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An Innate Template for Enemy Recognition in Red Wood Ants

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Abstract—The hypothesis of the innate template for perception and recognition of the enemy image in red wood ants *Formica aquilonia* Yarr. was tested by initiating conflicts between ants and predatory ground beetles, their competitors for space. Live beetles and their models with different characters were used. In nature, ants respond selectively to such features of competitors as dark coloration, the presence of "outgrowths" (legs, antennae), body symmetry, the rate of movement, and scent. Comparison of behavior of ants from natural colonies and "naive" (laboratory reared) ones showed that ants having no experience of encounters with competitors responded aggressively to an integral and sufficiently realistic enemy image. This suggests that red wood ants possess an innate template for recognition of potential competitors. At the same time, the ability to single out the key features and complete the integral image seems to require accumulation of experience.

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One of the vital tasks faced by animals is the need to make immediate decisions as to the category of the encountered objects: whether it is prey, a dangerous predator, a competitor which should be driven away, or a possible mate. The ability to recognize vitally important objects is often based on specific key stimuli that "trigger" the corresponding responses. The key stimulus may be some feature of the object, perception of which is genetically determined (for review, see Zorina et al., 1999). The complex of characters may form a certain innate pattern, such as an "enemy image." The integrity of such patterns implies not the mere presence of a set of characters but also certain relations between them. Perception of vital objects based on innate templates is a phenomenon known for many species including man (for review, see Reznikova, 2007a). For example, it was recently shown that schematic drawings of spiders (presumably objects of "inborn fear") attracted the attention of five month old infants more than schemes chaotically composed of the same elements (body, head, extremities). Yet children who had seen realistic pictures of spiders paid greater attention to "incorrect" images. Actualization of the innate pattern resulted in singling out its characteristic features which began to be recognized and compared with the template (Rakison and Derringer, 2008).

The question how actualization of innate templates in the course of development occurs and how this process influences "decision making" in animals is one of the most interesting and least studied in modern behavioral ecology. The problem of decision making in variable and hardly predictable environment is very important for insects. At the same time, numerous recent investigations on fruit flies showed that the ability to learn in insects may be "costly": selection of strains for the corresponding behavioral traits showed that quickly learning insects had low viability according to many parameters (for review, see Dukas, 2008). This demonstrates how essential it is for insects to strike a balance between flexible behavior and genetically programmed responses. Recognition of innate images in insects was mainly studied for species feeding on plants and in particular concerns preferences of shape and size of flowers (Reznikova et al., 2007; Giurfa and Menzel, 1997; Kelber, 2002). As far as we know, recognition of the "enemy image" in predaceous insects has not been studied. It should be noted that the innate recognition of a "typical competitor" (enemy) has remained unstudied for vertebrates as well. Ants can be a convenient model for studying the role of innate "enemy image" in the process of decision making in animals since they possess complex and various behaviors in many ways similar to those of vertebrates (Dlussky, 1984; Reznikova, 2007b).

This communication is devoted to abilities of the ants *Formica aquilonia* for recognizing the enemy image, studied by the example of competitive relations between red wood ants and predaceous ground beetles. The ethological mechanisms of topical competition between these insects were studied earlier (Dorosheva and Reznikova, 2006a). The field and laboratory experiments showed that red wood ants actively attacked or deterred beetles and forced them out of their foraging territory. At the same time they were able not only to differentiate ground beetles from darkling beetles but also predatory species (in particular, *Pterostichus magus*, *P. oblongopunctatus*, *P. melanarius*, and *Carabus regalis*) from mixophytophages (*Amara nitida*, *Harpalus pygmaeus*) (Dorosheva and Reznikova, 2006b). It seems hardly probable that each ant encountering a ground beetle within the protected territory should rely entirely on the history of its individual and/or social experience.

We have suggested that red wood ants possess an inborn template for enemy recognition. In this work this hypothesis was tested by comparative assessment of behavioral responses of ants towards live enemies and models possessing different characters. This method, widely used in ethology and behavioral ecology, was for the first time applied to studying the behavior of insects by Tinbergen (1951). We were interested in how detailed the innate enemy image is and how it is actualized during early imaginal ontogenesis. To answer these questions we revealed the set of key features triggering the response of ants from natural colonies and those reared in the laboratory without contacts with "mature" ants or with their potential enemies and prey.

