

Heterospecific colony fusion in two *Temnothorax* (Hymenoptera: Formicidae) sibling ants

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Abstract

The two monogynous (single-queened), parapatric ant species *Temnothorax nylanderi* (FÖRSTER, 1850) and *T. crassispinus* (KARAVAJEV, 1926) are both characterized by a rather inefficient, environment-based nestmate recognition system, which does not prevent alien colonies from moving in with an unrelated colony when their own nest has decayed. Colony fusion results in a genetically heterogeneous colony, in which later one of the two queens is eliminated. The sporadic occurrence of mixed colonies with workers from both species or from a parental species and a presumed hybrid colony suggested that interspecific fusion may occasionally occur in the narrow contact zone in the Franconian Jura. Colony fusions could be observed after initial aggressions in one third of our laboratory experiments. One of the two queens was usually killed within a few days. Queens of the two species did not differ in their survival rate. Apparently, the close relatedness between the two species and the environment-based recognition system facilitate such heterospecific fusion.

Key words: *Temnothorax nylanderi*, *T. crassispinus*, nestmate recognition, colony odour, queen survival rate, aggressive interactions.

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Introduction

The colonies of social insects usually consist of more or less extended family groups that are closed to unrelated freeloaders and parasites through an efficient nestmate recognition system. All nestmates share a common colony odour consisting of chemical cues that are produced by and exchanged among colony members, often with considerable contributions from nest material, food or other environmental sources (HÖLDOBLER & WILSON 1990, VANDER MEER & MOREL 1998, LENOIR & al. 1999). Despite its evolutionary robustness, nestmate recognition may fail under special circumstances, e.g., when social parasites manage to acquire or mimic the colony odour of their hosts (LENOIR & al. 2001) or when unrelated colonies share genetic odour cues due to a loss of genetic diversity and / or selection against heterogeneity (TSUTSUI & al. 1999, GIRAUD & al. 2002). In the latter situation, individual nests are no longer distinct entities but form huge "supercolonies" amongst which individuals are freely exchanged, as in the Argentine ant, *Linepithema humile* (MAYR, 1868).

Previous research has also suggested a lack of variability in genetic odour determinants in *Temnothorax nylanderi* (FÖRSTER, 1850), one of the most common ant species of deciduous woodlands throughout Central and Western Europe. *T. nylanderi* nests in cavities in plant material, such as hollow acorns, hazelnuts, and rotting branches. Aggression assays in the laboratory showed that nestmate recognition relies predominantly on transient, environment-derived odour cues, and that unrelated colonies living in the same nesting material easily merge in a single nest site (HEINZE & al. 1996).

Sexuals of both sibling species, *T. nylanderi* and *T. crassispinus* (KARAVAJEV, 1926), mate during nuptial flights and colonies are founded by single-mated queens (mon-

andry) either solitarily, by pleometrosis, or by usurpation of alien nests. Though both species are monogynous (single queen per colony; BUSCHINGER 1968, PLATEAUX 1970), their colonies may occasionally contain workers from several matrilineal lines. This heterogeneity results from natural colony fusion and the usurpation of mature colonies by founding queens when populations are dense and suitable nest sites are rare (SEIFERT 1995, FOITZIK & al. 1997, FOITZIK & HEINZE 1998, 2000, 2001, FOITZIK & al. 2003, STRÄTZ & HEINZE 2004). Fighting among queens quickly leads to the restoration of monogyny (STRÄTZ & al. 2002).

While *T. nylanderi* and *T. crassispinus* are parapatrically distributed throughout most of their range, they meet and hybridize in a narrow contact zone close to the Elbe River in Northern Germany and near the continental divide in the Franconian Jura in Southern Germany (SEIFERT 1995, PUSCH & al. 2006). The two species are morphologically very similar and, though they can be discriminated by detailed morphological analysis (SEIFERT 1995), are more easily determined by their different electromorphs of the enzyme glucose-6-phosphate isomerase (*GPI*; SEIFERT 1995, PUSCH & al. 2006). In one particularly well-studied population, a few colonies simultaneously contained workers with the *GPI* genotypes of *T. crassispinus*, *T. nylanderi*, and heterozygous workers, presumably hybrids (PUSCH & al. in press).

