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Quantitative assessment of ant aggressiveness: *Formica uralensis* Ruzsky, 1895 vs. *Formica aquilonia* Yarrow, 1955 (Hymenoptera, Formicidae)

Количественная оценка агрессивности муравьёв: *Formica uralensis* Ruzsky, 1895 в сравнении с *Formica aquilonia* Yarrow, 1955 (Hymenoptera, Formicidae)

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Ключевые слова: муравьи, поведение, агрессивность, муравьи, метод оценки, универсальная шкала, простой искусственный раздражитель, рыжие лесные муравьи.

Abstract. Aggressive behaviour plays an important role in the stability of ant colonies and the success of ant species. To adequately assess the potential capabilities and competitiveness of individual ant species, and to predict their behaviour in different situations, it is important to have a clear understanding of their levels of aggressiveness, assessed on a single, «universal scale». An easy-to-use and sufficiently effective method for rapid assessment of ant aggressiveness, suitable for both laboratory and field use, has been refined and tested. The method is based on recording the responses of ant workers to a simple artificial stimulus and using a universal 9-point scale to quantify aggression. A comparative analysis of the aggressiveness of two ant species of the genus *Formica* Linnaeus, 1758 was carried out in the Central Altai (Russia, Altai Republic, Ust-Koksinsky District, Yustik Village) within the territories of large nest complexes of *Formica aquilonia* Yarrow, 1955 and *F. uralensis* Ruzsky, 1895. Tests were carried out on ant nest mounds and foraging trails (on tree trunks) for five nests of each ant species. The aggressiveness of *F. uralensis* workers, even from a large nest complex (more than 120 nests), was significantly lower than that of *F. aquilonia*, both in general and separately on foraging trails and nest mounds. Similar results were obtained both for the tested ant colonies as a whole and separately for large nests (mound diameter of about 80–100 cm). The results obtained are consistent with the data on the lower competitiveness of *F. uralensis* compared to representatives of the *Formica rufa* group. Despite lower aggressiveness compared to red wood ants, it is *F. uralensis* that usually dominates in mountain-valley forests of the central part of Altai (e.g., in birch-spruce-larch streamside forests), forming huge complexes of more than 200 (sometimes up to 400 or more) nests. In spring, such habitats often have extreme conditions for ants due to the prolonged inundation of the area during floods. We assume that *F. uralensis* has a sufficient (perhaps even unique) reserve of «physiological strength» to survive in such conditions, and that it is apparently superior to members of the *Formica rufa* group in this respect. However, this issue requires focused and detailed study.

Резюме. Агрессивное поведение играет важную роль в обеспечении стабильности существования семей и успешности видов муравьёв. Для адекватной оценки потенциальных возможностей и конкурентоспособности отдельных видов муравьёв, а также прогнозирования их поведения в различных ситуациях, важно иметь чёткое представление о диапазоне значений агрессивности рабочих особей по единой, «универсальной шкале». Доработан и апробирован простой в применении и достаточно эффективный метод оперативной оценки агрессивности муравьёв, подходящий для использования как в лаборатории, так и в природе. В основе метода лежит регистрация ответных реакций рабочих особей на простой искусственный раздражитель и использование универсальной 9-балльной шкалы для количественной оценки агрессивности. Сравнительный анализ агрессивности двух видов муравьёв рода *Formica* Linnaeus, 1758 проведён в Центральном Алтае (Россия, Республика Алтай, Усть-Коксинский район, с. Юстик) на территории крупных комплексов гнёзд *Formica aquilonia* Yarrow, 1955 и *F. uralensis* Ruzsky, 1895. Тестирование проводили на куполах муравейников и фуражировочных дорогах (на стволах кормовых деревьев) для 5 модельных гнёзд каждого вида. Агрессивность рабочих муравьёв *F. uralensis* даже из крупного поселения (более 120 гнёзд) оказалась значительно ниже, чем *F. aquilonia* как в целом, так и отдельно на кормовых дорогах и куполах муравейников. Аналогичные результаты получены как для протестированных семей в целом, так и отдельно для крупных гнёзд с диаметром купола 80–100 см. Полученные результаты согласуются с данными о более низкой конкурентоспособности *F. uralensis* по сравнению с представителями группы *Formica rufa.* Несмотря на более низкую степень агрессивности по сравнению с рыжими лесными муравьями, именно *F. uralensis* как правило доминирует в горно-долинных лесах центральной части Алтая (например, в прирусловых берёзово-елово-лиственничных), образуя гигантские комплексы размером более 200 (иногда до 400 и более) гнёзд. В весенний период в таких местообитаниях нередко складываются экстремальные условия для обитания муравьёв из-за опасности длительного затопления территории во время паводка. Мы предполагаем, что *F. uralensis* обладает достаточным (возможно, даже уникальным) запасом «физиологической прочности», позволяющем ему выживать в подобных условиях, и, по-видимому, превосходит в этом плане представителей группы *Formica rufa*. Однако этот вопрос требует отдельного детального изучения.

