

FILIPPO FRIZZI (\*) - SARA PANICHI (\*) - ALICE RISPOLI (\*)  
ALBERTO MASONI (\*) - GIACOMO SANTINI (\*)

## SPATIAL VARIATION OF THE AGGRESSIVE RESPONSE TOWARDS CONSPECIFICS IN THE ANT *CREMATOGASTER SCUTELLARIS* (HYMENOPTERA FORMICIDAE) <sup>(1)</sup>

(\*) Department of Biology, University of Florence, Via Madonna del Piano, 6, 50019 Sesto Fiorentino, Italy; e-mail: [filippo.frizzi@unifi.it](mailto:filippo.frizzi@unifi.it)

Frizzi F., Panichi S., Rispoli A., Masoni A., Santini G. - Spatial variation of the aggressive response towards conspecifics in the ant *Crematogaster scutellaris* (Hymenoptera Formicidae).

In this study we investigated the relationship between spatial distance and intraspecific aggression in the Mediterranean tree-nesting ant *Crematogaster scutellaris*. Aggression tests were carried out in the field confronting group of workers (20 from each nest) collected from pairs of nests located at increasing distances one from the other (5, 10, 20, 40 and 80 meters). For each distance, 6 replicate tests, using different pairs of nests, were carried out. The probability of aggression and the time of the first aggressive event were recorded and modelled as a function of the distance between two nests using generalised linear models. Results showed that both the probability of aggression and the time of first attack were correlated to spatial distance in a complex way, having their maximum at intermediate distances between nests. The observed relationship cannot be simply interpreted according to the “dear enemy” or the “nasty neighbour” effects and contains elements in favour of both.

KEY WORDS: Ants, nest mate recognition, intraspecific aggression, dear enemy effect, nasty neighbour effect

### INTRODUCTION

Nest mate recognition plays an important role in the biology of social organisms (STRASSMANN *et al.*, 2011). In ants, the capacity of discriminating nest mates is well developed and individuals coming from a foreign nest are generally aggressed and rejected (CROZIER and PAMILO 1996; STURGIS and GORDON, 2012). The degree of aggressiveness towards non-nest mates may, however, be highly variable among species and also within the same species (D’ETTORRE and LENOIR, 2010). Nest mate recognition is predominantly based on chemical cues, among which cuticular hydrocarbons play a fundamental role (e.g. BONAVITA-COUGOURDAN *et al.*, 1987; GREEN and GORDON, 2003; HOWARD and BLOMQUIST, 2005) but also other important factors are involved. The relative importance of chemical, genetic and environmental parameters in conspecific recognition is still a debated issue (D’ETTORRE and LENOIR, 2010).

Aggression toward non-nest mate conspecifics may be locally influenced by a number of factors, such as nutrient availability (e.g. GROVER *et al.*, 2007) or previous fighting experience (RAVARY *et al.*, 2007; VAN WILGENBURG *et al.*, 2010). Moreover, this type of aggression can have important ecological significance in drawing boundaries between ant colonies, promoting cascade effects on the dynamics of ecosystems (HAERING and FOX, 1987; DAVIDSON, 1998; GROVER *et al.*, 2007; DRESCHER *et al.*,

2011, SANTARLASCIO *et al.*, 2014). Several studies revealed a dependence of aggression on the spatial proximity between colonies, with an increase in aggression probability for increasing distances (e.g. HEINZE *et al.*, 1996; LANGEN *et al.*, 2000; DIMARCO *et al.*, 2010). This relationship, which may simply be due to the fact that more distant colonies are more likely to be genetically unrelated, may be complicated in two contrasting ways, known as “dear enemy” and “nasty neighbour” effects (TEMELES, 1994), respectively. The “dear enemy” effect occurs when an animal responds less aggressively against a neighbouring conspecific than to a stranger and it is thought as a mechanism to reduce the cost of fighting against well established competitors (YDENBERG *et al.*, 1998). On the other side, the “nasty neighbour” effect occurs when aggression is stronger against a neighbour than a stranger, given that this is likely to use the same resources as the resident (TEMELES, 1994, MULLER and MANSER, 2007).

*Crematogaster scutellaris* is a common ant species widely distributed both in natural and human-managed ecosystems throughout the Mediterranean basin (BERNARD, 1968; BARONI URBANI, 1971). Nests are excavated in tree trunks or dead logs and can host up to several thousand workers (BERNARD, 1968; CASEVITZ-WEULERSE, 1972). Previous investigations showed that *C. scutellaris* is one of the most highly ranked competitors in Mediterranean ant assemblages and may have a pivotal role in the assembling of arthropod communities (CAMMELL *et al.*, 1996; WAY *et al.*, 1997; SANTINI *et al.*, 2007; OTTONETTI *et al.*, 2008). In the last year, this species received increasing attention. This was probably due not only for its ecological traits but also to the potentiality of *C. scutellaris* as candidate for the biomonitoring of pollutants (trace metals) in both natural and anthropic habitats (GRAMIGNI *et al.*, 2011, 2013).