MATERIALS AND METHODS

The study was carried out in 2000–2008 in the recreational park zone of Akademgorodok (Novosibirsk). The field experiments were carried out in the territory of a colony of *Formica aquilonia* (Yarrow). Groups of ants for laboratory experiments were taken from the same colony. We established a control colony with 1 thousand workers and a female taken from nature, and a "naive" colony (500 workers and a female) consisting of ants reared in the laboratory from pupae, which had never met either "mature" worker ants or prey, predators or competitors. The colonies occupied artificial nests (25 × 10 × 2 cm) located in separate arenas of 0.8 × 1.5 m. The ants received sufficient quantities of carbohydrate and proteinaceous food.

Distinguishing the key features by which ants recognize the "enemy image." The ants were presented with models of ground beetles on foraging trails of large (more than 100 cm in diameter) nests at a distance of 6–10 m from the nest. The experiments were carried out from 9.30 to 11.30 a.m. and from 3.00 to 6.00 p.m. during the periods of maximal seasonal activity of ants (June–July).

Six models of beetles were used, made of chamois and differing in a set of characters: size (1.5 and 3.0 cm in length), color (white and dark), symmetry (symmetrical, repeating the characteristic outline of a predatory beetle, and asymmetrical), the presence of "legs" and "antennae." The choice of the size of the models was determined by the average body length of the mass species of predatory ground beetles found in the territory of the studied colony (1.5 cm in *Pterostichus* and 3.0 cm in *Carabus*). Each model was placed 20 times on the foraging trail and also dragged 20 times on a thread along the trail at a rate of 6–7 cm/s. Attractiveness of the model to ants was determined as the number of ants attracted to the model (examining, biting, or dragging it) per minute. The differences in attractiveness of six models with different sets of characters, immobile and dragged (12 altogether) to ants was determined by Scheffe's multiple comparison test (Pollard, 1982). Together with the chamois of models, the ants were also presented with dead beetles *Pterostichus magus* with legs and antennae and without them; with live beetles *C. regalis* in their natural state and painted white with chalk; with live beetles *C. regalis* wrapped up in white and dark cloth and balls of similar size made of the same cloth. Dead beetles and cloth balls containing different objects were placed on the trail, whereas live beetles were released in the central part of the trail. All these objects were compared pairwise (differing in a single character) in their attractiveness to ants, using Student's test. In all, 420 tests with 15 objects were carried out.

The study of development of response to the "enemy image" during imaginal ontogenesis. Ants from laboratory colonies were tested individually in small arenas (15 × 15 cm) by presenting them with a real beetle or a chamois model (7 × 15 mm). In the laboratory experiments 4 variants of models were used (Fig. 1). In the arenas the model were moved with the same speed as on the foraging trails. All the behavioral responses of ants to the models were recorded. In addition, live beetles *Pterostichus magus* and *P. oblongopunctatus* were used as test objects. These common competitors were previously shown to evoke the most aggressive response in ants (Reznikova and Dorosheva, 2004).

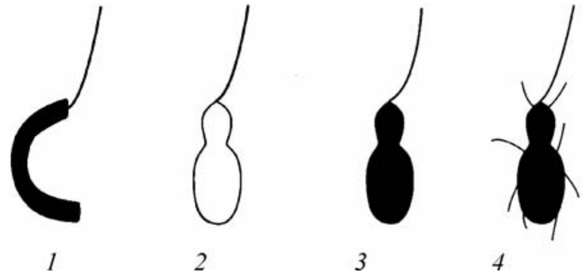

Fig. 1. Models presented to ants: asymmetrical, dark (*1*); symmetrical: white (*2*), dark (*3*), dark with outgrowths (“legs” and “antennae”) (*4*).

Two groups of 20 ants each were used in the experiments: an undifferentiated group of “naive” ants aged 3–5 weeks and a group of “guards” from the control colony. At the age of 3–5 weeks, at least under experimental conditions, the workers start to leave the nest for foraging (Reznikova and Novgorodova, 1998). Each ant was tested only once. The “guards” were sampled from the cover of the artificial nest and near the entrances by moving a preparation needle over the ants and collecting those which attacked it most aggressively. Single ants were placed together with a live beetle and all their behavioral responses were recorded for 15 min. The same ants were tested 5–15 min later on models which were presented randomly, one at a time, during 15 min. In all, 200 such tests were carried out and the number and sequence of responses of 40 ants was recorded. The frequency of a particular response for each group was calculated as the number of individuals which showed this response at least once in the test with a particular model or beetle, related to the total number of ants tested (20 ind.). The mean value and standard deviation of the number of responses for the group were determined. Hypotheses on the non-equal frequencies of responses to different objects in each group and between groups were tested by Fisher’s algorithm for sampling fractions and by χ^2 test. The mean values of responses shown by ants in tests with different objects and between groups of ants were compared using Wilcoxon test (Dytham, 2005).