Introduction

Multispecies associations and communities of ants are characterised by a clear hierarchical structure, which is determined by the population density of ant species in a certain area, as well as their social and territorial organisation [Dlussky, 1967; Demchenko, 1975; Reznikova, 1983; Seima, 2008; Zakharov, 2015]. The success and prosperity of a species, including its abundance, position in the hierarchical structure of multispecies associations and ability to form large colonies and nest complexes, is directly related to its willingness not only to find food resources quickly, but also to protect them from competitors. The ability of ants to do this depends largely on their level of aggressiveness [Zakharov, 2021]. One of the most striking examples is the rapid occupation of territories by invasive species with high levels of aggression (e.g., the Argentine ant *Linepithema humile* (Mayr, 1868)), which allows these ants to easily displace native species [Rowles, O'Dowd, 2007; Carpintero, Reyes-López, 2008; Bertelsmeier et al., 2015] and form huge settlements (supercolonies) consisting of hundreds or thousands of nests [Abril, Gómez, 2011]. The higher the threshold of maximum values of aggressiveness of the workers of a particular ant species, the greater the chances for its colonies/supercolonies to successfully cope with any threats and maintain control over the foraging territory and food resources. Therefore, the efficiency of defence of aphid colonies against their natural enemies is directly dependent on the presence of aggressive individuals in the ant foraging teams that care for aphids and collect honeydew [Novgorodova, Gavrilyuk, 2012]. This provides foraging stability and an additional advantage over other members of the multispecies ant association and the community of ants as a whole.

Among the Holarctic ant species of the genus *Formica*, members of the *Formica rufa* group, with numerous colonies (10^4–10^7 workers) and complex social and territorial organization, are considered to be the most successful and prosperous [Dlussky, 1967; Reznikova, 1983; Zakharov et al., 2013; Zakharov, 2015]. In multispecies associations of anthills, these species fulfil the role of obligate dominants [Seima, 2008; Zakharov et al., 2013; Zakharov, 2015; Dyachenko, 2017], while they actively control and rather strictly regulate the number (colony size and dynamic density) of subdominant ants of *Formica fusca* and *F. rufibarbis* groups [Reznikova, 1999, 2003, 2018; Bugrov, 2015; Zakharov, 2021].

Formica uralensis Ruzsky, 1895, is also considered an obligate dominant [Zakharov et al., 2013]. This species resembles red wood ants and *F. pratensis* Retzius, 1783 in the appearance of workers, the character of nest construction, the presence of a developed system of foraging trails [Dlussky, 1967] and the ability to form large nest complexes [Rosengren, 1969; Chesnokova, Omelchenko, 2018]. However, in the European part of its range, *F. uralensis* usually leaves the territory when members of the *Formica rufa* group appear on it [Rosengren, 1969; Zakharov et al., 2013; Wegnez, Mourey, 2016]. The assumption of Rosengren [Rosengren, 1969] that *F. uralensis* cannot withstand competition from ants of this group because of its low level of aggressiveness seems quite logical, but still needs to be verified and confirmed. This is because ant behaviour, including aggression, may depend on the dynamic density of individuals and the size of their colonies [Reznikova et al., 1978; Reznikova, Shillerova, 1979; Reznikova, 1983, 2018; Zakharov, 1975, 1991, 2005]. In 2014, a huge nesting complex of *F. uralensis*, including more than 400 anthills, was found in Altai, along with large settlements of *F. aquilonia* typical of this region [Chesnokova, Omelchenko, 2018]. Since then, the question of the potential of *F. uralensis*, including the maximum possible values of aggressiveness of its workers, has become particularly relevant. The finding of such a large nest complex indicates that the potential of *F. uralensis* in terms of its competitiveness is still insufficiently studied, and the mechanisms of formation of large settlements of this species near nest complexes of red wood ants require a separate study.

In order to better understand the potential capabilities of ants, their competitiveness, possible limits in terms of dominance in the community, as well as the mechanisms of formation of the structure of multispecies ant communities, it is first necessary to have a clearer idea of the range of aggressiveness values, including their maximum.

