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Latitudinal Variation in Cold Hardiness and Body Size in the Boreal Ant species Leptothorax acervorum (Hymenoptera: Formicidae)

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--- [Article]

The Myrmicinae species *Leptothorax acervorum* (Fabricius 1793) is among the ants ranging farthest north in both North America and Eurasia. Workers from 3 populations living in areas with greatly different climates differ in cold hardiness and individual size. Workers from Cape Kindo on the shore of the White Sea (66° 33' N) survived best at low temperatures and were significantly larger than workers from Nürnberg Reichswald (49.5°N), with workers from the St. Petersburg area (60°N) lying between the two extremes. The significance of the findings is discussed.

Key words: *Leptothorax acervorum* (Fabricius 1793) - Myrmicinae - Bergmann's rule - hibernation - supercooling

HEINZE J & FOITZIK S [Inst Zool I, Univ Erlangen-Nürnberg, D-91058 Erlangen, KIPYATKOV VE & LOPATINA EB [Inst Entomol, Univ St Petersburg, St Petersburg, 199034, Russ Föderation]: **Geographische Unterschiede in Kälteresistenz und Körpergröße bei der borealen Ameisen-Art Leptothorax acervorum (Hymenoptera: Formicidae).** Entomol Gener **22**(3/4): 305-312; Stuttgart 1998-04.

--- [Abhandlung]

Die Myrmicinae-Species *Leptothorax acervorum* (Fabricius 1793) gehört zu den am weitesten nach Norden verbreiteten Ameisen in Nord-Amerika und Eurasien. Arbeiterinnen von drei Populationen aus unterschiedlichen Klimagebieten unterscheiden sich deutlich voneinander in ihrer Kälteresistenz und Körpergröße. Arbeiterinnen von Kap Kindo an der Küste des Weißen Meeres (etwa 66°N) überlebten tiefe Temperaturen am besten und waren signifikant größer als Arbeiterinnen aus dem Nürnberger Reichswald (49.5°N); Arbeiterinnen aus der Umgebung von St Petersburg (60°N) lagen in beiden Parametern zwischen den beiden anderen Populationen. Die Bedeutung der Untersuchungsergebnisse wird diskutiert.

Schlüsselbegriffe: *Leptothorax acervorum* (Fabricius 1793) - Myrmicinae - Bergmann'sche Regel - Überwinterung - Unterkühlung

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1 Introduction

Only very few ants are adapted to cold climate and survive in areas with long winters and hard frost. The tiny myrmicine *Leptothorax acervorum* is among the ant species ranging farthest north, both in Eurasia and North America.

It can be found close to the North Cape in Norway, in Northern Alaska, and in the tundra of Eastern and Northern Siberia [HOLGERSEN 1942, ARNOL'DI 1968, NIELSEN 1987, HEINZE 1993, HEINZE & HÖLLDOBLER 1994]. This is especially surprising as in contrast to other boreal ant genera, such as *Formica*, *Camponotus*, and *Myrmica*, *Leptothorax* do not move into the soil for hibernation but stay year-round in their nests in fallen branches and rotting logs, and thus may be exposed to very deep temperature. Siberian scientists have measured temperatures [**TT**] of as low as -25°C in *Leptothorax* colonies under a thick layer of snow at air **T** of -57°C. They found that the supercooling point of hibernating *Leptothorax* was near -40°C [BERMAN et al 1982, LEYRIKH 1989].

Leptothorax acervorum is, however, not restricted to this very harsh environment but may be found in rather high densities in coniferous forests in Central Europe, the Appenine, and the Ilgaz Daglari in Northern Turkey, where **TT** rarely are that low. We therefore investigated whether *L acervorum* workers from 3 different geographical latitudes respond differently to **TT** below the freezing point. Furthermore, as previous studies suggested that individual size of ant workers increases with latitude [CUSHMAN et al 1993], a correlation which is more generally known as Bergmann's rule, we examined the body size of workers from the same 3 populations.

2 Material and Methods

Complete colonies of *Leptothorax acervorum* were collected from their nests in driftwood and logs in June 1996 in Cape Kindo near Poyakonda on the Western coast of the White Sea (WS, just on the Polar Circle, 66° 33' N, Karelian Republic, Russian Federation), from rotting branches in August 1996 in Vyritsa and Pervomajskoe near St Petersburg (SP, 60°N, Russian Federation), and again from rotting branches in September 1996 in Reichswald around Nürnberg and Erlangen (RW, 49.5°N, Germany). In the laboratory, the ants were housed in 3-chambered plastic boxes as previously described [HEINZE & ORTIUS 1991] and kept at room **T**. After placing the nests into artificial hibernation conditions (10h 5°C, 14h 0°C) in an incubator, those ants which were used for studies on cold hardiness were transferred in groups of 10 workers each in Eppendorff vials with a perforated lid and exposed to low **TT** in a Frykavision B30-40 cryostat. Previous observations had shown that the duration of exposure to room **T** does not affect winter mortality.