RESULTS

The key features by which ants recognize the “enemy image.” In field experiments the dark color and movement increased the model’s attractiveness ($p < 0.01$ for all the pairs; see Tables 1, 2). Light objects almost never attracted ants regardless of their size, motility, and the presence of scent (a beetle wrapped up in white cloth). The presence of “legs” and “antennae” increased the number of aggressive responses of ants as compared to exploratory ones. Asymmetrical models proved to be more attractive than symmetrical ones of the same size (5.0 ± 1.5 and 27.5 ± 2.5 for dragged models, 3.8 ± 1.2 and 23.7 ± 1.9 for immobile ones, respectively; $p < 0.01$).

Dead beetles (immobile objects), both with and without extremities equally attracted ants (24.4 ± 1.8 and 25.2 ± 2.2 ind./min). These values were smaller than in case of dragged models 1.5 cm long, with or without extremities ($p < 0.01$). It should be noted, however, that most of the ants presented with a model explored it for only 0.5–1.5 s and continued on their way, whereas those presented with a dead beetle started biting and dragging it. The models attracted 1–4 ants at a time, whereas up to 17 ants gathered around the beetle by the end of the 1-min test. Light painted live *C. regalis* was prone to fewer attacks than a naturally colored beetle (8.4 ± 1.1 and 26.9 ± 3.3 ants, respectively; $p < 0.01$). The ants approached *C. regalis* wrapped up in dark cloth more often that a similarly sized cloth ball without the scent of the ground beetle (15.8 ± 1.4 and 6.7 ± 1.1 ants, respectively; $p < 0.01$). The ants not only explored the cloth ball with a beetle inside but also bit it and tried to drag it, though not for long (several seconds, i.e., less than when dead beetles were presented). No differences were revealed between the number of ants attracted to *C. regalis* wrapped up in white cloth and a ball made of white cloth (4.1 ± 1.2 and 5.5 ± 1.4 ind./min, respectively; $p = 0.07$). In both cases this number was three times fewer than that for a dark ball with a beetle.

Table 1. The number of ants which responded to different chamois models (20 tests of 1 min each, $X \pm m$)

Models	Symmetrical					Asymmetrical
	1	*2*	*3*	*4*	*5*	*6*
Mobile	27.5 ± 6.4	7.3 ± 3.9	30.1 ± 5.2	6.9 ± 3.1	34.3 ± 5.4	5.0 ± 3.8
Immobile	23.7 ± 4.9	3.1 ± 3.0	12.9 ± 3.6	2.8 ± 2.8	15.6 ± 6.1	3.8 ± 3.1

Notes: Models: dark, 3 cm long (*1*); white, 3 cm long (*2*); dark, 1.5 cm long (*3*); white, 1.5 cm long (*4*); dark, 1.5 cm long with “legs” and “antennae” (*5*); dark, 3 cm long (*6*).

Table 2. Significance of differences in preference of different models by the ants (Scheffe's multiple comparison test)

No	1m	1i	2m	2i	3m	3i	4m	4i	5m	5i	6m	6i
1m		0.97	*	*	0.47	*	*	*	*	*	*	*
1i	0.97		*	*	*	*	*	*	*	*	*	*
2m	*	*		0.36	*	0.04	1.00	0.27	*	*	0.98	0.67
2i	*	*	0.36		*	*	0.54	1.00	*	*	0.99	1.00
3m	0.47	*	*	*		*	*	*	0.38	*	*	*
3i	*	*	0.04	*	*		0.02	*	*	0.93	*	*
4m	*	*	1.00	0.54	*	0.02		0.43	*	*	0.99	0.82
4i	*	*	0.27	1.00	*	*	0.43		*	*	0.98	1.00
5m	*	*	*	*	0.38	*	*	*		*	*	*
5i	*	*	*	*	*	0.94	*	*	*		*	*
6m	*	*	0.98	0.99	*	*	0.99	0.98	*	*		1.00
6i	*	*	0.67	1.00	*	*	0.82	1.00	*	*	1.00	

Notes: The models are numbered as in Table 1; the mobile models (dragged on a thread) are designated with *m*, and immobile ones, with *i*; * marks significant differences in a pair of models as to the number of ants attracted, $p < 0.01$.

