

Apropos *Formica (Coptoformica) suecica* ADLERZ, 1902: Aggressive behaviour as a cue for colony structure and additional comments on the biology (Hymenoptera, Formicidae)

Martin-Carl Kinzner, Magdalena Tratter & Herbert Christian Wagner

Abstract

More than 90% of *Formica suecica* colonies are monodomous. One of the two only known populations of Central Europe in Obergurgl (Ötztal, Tyrol, Austria) is a possible candidate for polydomy, because of spatial vicinity of nests. Thus, we investigated the behaviour within nests, among nests and among *F. suecica* and a codominant species with special focus on aggression by one-on-one behavioural experiments. We showed that the intraspecific aggression was generally low, but intranest aggressiveness was lowest, interspecific highest and the intraspecific aggression increased by distance of nests. Additionally, we found a mixed monodomous colony of *F. suecica* and *F. lemani*, verifying that the latter is the host species of the former for social parasitic colony foundation. We suggest that the 8 nests of *F. suecica* in spatial vicinity within the same habitat are at least 1 monodomous and some polydomous or only monodomous colonies more or less fused to a supercolony with decreasing olfactory similarity by distance. We recommend population genetics and analysis of recognition chemicals to test which of our hypotheses, only monodomous or a combination of monodomous and polydomous colonies, is correct. Moreover, we suggest *F. suecica* in Obergurgl as ideal study object for investigations on the evolution of supercolonies.

Keywords: *Coptoformica*, aggression, supercolony, social parasitism, ants, Formicidae

1 Introduction

As eusocial insects, ants (Formicidae) build colonies with more or less distinctive boundaries and normally, they are able to discriminate between colony members and aliens (SEIFERT 2007). Colonies consist either of a single nest with a single queen (monodomous-monogynous), a single nest with more than one queen (monodomous-polygynous), several nests with a single queen (polydomous-monogynous) or of several nests with several queens (polydomous-polygynous) (SEIFERT 2007, STEINER et al. 2010). Hence, the colony structure has a deep impact on the relatedness, i.e., recognition odours, within a colony, e.g., in colonies with a single queen all the workers are highly related due to the fact that they are all offspring of the same mother. Monogynous colonies are known to be more aggressive. In contrast, in polygynous colonies the workers are offspring of different queens, and thus less related (STEINER et al. 2010). Polydomous-polygynous colonies can rise from a monodomous colony by budding and related young queens build the reproductive units in the new nests, or by a cooperative colony foundation

of several queens (PEETERS & MOLET 2010, STEINER et al. 2010). Due to the similarities of recognition odours, normally, workers are not aggressive between different nests of a single colony, although it was shown, that the relatedness decreases by spatial distance of nests whereas aggression increases (BEYE et al. 1998). A completely other situation arises when ant colonies cooperate, related or not, and form a supercolony without (STEINER et al. 2007) or with low aggression (KISS & KOBORI 2011) among the connected colonies.

Formica suecica ADLERZ, 1902 is a boreo-alpine species of the subgenus *Coptoformica*. It seems to have a very disjunct distribution with bigger connected areas only in Fennoscandia (SEIFERT 2000). One of the two known populations of this species in Central Europe was found in Obergurgl (Ötztal, Tyrol, Austria), the other is nearby in the Venter valley and both are considered to be a postglacial relict in the Alps (GLASER & SEIFERT 1999, GLASER et al. 2010). SEIFERT (2000) presumed a wider distribution range due to possible confusions with other *Coptoformica* species and moreover, there is a general lack of knowledge and investigations on *F. suecica*. GLASER & SEIFERT (1999) assumed that *Formica (Serviformica) lemani* is the host species of *F. suecica* for colony foundation, because it is the only known species of the subgenus *Serviformica* in Obergurgl, although this has never been verified by direct observations of mixed nests.

