Low intraspecific aggression among polydomous colonies of *Formica exsecta* (Hymenoptera: Formicidae)

Klára Kiss & Ottília Tímea Kóbori

Summary: Aggressive behaviour of *Formica exsecta* was studied on pastures near the După Luncă Marsh (Harghita County, Romania). More than 3000 *Formica exsecta* nests can be found here. Three small nest aggregations situated at different distances from each other were chosen for the purpose of our research. The aim of our study was to find out if there are any differences in the aggressive behaviour between the nest complexes and if so, then can there be found any differences regarding the level of aggression between workers from different distances. The behaviour of ants was tolerant, even if they were from nests aggregations situated at bigger distances from each other. Lower level of aggression was found between nest aggregations situated closer to each other, however this could be shown only in spring.

Key words: *Formica exsecta*, aggressive behaviour, polydomous colonies

Introduction

Ants are known as social insects which can discriminate nestmates from non-nestmates and defend aggressively their colony from aliens. It is demonstrated that the clue of nestmate recognition is the cuticular hydrocarbon profile, of specimens in many ant species (Suarez *et al.* 2002, Sorvari *et al.* 2007, Martin *et al.* 2008, Martin and Drijfhout 2009, Martin *et al.* 2009). CHC profile has a genetically and an environmentally determined component. If the CHC profile of the individuals is different the aggression level between them will be higher (Torres *et al.* 2007). In some species the level of aggression is increasing with decreasing genetic relatedness (Beye *et al.* 1998, Holzer *et al.* 2006, Tripet *et al.* 2006, Drescher *et al.* 2007). Social structure also affects the level of aggression in ants. Workers from monodomous colonies are often more aggressive toward aliens than those from polydomous colonies (Pisarski 1982a). The lack of aggression among non-nestmate workers within polydomous systems is well-known in many ant species (Chapuisat *et al.* 2004, Holzer *et al.* 2006, Katzerke *et al.* 2006, Debout *et al.* 2007, Thomas *et al.* 2007, Martin *et al.* 2009).

Formica exsecta is an aggressive territorial ant species (Pisarski 1982b). It is able to form both polydomous and monodomous colonies (Pisarski 1982b, Katzerke *et al.* 2006, Debout *et al.* 2007, Martin *et al.* 2009). It is not really frequent in Romania; there are only few mentions in the Romanian ant fauna list (Markó *et al.* 2006). One of its known occurrences is near the După Luncă Marsh (Harghita County, Romania). There are more than 3000 *Formica exsecta* nests here in a relatively restricted area, distributed in several different types of habitats. In most cases they form polydomous systems (Martin *et al.* 2009, Erős *et al.* 2009). It is demonstrated that the intranest relatedness is low in *F. exsecta* population living here (Goropashnaya *et al.* 2007), but the CHC profile of workers is very similar (Martin *et al.* 2009). The similarity of CHC profil predicts low level of aggression but the low genetic relatedness forecasts hostility between supercolonies. The aim of our study was to find out if there is any aggression between the different nest complexes and if so, then is there any difference in the level of aggression among ants from nest complexes situated in different spatial distance.

Materials and methods

Study area Our field study was carried out near the După Luncă Marsh (Harghita County, Romania, 46°36N, 25°36E, ~780 m a.s.l.) from July 2005 to May 2007. The area is situated in one of the coldest regions in Romania, in the southern part of Giurgeului Depression. Different sized *Formica exsecta* nest-complexes can be found here. The largest has more than 1000 nests, smaller aggregations contain around 10 nests (Erős *et al* 2009). We chose three small-sized complexes. The nest complexes were situated close to the creek on wet pastures where there are small trees (*Betula pubescens*, *Salix* spp.) scattered