Since in both species, nestmate discrimination seems to rely mainly on environmental cues and genetic data indicated occasional heterospecific fusion (PUSCH & al. in press), we conducted laboratory experiments in arenas with only one nest available for two heterospecific colonies (see FOITZIK & HEINZE 1998) to determine in more detail how readily such fusion occurs.

Material and methods

Complete ant colonies were collected in September 2004. *Temnothorax crassispinus* colonies came from beech sticks and acorns in a beech-oak-pine forest in Pentling near Regensburg (Germany). *Temnothorax nylanderi* colonies were sampled from beech sticks in three beech-oak forests close to Altdorf, east of Nuremberg (Germany). Five additional *T. nylanderi* colonies were collected from beech twigs in a beech-pine forest near Velburg (Germany) and exclusively used in series 1 (see below). All colonies were left in their natural nests until the experiments to avoid a change in their natural colony odour profile. Ants were fed twice a week with undiluted honey and pieces of cockroach. We counted all ants per colony and individually marked their queens with Edding paint marker.

The fusion experiments were conducted in 19 × 19 cm arenas. In each we provided one standard laboratory nest (HEINZE & ORTIUS 1991), into which one colony was allowed to move in (series 1: N = 21, resident species = *T. nylanderi*; series 2: N = 20, resident species = *T. crassispinus*). One day after the first colony had successfully moved into the provided nest site, we released the second colony directly in front of the nest entrance. Colonies varied considerably in size and consisted of about 40 to almost 300 workers. We therefore chose pairs of queenright colonies of similar size (= number of workers; Wilcoxon matched pair test; series 1: Z = 1.25; p = 0.21; series 2: Z = 0.02; p = 0.99) to avoid any effect from differing colony size. Behaviour was observed for 30 minutes after the second colony had been added. Every ten minutes, all aggressive interactions (= mandible opening, biting, and fighting) among workers, between queens and workers, and between queens were scanned for two minutes. For each experiment, the number of aggressive interactions recorded during the 30 minutes in three two-minute observations was summed and divided through the number of scans. On the following days, the colonies were scanned once per day for two minutes to check for aggression or queens' death. All statistical analyses were conducted with Statistica 6.0 (Statsoft, Tulsa, OK, USA). Data that were not normally distributed were analysed with non-parametric statistics.

Results

In all experiments, workers of the newly added colony discovered the entrance of the nest site within the first 30 min and entered the nest without being attacked, as the nest entrance was usually left unguarded (see also FOITZIK & HEINZE 1998). Six of 21 *T. crassispinus* colonies moved into a nest site occupied by *T. nylanderi* and eight of 20 *T. nylanderi* colonies moved into a nest site inhabited by *T. crassispinus* always resulting in colony fusion (Fisher's exact test: p = 0.51). In the other experiments, the introduced colonies did not manage to successfully usurp the inhabited nest within 20 days and in most of the experiments instead settled in one corner of the arena. Which species initially inhabited the nest site did not influence the time until fusion (Gehan's Wilcoxon test, test statistic = 0.826, p = 0.409).

Workers of the confronted colonies behaved aggressively towards each other only after direct contact. Aggressions were frequent on the day when the second colony was released into the arena, became much less intense the following two days and in almost all experiments ceased completely after five days. The frequency of worker-worker ag-

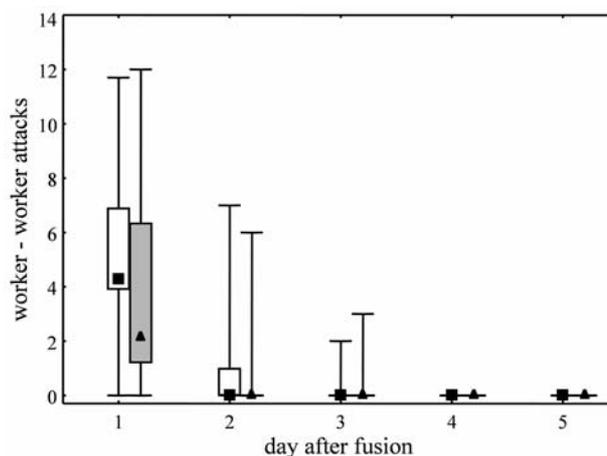


Fig. 1: Aggressive interactions (median, 25 % - 75 % quartiles, number of all aggressions per scan) among workers of the ant species *Temnothorax nylanderi* and *T. crassispinus* observed in all experimental setups during five days after one colony without nest was added into an arena with a nest containing a colony of the other species.

gression on the first day of the experiment did not differ between the two series (two-tailed t-test: t = 1.1, p = 0.28, Fig. 1).

