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Individual specific trails in the ant *Leptothorax affinis* (Formicidae: Myrmicinae)

U. Maschwitz[1], S. Lenz and A. Buschinger

Zoologisches Institut der Universität, D-6000 Frankfurt/M and Zoologisches Institut der Technischen Hochschule, D-6100 Darmstadt (Federal Republic of Germany), 31 October 1985

Summary. Leptothorax affinis lays chemical trails during nest emigration. Workers which carried colony members during nest movement refused trails of nest mates and searched for their own trails. The origin of the individual specific trail substance could not be localized.
Key words. Individual specific pheromone trails; *Leptothorax;* nest emigration; ant.

The myrmicine ants of the genus *Leptothorax*, which characteristically live in small colonies, possess two recruitment systems: tandem running for recruitment to food, and social carrying for nest emigrations (which are initiated by a brief phase of tandem running). As we demonstrated in marking experiments, individual *Leptothorax* workers do not follow narrowly defined trails but instead walk to their destinations over a broad area unless restricted by a narrow guiding structure like, e.g. a slender twig. On account of these observations we[2] at first did not look for the existence of chemical trails in *Leptothorax*. Chemical trails were demonstrated subsequently by Lane[3] in *Leptothorax unifasciatus*. We took up the problem of trail laying in *Leptothorax* again during recent investigations of territorial behavior in this genus. To our great surprise we discovered not only colonyspecific trials but also individualspecific trails in the tree-inhabiting *Leptothorax affinis*.

Our experimental colonies were maintained in artificial nests consisting of two small glass slides separated by a frame of cardboard. For our experiments we opened the old nest chamber and connected it to a new nest circa 90 cm away via a system of five 15-cm long wooden bridges linked together and supported by raised platforms. Soon the first scouts detected the new nest and – after an initial phase of tandem running – began to transport other workers and brood into it. Now we replaced the original marked bridge with a new unmarked one. The old bridge remained connected to the platform parallel to the former position but shifted aside by 4 cm. During the first half of the experiment, carrying workers which had already learned the route to the new nest became disoriented when encountering the new bridge. After initially palpating it with their antennae they either did not walk onto it at all or they turned around after walking on it for only a few cm. They kept walking around on the platform until they found the old bridge which they recognized instantly and crossed without hesitation even though it was lying in a different position. This indicates that the carrying workers used a chemical trail for orientation (15 tests with 6 colonies: 144 out of 158 workers refused). Carrying individuals which had frequently walked the distance between the old and the new nest, as well as noncarriers which had been walking back and forth, were only partially disoriented by the new bridge. Apparently they used additional cues for orientiation.

When we exchanged the bridges of two different colonies we noted that carrying workers which refused new unmarked bridges refused bridges marked by the other colony as well. In three experiments 39 out of 44 workers refused the foreign bridge and instead searched for their own bridge which they crossed without hesitation after they had detected it in its new position ($p < 0.001$). Thus it can be concluded that carrying *Leptothorax affinis* workers prefer trails of their own colony to trails of other conspecific colonies.

Next we tested whether *Leptothorax affinis* produces individual specific trials, a behavior characteristic which was recently discovered in the ponerine ant *Pachycondyla tesserinoda*[4]. In order to investigate this question we caused small colony fragments consisting of only a few individuals and brood each to move to new nest sites. We allowed one marked worker of each colony fragment to mark a bridge during at least five successive runs. Other scouts were removed. Then we exchanged the marked bridges according to the method described earlier. We made sure that the trails tested were no older than those laid by the workers of the other colony fragments used for the tests. It turned out that the carrying workers used only those bridges that they themselves had marked for their emigration. Bridges marked by their nestmates were refused as were unmarked bridges (6 tests with 6 different colonies: 16 out of 17 workers refused to walk on bridges marked by their nestmates, $p < 0.001$). In another series of experiments we again offered bridges to carrying workers of small colony fragments. These bridges had been used by a large number of their colony members but not by themselves. These strongly marked bridges, too, were refused significantly as they did not contain the trails of the workers tested (6 tests: 10 out of 12 workers refused the strongly marked trails of their own colonies, $p = 0.019$).

The fact that workers follow mainly their own trails may explain our inability to localize the origin of the trail substance. During our search we tested three colonies on bridges marked with extracts of following body parts from some of their own workers: legs, sting glands and sting sclerites, recta and all gastral sclerites. For each test the different glands and body parts of ten workers were thoroughly ground in 0.04 ml water. The whole extracts were applied with brushes to the bridges. These bridges were offered to workers which carried nestmates.

In earlier experiments water had proved to be a good solvent for pheromones of the sting glands. Results: own marked bridges: 217 tested workers, none refused; control unmarked bridge: 34 workers tested, 30 refused; legs: 42 workers tested, 39 refused; sting glands and sting sclerites: 62 workers tested, 59 refused; remaining sclerites of the gaster: 43 workers tested, 37 refused; rectum: 35 workers tested, 30 refused.