Development of response to the "enemy image" in imaginal ontogenesis. On the basis of the features of ground beetles most important for ants, revealed in the course of field experiments, we investigated the process of actualization of the "enemy image" in the laboratory. Having analyzed 200 ethograms of control and naive ants, we distinguished the following responses of ants ranged by the increasing degree of aggressiveness.

(1) Lunge: a lunge towards the moving object with mandibles open, sometimes clicking the empty mandibles;

(2) A quick bite: a bite lasting less than 5 s;

(3) A prolonged bite: a bite lasting 5 s or more;

(4) "Deadlock": the ant seizes the object clasping it with its legs, squirts acid on it, without letting it go for a minute or more.

(5) Chase: the ant pursues the object keeping not more than 6 cm behind it, sometimes lunging at it or giving it quick bites.

Comparison of ethograms of ants from control and naive colonies showed that when interacting with live beetles they demonstrated similar ranges of behavioral responses (Table 3). The exception was the "deadlock" response which was not observed in naive ants, whereas 20% of ants from the control colony showed it when meeting a beetle. With the general similarity of responses, quantitative differences were observed between members of the control and naive colonies. Prolonged bites and chase of the enemy were observed in fewer individuals in the naive group than in the control (25 and 45% vs. 70 and 100%, respectively; $p < 0.05$).

Most individuals from both groups (from 85 to 100%) made lunges and quick bites in tests with a live beetle and dark symmetrical models with and without "extremities." Members of the control group, when interacting with a live beetle, made considerably more quick bites than naive ants (21.1 ± 6.7 and 11.6 ± 2.9, respectively; $p < 0.01$). At the same time, naive ants made more lunges than ants from the "wild" colony (12.5 ± 5.3 and 8.9 ± 3.7, respectively; $p < 0.01$). The members of both colonies made more quick bites at the live enemy, as compared to the dark symmetric model with "extremities" (control group: 21.1 ± 6.7 and 6.2 ± 4.1, $p < 0.01$; naive group: 11.6 ± 2.9 and 3.8 ± 2.1, $p < 0.01$) (Table 4). In tests with models naive ants demonstrated only lunges and quick bites, whereas members of the control colony, besides these responses, made prolonged bites (Fig. 2).

Table 3. Occurrence (%) of different responses in groups of naive and control ants when interacting with a beetle

Colony	Behavioral responses				
	1	*2*	*3*	*4*	*5*
Naive	100	100	25	0	45
Control	100	100	70	20	100

Note: Responses: lunge (*1*), quick bite (*2*), prolonged bite (*3*), "deadlock" (*4*), chase (*5*).

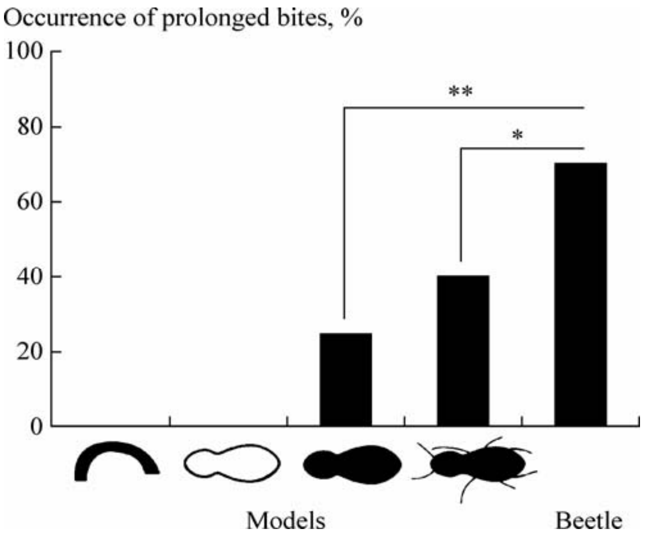

Fig. 2. Occurrence of prolonged bites in tests with models and a real beetle in the control group of ants (Fisher's test, * $p < 0.05$, ** $p < 0.01$).

