workers (BUSCHINGER & KLUMP 1988). In contrast, the elimination and replacement of host queen/s during colony foundation is the rule for temporary parasitic and slave-making (or dulotic) ants. Young queens of temporary parasites are assisted by the host workers only during the initial stage of colony establishment. In slave-making species, the host workers rear the offspring of the parasite until its worker population is sufficiently large to supplement the slave force by slave raids. During the raid, the dulotic workers attack other nests of the host species and pillage the brood from which the slaves emerge (BUSCHINGER 1986, BUSCHINGER & KLUMP 1988, HÖLLDOBLER & WILSON 1990, TOPOFF 1990).

The formicine genus *Polyergus* comprises five species living in obligatory dependence upon workers of the genus *Formica* for food, brood rearing, and colony maintenance (HÖLLDOBLER & WILSON 1990). Moreover, *Polyergus* queens are unable of rearing even their first brood and must rely on a form of dependent foundation named usurpation in which, before laying eggs, they must locate and invade a colony of the host species and usurp the role of reproductives. Host-colony usurpation has been exhaustively described in the American amazon ant *P. breviceps* (TOPOFF et al. 1988, 1990; TOPOFF & MENDEZ 1990; TOPOFF & ZIMMERLI 1993), and in the European amazon ant *P. rufescens* (EMERY 1908, 1909, 1911, 1915; FOREL 1920; ZAAVER 1967; MORI et al. 1995).

The critical phase of usurpation is the entrance into the target nest, since the parasitic queen must avoid the defensive behaviour of the *Formica* workers. In particular, laboratory observations have shown that when young queens of *P. rufescens* are introduced into artificial host colonies, the residents become alarmed and immediately attack the invading female. The queen usually attempts to avoid combat, but may also retaliate (MORI et al. 1995). In a successful usurpation, the parasite female detects the resident queen and kills her in a short time with her sharp mandibles. The lack of the resident queen causes a drastic change in the behaviour of the resident workers. The attacks by the host workers against the usurper become both less frequent and less violent, and begin to alternate with grooming. During this final stage of colony usurpation, the invader performs a typical vibration of the gaster that induces attraction and grooming by host workers. In few hours, the *P. rufescens* female stands on the host brood surrounded with a court of *Serviformica* worker slaves (MORI et al. 1995).

A morphological and bioacoustical analysis did not reveal the presence of a stridulatory organ and sound emission (MORI et al. 1998a). Therefore, we suggested that this gastral vibration favours the release of chemical substances that function as appeasement and/or attractant allomones (cf. BROWN et al. 1970) on the host species. Further experiments have confirmed that the reduction in aggression during host-colony take-over is likely the result of the secretion of an appeasement allomone by the *P. rufescens* queens (MORI et al. 1998b, 2000). This particular strategy occurs also in *P. breviceps* (TOPOFF et al. 1988) and thus appears to increase usurpation success by parasitic queens. This may occur by the queen acquiring an odour similar to that of the host queen, or by reducing aggression of the host workers until she is able to acquire the colony odour from the environment of the invaded nest.

The effect of the secretion of different glands of newly-mated females of *P. rufescens* on the behaviour of workers of its common host species *Formica cunicu-*

laria has been analysed in the laboratory (MORI et al. 1998b, 2000). The results of a set of aggression test showed that the secretion of the mandibular, pygidial, rectal, and poison glands do not alter the characteristic aggressive reactions generally performed by resident workers against alien ants. In contrast, the Dufour's gland secretion played a crucial role in the appeasement of residents of the target host colony. In fact, its secretion drastically lowered the degree of overt aggression shown by *F. cunicularia* workers against the intruders. This is the first evidence of the involvement and the role of Dufour's gland in the process of colony founding in *P. rufescens* (MORI et al. 1998b, 2000).

The Dufour's gland is a sac-like structure closely associated with the sting of queens and worker castes of all social Hymenoptera (BILLEN 1987). Although the primary purpose of the Dufour's gland in the Formicidae is not completely known, many recent studies in ants have shown that it performs various (probably secondary) functions, containing substances that serve as pheromones. The volatile compounds stored in the Dufour's gland frequently have a communicative function (PARRY & MORGAN 1979): in some cases it is involved in alarm, recruitment, propaganda, clustering or sexual attraction, as a source of trail pheromones or territorial markers (cf. MORGAN 1984, HÖLLDOBLER & WILSON 1990, BILLEN & MORGAN 1998).