Comparative analyses of aggression in different species usually involve conducting experiments on ant collisions under laboratory conditions (individual interaction, group confrontation) [Grangier et al., 2007; Bertelsmeier et al., 2015] or modelling similar situations in field experiments [Rowles, O'Dowd, 2007]. In these cases, the observers record the features of the individuals' collision/contact behaviour, usually noting the frequency or duration of different units of behaviour and the final outcome of the collisions [Carlin, Hölldobler, 1986; Errard et al., 2006; Rowles, O'Dowd, 2007; Carpintero, Reyes-López, 2008; Bertelsmeier et al., 2015]. For specific tasks, this approach to assessing aggressiveness is justified and quite effective, but it has a number of limitations. In particular, conducting individual grafting experiments requires additional time and energy; the situation is further complicated when more than two species need to be compared. The removal of ants from their natural habitat and the conditions of both housing and laboratory experiments can affect the results of the study to some extent. In addition, data obtained by different experimenters cannot be compared due to differences in the empirical aggression scales developed according to the requirements of the particular study. To avoid such problems, a universal «tool» is needed that can quickly assess ant aggressiveness using a single scheme and that is suitable for use directly in the field. A good alternative to pairwise interaction experiments is to record responses to a simple artificial stimulus that can be presented to individuals or groups of individuals, either in the laboratory or in their natural habitat [Reznikova, Novgorodova, 1998; Novgorodova, 2009, 2015].

The aim of this work is (i) to refine and test a universal method for the comparative analysis of the aggressiveness of different ant species, based on existing methodological approaches; (ii) to assess the degree of aggressiveness of *F. uralensis* workers from large nest complexes in comparison with members of the *Formica rufa* group.

Materials and methods

The study was conducted in July 2020 in the vicinity of Yustik Village (Altai Republic, Ust-Koksinskiy district; 50°23' N, 85°14' E, 1050 m a.s.l.).

Species studied

We carried out a comparative analysis of the aggressiveness of two ant species of the genus *Formica* Linnaeus. A brief description of the species is given below.

Formica uralensis Ruzsky, 1895 is a trans-Palaearctic species, distributed from Northern Europe to Primorye. In Europe and the northern Urals, it usually lives in swamps, in the south of Siberia, the north of Mongolia and Kazakhstan, and in Primorye, it is found on the edges of pine forests and larch forests, in steppe, flood meadows and wet peatlands. *F. uralensis* builds nest mounds similar to those of the *Formica rufa* group. The number of individuals in large nests may reach several hundred thousand [Zakharov et al., 2013]. This ant is an active herpetobiont-zoophage, and has by well-developed trophobiotic relationships with aphids. The foraging territory is protected and there is often a well-developed network of foraging trails, which in some cases may be deepened [Zakharov et al., 2013]. *F. uralensis* may either live in a solitary anthills or form nest complexes and supercolonies [Rozengren, 1969; Dmitrienko, Petrenko, 1976; Reznikova, 1980]. In the study area, large nest complexes were found in larch-birch-spruce forests [Chesnokova, Omelchenko, 2018] and birch-spruce-larch forests.

Formica aquilonia Yarrow, 1955 is a trans-Palaearctic forest ant species. It belongs to the *Formica rufa* group (red wood ants). This ant has a complex social and territorial organization and builds dome-shaped nests. The number of individuals in large nests can reach several million. This ant is an active zoophagous herpetobiont, and has well-developed trophobiotic relationships with aphids. Typically, *F. aquilonia* has an extensive protected foraging territory with a well-developed network of foraging trails. This species is characterised by a complex foraging territory, with secondary subdivision, when areas are controlled by individual foragers or small groups of foragers. Among the inhabited biotopes, it prefers spruce forests, where it forms large complexes (more than 100 anthills). The most mobile among red wood ants, it easily forms complex structures of different levels, including supercolonies or according to A.A. Zakharov, secondary federations [Zakharov, 1991, 2005; Zakharov et al., 2013]. In the study area, a sparse nest complex of *F. aquilonia* was found in park larch forests in the southern part of the Abai intermountain basin. Nests of this species were often found quite close (<1 km) to large nest complexes of *F. uralensis*.

It is known that as colony size increases, ants can exhibit changes in social structure and behaviour, including the emergence of a protected territory and increased aggression of workers [Zakharov, 1975, 2005, 2021; Reznikova et al., 1978; Reznikova, Shillerova, 1979; Reznikova, 2017]. Thus, the highest level of aggression is expected in ants from large colonies and large nest complexes. In order to gain a better understanding of the potential of *F. uralensis*, including the maximum levels of aggressiveness characteristic of individuals of this species, the study was carried out using colonies from large nest complexes of the tested species.

The nest complex of *F. uralensis* tested consisted of more than 120 anthills. Five nests of each species were selected for testing (Table 1). Ant aggressiveness was assessed on foraging trails and nest mounds during the period of high ant activity in the first half of the day (from 10 to 12 a.m.) under similar weather conditions (cloudy with clearing).