The supercooling point of arthropods is often determined by quickly decreasing the temperature of a vial containing a study animal. As it has repeatedly been questioned whether this point indeed shows the lowest temperature at which an animal can survive, we allowed the ants to acclimate and **TT** were reduced stepwise at a rate of -5°C per day until the minimal temperature was reached [HEINZE et al 1996]. Ants were thus exposed to 0°C for 7d (0°C), to 0°C for 1d and to -5°C for 6d (-5°C), to 1d 0°C, 1d -5°C, and 5d -10°C (-10°C) etc. For each tested temperature (-0°C to -20°C at 5°C intervals), eight vials with ten ants each from all populations were used in the cooling experiment. The percentages of surviving ants in the ten groups were compared between the 3 populations. Each worker was used only in one experiment. The experiments were performed directly after the ants had been transferred into the winter incubator and repeated with new ants after two months of artificial hibernation.

Fig 1| ⇒ page 307|: Mortality of workers of the ant species *Leptothorax acervorum* (Fabricius 1793) [Hymenoptera: Formicidae: Myrmicinae] from 3 different populations subjected in eight groups of ten workers each to one week at low **TT** shortly after the beginning of hibernation (0°C, -5°C, -10°C, -15°C on the left side of each graph) and after two months in winter condition (0°C, -10°C, -15°C, -20°C on the right side of each graph). RW: Nürnberger Reichswald (latitude 49.5° N); SP: St. Petersburg area (60° N); WS: Cape Kindo, White Sea (66° 33' N). For details see text. [⇒ **307**].