In the case of successful nest usurpation, both resident and usurping queens were attacked. As workers were not marked and the two species are difficult to distinguish by eye it remains unclear, whether queen-worker aggression always involved pairs of heterospecific individuals. Worker aggression towards queens became less intense after the first day of the encounter and almost completely ceased on day five. Interestingly, aggression among queens and workers was considerably more frequent on day 1 of the experiment when *T. nylanderi* occupied the nest (Mann-Whitney U-test: median, quartiles, series 1: 1, 0.3, 1.9; series 2: 0, 0, 0.3; U = 92, p < 0.02, Fig. 2). However, this did not affect the outcome of the fusion experiment.

In nine of 14 colonies, only one queen survived colony fusion, whereas the other was finally killed by the workers. Queen-queen aggression was not observed. In series 1 with *T. nylanderi* as residents, the *T. nylanderi* queen was killed in one colony, the *T. crassispinus* queen was killed in three colonies, both queens died in one colony, and both queens stayed alive in one colony. In test series 2 with *T. crassispinus* as residents, two *T. nylanderi* queens and three *T. crassispinus* were killed, both queens died in one setup and both survived in two experiments. Resident queens did not survive better than intruder queens (Fisher's exact test, n₁ = n₂ = 14, p = 0.500). As we stopped the experiment after 20 days, we could not observe if both queens permanently staid in the nest and laid eggs.

Discussion

Our experiments corroborate field data, which indicate that colonies of the two sibling species *T. crassispinus* and *T. nylanderi*, and presumably also colonies of their hybrids, may occasionally fuse when nest sites are scarce and that they may subsequently form stable, mixed colonies. Heterospecific colonies fused in only one third of the experiments. This contrasts with an earlier observation that colonies fused in 30 of 38 experiments with two *T. nylanderi*

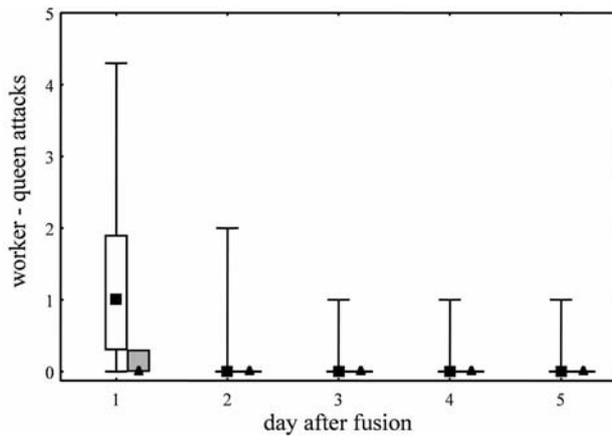


Fig. 2: Aggressive interactions (median, 25 % - 75 % quartiles, number of all aggressions per scan) between workers and queens observed in the fusion experiments. The left (open) bars indicate experiments with *T. nylandereri* as resident colony and *T. crassispinus* as intruders, the right (hatched) bars experiments with *T. crassispinus* as residents and *T. nylandereri* as intruders.

colonies (FOITZIK & HEINZE 1998), in eight of eight (K. Pusch, unpubl.) and in 24 of 30 (TICHÁ 2002, TICHÁ & ŠTYS 2002) experiments with paired *T. crassispinus* colonies. The difference may be explained by the higher similarity of conspecific colonies and our usage of colonies from different populations. Consistent with previous experiments with *T. nylandereri* (FOITZIK & HEINZE 1998) and *T. crassispinus* (K. Pusch, unpubl.), in most cases (nine of 14 merged colonies), only one queen survived, independent of whether the queen was nest resident or not. In a study on colony usurpation by founding queens of *T. nylandereri*, STRÄTZ & al. (2002) suggested that workers choose the younger and / or more fertile queen and eliminate the other. At present it is not known how workers in merged colonies chose the queen to be eliminated and whether only heterospecific workers attacked and killed the queen. *T. nylandereri* and *T. crassispinus* are very closely related and queen fertility signals might perhaps be sufficiently conserved to be understood across species borders and lead to maladaptive favouring the more fertile queen even when it belongs to the wrong species. Queen-queen interactions may have preceded worker aggression, as in *T. nylandereri* (FOITZIK & HEINZE 1998, STRÄTZ & al. 2002). The takeover of a colony with the subsequent elimination of one queen reminds of interspecific social parasitism, a life history that has convergently evolved in several clades of the ant tribe Formicoxenini, including the genus *Temnothorax* (BUSCHINGER 1986, BEIBL & al. 2005).