It is known that more than 90% of the *F. suecica* colonies are unambiguously monodomous, the remaining 10% are unknown, and that their tendency to build polydomous-polygynous colonies is very low (SEIFERT 2000). Although GLASER & SEIFERT (1999) supposed possible polydomous-polygynous colonies in Obergurgl, because of a minimum nest vicinity of 1-2 meters and reduced aggression between nests during experimental nest mixing, an in-depth investigation of the colony structure of the Austrian *F. suecica* population is still missing. Based on this, we formulated 5 hypotheses for a possible colony structure of *F. suecica* in Obergurgl:

- (i) 1 single polydomous colony without any aggression among nests.
- (ii) 1 single polydomous colony with increasing aggression by distance due to decreasing similarity of odours.
- (iii) Several polydomous colonies, each consisting of a few neighboured nests, with low aggression within and high among colonies.
- (iv) Every nest as monodomous colony with high aggression among the colonies due to low similarity of odours.
- (v) Every nest as monodomous colony or there are some polydomous colonies, but all colonies are more or less fused to a supercolony with no or low aggression among the colonies independent from similarity of odours.

To test which of these is the likeliest hypothesis, we used behaviour assays by one-on-one encounters of ant workers focusing 5 nests of *F. suecica* situated in the same habitat within a radius of 40 m.

2 Methods

2.1 Study system

The study was conducted in Obergurgl (Tyrol, Austria, 46.859°N, 11.021°E) at 2060 m a.s.l. on a small area characterised mainly by *Juniperus sibiricus*, *Rhododendron ferrugineum*, *Vaccinium myrtillus*, *V. uliginosum*, *V. vitis-idaea*, *Larix decidua*, *Pinus cembra* and different grasses (Figure 1A, see also GLASER & SEIFERT 1999). This north-west exposed biotope is completely surrounded by slopes with intensive ski tourism in winter, where also the study area is used as off-piste skiing side. In summer the whole skiing area is used as willows for cattle and hiking tourism is intensive. From the nests of *Formica* (*Coptoformica*) *suecica* ADLERZ, 1902, *F.* (*Formica* s. str.) *lugubris* ZETTERSTEDT, 1838 and *F.* (*Serviformica*) *lemanii* BONDROIT, 1917, situated in the same habitat (Figure 1B), 5 *F. suecica* nests (S1-5) and 1 *F. lugubris* nest (L) were selected. The distance between nests was measured as shortest line along the surface including ground unevenness, e.g., vegetation, rocks, small hills or holes, from the nearest edges of one nest to the other (Table 1).

Table 1: Nest combinations of *Formica suecica* (S1-5) and *Formica lugubris* (L) with the nest distance and the median of behavioural response index (RI) of the 4 replica.

Nest A	Nest B	Distance [m]	RI median
S1	S1	0.00	1.982
S1	S2	2.70	2.016
S1	S3	24.90	2.092
S1	S4	71.30	2.145
S1	S5	73.20	2.130
S2	S2	0.00	1.975
S2	S3	27.80	2.000
S2	S4	72.30	2.141
S2	S5	75.00	2.235
S3	S3	0.00	1.974
S3	S4	56.20	2.069
S3	S5	59.50	2.060
S4	S4	0.00	1.966
S4	S5	9.10	2.020
S5	S5	0.00	1.974
L	S1	14.40	2.833
L	S2	15.40	2.429
L	S3	21.70	2.429
L	S4	67.50	2.250
L	S5	69.00	4.237