Table 1. Main characteristics of ant nests used to assess the aggressiveness of *Formica uralensis* (Ur_1–Ur_5) and *F. aquilonia* Yarrow (Aq_1–Aq_5)

Таблица 1. Основные характеристики гнёзд, использованных для оценки агрессивности муравьёв *Formica uralensis* Ruzsky (Ur_1–Ur_5) и *F. aquilonia* Yarrow (Aq_1–Aq_5)

Ant nests	D, cm	d, cm	H, cm	h, cm
Ur_1	150	90	40	30
Ur_2	200	100	45	30
Ur_3	100	50	50	35
Ur_4	120	90	50	40
Ur_5	100	90	35	25
Aq_1	300	180	100	50
Aq_2	250/300	150/280	40	20
Aq_3	100	40	35	5
Aq_4	120	80	35	10
Aq_5	150	100	45	10

Note. Characteristics: D — total diameter of the anthill, including the soil rim around the central dome-shaped part built of plant residues; d — diameter of nest mound built of plant residues; H — total height of the anthill, including the soil rim; h — height of the nest mound built of plant residues (h) [Zakharov et al., 2013].

Примечание. Характеристики: D — общий диаметр муравейника с учётом земляного вала; d — диаметр купола гнезда из растительных остатков; H — общая высота муравейника; h — высота купола из растительных остатков [Zakharov et al., 2013].

Assessment of aggressiveness

The study is based on a previously developed method, the main principle of which is to record the ants' responses to the same type of stimulus and to use a universal scale for quantitative assessment of aggressiveness [Novgorodova, 2009, 2015]. The spectrum of all possible reactions of ants to various stimuli (competitors, natural enemies, aphidophages, etc.) was revealed in the course of many years of research on the behaviour of different ant species (*Formica* — 10, *Myrmica* — 4, *Camponotus* — 2, *Lasius* — 3) and became the basis for the ranking and formation of a universal scale of ant aggressiveness [Novgorodova, 2009, 2015]. The nine-point scale reflects the order of increasing ant aggression in response to a stimulus (Table 2).

To simplify the testing process and improve the efficiency of the previously developed method for assessing the aggressiveness of not only individuals but also of groups of foragers on trails and nest mounds, some additions were made to the method with regard to the choice of stimulus and the testing procedure.

Choice of stimulus. For a comparative analysis of the aggressiveness of different ant species, a simple artificial stimulus is sufficient. The stimulus should be applied once or treated after each application to remove any odour marks made left by the tested individuals. This is particularly important in the case of *Formica* ants, which can splash acid on an irritant from a distance, which can increase the aggression of other individuals towards the marked object. To avoid the influence of the ants' odour markings and also the regular cleaning instruments from applied formic acid, we recommend using ordinary toothpicks (without any coating, menthol or other) or matches (4 cm long) as a simple artificial (disposable) irritant. Any toothpick or match (hereinafter referred to as stimulus) is applied once.

Testing. During the test, the stimulus removed from the box with tweezers was brought to the ants at a distance of about 1 cm. This distance is sufficient for the ant to notice and grasp the object. Ant responses to the stimulus were recorded using a previously developed 9-point scale (Table 2). If an ant showed a range of responses, each behavioural reaction was recorded in turn, with the most aggressive response used in the analysis.

In order to gain a more complete understanding of the aggressiveness of the ant species studied, tests were carried out for each ant colony both on the main foraging trails and on the nest mounds. The aggressiveness of individuals (at least 20 workers for each colony) was assessed on the largest trails on foraging trees (spruce, larch) closest to the nest (< 1.5 m). The irritant was held at one end with tweezers and presented to the ant once with the other end (if a match is used, it is better to hold it close to the sulphur head). The tests were conducted on tree trunks, with approximately equal proportions of individuals ascending and descending trees among tested foragers.

We also tested ants on the nest mound. Due to the high dynamic density of ants on the mound surface, when a stimulus was presented, the aggressiveness of all individuals that paid attention to the stimulus and showed reactions with different degrees of aggressiveness towards the new object was assessed. Individuals that did not pay attention to the stimulus were not counted, in cases where it was unclear whether the ant noticed the stimulus or not. The irritant was held in the middle with tweezers and held parallel to the surface of the anthill at a distance of about 1 cm from the ants. We recorded ant responses for 20 seconds. The choice of such an interval for testing allows us not only to estimate the aggressiveness of the ants in the immediate vicinity of the stimulus at the moment it is brought to the surface of the mound, but also to determine the number of aggressive individuals able to mobilise quickly to resist the potential enemy. For large nests with a mound diameter (d) greater than 1.5 m, testing was conducted from different sides in 3–4 sectors (2 to 3 minutes apart). For small nests (d < 60 cm), testing was carried out once.

A total of 156 individuals of *F. uralensis* from 5 nests (102 workers — on trails, 54 — on nest mounds) and 296 individuals of *F. aquilonia* from 5 nests (110 workers — on trails, 186 — on nest mounds) were tested.