Aggression both between workers and queens and among workers was frequent and intense only on the first day of the experiment and later decreased rapidly. This might suggest a change in colony odour. Environmental odour cues appear to play an important role in *T. nylandereri*, where individuals from different colonies reacted more aggressively towards each other when the colonies inhabited nests of different material (HEINZE & al. 1996). Chemical analyses of cuticular hydrocarbons suggested that colony odours are not strongly differentiated and also do not reliably differ between the two sibling species: 3.3 % of *T. nylan-*

deri individuals and 6.7 % of *T. crassispinus* individuals were incorrectly classified as belonging to the respective sibling species by discriminant analysis (FOITZIK & al. in press). The predominance of environmental odour cues in nestmate recognition might be associated with the surprisingly low genetic variation of both species (PUSCH & al. 2006).

To conclude, interspecific fusion of colonies of *T. nylandereri* and *T. crassispinus* appears to be possible where the two species co-occur. The ease of fusion might therefore explain previous data on the genetic composition of colonies in a population near Velburg, Bavaria (PUSCH & al. in press), where hybrid colonies frequently also contained non-hybrid workers of one parental species.

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Zusammenfassung

Die beiden monogynen, parapatrisch vorkommenden Arten *Temnothorax nylandereri* (FÖRSTER, 1850) und *T. crassispinus* (KARAVAJEV, 1926) zeichnen sich durch ein ineffizientes, durch Umweltfaktoren geprägtes Kolonieerkennungssystem aus. Dies ermöglicht Kolonien, in ein bereits von einer fremden Kolonie der gleichen Art bewohntes Nest einzuziehen, wenn ihr eigenes Nest unbewohnbar wird. Dadurch entstehen genetisch heterogene Kolonien, in denen jedoch eine der beiden Königinnen getötet wird. Das Auftreten gemischter Kolonien mit Arbeiterinnen beider Arten bzw. Arbeiterinnen einer Art und Hybriden innerhalb der schmalen Kontaktzone beider Arten im fränkischen Jura zeigt, dass gelegentlich interspezifische Fusionen vorkommen. In einem Drittel der Laborexperimente fusionierten die Kolonien nach anfänglicher Aggression und in den meisten Fällen wurde nach wenigen Tagen eine der beiden Königinnen getötet. Unterschiede in der Überlebensrate der Königinnen beider Arten konnten nicht gefunden werden. Offenbar ermöglichen die nahe Verwandtschaft beider Arten und ihr umweltbedingtes Kolonieerkennungssystem heterospezifische Fusionen.

References

- BEIBL, J., STUART, R.J., HEINZE, J. & FOITZIK, S. 2005: Six origins of slavery in formicoxenine ants. – *Insectes Sociaux* 52: 291-297.
- BUSCHINGER, A. 1968: Mono- und Polygynie bei Arten der Gattung *Leptothorax* MAYR (Hymenoptera Formicidae). – *Insectes Sociaux* 15: 217-226.
- BUSCHINGER, A. 1986: Evolution of social parasitism in ants. – *Trends in Ecology and Evolution* 1: 155-160.
- FOITZIK, S., HABERL, M., GADAU, J. & HEINZE, J. 1997: Mating frequency of *Leptothorax nylandereri* ant queens determined by microsatellite analysis. – *Insectes Sociaux* 44: 219-227.
- FOITZIK, S. & HEINZE, J. 1998: Nest site limitation and colony takeover in the ant *Leptothorax nylandereri*. – *Behavioral Ecology* 9: 367-375.
- FOITZIK, S. & HEINZE, J. 2000: Intraspecific parasitism and split sex ratios in a monogynous and monandrous ant *Leptothorax nylandereri*. – *Behavioral Ecology and Sociobiology* 47: 424-431.
- FOITZIK, S. & HEINZE, J. 2001: Microgeographic genetic structure and intraspecific parasitism in the ant *Leptothorax nylandereri*. – *Ecological Entomology* 26: 449-456.