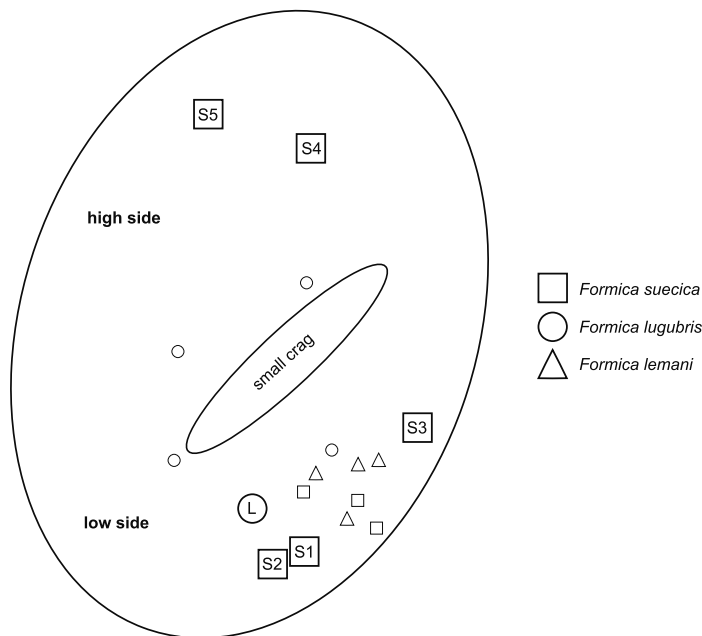


Figure 1: (A) Habitat structure and vegetation from low side view and (B) spatial position of the nests in the study area in Obergurgl. The 5 nests of *F.suecica* (S1-5) and the nest of *F.lugubris* (L) used in this study are labelled. Smaller symbols indicate nests, which were not used for the assays. The rocky area in (A) is indicated as "small crag" in (B).

2.2 Behaviour assays

For every possible combination of *F.suecica* nests 2 individuals were confronted in a one-on-one worker approach for 3 minutes. The two workers were simultaneously given in a plastic arena with 7 cm diameter and the frequencies of the following behaviours were counted: trophallaxis, grooming, antennation, aggressive posture, biting and grappling

(Table 2). Each observed behaviour was counted as 1 event. If any behaviour had duration of 5 seconds, it was counted as an additional event and so on for each following period of 5 seconds. Air temperature and relative humidity were measured at the end of every confrontation and the arena was cleaned using a customary tissue. Assays were video recorded to control potential ambiguous behaviours or critical situations. The behaviour assays were repeated 4 times per nest combination with other individuals of each nest (internest, 4 replicates). The same experiments were done using individuals from the same nest (intranest, 4 replicates) and confronting *F. suecica* with *F. lugubris* (interspecific, 5 replicates). The assays were performed in a random sequence daily from 04.-11. August 2013 ranging from 9.00-16.00. The weather during behavioural tests was sunny to cloudy with a mean temperature of 19°C, avoiding direct sunlight on the arena. Similar to the study of NEWEY et al. (2010), we assigned the behaviours a value from -2 to +3 (Table 2), reflecting the energetic costs for an individual: trophallaxis is energetically most beneficial, whereas grappling is most cost intensive.

Table 2: Types of observed behaviours with short descriptions and the score values. A high value indicates high energetic costs of the concerning behaviours, negative values indicate supporting behaviours.

Behaviour	Description	Score
Trophallaxis	Oral exchange of food	-2
Grooming	Cleaning or "licking" one another	-1
Antennation	Contact with antennae	0
Aggressive posture	Directional opening of mandibles	1
Bite	Biting	2
Grapple	Wrapping or rolling while biting	3

2.3 Statistical analysis

A behavioural response index (RI) was calculated according to NEWEY et al. (2010):