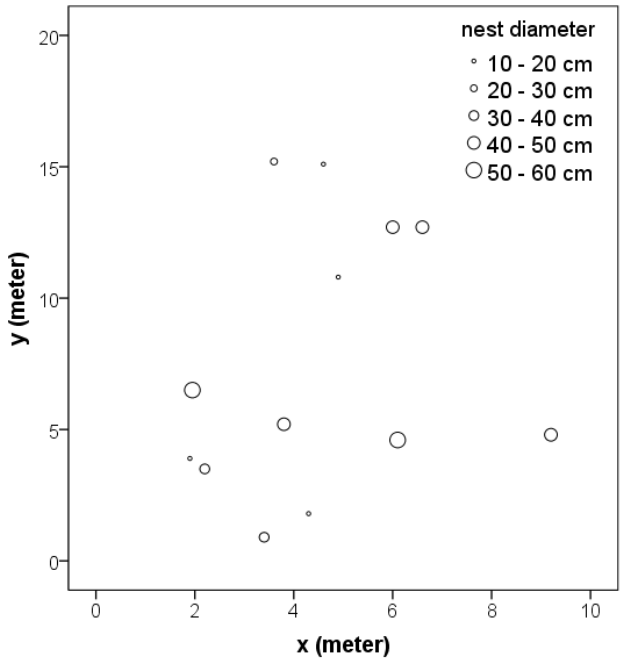

Fig. 1. Map of the first nest complex in summer 2005.

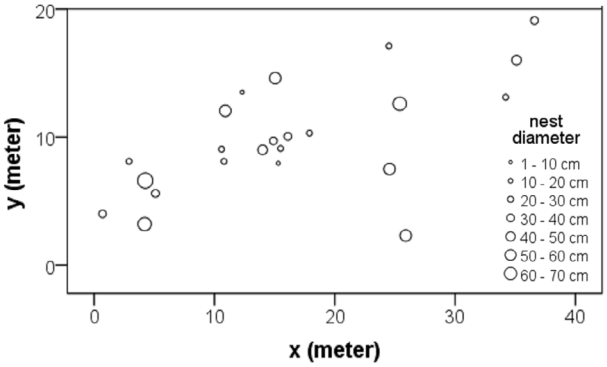

Fig. 2. Map of the second nest complex in summer 2005.

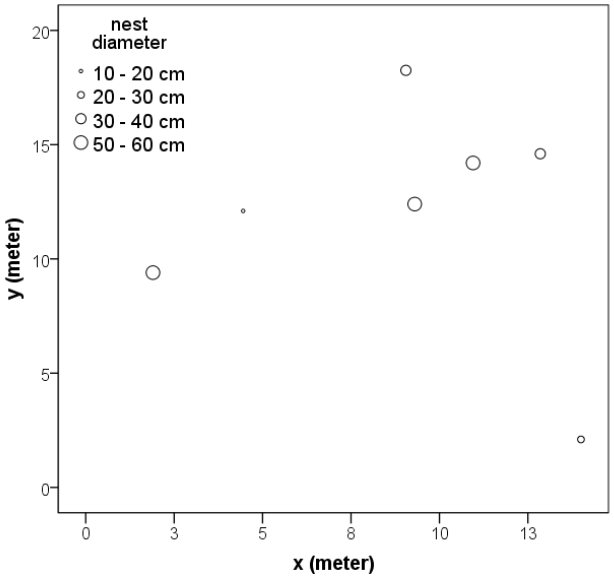

Fig. 3. Map of the third nest complex in summer 2005.

all over. Human interference was small in our study sites. Two of these nest aggregations (Figs. 1-2) were close neighbours; the distance among them was 200 meters. The third one (Fig. 3) was located 600 meters from these two on the other side of the creek. First nest complex was the reference and regarding from here the second was close neighbour the third was far neighbour. The number of *Formica exsecta* nests within each complex changed slightly during the study years (Table1).

Table 1. The number of nests in the studied nest complexes in the course of the research.

Year	Nest complex I	Nest complex II	Nest complex III
2005	7	23	13
2006	11	13	10
2007	11	15	11