Table 2. Ant aggressiveness scale
Таблица 2. Шкала агрессивности муравьёв

Responses of ant towards stimulus	Points
Avoidance — dropping down or running away	0
Tolerance — neutral reaction (ants do not react)	1
Antennation — investigation of the irritant using antennae	2
Alert pose — standing still with mandibles slightly open and antennae slightly extended towards the irritant	3
Aggressive pose — the stance adopted by ants before an attack (stilt-legged posture; mandibles widely open, antennae directed towards the irritant or slightly upwards; in the ants of the genus *Formica*, often with gaster extended forwards in order to spray acid)	4
Threatening lunges — usually repeated rapid lunges towards the irritant with open mandibles, but without contacting it	5
Hit-and-run attack — sudden attack on the irritant (≤ 1 s)	6
Biting — short bites (less than 5 s)	7
Death grip — a prolonged biting / stinging fight (ant seizes the irritant and does not loosen its grip for more than 5 s)	8

Data analysis

The Mann-Whitney test was used for the comparative analysis of the aggressiveness of workers of different species and of each species on the nest mounds and foraging trails. The comparative analysis of the aggressiveness of *F. uralensis* and *F. aquilonia* was carried out both for the combined data obtained during the tests on mounds and trails, and for each of the variants separately.

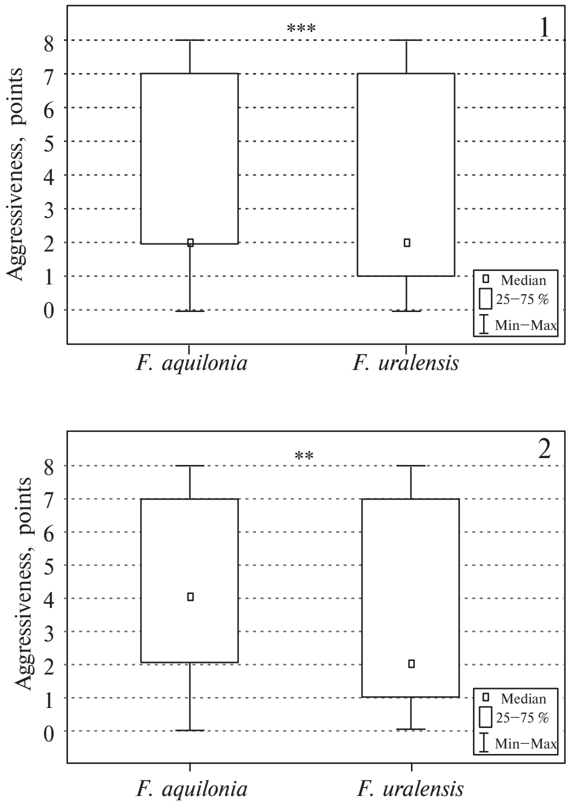
The size characteristics of ant nests (diameter of the nest mound built of plant residuals without a soil rim that typically surrounds the central part of the nest (d)) are closely related to the size of ant colonies [Zakharov, 1978; 2015; Dyachenko, 2017], which may have had some influence on the results of the study. In order to take into account the possible influence of this factor, the data were analysed both for all nests tested and separately for nests of a similar size category with a mound diameter (d) of 80–100 cm (Table 1). The Yates corrected Chi-square (χ^2) test was used to compare the proportions of ant responses to the stimulus. The relationship between the aggressiveness of the studied species and the diameter of nest mounds built of plant residues (d), which reflects colony size [Zakharov, 1978; Dyachenko, 2017], was assessed using Spearman's Rank correlation. Data were analysed using STATISTICA v.8.0.725 (StatSoft, Tulsa, OK, USA).

The present work is registered in ZooBank (www.zoobank.org) under LSID urn:lsid:zoobank.org:pub:B25857E0-9C53-4A45-968B-33E53616F11C

Results

Analyses of the pooled data (tests on nest mounds and foraging trails) showed that ant aggressiveness was highly species-dependent, with significantly higher levels observed for *F. aquilonia*, both for all anthill tested overall and for large nests of a similar size category with a mound diameter (d) of 80–100 cm (Figs 1–2).

A similar situation was observed when data from the nest mound and foraging trail tests were analysed separately. When analysing data from all nests tested, the aggressiveness of *F. uralensis* was significantly lower than that of *F. aquilonia* in both cases (Table 3). When analysing data from large nests of a similar size category, significant differences between species were found only in tests on nest mounds, while differences on trails were not as clear, p = 0.06 (Table 3).



Figs 1–2. Comparison of aggressiveness of two ant species using pooled data. 1 — from all anthills; 2 — from anthills of similar size category with a mound diameter of 80–100 cm. Mann-Whitney test: ** — $p < 0.01$; *** — $p < 0.001$.