In all the groups of ants the white color of the model significantly reduced the frequency of lunges ($p < 0.05$) and quick bites ($p < 0.05$; Fig. 3). The asymmetrical shape of the model reduced lunge occurrence only at the level of trend; significant differences in the number of lunges were revealed only for the pair "asymmetrical model"–"black symmetrical model without extremities" ($p < 0.05$; Fig. 3). Addition of "extremities" caused a non-significant increase in frequency of prolonged bites in the control group (Fig. 2). The average number of lunges for ants from the control group increased consistently, from the asymmetrical to the symmetrical model without "legs" and "antennae" and to the model with "extremities" (Table 4). The naive group did not show a linear increase of the lunge frequency in this series of models (0.8 ± 0.7, 4.9 ± 2.6, and 3.2 ± 1.2 lunges, respectively).

In experiments with a white and asymmetric models, color turned out to be a more significant character during recognition of the "enemy image" for naive ants as compared to the control ($p < 0.05$), whereas symmetry was equally important for both groups (Fig. 4).

DISCUSSION

According to the data from field and laboratory experiments, the features attracting ants in the models of competitors are recognized in a certain sequence. The dark color and the presence of bilateral symmetry serve as the first visual characters. They attract the ants' attention and "switch on" their responses to movement, size, and scent of a beetle, which are not evoked in case of white models. For dark symmetrical models, such characters as movement and the presence of extremities appeared to be additive as to their action on the ant behavior. The distinct responses of ants to beetles wrapped up in dark cloth, and also to dead beetles with or without extremities point to the fact that ants largely rely on scent in recognizing their potential "enemies," ground beetles. It is possible that ants respond to "the smell of a predator." Response to universal scent signals associated with consuming animal food has been described for many species of invertebrates avoiding potential predators (for review, see Grostal and Dicke, 1999).

Naive ants at an age of 3–5 weeks, which have never encountered enemies or prey, show aggressive response on their first meeting with both a live beetle and its model. Naive ants demonstrated fewer "highly aggressive" responses (chase and prolonged bites) towards live beetles and never showed a "deadlock." This may be accounted for by "immature" aggressive behavior in ants of this age. At the same time, the number of quick bites was higher in wild ants, whereas naive ants made significantly more lunges than those from the control group. As shown in experiments with models, in recognizing potential enemies naive ants

Table 4. The number ($X \pm m$) of responses by ants from the control and naive colonies in 15-min tests with models and a real beetle

Colonies	Responses	Models				Beetle
		1	*2*	*3*	*4*	
"Naive"	Lunges	0.8 ± 0.7	0.3 ± 0.6	4.9 ± 2.6	3.2 ± 1.2	12.5 ± 5.3
	Quick bites	1.6 ± 1.3	0	3.4 ± 1.5	3.8 ± 2.1	11.6 ± 2.9
Control	Lunges	1.2 ± 0.9	1.4 ± 1.6	4.5 ± 2.8	7.4 ± 3.1	8.9 ± 3.7
	Quick bites	2.3 ± 1.5	0.8 ± 1.3	5.0 ± 4.0	6.2 ± 4.1	21.1 ± 6.7

Note: The models are numbered as in Fig. 1.

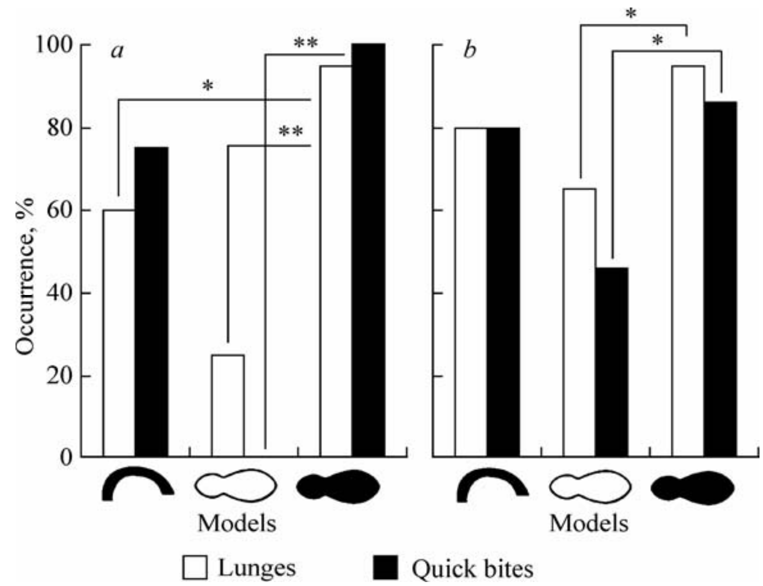

Fig. 3. Occurrence of lunges and quick bites in naive (*a*) and control (*b*) groups in tests with models (χ^2 test, * $p < 0.05$, ** $p < 0.01$).