Studies of the Dufour's gland secretion in various ant species show that it has a species-specific composition (MORGAN 1984, HÖLLDOBLER & WILSON 1990). It is a source of hydrocarbons and its derivatives such as long-chain acetates or ketones (BLUM & HERMANN 1978, ATTYGALLE & MORGAN 1984, MORGAN 1984). Analyses by gas chromatography-mass spectrometry show that the Dufour's gland of queens in *P. rufescens* produces a relatively simple secretion, consisting of a single hydrocarbon and several esters (VISICCHIO et al. 2000). The secretion is dominated by a single ester that was initially misidentified as decyl isobutyrate (VISICCHIO et al. 2000). We have since, after suggestions by HEFETZ & FRANCKE (pers. comm.), correctly identified this major component as decyl butyrate. This ester is present in large quantities (always more than 80%), with the other compounds being present in trace amounts. As far as we know, decyl butyrate has never been recorded in other insects. So, its occurrence in *P. rufescens* may be linked to the specialised life style of this species as a parasite.

As we previously reported, *P. rufescens* females use their Dufour's gland secretion during the crucial phase of colony usurpation, where it functions as an appeasement substance (MORI et al. 1998b, 2000). Based on the evidence that the secretion of the Dufour's gland is dominated by decyl butyrate, we have suggested that this single component may act as an appeasement allomone (VISICCHIO et al. 2000). The aim of the present work was to verify this hypothesis. Laboratory experiments involving bioassays to test the behavioural effect of decyl butyrate on workers of slave species were conducted. Additional trials were also performed in order to understand the nature of reaction released by decyl butyrate in host workers.

MATERIALS AND METHODS

Colony fragments of *Formica cunicularia* (the most common *Serviformica* species in our study area) were collected from a pure colony in Casola (province of Parma, Northern Italy) and housed in the laboratory in circular plastic boxes (20 cm diameter and 5 cm height), each consisting of about 60 adult workers and brood (larvae and pupae within cocoons). Conditions of temperature and relative humidity were kept under control (T 24-26 °C; RH 60-80%), and ants were fed with water, honey, and pieces of insects.

Decyl butyrate was synthesised and successively diluted (0.5%), using hexane as solvent. Aliquots of 1 μ l of this solution were put onto the thorax of workers of *Camponotus ligniperda* (collected in Parma) which under normal conditions evoke overt attacks by *F. cunicularia* ants. According to the procedure followed by TOPOFF and co-workers (1988), and MORI et al. (2000), we chose this indirect method of evaluation since it was impossible to use *P. rufescens* queens for bioassays of the substance's effectiveness, since we could not prevent them from secreting their own pheromones. Moreover, we used workers of *C. ligniperda* because their dimensions are similar to those of *P. rufescens* queens. Afterwards, the daubed *C. ligniperda* ants were individually introduced into artificial colony fragments of *F. cunicularia*. As controls, *C. ligniperda* daubed with only the solvent were tested in the same way.

A 10-min 'aggression test' (cf. LE MOLLI & PARMIGIANI 1981) was conducted to record the behaviour of resident workers towards the intruder. In particular, using a series of electronic counters, the following indices were measured in seconds: mutual investigation (MI), i.e. the time spent in reciprocal antennal inspection by the intruder and resident workers; latency to attack (LA), i.e. the time from the first contact to the first attack (when no attack occurred, 10-min latency was allocated); accumulated attacking time (AAT), i.e. the total time spent attacking.

The frequencies of some elements of aggressive behaviour were also recorded (WALLIS 1964, DE VROEY 1980, LE MOLLI & PARMIGIANI 1981): threat with open mandibles, gaster flexing, seizing, and dragging. Moreover, the number of attacks (No. Att) delivered was counted. A different group of *F. cunicularia* and *C. ligniperda* workers were used for each test.

