

Aggression, cooperation, and relatedness among colonies of the invasive ant, *Monomorium pharaonis*, originating from different areas of the world

Jan FROUZ^{1,5,6}, Radek JOHN², Václav RUPEŠ³, Gábor CECH⁴ & Károly MARIALIGETI⁴

¹Institute of Soil Biology, Biology Centre AS CR, Na Sádkách 7, CZ-37005, Czech Republic

²Charles University, Department of Ecology, Viničná 7, CZ-12000, Prague, Czech Republic

³Institute for Public Health, Šrobárova 48, CZ-10048, Prague, Czech Republic

⁴Eötvös Loránd University, Department of Microbiology, Pázmány Péter sétány 1/c, H-1117 Budapest, Hungary

⁵South Bohemian University, Department of Ecology, Branišovská 31, CZ-37005, Czech Republic

⁶Charles University, Institute for Environmental Studies, Benatska 2, CZ-12800 Prague, Czech Republic

Abstract: The cooperation and aggression between five laboratory colonies of *Monomorium pharaonis* were compared using an aggressiveness test and pupa-carrying test in laboratory arenas. The colonies were derived from field collections in different parts of Europe and USA. Generally, inter-colony aggressiveness was low and acceptance of pupae from other colonies was high. Workers from one colony (Lužiny, CZ), however, frequently displayed aggressive behavior when paired with workers from other colonies, and the Lužiny pupae were avoided by workers of other colonies in pupa-carrying tests. Behavioral tests were only partly consistent with the phylogenetic relatedness of ants because the Wisconsin colony (USA) grouped with the Lužiny colony (and not with the other three colonies) in the phylogenetic analysis but grouped with the other three colonies in the behavioral tests.

Key words: *Monomorium pharaonis*; kin selection; supercolony; genetic bottleneck; aggression, invasive species, ant

Introduction

Pharao ant *Monomorium pharaonis* (L., 1758) (Formicidae, Myrmicinae) is an invasive ant species with cosmopolitan distribution (Czechowski et al. 2002). It is an important indoor pest in homes and hospitals (Paacock & Baxter 1949; Lauterer 1971; Beatson 1972), where it can transmit a variety of diseases (Beatson 1972). This species is very thermophilous and is restricted to heated indoor locations in temperate areas (Beatson 1972) because it can not survive winter without protection. The species has cosmopolitan distribution, the original (natural) range of its distribution is not clear.

Several authors reported that *M. pharaonis* forms large colonies with a very low level of inter-colony aggressiveness, but data supporting this observation are lacking. The absence of inter-colony aggressiveness suggests that this species underwent a substantial reduction of genetic diversity during invasion, which might not only reduce inter-colony aggressiveness but even cause superclonality as described for some other invasive species, especially *Linepithema humile* (Mayr, 1868) (Formicidae, Dolichodema) (Holway et al. 1998; Tsutsui et al. 2000, 2003; Starks 2003).

The aim of this study was to measure inter-colony aggressiveness and potential inter-colony cooperation among five colonies of *M. pharaonis* collected from

different parts of the world. The cognation of these colonies was also tested by DNA techniques.

Material and methods

The five ant colonies used in this study were derived from laboratory-reared colonies maintained in Prague; the rearing system is described in Rupeš et al. (1978). These colonies were originally started as field collections from Wisconsin (USA), Mannheim (Germany), Lužiny (Prague, Czech Republic), Ústí nad Labem (Czech Republic, hereafter only “Ústí”), and Olomouc (Czech Republic). From each of these colonies we derived a smaller colony with 200–1000 workers and 3–10 queens, which were kept in plastic dishes (12 cm in diameter) with walls covered by fluon to prevent ants from escaping. Colonies were held at 28°C, 12:12 h L:D and supplied with boiled egg yolk, dead insects, and water ad libitum.

The design of a pair aggressiveness test was modified from Giraud et al. (2002). In each test a pair of workers, one from each tested colony, was placed into a well-ventilated circular arena 2.4 cm in diameter with walls covered by fluon. The ants were observed for 5 minutes and any aggressive behavior was recorded. Because the quality and strength of the aggressive behavior were rather uniform, we recorded only the presence or absence of aggression. Each test used a washed arena freshly covered by fluon. The aggression experiment was performed twice (trial 1 in October 2004 and trial 2 in March 2005), with five replicates

Table 1. Aggression between workers from different colonies (colony name indicates site of original collection) in the aggressiveness.