Aggressiveness tests The level of aggression among ants was determined by the use of aggressiveness tests. Various types of such behavioural tests are frequently used in the research of ants and other social insects. By the use of such tests it is possible to assess the level of discrimination among nestmates and non-nestmates, conspecifics and allospecifics (Le Moli and Mori 1990, Leponce *et al.* 1996, Beye *et al.* 1998, Pirk *et al.* 2001, Roulston *et al.* 2003, Chapuisat *et al.* 2004, Katzerke *et al.* 2006, Grover *et al.* 2007, Martin *et al.* 2009). Aggressiveness tests were carried out between workers from different nests but in the same nest complex to analyse if the nest complex is a supercolony or not. To detect the differences in the level of aggression caused by the distance between the supercolonies, tests with workers from neighbouring and distant nest aggregations were carried out. The reference was the first super colony (Fig. 1). To detect the differences between the intranest and internest aggression, aggressiveness tests with workers from the same nest were carried out as well. One worker marked on the back of the pronotum from one nest and three unmarked workers from the other nest were put in a plastic cup covered by net, which would allow air exchange. In all cases interactions of individuals with the marked, focal specimen were recorded. We also carried out aggression tests with unmarked workers to control for the effect of marking, but in this case only two workers were used. Thus the interactions in this case could also be analysed as behavioural acts toward one focal individual. Five behavioural categories were recorded: ignore, mutual feeding, mandible gapping, charge and fighting. The duration of each test was five minutes.

Aggressive *Formica* species often are more aggressive with each other in spring than in summer (Mabelis 1978, 1984). To avoid the false conclusions caused by the seasonal variation each type of test was

carried out in spring and in summer. Total number of tests was 343 (132 within nest complexes in spring, 167 between nest complexes, 23 intranest and 21 with unmarked workers).

Statistical analysis Percentage of different behavioural categories was analyzed. We compared the percentage of interaction types from aggressiveness tests with and without marking to determine the impact of marking. We also made comparison between the results of intranest and internest tests from the same territory to establish the differences between the intranest and internest aggression. The results of tests done in different combination were compared as it follows:

- tests within the nest complex and between nest complexes
- tests between neighbouring and distant nest complexes
- test carried out in spring and summer

All comparisons were effectuated with Mann-Whitney U-test. Statistical analysis was realized with SPSS for Windows 17.0.

Results

Aggression within and between nest complexes Marking had no significant effect on the behaviour of ants, neither the proportion of ignorance, nor those of mandible gaping interactions differed between marked intranest and unmarked intranest interaction sequences (Mann Whitney *U*-test, n_1 = 21, n_2= 18, z_{ignore}= -0.46, p_{ignore} = 0.686, $z_{mandible\ gapping}$= -0.579, $p_{mandible\ gapping}$= 0.626). No other interaction type was observed in the course of control tests.

An important fact is that the behaviour of *Formica exsecta* workers was very tolerant toward the conspecific individuals in hole course of our research. High percentage of ignores (Fig. 4) and low percentage of negative (mandible gapping, attack and fighting) interactions (Fig. 5) was observed at within and between nest complexes. The percentage of ignores was constantly higher than the percentage of all negative interaction forms (Figs. 4-5). Mutual feeding was also observed between workers from different nest complexes. Fatal interactions were not noticed at all. Fights were detected only in case of between nest complex test but their number was very small in all tests effectuated.

The level of intranest aggression did not differ from that of within nest complex aggression. No significant difference was found concerning the percentage of ignores between ants from the same nest and workers from different nests of the same complex (Mann Whitney *U*-test, n_1 = 23, n_2=30, z_{ignore}= -0.353, p_{ignore} =0.724). However both mutual feeding and few mandible gapping interactions were observed in both combinations. Attacks and fights were not observed at all.

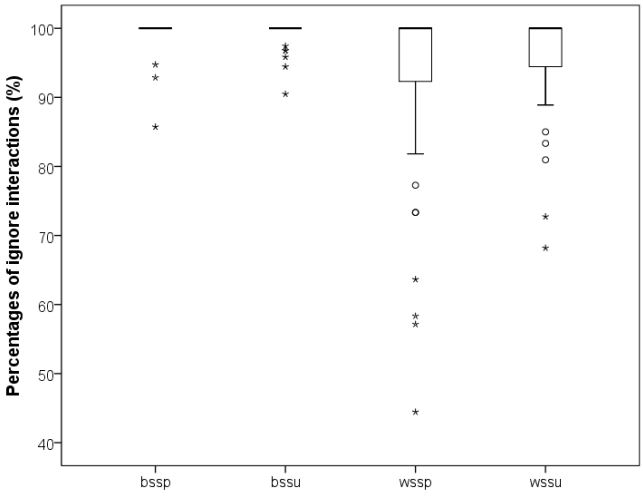

Fig. 4. Percentages of ignore interactions in different test combinations: bssp – between supercolonies in spring, N_{bssp} = 36, bssu – between supercolonies in summer N_{bssu} = 96, wssp – within supercolony spring N_{wssp} = 73, wssu – within supercolony in summer, N_{wssu} = 101.