- FOITZIK, S., STRÄTZ, M. & HEINZE, J. 2003: Ecology, life history and resource allocation in the ant, *Leptothorax nylanderi*. – *Journal of Evolutionary Biology* 16: 670-680.
- FOITZIK, S., STURM, H., PUSCH, K., D'ETTORRE, P. & HEINZE, J. in press: Nestmate recognition, genetic diversity and variation in cuticular hydrocarbon profiles in *Temnothorax* ants. – *Animal Behaviour*.
- GIRAUD, T., PEDERSEN, J.S. & KELLER, L. 2002: Evolution of supercolonies: The Argentine ants of southern Europe. – *Proceedings of the National Academy of Sciences of the United States of America* 99: 6075-6079.
- HEINZE, J., FOITZIK, S., HIPPERT, A. & HÖLLDOBLER, B. 1996: Apparent dear-enemy phenomenon and environment-based recognition cues in the ant *Leptothorax nylanderi*. – *Ethology* 102: 510-522.
- HEINZE, J. & ORTIUS, D. 1991: Social organisation of *Leptothorax acervorum* from Alaska (Hymenoptera: Formicidae). – *Psyche* 98: 227-240.
- HÖLLDOBLER, B. & WILSON, E.O. 1990: *The ants*. – Belknap Press of Harvard University Press, Cambridge, MA, 732 pp.
- LENOIR, A., D'ETTORRE, P., ERRARD, C. & HEFETZ, A. 2001: Chemical ecology and social parasitism in ants. – *Annual Review of Entomology* 46: 573-599.
- LENOIR, A., FRESNEAU, D., ERRARD, C. & HEFETZ, A. 1999: The individuality and the colonial identity in ants: the emergence of the social representation concept. In: DETRAIN, C., DENEUBOURG, J.L. & PASTEELS, J. (Eds.): *Information processing in social insects*. – Verlag Birkhäuser, Basel, pp. 219-237.
- PLATEAUX, L. 1970: Sur le polymorphisme social de la Fourmi *Leptothorax nylanderi* (FÖRSTER). II. - Activité des ouvrières et déterminisme des castes. – *Annales Scientifiques Naturelles (Zoologiques)* 13: 1-90.
- PUSCH, K., FOITZIK, S., SEIFERT, B. & HEINZE, J. 2006: Distribution and genetic divergence of two parapatric sibling ant species in Central Europe. – *Biological Journal of the Linnean Society* 88: 223-234.
- PUSCH, K., HEINZE, J. & FOITZIK, S. in press: The influence of hybridisation on colony structure in the ant species *Temnothorax nylanderi* and *T. crassispinus*. – *Insectes Sociaux*.
- SEIFERT, B. 1995: Two new Central European subspecies of *Leptothorax nylanderi* (FÖRSTER, 1850) and *Leptothorax sordidulus* MÜLLER, 1923 (Hymenoptera: Formicidae). – *Abhandlungen und Berichte des Naturkundemuseums Görlitz* 68: 1-18.
- STRÄTZ, M. & HEINZE, J. 2004: Colony structure and sex allocation ratios in the ant *Temnothorax crassispinus*. – *Insectes Sociaux* 51: 372-377.
- STRÄTZ, M., STREHL, C.-P. & HEINZE, J. 2002: Behavior of usurping queens in colonies of the ant species *Leptothorax nylanderi* (Hymenoptera: Formicidae). – *Entomologia Generalis* 26: 73-84.
- TICHÁ, K. 2002: Nestmate discrimination in *Leptothorax crassispinus* (Hymenoptera: Formicidae). – *Acta Universitatis Carolinae Biologica* 46: 325-336.
- TICHÁ, K. & ŠTYS, P. 2002: Monogyny in *Leptothorax slavonicus* (Hymenoptera: Formicidae). – *Acta Societatis Zoologicae Bohemicae* 66: 151-160.
- TSUTSUI, N.D., SUAREZ, A.V., HOLWAY, D.A. & CASE, T.J. 2000: Reduced genetic variation and the success of an invasive species. – *Proceedings of the National Academy of Sciences of the United States of America* 97: 5948-5953.
- VANDER MEER, R.K. & MOREL, L. 1998: Nestmate recognition in ants. In: VANDER MEER, R.K., BREED, M.D., ESPELIE, K. & WINSTON, M.L. (Eds.): *Pheromone communication in social insects. Ants, wasps, bees and termites*. – Westview Press, Boulder, CO, pp. 79-103.