Colony	Colony				
	Wisconsin	Mannheim	Ústí	Lužiny	Olomouc
Wisconsin USA		2/10	2/10	4/10*	1/10
Mannheim G	0/5		0/10	2/10	3/10*
Ústí CZ	0/5	0/5		4/10*	2/10
Lužiny CZ	0/5	0/5	2/5		0/10
Olomouc CZ	0/5	0/5	0/5	0/5	

Explanations: Fractions indicate the incidents of aggressive behavior divided by total number of observations (results from trial 1 have a denominator of 5 and those from trial 2 have a denominator of 10). An asterisk indicates that occurrence of aggressive behavior was more frequent than random according to χ^2 test, $P < 0.05$. No aggression was observed between workers from the same colony. USA – United States of America, G – Germany, CZ – Czech Republic.

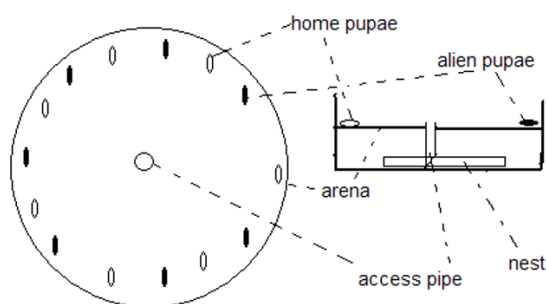


Fig. 1. Diagram of arena used for pupa-carrying cooperation test.

trial 1 and 10 replicates in trial 2 (Table 1). For each replicate different ants were used and no worker was used twice in aggressivity test. Controls, which consisted of two workers from the same colony, were replicated five times in trial 1 and 10 times in trial 2. The χ^2 test was used to determine whether the occurrence of aggression was more frequent than random.

The pupa-carrying test as initially described by Rosengren et al. (1994) and then modified by Maeder et al. (2005) was used to measure potential cooperation. An experimental arena was made from a Petri dish 6 cm in diameter. The wall of the arena was covered by fluon, and an access pipe connected the centre of the arena with the chamber below; chambers were constructed from two cover slides (17 × 61 mm) with wooden “walls” on the periphery that maintained a clearance between cover slips of about 1 mm. To start a test, eight pupae from one colony (colony A) and eight from a different colony (colony B) were alternated at the periphery of the arena. About 20 workers from colony A were then placed in the chamber. The workers could then use the access pipe to enter the arena and carry out pupae (Fig. 1). When a worker started to carry a pupa, we recorded whether the pupa was from the “home” colony (worker from colony A selects pupa from colony A) or “alien” colony (worker A selects pupa B); that worker was then removed and the selected pupa was replaced with a new pupa. Data were recorded for the first 10 workers that selected pupae. Then the same experiment was repeated with new set of pupae from colonies A and B as above but in this second run

workers from colony B were placed in the chamber and allow to choose pupae. So for each pair of colonies we have two experiments in which both colonies were used as both “home” and “alien” colony. Experiments were repeated with all combinations of the five colonies in pairs. The protocol developed by Maeder et al. (2005) was used to test the hypothesis that workers selected their own pupae (from the home colony) more frequently than random.

Ants used for the behavioral tests were subjected to DNA analysis. Genomic DNA from frozen ants was extracted using a QIAGEN DNeasyTM tissue kit (animal tissue protocol; Qiagen) and stored at 4°C. The *CO I* gene was amplified using the primers C1-J-2195 (Simon et al. 1994) and CW 3031 (Heinze et al. 2005) in a 50 µl reaction, which contained 3 µl of extracted genomic DNA, 10 µl 1 mM deoxy-ribonucleotide triphosphates (dNTPs, MBI Fermentas), 0.5 µl of each primer (32.5 µM), 5 µl 10X Taq buffer (MBI Fermentas), 4 µl 25 mM MgCl₂, 1 µl Taq polymerase (1 U) (MBI Fermentas), and 26 µl DEPC water. The PCR cycle consisted of an initial denaturation step of 98°C for 5 min; followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 90 s; and finally a terminal extension at 72°C for 7 min and storage at 4°C. PCR reactions were performed in an Applied Biosystems 2720 Thermal Cycler. The PCR products were subjected to electrophoreses in 1.0% agarose gels in Tris-Acetate-EDTA (TAE) buffer. The gels were stained with 1% ethidium bromide, and the PCR products were purified with the PCR-MTM Clean Up System (Viogene).