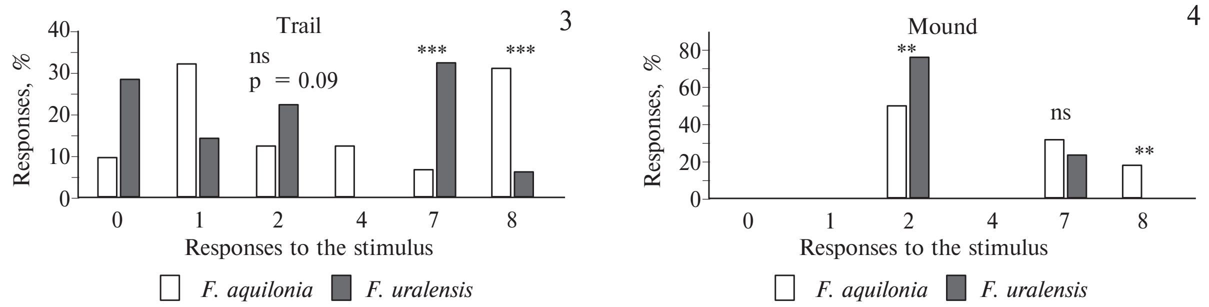
Рис. 1–2. Сравнение агрессивности двух видов муравьёв по объединённым данным. 1 — по всем муравейникам; 2 — по гнёздам сходной размерной категории с диаметром купола 80–100 см. Критерий Манна-Уитни: ** — p < 0,01; *** — p < 0,001.

The ranges of ant responses to the stimulus obtained when testing on nest mounds and foraging trails differed significantly, and in both ant species (Figs 3–4). In tests on nest mounds, ant workers demonstrated only three basic responses (antennation, biting and death grip), with no death grip observed in *F. uralensis*, individuals of this species were limited to biting (Figs 3–4). When tested on tree trunks, the spectrum of the most aggressive ant responses was much wider and included 6 out of 9 possible variants (Figs 3–4). Typically, the ants also demonstrated an alert pose,

Table 3. Results of a comparative analysis of the aggressiveness of two ant species of the genus *Formica* on nest mounds (Mounds) and on the trunks of foraging trees (Trails) (Mann-Whitney test; significant differences are in bold)

Таблица 3. Результаты сравнительного анализа агрессивности двух видов муравьёв рода *Formica* на куполах гнёзд (Mounds) и на стволах кормовых деревьев (Trails) (Критерий Манна-Уитни; значимые различия выделены жирным шрифтом)

Groups analysed (diameter of nest mounds of tested anthills, d)	Location	*F. uralensis*	*F. aquilonia*	U	p
Pooled data for all tested anthills (40–150/280 cm)	Mounds	2.0 [2.0; 2.0]	2.0 [2.0;7.0]	3532.50	**0.001**
	Trails	2.0 [0.0; 7.0]	2.0 [1.0; 8.0]	4542.00	**0.017**
Large anthills (80–100 cm)	Mounds	2.0 [2.0; 7.0]	7.0 [2.0; 7.0]	823.50	**0.002**
	Trails	2.0 [0.0; 7.0]	2.0 [1.0; 8.0]	1371.00	0.06



Figs 3–4. Responses to the same type of stimulus by workers of *Formica uralensis* and *F. aquilonia*. 3 — on trunks of forage trees; 4 — on the nest mounds. Designations: 0 — avoidance; 1 — tolerance; 2 — antennation; 3 — alert pose; 4 — aggressive pose; 5 — threatening lunges; 6 — hit-and-run attacks; 7 — biting; 8 — death grip. Yates corrected Chi-square (χ^2): ** — p < 0,01; *** — p < 0,001; ns — non-significant differences, p > 0.05.

Рис. 3–4. Ответные реакции на однотипный раздражитель рабочих особей *Formica uralensis* и *F. aquilonia*. 3 — на стволах кормовых деревьев; 4 — на куполе гнёзд. Обозначения: 0 — избегание; 1 — нейтральная реакция; 2 — исследовательское поведение; 3 — поза настороже; 4 — агрессивная поза; 5 — выпады; 6 — наскоки; 7 — укус(ы); 8 — мёртвая хватка. Критерий χ^2 с поправкой Йейтса: ** — p < 0,01; *** — p < 0,001; ns — различия незначимы, p > 0,05.

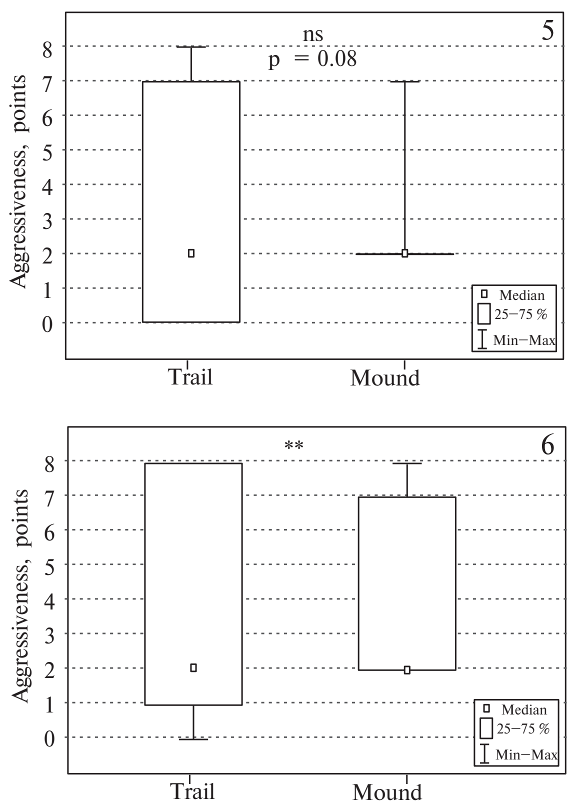
threatening lunges and hit-and-run attacks, but only in a series of rapidly changing behavioural reactions that ended in biting or death grip.