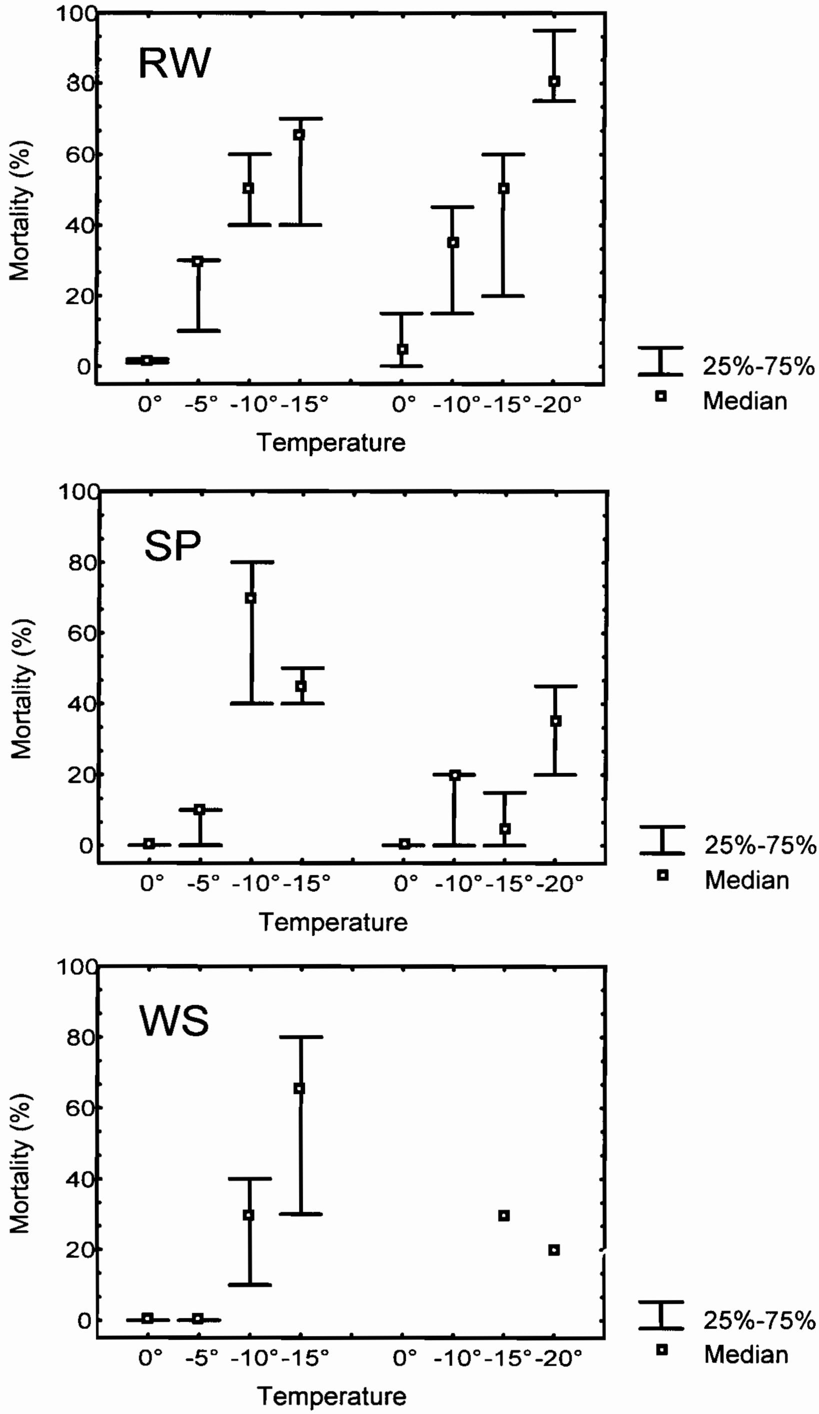


Fig 1: Mortality of workers of the ant species *Leptothorax acervorum* (Fabricius 1793) [Hymenoptera: Formicidae: Myrmicinae].- [Explanation on page ⇔ **306**].

Due to high mortality in colonies from the White Sea during prolonged artificial hibernation, not all experiments could be done with a sufficiently large sample size. In total, 930 workers were used in the experiments at the beginning of winter (RW 320, SP 320, WS 290), and 680 in the experiments two months later (RW 320, SP 320, WS 40) .

Body size (head width and Weber's thorax length) was determined by measuring workers under a binocular microscope at 50x magnification.

3 Results

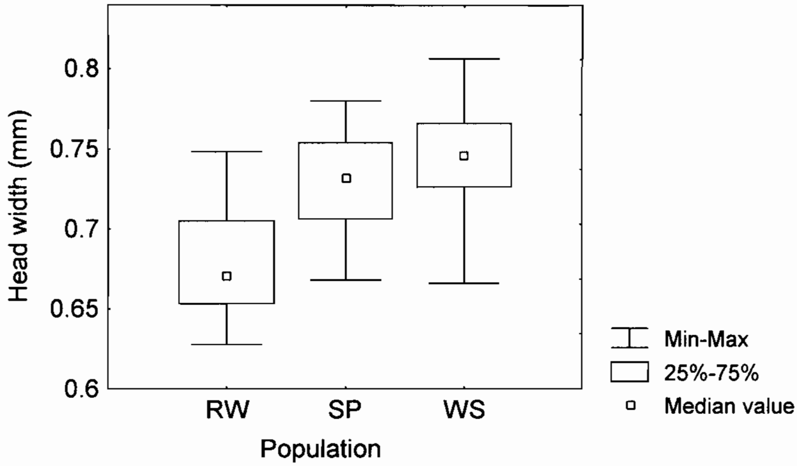
Most workers of *Leptothorax acervorum* survived when subjected to 1 week at 0°C shortly after their colonies had been transferred from room temperature to overwintering conditions, regardless of their provenance. Nevertheless, mortality was significantly higher among ants from Reichswald than in the Russian populations (Kruskal-Wallis H-test, df = 2, H = 13.96, p = 0.0009, post hoc comparison with Tukey HSD-test: RW - SP: p = 0.0006, RW - WS: p = 0.0004, SP - WS: p = 0.881). Similarly, more ants from Reichswald died when exposed for one week to minimally -5°C (H = 11.99, p = 0.0025; Tukey HSD-test: RW-SP: p = 0.039, RW-WS: p = 0.002, SP-WS: p = 0.251). Worker mortality strongly increased at lower **TT**, and about 60% of all workers were killed during one week at minimally -15°C. Worker mortality at lower **TT** did not differ between the 3 populations (-10°C: H = 5.204, p = 0.074; -15°C: H = 2.04, p = 0.36).

Worker mortality did not differ between samples subjected to 0°C at the beginning of hibernation and after approximately two months in winter conditions (Mann-Whitney U-test, RW: U = 25.5, p = 0.285), but workers survived better at **TT** of -10°C and -15°C after prolonged hibernation (-10°C, RW: U = 16.5, p = 0.06; SP: U = 1, p = 0.0011; -15°C, RW: U = 14, p = 0.04; SP: U = 0, p = 0.0006). Mortality now was not different between workers from Reichswald and Russia at 0°C (H = 4.38, p = 0.119) and -10°C (only workers from Reichswald and St. Petersburg: U = 15, p = 0.074), whereas it was significantly higher in the Reichswald population at lower **TT** (-15°C: H = 9.125, p = 0.014; Tukey HSD: RW-SP: p = 0.005, RW-WS: p = 0.949, SP-WS: p = 0.154; -20°C: H = 12.017, p = 0.0025; Tukey HSD: RW-SP: p = 0.0022, RW-WS: p = 0.0051, SP-WS: 0.685).