Purified PCR products were sequenced in both directions with the above-mentioned primers using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit with an ABI 310 Genetic Analyser. The forward and reverse sequences were aligned in BioEdit (Hall 1999) and ambiguous bases were clarified using corresponding ABI chromatograms. Nucleotide sequences were aligned with the software CLUSTAL W (Higgins et al. 1994). The alignment was corrected manually using the alignment editor of the software MEGA 3.1 (Kumar et al. 2004). DNA sequence similarities were calculated with the Sequence Identity Matrix of the software BioEdit. Phylogenetic calculations were performed with MEGA 3.1. The data were analyzed with neighbor-joining using the Tamura-Nei parameter (Tamura & Nei 1993) and with maximum parsimony. *Temnothorax duloticus* Wesson, 1937 (Formicidae, Myrmicinae) was selected as an outgroup.

Results

In each aggressiveness test, both workers typically met 20 to 40 times. Non-aggressive behavior during this meeting typically consisted of antennation. Aggressive behavior was very uniform: the attacking worker bit the opponent in the petiolus. No aggressive behavior was observed in control pairs where two workers from the same colony were used, and aggressive behavior was quite rare between workers from different colonies. In the first trial, only two cases of aggressive behavior were observed (both in the pair Ústí – Lužiny) but this incidence of aggressive behavior did not differ significantly from random incidence. In the second trial, the aggressive behavior was more common and its occurrence was significantly different from random in three cases, two

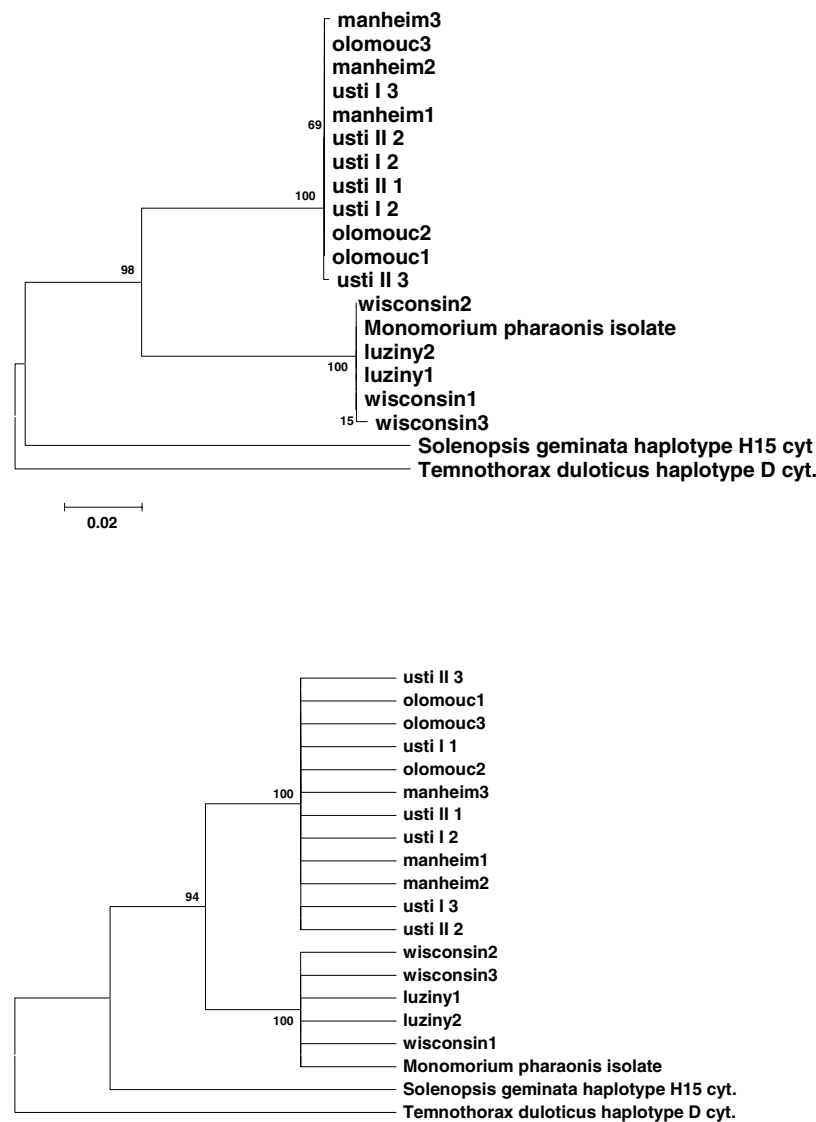


Fig. 2. Bootstrap consensus tree of relatedness based on nearest neighbour join (top) and maximum parsimony (bottom).