In contrast to *F. aquilonia*, the range of basic responses of *F. uralensis* on trails lacked the aggressive pose; bites prevailed among the most aggressive reactions, and the proportion of death grip was much lower (Figs 3–4). At the same time, the proportion of *F. uralensis* workers investigating the irritant with their antennae (antennation) was higher than in *F. aquilonia* (Figs 3–4). In general, the aggressiveness of *F. aquilonia* was significantly higher on the surface of the nest mounds than on foraging trails, while no significant differences were found for *F. uralensis* (Figs 5–6).

A significant positive correlation between ant aggressiveness and the diameter of nest mound built of plant residues (d) was found only for *F. uralensis* when tested on the surface of the nest mound (Spearman's Rank Order Correlation, r = 0.47, p = 0.0003).

Discussion

Researchers are often faced with the need to quantify and compare the aggressiveness of individuals or animal species. This is particularly relevant for social insects, especially ants, with their complex organisation of multispecies communities and high capacity for invasion by some species. The assessment of aggressiveness is often used in works investigating the recognition of their conspecifics [Carlin, Hölldobler, 1986; Stuart, Herbers, 2000; Roulston et al., 2003; Murata et al., 2017], the effectiveness of ants in protecting their symbionts [Novgorodova, Gavrilyuk, 2012], as well as interspecific interactions [Langen et al., 2000; Grangier et al., 2007; Tanner, Adler, 2009]. For a deeper understanding of the mechanisms of formation and functioning of multi-species associations and communities of ants, it is extremely important to have a more accurate idea of the potential capabilities of different species, including their aggressiveness. When settling new territories, not only the success, but also the behavioural strategy of a species is highly dependent on the level of aggressiveness of worker individuals [Bertelsmeier et al., 2015]. Quantitative assessments of the aggressiveness of different ant species allow more accurate predictions of not only of collision outcomes, but also of the choice of behavioural strategy. Both the experimental design and the scale used are important for a proper comparative analysis.



Figs 5–6. Aggressiveness of the ants on the trunks of foraging trees and on the ant mounds. 5 — *Formica uralensis*; 6 — *F. aquilonia*. Mann-Whitney test: ** — p < 0,01; ns — non-significant differences, p > 0.05.

Рис. 5–6. Агрессивность муравьёв на стволах кормовых деревьев и на куполах гнёзд. 5 — *Formica uralensis*; 6 — *F. aquilonia*. Критерий Манна-Уитни: ** — p < 0,01; ns — различия незначимы, p > 0,05.

Typically, the level of aggression is assessed by measuring the frequency or duration of various behavioural registration units in response to some stimulus [Carlin, Hölldobler, 1986; Reznikova, Novgorodova, 1998; Errard et al., 2006; Bertelsmeier et al., 2015]. Various objects, both natural and artificial, can act as irritants. In particular, natural objects include various competitors that ants encounter in nature (representatives of another species or colony of the same species; aphidophages; ground beetles, etc.). A dissecting needle can be used as a simple artificial irritant [Reznikova, Novgorodova, 1998; Novgorodova, 2009, 2015]. More complex artificial stimuli include models that simulate the «enemy image» [Dorosheva et al., 2011; Reznikova, Dorosheva, 2013]. The stimulus is usually selected according to the aims and objectives of the study, and the empirical scale of aggressiveness in points is developed according to the requirements of a specific study and for a particular ant species [Roulston et al., 2003; Grangier et al., 2007; Chirino et al., 2012]. However, when it comes to comparing ants of different species or their responses to different types of stimuli, there is an urgent need for uniform ranking and obtaining a single scale of aggressiveness.

We have refined and tested a simple and at the same time effective method for assessing the response of worker individuals to a simple artificial stimulus, which can be used as a universal «tool» for the rapid quantitative assessment of ant aggressiveness under both laboratory and natural conditions. The 9-point scale used in the study was previously formed based on the results of long-term studies of the behaviour of ants of 19 species from 4 genera of two subfamilies (Formicinae, Myrmicinae) and includes the spectrum of all possible reactions of ants to various stimuli (competitors, natural enemies, aphidophages, etc.) [Novgorodova, 2009, 2015]. The order of reactions in increasing aggression, starting from jaw opening (alert pose) to death grip, was determined according to how the reactions in the behaviour of tested individuals changed as their aggression increased. A quite complete set of ant behavioural responses was ranked, from avoiding contact to a series of bites and death grip (9 responses in total). The resulting scale is universal and can be used to quantitatively assess ant aggressiveness in a variety of testing options. This scale has previously been successfully used to assess the aggressiveness of honeydew collectors of different species [Novgorodova, 2015].