Body size (head width and Weber's thorax length) was measured in 40 workers from each population. As expected from Bergmann's rule, workers from the White Sea were largest and those from Nürnberg Reichswald near Erlangen were smallest (Weber's thorax length: WS 1.14 ± SD 0.053mm, SP 1.13 ± 0.05mm, RW 1.04 ± 0.04mm; head width: WS 0.74 ± 0.03mm, SP 0.73 ± 0.029mm, RW: 0.68 ± 0.03mm. Size differences between the 3 groups were statistically significant (ANOVA, thorax length $F(2, 117) = 60.10$, $p < 0.0001$, headwidth $F(2, 117) = 48.49$, $p < 0.0001$), which according to posthoc comparisons is mainly due to the difference between the two Russian populations and the Reichswald population (Tukey-HSD-test, thorax length: RW-SP p = 0.0001, RW-WS p = 0.0001, SP-WS p = 0.132; headwidth RW-SP p = 0.001, RW-WS = 0.001, SP-WS p = 0.286; Fig 2).

4 Discussion

As expected, mortality of workers of *Leptothorax acervorum* which were subjected to **TT** below 0°C showed temporal and geographical variation. Workers survived low **TT** better after having acclimated to winter conditions, and the mortality was lower in populations from Russia than in the Reichswald population (Fig 1).



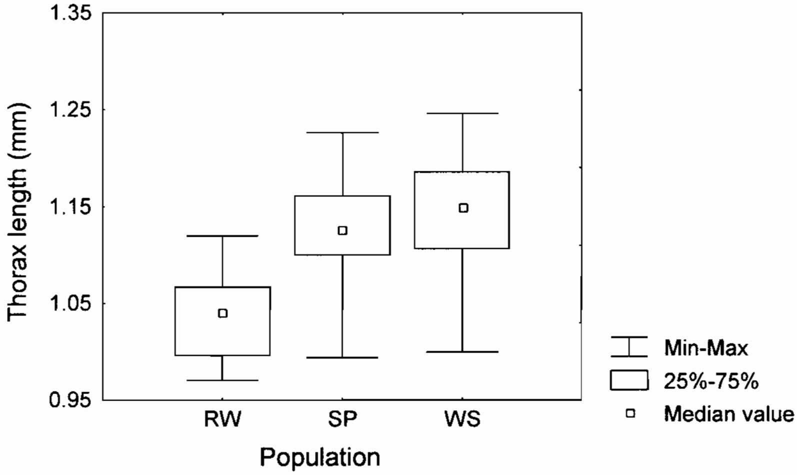


Fig 2 (above): Head width in workers of *Leptothorax acervorum* (Fabricius 1793) [Hymenoptera: Formicidae: Myrmicinae] from 3 different latitudes (**RW** Reichswald, **SP** St Petersburg, **WS** Cape Kindo / White Sea). In each population, the head width of 40 workers was measured.

Fig 3 (below):Weber's thorax length in workers of *Leptothorax acervorum* (Fabricius 1793) [Hymenoptera: Formicidae: Myrmicinae] from 3 different latitudes (**RW** Reichswald, **SP** St Petersburg, **WS** Cape Kindo / White Sea). In each population, the thorax length of 40 workers was measured.

Probably because of the low number of colonies from the White Sea available for our study, which limited the number of possible comparisons, a difference in cold hardiness between the two Russian populations could not be substantiated despite differences in mean **TT** in January and the duration of frost between the two localitites.

It would therefore be premature to conclude from this study that the difference between the cold hardiness of ants from different populations results from adaptation to different climate: The Reichswald population could be more sensitive to frost by causes unrelated to geographical latitude, though certainly different average winter **TT** are the most likely explanation for our result. Geographical and seasonal variation in cold hardiness has been previously reported for a number of arthropods [e.g: BAUST & MILLER 1970, TANAKA 1996]. LEYRIKH [1989] found that the supercooling points of ants drop significantly from summer to winter. In *L acervorum* from East Siberia, e.g, it falls from -8°C in June to close to -40°C in February owing to an increase in the concentration of polyols in the haemolymph. Supercooling points differed also with longitude: supercooling points in hibernating *L acervorum* were approx -25°C in nests from Southern Finland but near -40°C in nests from the upper Kolyma area in East Siberia [BERMAN & ZHIGULSKAYA 1995].