In order to understand the nature of reaction of the residents towards decyl butyrate, we performed another kind of experiment. Two pieces of filter-paper were introduced into the arena simultaneously: one (experimental) was daubed with 1 μ l of decyl butyrate solution, the other (control) with a same quantity of the solvent (hexane). During the successive 5 min, the number of individuals antenning the two pieces of paper (No. Ind) and the antennal investigation time (AIT) were counted.

RESULTS

The effect of decyl butyrate on the aggressive behaviour of *F. cunicularia* workers towards treated *C. ligniperda* is illustrated in Figs 1-2, and in Table 1. *F. cunicularia* workers always showed fierce aggressive reactions against intruders daubed with the solvent. In fact, the alien ant (used as a vehicle for the chemical substance) was immediately attacked by the residents. The time spent in mutual investigation and latency to attack was very short. In contrast, the accumulated attacking time and the number of attacks delivered were high (cf. Fig. 1). Fifteen minutes after the end of the experimental time, 3 (25%) *Camponotus* workers were found dead, 5 (41.7%) were injured (in particular they had lost antennae and/or legs), and 4 (33.3%) were still being attacked by the residents. Moreover, several *F. cunicularia* individuals were killed or injured by the intruder.

Decyl butyrate protected the intruding ants from the incessant aggression by resident *Serviformica* workers. When *C. ligniperda* masked with this ester were introduced into *Serviformica* colony fragments, the time spent in the combat with the intruder (AAT) was statistically lower ($P < 0.002$) in comparison with the control, as were the number of attacks delivered ($P < 0.02$). Prolonged latency to attack and a longer antennal inspection were also recorded, being statistically higher ($P < 0.002$) in comparison with the controls (cf. Fig. 1). Finally, it is worth noting that *C. ligniperda* daubed with decyl butyrate were free to move inside the artificial colony and even allowed to remain near the brood. Moreover, 15 min after the end of the experimental time, no killed or injured *Camponotus* workers were found.

The use of decyl butyrate also caused a less frequent expression of all the elements of aggressive behaviour. This was particularly evident for the patterns typical

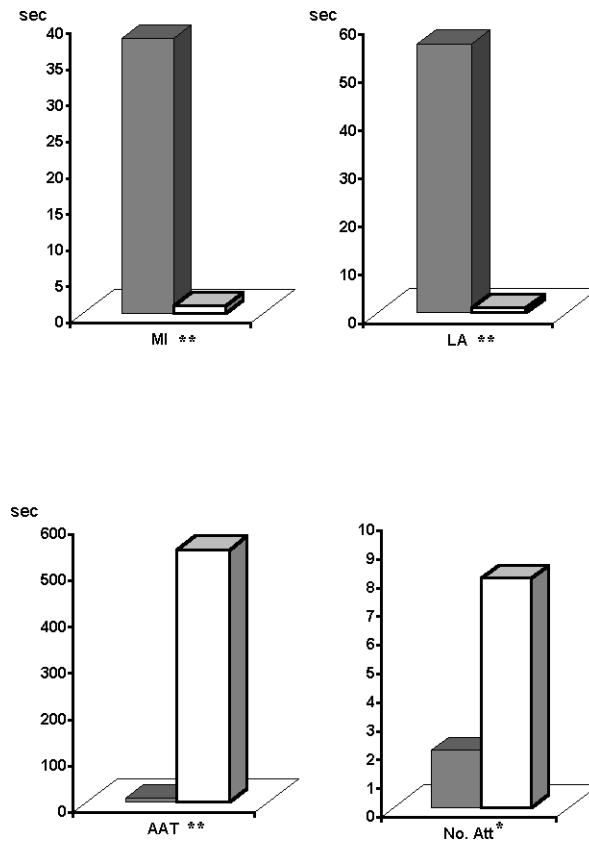


Fig. 1. — Comparison between median values of MI (mutual investigation), LA (latency to attack), AAT (accumulated attacking time), and No. Att. (number of attacks) recorded in experimental ($n = 12$; grey bars) and control ($n = 12$; white bars) tests. (*) $P < 0.02$; (**) $P < 0.002$ (two-tailed Mann-Whitney 'U' test throughout).