Table 2. Cooperation between colonies as indicated by the pupa-carrying test.

Home colony	Alien colony				
	Wisconsin	Mannheim	Ústí	Lužiny	Olomouc
Wisconsin		ns	ns	$P < 0.05$	ns
Mannheim	ns		ns	$P < 0.05$	$P < 0.05$
Ústí	ns	ns		$P < 0.05$	ns
Lužiny	ns	ns	ns		ns
Olomouc	ns	ns	ns	$P < 0.05$	

Explanations: In this test, workers selected between pupae from their own colony (the home colony) and from a different colony (the alien colony). $P < 0.05$ indicates that workers carried pupae from the home colony significantly more often than pupae from alien colony, while ns indicates that workers did not distinguish between home and alien pupae.

of which included the Lužiny colony (Table 1). Overall, however, aggressive behavior was rare in both trials.

In the pupa-carrying test, workers rarely differen-

tiated between their own and alien pupae. An exception was that workers from all other colonies carried fewer Lužiny pupae than pupae from their own colonies (Table 2). On the other hand, Lužiny workers did not differentiate between their own and alien pupae (Table 2). Workers significantly preferred pupae from their own colony in only one other case, which was with Mannheim workers and Olomouc pupae.

From the cytochrome oxidase I gene, approximately 750 base pairs were amplified but only 724 base pairs were used in the phylogenetic calculations because of gaps at the alignment of outgroup sequences. Among the *Monomorium* sequences, there were no insertions or deletions. Both the neighbor-joining and maximum parsimony trees supported the phylogenetic separation of the Lužiny and Wisconsin colonies from the other colonies; bootstrap values were 98% and 94%, respectively. The group level distance was 12.7% (i.e., 12.7 bases differ in 100 base-pair gene length). The only *Monomorium* sequence in the GenBank grouped with the Lužiny and Wisconsin ant genes.

Discussion

In agreement with the previous assumption (Hölldober & Wilson 1990), the aggressiveness and pupa-carrying tests demonstrated a low level of aggressiveness and a high level of potential cooperation between *M. pharaonis* colonies originating from different parts of the world. The level of cooperation and aggressiveness was not related to the geographic distance between the locations where the colonies were collected. Most European colonies showed no aggressiveness towards American colonies, but the Lužiny colony from Prague was aggressive when confronted with some colonies from other places of the Czech Republic. The behavioral tests were consistent in that all colony pairs showing aggressive behavior also showed pupal selection in the pupa-carrying test. The pupa-carrying test, however, indicates that pupal differentiation can be a one-way process because in all pairs where workers of one colony preferred their own pupae to the alien pupae, the worker of the other colony did not differentiate between their own and alien pupae. The reason for this phenomenon is not clear and requires more study. Behavioral tests were only partly consistent with the phylogenetic analysis because the Lužiny and Wisconsin colonies fell in a different cluster from all of the other colonies based on genetic data but the Wisconsin colony was grouped with the other colonies and not with the Lužiny colony based on the behavioral data. An inconsistency between behavioral and phylogenetic analysis was also demonstrated by the aggressiveness between Olomouc and Mannheim nests.

According to phylogenetic analysis, the *Monomorium CO I* gene sequences form two distinct groups separated at a distance of 12.7%, which in other related ant genera (e.g., *Temnothorax*, *Cardiocondyla*, *Solenopsis*) denotes species-level differences. Based on this information, a taxonomic revision of the species *Monomorium pharaonis* is highly recommended.

The results suggest that during the invasion of this species there were only a few, but at least two, colonization events. This bottleneck apparently caused a decrease in the genetic variability of the two lines of the species and resulted in the relatedness of recent world populations. A similar phenomenon was observed in another species of invasive ants, *Linepithema humile* (Starks 2003; Tsutsui et al. 2000, 2003). However, that a decrease in inter-colony aggressiveness does not correspond completely with colony relatedness suggests that factors other than relatedness affect inter-colony behaviour.

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