$$RI = \frac{\sum f_i s_i}{T} + 2$$

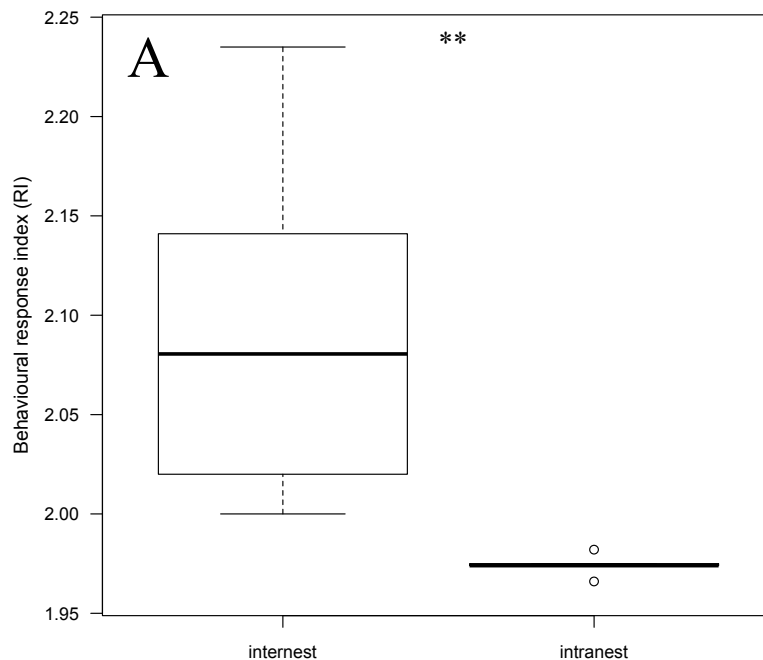
where f_i is the frequency of the behaviour i , s_i is the score of the behaviour i and T is the total number of observed behaviour events, i.e., the bigger the behavioural response index the higher was the aggressive behaviour. We added "2" to avoid negative values.

The median of the RI of the 4 replicates was used for further statistical analyses because of non normally distributed data. Spearman's rank correlation and Mann-Whitney-U test were performed using PAST version 2.17 (HAMMER et al. 2001) and Analysis of Similarities (ANOSIM) using PRIMER version 6.1.15 (Plymouth Marine Laboratory, Plymouth, United Kingdom) with Bray-Curtis-similarities and 9,999 permutations.

3 Results & Discussion

The observed behavioural response indices (RI) for *F. suecica* ranged from 1.966 (within S4), which indicated more supporting behaviour, to 2.235 (S2-S5), which indicated more aggressive behaviour, but the intraspecific aggression behaviour of *F. suecica* was generally low (Table 1). The intranest aggressiveness was significantly lower than among nests (Figure 2A, Mann-Whitney-U, $p = 0.0027$; ANOSIM, $R = 0.411$, $p = 0.009$). Furthermore, the aggressive behaviour was much higher among *F. suecica* and *F. lugubris* (Figure 2B, Mann-Whitney-U, $p = 0.0027$; ANOSIM, $R = 0.749$, $p = 0.0003$), including the highest RI of 4.237 due to an intensive fight for more than 2 minutes. None of the encounters resulted in dead ants. The behaviour was not dependent on air temperature (Spearman's rank correlations, $p = 0.204$) or humidity (Spearman's rank correlations, $p = 0.172$). Based on these outcomes, we conclude that our methodology was appropriate and that the aggressive behaviour is lowest between nestmates because of their olfactory similarity, which is an indicator for high relatedness (BEYE et al. 1998, HOLZER et al. 2006).

A Spearman's rank correlation of the nest distance and the RI (Figure 3) was very significant ($R = 0.806$, $p = 0.0049$). This result verifies our hypothesis of increasing aggressiveness by distance and thus, in combination with the lower aggression within nests, we reject the hypotheses (i) of a single colony without any aggression. Moreover, if each nest was a monodomous colony we would expect high aggression among nests and no increasing aggressiveness by distance, but *F. suecica* workers showed generally low intraspecific aggression and considerably higher aggressive behaviour against *F. lugubris*. Hence, hypothesis (iv) of single isolated monodomous colonies was also discarded. Similar results were found in a study on the related species *F. (Coptoformica) exsecta*, where the intraspecific aggression was also generally low, although this species is known to be normally very aggressive (KISS & KOBORI 2011).