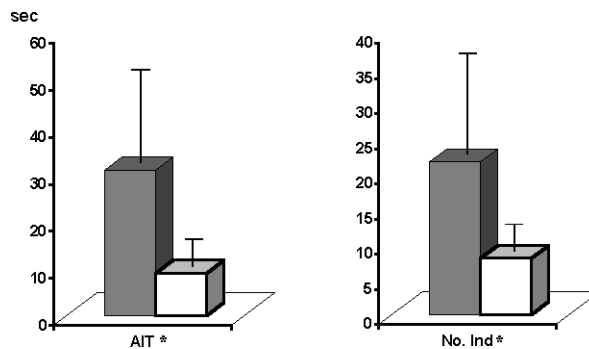


Fig. 2. — Comparison between mean values (\pm SE) of antennal investigation time (AIT) and number of ants (No. Ind) inspecting experimental (grey bars) and control (white bars) pieces of filter-paper recorded in tests ($n = 12$). (*) $P < 0.01$ (Wilcoxon test throughout).

Table 1.

Number of elements of aggressive behaviour recorded in trials ($n = 12$) between resident workers (*F. cunicularia*) and an alien ant (*C. ligniperda*) daubed either with solvent (control) or with decyl butyrate solution (experimental). *P*: probability level, Fisher exact probability test.

	Threat	Gaster flexing	Seizing	Dragging
Control	11	12	11	12
Experimental	4	4	3	1
<i>P</i>	< 0.005	< 0.001	< 0.002	= 0

of overt aggression (i.e. gaster flexing, seizing, and dragging) that frequently may determine injuries or killings. However, the frequencies of all the elements of aggression were statistically different in experimental and control trials (cf. Table 1).

When pieces of filter paper soaked with decyl butyrate or hexane were offered, *F. cunicularia* resident workers were more attracted by the pieces treated with the ester. The time devoted to the antennal investigation of the experimental lures and the number of ants inspecting them was statistically higher than that devoted to the control lures (cf. Fig. 2).

DISCUSSION

Whereas facultative slave-making ants can rely on a broad range of strategies during the foundation of new colonies, usurpation is the only system of colony founding adopted by the obligatory slave-makers of the genus *Polyergus* (HÖLLDOBLER & WILSON 1990). Such a strong specialisation in the parasite habitus is guaranteed by morphological, physiological, and behavioural adaptations. In this context, not only the evolution of sharp mandibles and a thick integument, but also the development of a sophisticated chemical strategy may have allowed *Polyergus* queens to become very efficient parasites. We have indeed confirmed this in *P. rufescens* by showing that the Dufour's gland produces an appeasement allomone during the crucial phase of host-colony usurpation (MORI et al. 1998b, 2000). Furthermore, based on the evidence of our gas chromatography-mass spectrometry analysis (VISICCHIO et al. 2000), it is likely that only a single component (the ester decyl butyrate) of the secretion acts as the appeasing substance.

The outcome of the present experiments, involving the use of bioassays to verify the behavioural effect of decyl butyrate on workers of the slave species *F. cunicularia*, confirm our hypothesis. In fact, the results of the laboratory 'aggression test' indicate that the aggressiveness of the *F. cunicularia* residents towards the *Camponotus* intruders daubed with the decyl butyrate, decreases drastically in comparison with that shown against intruders treated with solvent only (hexane). In particular, the time spent in combat was statistically longer in the controls, where *Camponotus* workers introduced into host colony fragments were immediately recognised as aliens and fiercely attacked by the *Serviformica* individuals without mutual inspection. In contrast, *Camponotus* intruders masked with decyl butyrate provoked less and shorter attacks by the residents. Additionally, when combats occurred, they were generally started by the *Camponotus* intruders, probably engaged in a defensive attack due to the unknown environment. It is worth noting that at the beginning of the test, *F. cunicularia* workers devoted their time to antennal inspection of

the alien ants. Therefore, the intruders were not ignored but actively investigated and successively adopted into the *Serviformica* colony. In fact, they were free to move among the residents and even tolerated in proximity of the host brood.