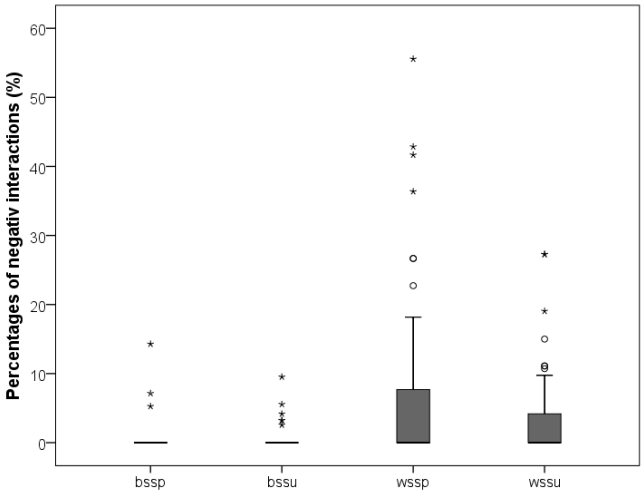

Fig. 5. Percentages of negative interactions in different test combinations: bssp – between supercolonies in spring, N_{bssp} = 36, bssu – between supercolonies in summer N_{bssu} = 96, wssp – within supercolony spring N_{wssp} = 73, wssu – within supercolony in summer, N_{wssu} = 101.

Comparing the percentage of different interaction types between workers from different nest complexes and the same nest complex we observed that ants from different nest complexes were more aggressive towards each other, than ants from the same nest system. The percentages of mutual feedings and ignores were significantly higher in within nest-complex aggressiveness tests (Fig. 6), while the percentage of mandible gapping was significantly higher between workers from different nest complexes (Fig. 7). Attacks and fights were found only in course of tests between ants from different nest complexes.

Neighbourhood effect We tried to find out whether the level of aggression between nest systems could be distance related. Our data from spring showed that workers from neighbouring nest complexes were more tolerant to each other, than workers from distant nest-complexes (Fig. 8). The percentage of negative interactions were significantly higher between ants

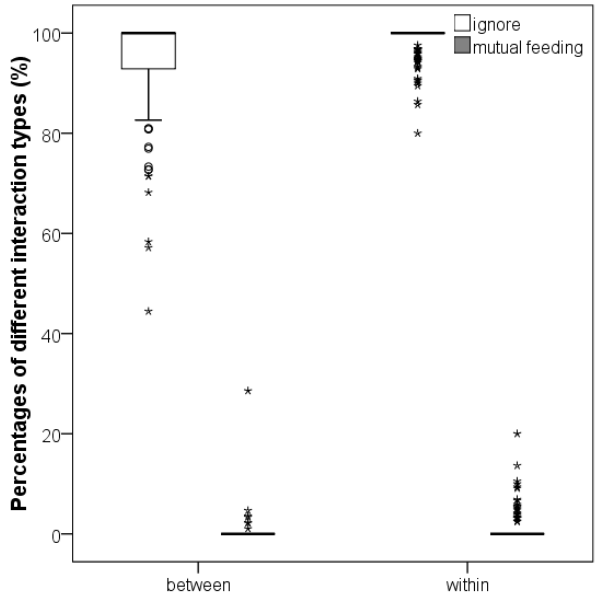

Fig. 6. Percentages of ignore and mutual feeding interactions in between and within nest-complex aggressiveness tests: Mann-Whitney *U*-test $N_{between} = 183$, $N_{within} = 132$, $z_{ignore} = -4.841$, $p_{ignore} < 0.001$, $z_{mutual\ feeding} = -4.296$, $p_{mutual\ feeding} < 0.001$.