This stands in contrast to Lane's results on *Leptothorax unifasciatus*[3] where the poison gland secretion is said to release trail following. According to our investigation[2] the poison gland secretion functions as releaser for tandem running and as a following signal for conspecifics. So far we lack any hint as to where the trail secretion might originate from. During tandem running the sting chamber is open with the sting sometimes visibly protruding. No comparable behavior was detected in trail laying individuals. Trail laying individuals pressed their gasters upon the substrate. On smoked glass plates they thereby generated tracks in which the imprints of gastral hairs but no sting marks could be recognized.

We interpret our finding that *Leptothorax affinis* lays individual trails as follows: the colonies of this ant organize their foraging and other outside activities mainly individually. Therefore it is important for the workers to be able to find their own trails quickly and securely and to be able to distinguish them from the trails of other colony members. Recruited individuals come to know their routes directly by tandem running or social carrying without the need to use the trails of nestmates. Our investigations will be continued.

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Teratogenic effects of cadmium on *Bufo arenarum* during gastrulation[1]

C.S. Pérez-Coll[2], J. Herkovits and A. Salibián[3]

Instituto de Biología de la Reproducción y Desarrollo Embrionario, Universidad Nacional de Lomas de Zamora, C.C.95, 1832 Lomas de Zamora (B.A., Argentina), 1 October 1985

Summary. Developing *Bufo arenarum* embryos were treated during gastrulation with cadmium chloride in concentrations ranging from 6×10^{-7} to 1.5×10^{-5} M Cd^{++} at 20 and 30 °C. Initial failures at gastrulation result mainly in axial incurvations, microcephaly, hydropsy and abnormal tail formation. The higher temperature has a dual effect: at high concentrations of Cd early malformations are significantly increased, whereas at low concentrations the higher temperature prevents alterations.
Key words. Teratogenesis; cadmium; temperature dependence; amphibian; gastrulae.

The increasing incorporation of cadmium into ecosystems as a result of its negligent use in industry and agriculture has been repeatedly reported[4,5]. Its teratogenic effects on vertebrates include skeletal malformations, behavioral disorders, delayed development and reduced body size[6]. Particularly in amphibians, Cd applied continuously from the 2-cell stage onwards produces cellular dissociation, alterations in ectodermal tissues and in the formation of gills and fins[7,8]. In order to establish whether any of these effects are related to the particular stage of development at which Cd is applied, we studied the alterations caused by Cd in *Bufo arenarum* embryos, exposing them at the gastrula stage and analyzing embryocidal and teratogenic effects. Gastrulation involves displacements of cells, changes in cellular adhesiveness and inductions which establish the basic pattern of the organism[9].

Considering that alterations of temperature might change the susceptibility of embryos to Cd[10], we also studied the effects of exposing the embryos to different concentrations of this heavy metal at 20 and 30 °C.

Material and methods. Ovulation was induced by injecting an adult female toad with a suspension of homologous hypophysis. Oocytes were fertilized in vitro and jelly coats were removed with 2% thioglycolic acid pH 7.2. Batches of 30 embryos were treated at the onset of gastrulation (stage 10, S. 10)[11] with 10% Holtfreter's solution containing 6×10^{-7}, 3×10^{-6} and 1.5×10^{-5} M Cd^{++} at 20 and 30 °C until controls reached the late gastrula stage (S. 12) and then maintained in Holtfreter's solution at 20 °C. Controls were untreated embryos maintained at the two temperatures between S. 10 and S. 12 and then at 20 °C until they completed their development. Teratogenic effects were observed

Effects of cadmium (chloride) at 20 and 30 °C of *Bufo arenarum* gastrulation. Survival (A), intense malformations (B) and slight malformations (C); data collected at the neural fold stage (S. 14), cardiac beating stage (S. 19) and complete operculum stage (S. 25) of *Bufo arenarum* embryos exposed to cadmium (chloride) during gastrulation. Data are expressed in percentages

Cd^{++} concentration (M)	°C	Stages 14			19			25		
		A	B	C	A	B	C	A	B	C
Control	20	100	0	0	100	0	0	96.7	0	0
6×10^{-7}		100	0	0	90	3.3	86.7	90	3.3	86.7
3×10^{-6}		100	0	0	90	3.3	86.7	90	3.3	86.7
1.5×10^{-5}		100	0	0	100	6.7	93.3	90	0	90
Control	30	100	0	0	100	6.7	3.3	100	6.7	3.3
6×10^{-7}		100	0	0	96.7	3.3	10	93.3	3.3	6.7
3×10^{-6}		100	0	0	93.3	6.7	13.3	93.3	6.7	13.3
1.5×10^{-5}		100	30	0	96.7	33.3	63.3	96.7	40	56.7