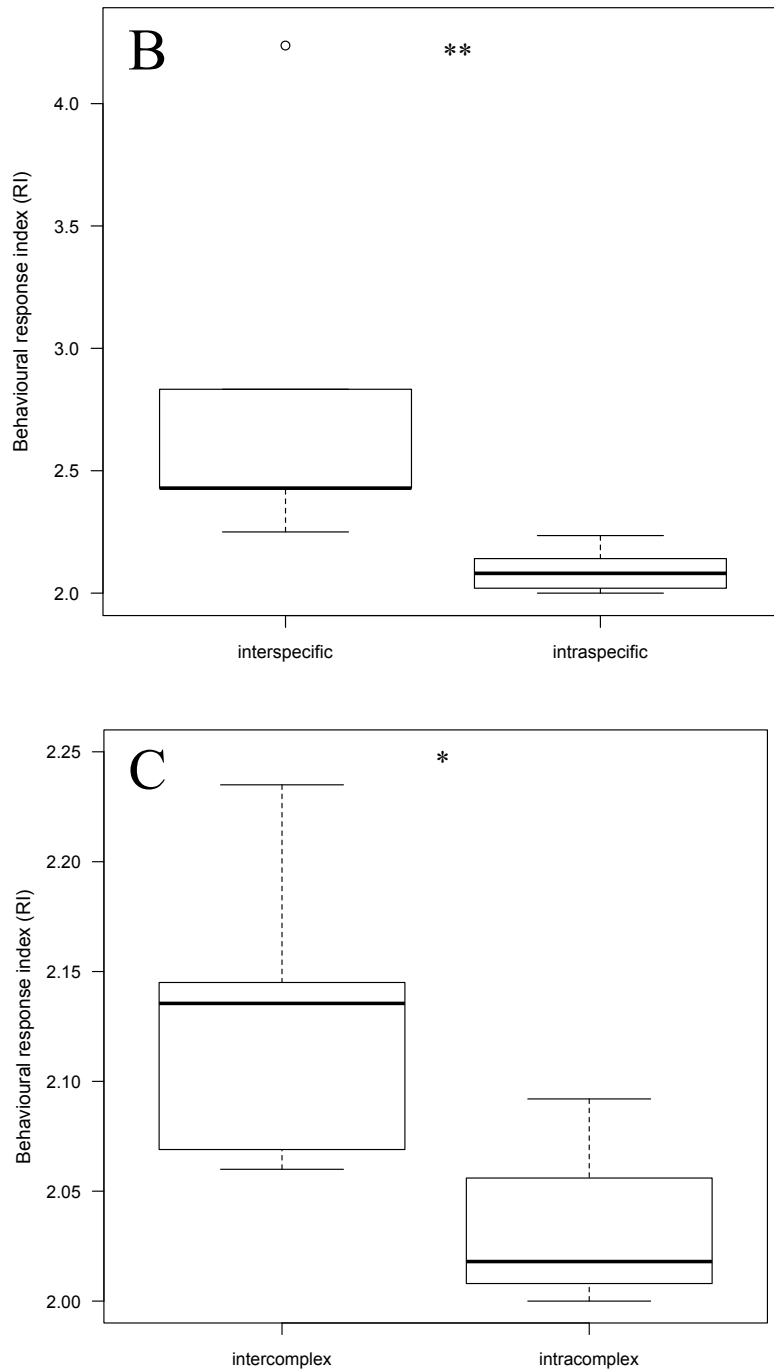


Figure 2: Behavioural response index (RI) comparing (A) internest – intranest, (B) interspecific – intraspecific, and (C) intercomplex – intracomplex. A small crag divided the habitat into a low side complex (S1-3) and a high side complex (S4-5). *...significantly different, **...very significantly different (Mann-Whitney-U).