The nature of the reaction released by decyl butyrate on *Serviformica* workers is extremely interesting. When pieces of filter paper treated with the ester or hexane, respectively, were offered to the resident ants, a great portion was attracted by the lures treated with decyl butyrate. Host workers were observed to approach the filter paper, and often remained on and antennated them. The interest towards the lures soaked with the solvent was significantly lower.

We can conclude that decyl butyrate really acts as an appeasement allomone which likely reduces the aggression of the resident workers of the host colony until the usurper queen acquires the odour from the environment of the invaded nest. Being an ester and having a moderate volatility (molecular weight of 256), this compound would persist in an enclosed environment at low concentrations for a significant amount of time. Therefore, the use of decyl butyrate by *P. rufescens* queens during the critical phase of host-colony usurpation, seems to represent a case of exploitation by the parasite of an unknown odour that is attractive to the host (cf. VISICCHIO et al. 2000).

ACKNOWLEDGEMENTS

We wish to thank Abraham Hefetz (Tel-Aviv University) and Wittko Francke (Hamburg University) for their comments and suggestions allowing us to correctly identify the decyl butyrate (see Errata-Corrige in *Ethology Ecology & Evolution* 12: 221, 2000). This research was supported by grants from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (ex-40 and 60% funds) assigned to F. Le Moli and S. Turillazzi. Funding was also obtained through the research network "Social Evolution" of the Universities of Aarhus, Firenze, Keele, Sheffield, Uppsala, Würzburg and the ETH Zürich, financed by the European Commission via the Training and Mobility of Researchers (TMR) programme.

REFERENCES

- ATTYGALLE A.B. & MORGAN E.D. 1984. Chemicals from the glands of ants. *Chemical Society Reviews* 13: 245-278.
- BILLEN J. 1987. New structural aspects of the Dufour's and venom glands in social insects. *Naturwissenschaften* 74: 340-341.
- BILLEN J. & MORGAN E.D. 1998. Pheromone communication in social insects: sources and secretions, pp. 3-33. In: Van der Meer R.K. et al., Edits. Pheromone communication in social insects. Ants, wasps, bees, and termites. *Boulder: Westview Press*.
- BLUM M.S. & HERMANN H.R. 1978. Venoms and venom apparatuses of the Formicidae: Myrmeciinae, Ponerinae, Dorylinae, Pseudomyrmecinae, Myrmicinae, and Formicinae, pp. 801-869. In: Bettini S., Edit. Arthropods venoms. *Berlin, Heidelberg: Springer Verlag*.
- BOURKE A.F.G. & FRANKS N.R. 1995. Social evolution in ants. *Princeton, New Jersey: Princeton University Press*.
- BROWN W.L., EISNER T., WHITTAKER R.H. 1970. Allomones and kairomones: transspecific chemical messengers. *BioScience* 20: 21-22.
- BUSCHINGER A. 1986. Evolution of social parasitism in ants. *Trends in Ecology & Evolution* 1: 155-160.
- BUSCHINGER A. & KLUMP B. 1988. Novel strategy of host-colony exploitation in a permanently parasitic ant, *Doronomyrmex goesswaldi*. *Naturwissenschaften* 75: 577-578.
- DE VROEY C. 1980. Mesure de l'agressivité chez *Myrmica rubra* (*M. laevinodis*, Formicidae). *Biology of Behaviour* 5: 37-46.