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In this study we investigated the relationship between spatial distance and intraspecific aggression in *Crematogaster scutellaris*, confronting workers from nests located at increasing distances one from the other.

## MATERIAL AND METHODS

The study was carried-out near Florence (WGS84: 43° 53' 15.77" N, 11° 09' 53.16" E), Italy, during the summer 2008. Climate of the study area is Mediterranean with hot dry summers and mild, wet winters. In particular, ants were sampled in a managed woodland, a mixed stand mainly composed by downy oak (*Quercus pubescens*) and maritime pine (*Pinus pinaster*). Ants were searched for on tree trunks and dead logs and presence of *C. scutellaris* nests was assessed by repeated hammering on the trunk. A trunk was scored as hosting a nest only if a defensive swarming of ants from nest holes was elicited. Isolated ants scouting on trees were not considered.

### AGGRESSION TESTS

Ants from each nest were confronted with ants from nests placed at increasing spatial distance. The distances assessed were: 5, 10, 20, 40, 80 ( $\pm 1$ ) meters and six replicate tests for each distance were carried out. Additionally, 6 control tests (distance 0) were also performed, confronting groups of ants collected from the same nest. Before the test started, 20 workers were attracted to plastic containers (height = 8.5 cm, diameter = 8 cm) pinned on the trunk in the proximity of a nest exit. At the beginning of each test, containers from paired nests were joined to form a single arena. Then, ants were observed for three consecutive minutes and behaviour recorded according to two categories: 0) no aggression, when ants from different groups interacted with no evident behavioural change; 1) aggressive behaviour, which included both aggressive displays (e.g. open mandibles) and direct attacks towards non-nest mates. The time of first aggression was also measured as the time elapsed from the beginning of the experiment to the first observed aggressive behaviour.

Aggression probability and the time of first aggression were modelled as a function of spatial distance using generalised linear models, with a binomial and Gaussian error distribution, respectively (CRAWLEY, 2007). Four different models for these relationships were compared: 1) model with a sigmoidal increase of aggression with distance, 2) a quadratic model, describing a humped relationship with aggression probability being maximal for intermediate distances and lower at both short and long distances, 3) a logarithmically transformed quadratic model, similar to the previous but with peak of aggression skewed to the left and 4) a null model, indicating that aggression does not vary with distance. Models were ranked on the basis of their AICc values (AIC values corrected for small sample size) following BURNHAM and ANDERSON (2002) and the 'best' model was chosen as the one having the lowest AICc value. All the analyses were carried out using the R (ver. 3.0.2) statistical software.

## RESULTS AND DISCUSSION

Aggression probability was strongly related to distance and increased rapidly with increasing distance, remained stable around a maximum value ( $\sim 0.8$ ) between 10 and

40 meters and then decreased again at greater distances (Fig. I). The best model in describing this pattern was model 3 (log-transformed quadratic model) which significantly outperformed all other models.  $\Delta AICc$  of alternative models were always  $>6$ , indicating that these had no support (Table 1, A). A comparable trend was observed when considering the time of first aggression (TFA, Fig. II), where model 2 (quadratic model) outperformed all other models (Table 1, B). TFA steadily increased with distance, reached a maximum around 40 m and then decreased again. As for example, aggressive encounters, when present, started on average 25 seconds after ants of two nearby nests (5 m distance) were placed in contact but it took  $\sim 90$  seconds to elicit an aggression among nests located 40 m apart.

Overall the results of this study showed that aggression probability was maximal at intermediate distances and lower at both shorter and larger distances. The lower levels of aggression at short distances may be explained in two different ways. First, previous studies suggested that this species may adopt a polydomous arrangement of colonies with a spatially clumped distribution of nests (SCHATZ and HOSSAERT-MCKEY, 2003; SANTINI *et al.*, 2011). The low aggressiveness observed at short distances may hence be due to the fact that some of the tested nests were simply subunits of the same colony, although care was taken to choose nests not directly connected by evident trails of workers. A second likely explanation deals with the "dear enemy" mechanism, according to which an animal responds less aggressively against a neighbouring (non-nest mate) conspecific than to a stranger (e.g. HEINZE *et al.*, 1996; LANGEN *et al.*, 2000). Further investigations, with use of molecular tools to assess the degree of relatedness among colonies are therefore needed (FRIZZI *et al.*, 2009).