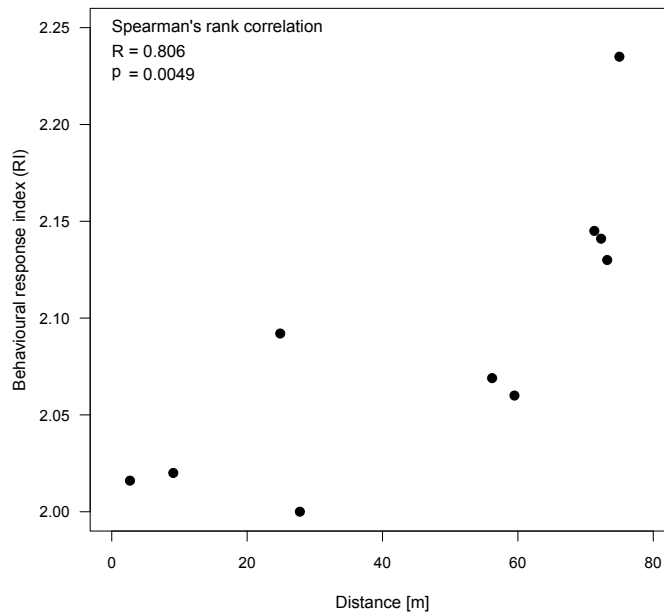


Figure 3: Spearman's rank correlation of behavioural response index (RI) and nest distance of *F. suecica* was very significant ($R = 0.806$, $p = 0.0049$).

The small crag in the study area (Figure 1) could represent a natural barrier for exchange between two complexes of *F. suecica* nests (low side: S1-3, high side: S4-5). By comparing the behaviour within the complexes (intracomplex) with that between them (intercomplex), a significant difference was detectable (Figure 2C, Mann-Whitney-U, $p = 0.0428$; ANOSIM, $R = 0.321$, $p = 0.043$). This is an indication that the two clusters form at least 2 separated colonies. However, we suggest that, due to the much higher interspecific aggressiveness, this difference among the complexes is an artefact of the high spatial distance (Table 1), although we cannot unambiguously reject or confirm hypothesis (iii) of clusters of colonies.

In the *F. suecica* nest S2 we found several *F. lemani* workers, without any observable aggression between the species in the nest and this nest was the smallest (diameter: 20-30 cm, height: ca. 3 cm) of the 8 *F. suecica* nests in the study area. With this first finding of a mixed nest of *F. suecica* and *F. lemani* we verified the hypothesis of GLASER & SEIFERT (1999) that *F. lemani* is the host species of *F. suecica* for colony foundation. Moreover, we suggest that this nest was a young monodomous colony founded not by budding but by temporary social parasitism, although the nest S1 was nearby (2.70 m) and the aggression between individuals of these 2 nests was low ($RI = 2.016$, Table 1). This result supports the hypothesis (v) that in *F. suecica* monodomous colonies could fuse to a supercolony.

Based on the combination of low intraspecific aggression, relative to the high aggressiveness against a codominant ant species and against collectors, and of the lower aggressive

behaviour within nests than among nests, we assume that the nests of *Formica suecica* in Obergurgl are monodomous colonies or monodomous and polydomous colonies more or less fused to a supercolony. One nest (S2) has to be treated for sure as a monodomous colony because of temporary social parasitism. The supercolony hypothesis does not categorically require high relatedness among the single colonies (STEINER et al. 2007). The low observed aggression can be explained by decreased recognition ability or increased olfactory similarities of neighboured nests, which decrease by distance (BEYE et al. 1998, GIRAUD et al. 2002). We recommend population genetics in combination with chemical analysis of recognition components to resolve the colony structure of *Formica suecica* in Obergurgl.

In addition to the behaviour assays, 50 individuals (S1-3) and 100 individuals (S4, S5) of the five *F. suecica* nests were marked with different colours using UNI Posca permanent markers. Every second day the nests were scoured for marked individuals, especially for individuals marked with alien-nest colours to investigate a possible exchange of individuals between nests, but after a period of 9 days we could find no stranger in any of the *F. suecica* nests. This result indicates that there possibly is no exchange between the nests, although it could yield from unconsciously marking workers which were not involved in the exchange or the exchange takes place at times when we could not observe it (e.g., in spring).