- EMERY C. 1908. Osservazioni ed esperimenti sulla formica amazzone. *Rendiconti delle Sessioni della Regia Accademia di Scienze dell'Istituto di Bologna* 12: 49-62.
- EMERY C. 1909. Über den Ursprung der dulotischen, parasitischen und myrmekophilen Ameisen. *Biologisches Centralblatt* 29: 352-362.
- EMERY C. 1911. Ulteriori osservazioni ed esperienze sulla formica amazzone. *Rendiconti delle Sessioni della Regia Accademia di Scienze dell'Istituto di Bologna* 15: 60-75.
- EMERY C. 1915. La vita delle formiche. *Torino: Fratelli Bocca*.
- FOREL A. 1920. Les fourmis de la Suisse. *La Chaux-de-Fonds: Imprimerie Coopérative*.
- HÖLLDOBLER B. & WILSON E.O. 1990. The ants. *Berlin, Heidelberg: Springer Verlag*.
- KELLER L. 1991. Queen number, mode of colony founding, and queen reproductive success in ants (Hymenoptera Formicidae). *Ethology Ecology & Evolution* 3: 307-316.
- LE MOLI F. & PARMIGIANI S. 1981. Laboratory and field observations of attack by the red wood ant *Formica lugubris* Zett. on *Formica cunicularia* Latr. (Hymenoptera: Formicidae). *Aggressive Behavior* 7: 341-350.
- MORGAN E.D. 1984. Chemical words and phrases in the language of pheromones for foraging and recruitment, pp. 169-194. In: Lewis T., Edit. *Insect communication*. *London: Academic Press*.
- MORI A., D'ETTORRE P. & LE MOLI F. 1995. Host nest usurpation and colony foundation in the European amazon ant, *Polyergus rufescens* Latr. (Hymenoptera: Formicidae). *Insectes Sociaux* 42: 279-286.
- MORI A., GRASSO D. A. & LE MOLI F. 1998b. Raiding behaviour, mating strategies and colony founding in two species of slave-making ants, p. 325. In: Schwarz M.P. & Hogendoorn K., Edits. *Social insects at the turn of the millennium*. Proceedings of the XIII International Congress of I.U.S.S.I. (International Union for the Study of Social Insects). *Adelaide: Flinders University Press*.
- MORI A., GRASSO D.A., LE MOLI F., GIOVANNOTTI M., PAVAN G. & PRIANO M. 1998a. Morphological and bioacoustical evidence for lack of sound production by queens of *Polyergus rufescens* during host colony usurpation (Hymenoptera, Formicidae). *Fragmenta Entomologica* 30: 191-200.
- MORI A., GRASSO D.A., VISICCHIO R. & LE MOLI F. 2000. Colony founding in *Polyergus rufescens*: the role of the Dufour's gland. *Insectes Sociaux* 47: 7-10.
- PARRY K. & MORGAN E.D. 1979. Pheromones of ants: a review. *Physiological Entomology* 4: 161-189.
- TOPOFF H. 1990. Slave-making ants. *American Scientist* 78: 520-528.
- TOPOFF H., COVER S., GREENBERG L., GOODLOE L. & SHERMAN P. 1988. Colony founding by queens of the obligatory slave-making ant, *Polyergus breviceps*: the role of the Dufour's gland. *Ethology* 78: 209-218.
- TOPOFF H. & MENDEZ R. 1990. Slave raid by a diminutive colony of the socially parasitic ant, *Polyergus breviceps* (Hymenoptera: Formicidae). *Journal of Insect Behavior* 3: 819-821.
- TOPOFF H., WEICKERT T. & ZIMMERLI E. 1990. A comparative study of colony takeover between queens of facultative and obligatory slave-making ants (Hymenoptera: Formicidae). *Journal of Insect Behavior* 3: 813-817.
- TOPOFF H. & ZIMMERLI E. 1993. Colony takeover by a socially parasitic ant, *Polyergus breviceps*: the role of chemicals obtained during host-queen killing. *Animal Behaviour* 46: 479-486.
- VISICCHIO R., SLEDGE M.F., MORI A., GRASSO D.A., LE MOLI F., TURILLAZZI S., MONETI G., SPENCER S. & JONES G.R. 2000. Dufour's gland contents of queens of slave-making ant *Polyergus rufescens* and its host species *Formica cunicularia*. *Ethology Ecology & Evolution* 12: 67-73.
- WALLIS D.I. 1964. Aggression in social insects, pp. 15-22. In: Carthy J.D. & Ebling F.J., Edits. *The natural history of aggression*. *London: Academic Press*.
- ZAAYER P.M. 1967. Paarung und Koloniegründung von *Polyergus rufescens* Latr. im Kunstnest (Hymenoptera, Formicidae). *Zeitschrift der Arbeitsgemeinschaft Österreichischen Entomologen* 19: 1-9.