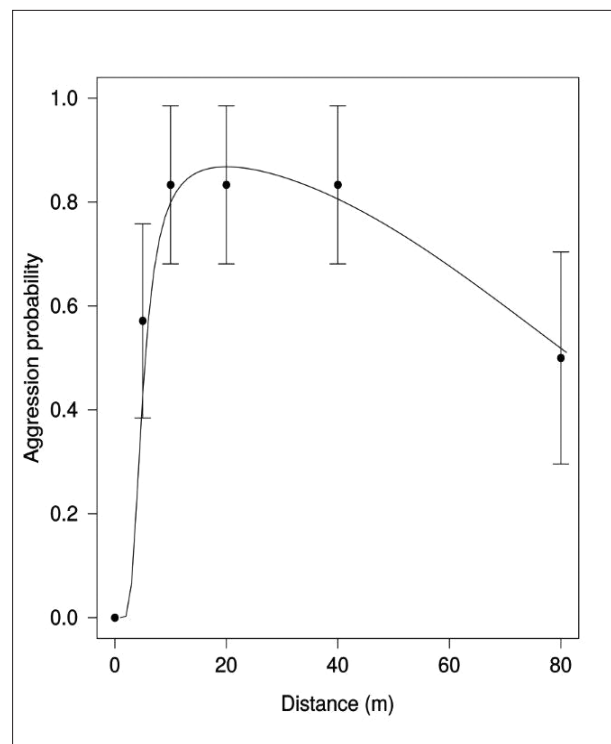


Fig. I – Spatial variation of aggression probability. Continuous line represents predicted values (log-transformed quadratic model), dots are observed values (mean  $\pm$  SE).

Table 1 - Results of fitting glm models to data. Models are ranked in increasing order of AICc, from best to worst.  $\Delta$ AICc is the difference in AICc value between each model and the best one. A) Probability of aggression, B) time of first aggression.

	Model (number)	AICc	$\Delta$ AICc
A) Aggression probability			
	log-quadratic (3)	40.91	0.00
	quadratic (2)	47.46	6.55
	null (4)	53.84	12.93
	sigmoidal (1)	55.37	14.46
B) Time of first aggression			
	quadratic (2)	240.79	0.00
	log-quadratic (3)	244.32	3.53
	null (4)	244.75	3.53
	sigmoidal (1)	246.00	5.21

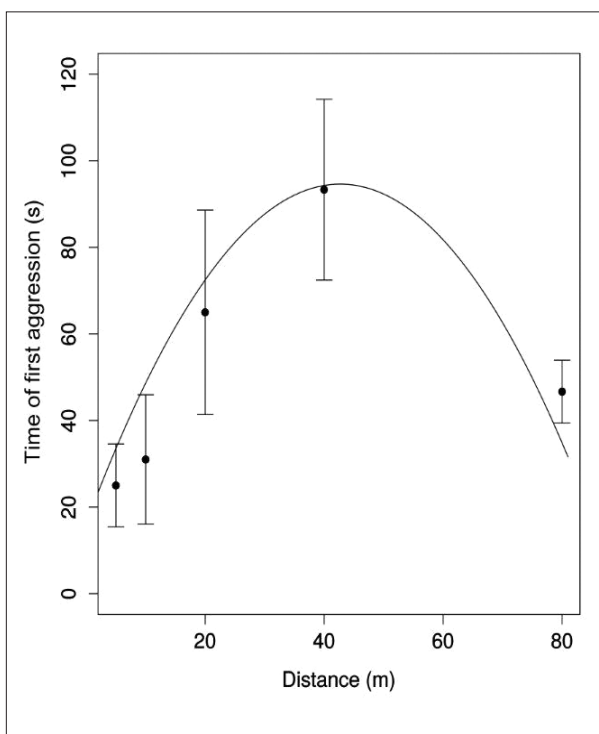


Fig. II – Spatial variation of time of first aggression (TFA). Continuous line represents predicted values (quadratic model), dots are observed values (mean  $\pm$  SE).

On the opposite side, the decrease in aggression probability at larger distances is broadly compatible with a “nasty neighbour” behaviour, as already described in other ant species, such as *Pogonomyrmex barbatus* (GORDON, 1989), *Cataglyphis fortis* (KNADEN and WEHNER, 2003), *Pristomyrmex pungens* (SANADA-MORIMURA *et al.*, 2003), *Iridomyrmex purpureus* (VAN WILGENBURG *et al.*, 2007), *Linepithema humile* (THOMAS *et al.*, 2005), *Oecophylla smaragdina* (NEWAY *et al.*, 2010) and *Formica pratensis* (BENEDEK and KÖBORI, 2014). However, even in this case, an assessment of the actual relatedness among colonies is mandatory before any firm conclusion can be reached.

The time of first aggression showed a similar variation with distance: an increase with increasing distance up to

40 m and a decrease for larger values. The observed increase in reaction time may suggest that ants take longer to recognize more distant and probably unknown opponents although the decrease observed for greater distances seems to contradict this interpretation. This latter decrease, however, could be partly influenced by a bias in the duration of observation. Interactions between colonies were in fact observed for three consecutive minutes, and aggressive responses taking longer times to be triggered, could have been simply overlooked (ROULSTON *et al.*, 2003; JAQUIÈRY *et al.*, 2005).

In conclusion, this preliminary study provided a first description of how aggressiveness between *C. scutellaris* colonies varies with spatial distance, showing a complex relationship that cannot be simply interpreted according to the “dear enemy” or the “nasty neighbour” effects, but rather to a mix of the two. This study also raised a number of questions, stimulating new research directions. A deeper understanding of the observed patterns of aggression will require, in fact, a thorough assessment of the genetic relatedness among colonies using molecular tools (FRIZZI *et al.*, 2009), but also a characterisation of their cuticular chemical profiles (MENZEL *et al.*, 2010). Answering these basic questions will deepen our comprehension of the biology and ecology of this important ant species.

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