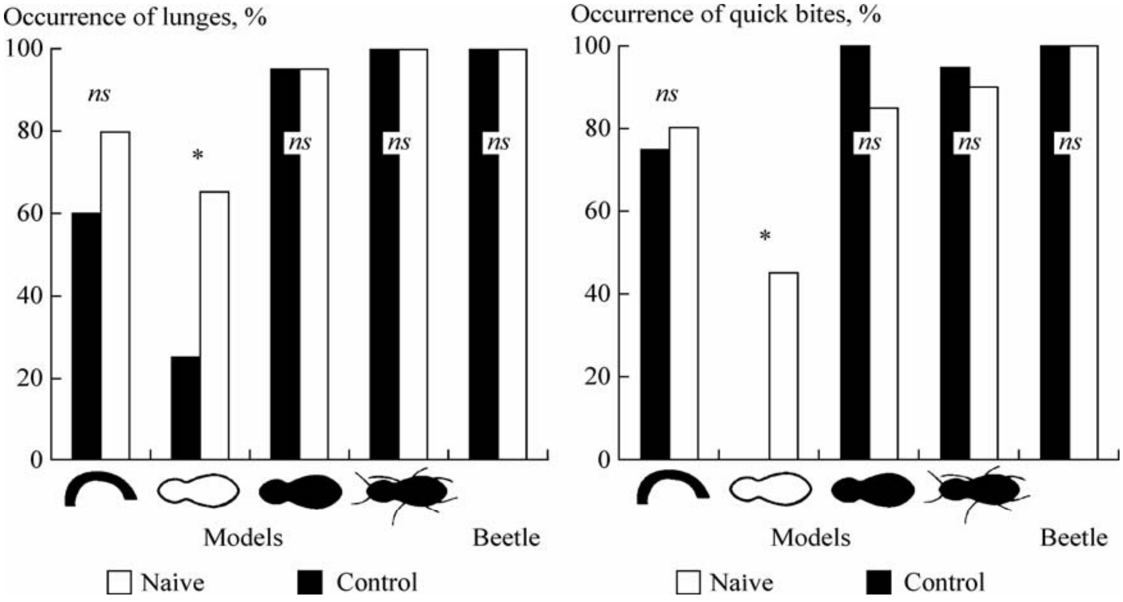

Fig. 4. Occurrence of lunges and quick bites in response to models and a real beetle in naive and control groups of ants (χ^2 test, * $p < 0.05$).

mainly use the same visual characters as members of natural colonies. The dark color and bilateral symmetry of models attract more attention than the white color and asymmetrical shape of models. This suggests the presence of the innate template of "enemy image" in red wood ants. However, this template age is less accurate in naive ants at an age of 3–5 weeks than in those from natural colonies. First of all, one should note a considerable difference in responses of naive ants to models and a live beetle. Although prolonged bites and chases occurred in a smaller fraction of the naive group than in control ants, they still did occur, and only toward a live enemy but not a model. Further, when comparing the attitude to the models in members of both groups, it may be concluded that the dark color of the model is a more significant character for naive ants than for controls, since its absence (a white model) almost completely "switches off" aggressive responses in the members of the first group. The naive ants proved to be unable to distinguish outgrowths imitating "antennae" and "legs" of a beetle as the key character of the enemy. Addition of extremities increased the model's attractiveness only for ants from the control group. Therefore, the ants which have never met ground beetles respond adequately to a live beetle with its combination of characteristic features, whereas their responses to separate features in the models differ from those of natural colonies.

On the whole, it may be supposed that at early stages of imaginal development corresponding to the age when foragers leave the nest, ants with no experience of encounters with different objects respond to the integral and sufficiently realistic "enemy image". A sufficient degree of correspondence between the object and the innate template seems to be required for the aggressive response to be triggered. Distinguishing of some key features and the ability to "grasp" and complete the integral image on their basis seem to require some tuning. Additional experiments are necessary to reveal the relative role of individual and social experience and physiological maturation in this process.

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