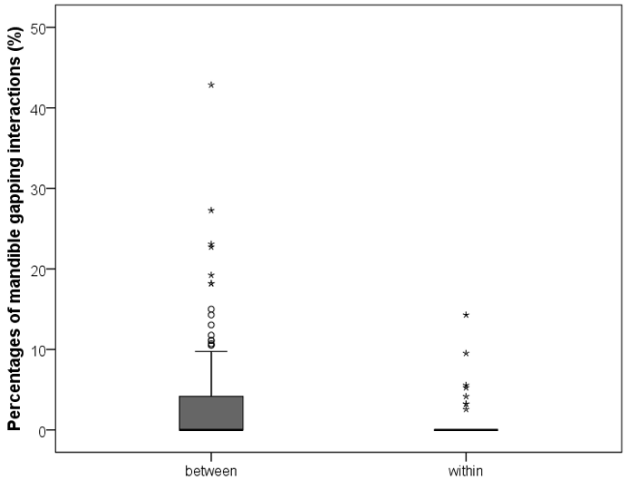

Fig. 7. Percentages of mandible gapping interactions in between and within nest-complex aggressiveness tests: Mann-Whitney *U*-test $N_{between} = 183$, $N_{within} = 132$, $z_{mandible\ gapping} = -5.993$, $p_{mandible\ gapping} < 0.001$.

from distant nest-complexes (Fig. 8). However, no significant effect of spatial distance was found on the level of aggression in summer (Fig. 9).

Seasonality *Formica exsecta* workers were more aggressive in spring, than in summer. Mutual feeding was detected only in summer. The percentage of all negative interactions was significantly higher in spring. No significant difference was found in the percentage of ignores (Fig. 10).

Discussion

The level of aggression is very low among non-nestmates belonging to the same nest-complex, which leads us to the conclusion that indeed, these nest-complexes are interrelated polydomous systems, supercolonies. Such supercolonies are quite frequent

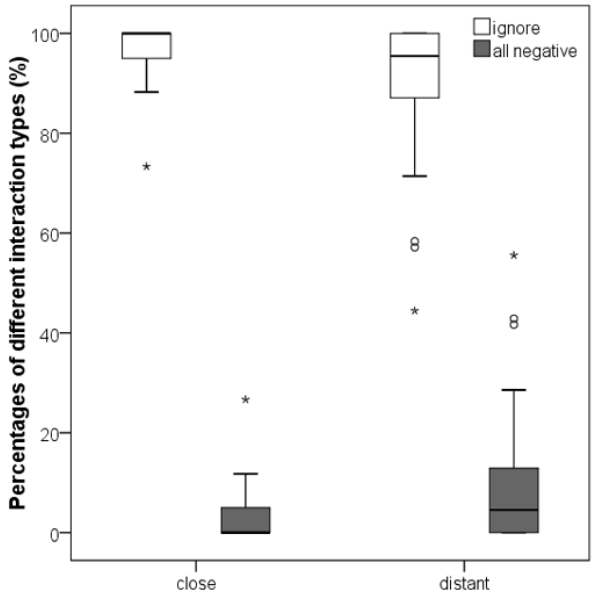

Fig. 8. Percentages of interaction types between ants from close and distant nest-complexes in spring: Mann-Whitney *U*-test, $N_{close} = 40$, $N_{distant} = 42$, $z_{ignore} = -2.936$, $p_{ignore} = 0.003$, $z_{all\ negative} = -2.936$, $p_{all\ negative} = 0.003$.