Individuals of *Coptoformica* have an efficient mandible and adductor muscle mechanism, for what reason their bites are painful to collectors and *Coptoformica* workers are known to be able to decapitate other ants (DIETRICH 1998, SEIFERT 2000). *Formica suecica* workers never reached the neck of their opponents during the whole behaviour approach of this study and no ant died. In a preliminary test, where 2 *F. suecica* workers of the same nest have fought with 1 individual of *F. lugubris*, one of the *F. suecica* workers directly attacked the neck of the *F. lugubris* worker, but during a period of 3 minutes the former was not able to decapitate the latter. This indicates that either the *F. suecica* workers had not enough time or they are generally not able to decapitate the much bigger *F. lugubris* workers.

We found alates in one of the five nests (S5) from 05.-10. August daily, but we could not observe mating flights. However, the dispersal capability of *F. suecica* seems to be very limited, concerning its disjunct distribution in general, because at least one nest was founded by social parasitism a few meters next to another, although adequate habitats not colonised by *F. suecica* were available about 100 m ahead. Moreover, the small size of *F. suecica* gynes (SEIFERT 2000) may speculatively indicate weak flight ability, and thus weak dispersal capability.

During our observations concerning the food import, *F. suecica* workers gathered mainly other ants (*Formica suecica*, *F. lugubris*, *F. lemani*, *Manica rubida*) and 2 beetles (Carabidae), although we cannot say if the food was carrion or living prey. ADLERZ (1902) reported *F. suecica* workers dragging other ants into their nests, too. No aphid visits on the vegetation have been seen during 9 days of observation, but root aphid visits are possible. We recommend a detailed investigation of the food source of *F. suecica* in a further study.

Conclusion

In Obergurgl *Formica suecica* nests had low aggression among each other, the aggression increased by distance of nests and was highest towards codominant *F. lugubris* workers. With our finding of a mixed nest we verified that *F. lemani* is the host species for social parasitic colony foundation of *F. suecica*. Finally, we suggest that the nests in Obergurgl are either all monodomous or some monodomous and some polydomous colonies. In any case, the nests are more or less fused to a supercolony with low intraspecific aggression within the location. We recommend population genetic investigations and analysis of recognition chemicals for resolving the enigma of colony structure of *F. suecica* nests in Obergurgl. Moreover, we suggest that *F. suecica* offers the possibility to investigate the evolution of supercolonies.

Zusammenfassung

Arbeiterinnen der *Formica suecica* Nester in Obergurgl zeigten wenig aggressives Verhalten zueinander, die Aggression stieg mit zunehmender Distanz der Nester und war gegenüber der kodominanten *F. lugubris* am höchsten. Durch unseren Fund eines Mischnests konnten wir die Hypothese verifizieren, dass *F. lemani* die Wirtsart für die sozialparasitische Koloniegründung von *F. suecica* ist. Alles in allem vermuten wir, dass die Nester in Obergurgl entweder nur aus monodomen oder aus monodomen und polydomen Kolonien bestehen. In jedem Fall sind die Nester bzw. Kolonien mehr oder weniger zu einer Superkolonie mit geringer innerartlicher Aggression verschmolzen. Wir empfehlen populationsgenetische Analysen und Untersuchungen der Erkennungsstoffe dieser Nester, um das Rätsel der Koloniestruktur von *F. suecica* in Obergurgl zu lösen. Des Weiteren glauben wir, dass *F. suecica* die Möglichkeit bietet, die Evolution von Superkolonien zu untersuchen.

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Authors' addresses:

Martin-Carl Kinzner, MSc
Magdalena Tratter, BSc
Mag. Herbert C. Wagner
Institute of Ecology
University of Innsbruck
Technikerstraße 25
A-6020 Innsbruck, Austria

Contacts:

martin-carl.kinzner@uibk.ac.at
magdalena.tratter@uibk.ac.at
heriwagner@yahoo.de

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