As for the testing process itself, the use of disposable irritants (unflavoured toothpicks, etc.) allows us to avoid the influence of additional factors on the results (e.g., odour marks, which increase ant aggression) without additional processing of the instruments, which saves a lot of time.

Testing ant workers on both foraging trails and nest mounds allows for a more accurate assessment of ant aggressiveness for individual colonies/species, including its maximum values. For example, the aggressiveness of *F. aquilonia* was significantly higher on ant mounds than on foraging trails.

It is known that the size characteristics of ant nests are closely related to the size of the ant colony [Zakharov, 1978; 2015; Dyachenko, 2017]. First of all, this concerns the diameter of the nest mound (d), therefore, in our study, a group of large nests of a relatively similar size category was selected precisely according to this parameter (d = 80–100 cm). The height of the nest mound of plant residues (h) does not always adequately reflect the situation, which may be due to the characteristics of individual species that have certain limitations on the height of the anthill. For example, according to the results of long-term observations of nest complex of *F. uralensis*, the height of its nests usually does not exceed 45 cm, while the diameter of the mound continues to increase with age and can reach 1 m [Rosengren, 1969], and in our data — 140 cm (unpublished data). Similar data were obtained for *F. uralensis* by other researchers, for example, in the territory of Central and Eastern Siberia [Dmitrienko, Petrenko, 1976], the Far East of Russia [Kupyanskaya, 1990].

Analysing the data obtained, we found that the aggressiveness of *F. uralensis* on the anthill surface increased significantly with increasing diameter of the nest mound, and, consequently, with the size of the ant colony. The lack of a significant correlation between aggression and colony size in *F. aquilonia*, as well as in *F. uralensis* on foraging trails, may be due to the insufficient amount of data on ant nests of different sizes, especially on small nests. Therefore, these need to be verified in further studies. Nevertheless, we have tried to take the possible influence of this factor into account when analysing the data.

A comparative analysis of the responses of the studied species to the stimulus showed that *F. uralensis* foragers behave less aggressively compared to *F. aquilonia* both in general, on foraging trails and on the surface of the nest mound. Similar results were obtained for large nests (d = 80–100 cm) of the species studied. The results obtained are consistent with our observations of ants during long-term studies. Thus, in the foraging areas of large colonies of *F. uralensis* (mound diameter more than 80 cm), individuals of this species actively attack the researcher only when performing any action on the surface of the nest mound or in the immediate vicinity of the anthill, as well as when collecting aphids in colonies protected by *F. uralensis*. With a similar dynamic density of ants, members of the *Formica rufa* group actively attack any moving object almost throughout the entire foraging territory of the ant colony. It is probably the relatively low level of aggressiveness of the workers compared to the *Formica rufa* group that explains the fact that *F. uralensis* is not among the active entomophages [Dmitrienko, Petrenko, 1976; Radchenko, 2016].

Overall, the results obtained confirm the assumption of Rosengren [1969] that *F. uralensis* is less competitive than members of the *Formica rufa* group due to its low level of aggressiveness. As for the mechanisms of formation of giant nest complexes by *F. uralensis* found in Altai, this issue requires a separate focused study. At this stage, we know that *F. uralensis* becomes an absolute dominant mainly in communities formed in river valleys (in particular, in streamside birch-spruce-larch forests). In spring and summer, these habitats are often characterised by extreme conditions for ants due to prolonged inundation of the area during floods. We assume that *F. uralensis* has a sufficient (perhaps even unique) reserve of «physiological strength» to survive under such conditions. The presence of rather large settlements of the more aggressive *F. aquilonia* in the immediate vicinity of *F. uralensis* nest complex suggests that the latter is superior to members of the *Formica rufa* group in this respect. This is indirectly confirmed by the results of studies by European colleagues, which show the displacement of *F. uralensis* by members of the *Formica rufa* group to bogs [Rosengren, 1969; Punttila, Kilpeläinen, 2009], i.e., to wetter habitats requiring specific physiological adaptations for survival. However, this hypothesis needs to be thoroughly tested in a separate detailed study.

In general, the study showed that the assessment of aggressiveness by recording responses to a simple artificial stimulus using a universal 9-point scale for quantitative assessment of aggressiveness is a simple to use and yet effective method for identifying the potential capabilities of ants. The method is sensitive to ranking ants (functional groups, colonies, and species) by aggressiveness. Besides, it is suitable for use in both laboratory and field conditions.

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