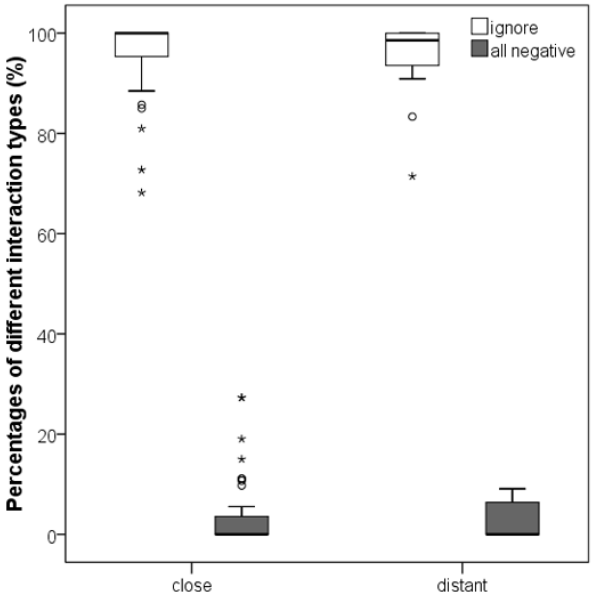

Fig. 9. Percentages of interaction types between ants from close and distant nest-complexes in summer: Mann-Whitney *U*-test, $N_{close} = 55$, $N_{distant} = 46$, $z_{ignore} = -0.346$, $p_{ignore} = 0.728$, $z_{all\ negative} = -0.725$, $p_{all\ negative} = 0.467$.

in *Formica exsecta* (Pisarski 1982, Holzer *et al.* 2006, Thomas *et al.* 2007, Zink *et al.* 2007, Chapuisat *et al.* 2004). Our results corroborate with the findings of Katzerke *et al.* (2006): aggression is higher among non-nestmates originating from different nest-complexes. However, in our experiment the level of aggression is generally low among such specimens irrespective of them being situated far from each other. The phenomenon has been observed in other ant species, too. Chapuisat *et al.* (2004) obtained similar results in the case of *Formica paralugubris*. This can be explained by the similarity of CHC profile of the *F. exsecta* workers living here (see Martin *et*

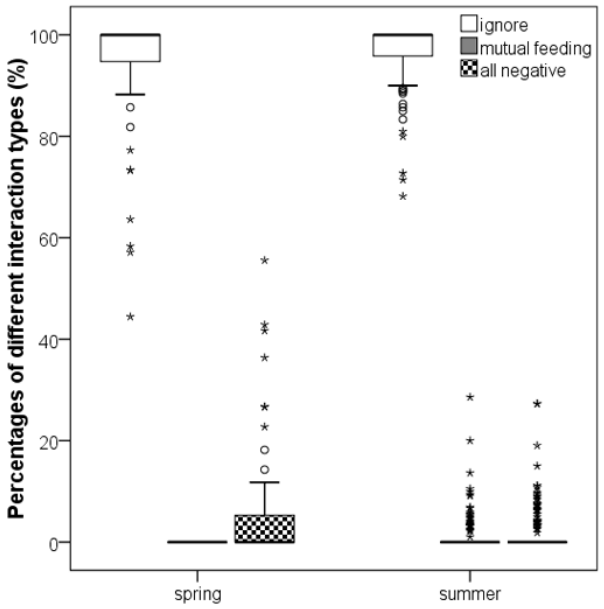

Fig. 10. Percentages of interaction types in spring and in summer, Mann-Whitney *U*-test, N_{spring} = 109, N_{summer} = 197, z_{ignore} = -0,102, p_{ignore} =0.919, $z_{all\ negative}$ =-2,640, $p_{all\ negative}$ = 0.008.

al. 2009). Regarding the low level of aggression we suppose that it could be connection between different colonies. Taking into consideration that our *Formica exsecta* population lives in one relatively restricted area this connection can be realized with females too however according to the results of Liautard and Keller (2001) dispersion of females between supercolonies is limited.

The level of aggression between neighbouring supercolonies was lower than between distant supercolonies. This could mean that the relation between the neighbouring supercolonies is stronger. It is possible that our two supercolonies situated close to each other have a common origin and they were part of one bigger supercolony. The effect of spatial distance on the level of aggression was demonstrated only in spring.

Although direct aggression was rare in both periods *F. exsecta* workers were more offensive in spring than in summer. The seasonal variation of aggressive behaviour was observed in many ant species (Ichinose 1991, Thurin and Aron 2007), more specifically in other territorial *Formica* species (Mabelis 1979, 1984) including *F. exsecta*, too (Katzerke *et al.* 2006). Further on the detailed analysis of the connection between cuticular hydrocarbon-profile and aggression is needed in order to elucidate more intricate mechanisms of the studied phenomenon. It would be interesting to find out whether less alterations of aggression are due to small variations of CHC profiles or explanation should be looked for elsewhere.

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