Mortality rates of Russian ants were similar to those of *L* cf *canadensis*, where about 50% of all workers were killed when subjected to minimally -20°C, and about 80% died at -25°C [HEINZE et al 1996]. Compared to supercooling points reported from *Leptothorax* from East Siberia [BERMAN et al 1982, LEYRIKH 1989], the **TT** survived by the ants in our experiments are rather mild. This might indicate that supercooling points do not always give a reliable estimate of the lowest **TT** animals can survive for longer periods, but it might also reflect much lower winter **TT** in East Siberia. Furthermore, a longer exposure to subfreezing **TT** might increase cold hardiness of ants more strongly than the two months at 0°C in our incubators and lead to increased survival of ants at extremely low **TT** in nature.

Though the ecological significance of Bergmann's rule in ectotherms is still controversely discussed [MOUSSEAU 1997, PARTRIDGE & COYNE 1997, VAN VOORHIES 1997], there is strong evidence that at least in some taxa body size increases with latitude. CUSHMAN et al [1993] reported that the average body size of ant workers in temperate and boreal Europe increases significantly with latitude. In their analysis they pooled data from different ant species; here we show that workers of *L acervorum* from the White Sea and St. Petersburg are significantly larger than workers from Erlangen. As data from additional populations [FOITZIK & HEINZE, unpublished] also suggest an increase of body size with latitude, our results probably corroborate Bergmann's rule, though workers from the White Sea are not significantly larger than workers from St. Petersburg with the conservative Tukey's HSD-test (using a less conservative Student-Newman-Keuls test for posthoc comparisons, differences in head width between workers from St. Petersburg and the White Sea are significant with $p = 0.044$).

The occurrence of Bergmann's rule in ectotherms was explained by both adaptive and non-adaptive hypotheses [e.g: ATKINSON & SIBLY 1997]. Whereas size at maturity is often correlated with fecundity, the growth rate of larvae and the size of adult ant workers does not necessarily determine their fitness; instead, colony growth rate and colony size appear to be of more importance. Indeed, KASPARI & VARGO [1995] have reported that colony size is smaller in ants from the tropics than in ants from temperate or boreal biomes. An analysis of intraspecific geographical variation in colony size would be helpful to understand the causes of this correlation, but it appears that *L acervorum* is not a good study model in this respect. Colony size appears to be strongly shaped by the type of nest the ants inhabit. Whereas colonies which nested in driftwood on the shore of the White Sea were very large (more than 100 workers), colonies collected from under flat rocks in Alaska very comparatively small, with a mean of 34.1 +/- SD 23.4 workers (9 colonies, [HEINZE & ORTIUS 1991]).

Body size might be related to the amount of fat stored and it was shown that large ant workers endure fasting much longer than small workers [KONDOH 1977, KASPARI & VARGO 1995]. Whether starvation avoidance is the major factor underlying the variation in worker size in boreal habitats in *L acervorum* is not yet completely clear, but it has been shown that starvation may indeed play an important role in hibernation success in ants [HEINZE et al 1996]. Future studies will focus also on the problem whether size differences are genetically mediated or caused by different environmental conditions.

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SMYTH J D: **Introduction to Animal Parasitology.** - [XX + 549 pp, numer fig + tab, 218 x 275 mm, softcover].- **Publ:** Cambridge University Press, Cambridge 1994; **ISBN:** 0-521-42811-4; **Pr:** Brit £ 27,95. --- [EGR-Nr 2325] Entomol Gener **22**(3/4): 312; Stuttgart 1998-04.

Die spektakulären Fortschritte in der Biologie, zu denen die Erkenntnisse der verschiedensten Disziplinen wie Ultrastrukturforschung, Biochemie, Molekularbiologie, Genetik und Immunologie geführt haben, haben auch das Wissen um die Parasiten in zahlreichen Aspekten deutlich erweitert. Beispielsweise ist auch der Artbegriff in einem neuen Licht zu sehen - gerade bei den hochpathogenen Parasitenspezies - seitdem sich viele bekannte Arten nach Analyse ihres Zymodems und ihrer Antigen-Eigenschaften als 'Artkomplexe' herausgestellt haben, welche intraspezifische Variationen in ihrem Metabolismus oder in ihrer Antigenität zeigen. Solche Unterschiede werden in der konventionellen Artbeschreibung nicht erfaßt.

Spezielle Aufmerksamkeit widmet der Autor denjenigen Parasitenarten, welche oft als Modellorganismen für spezifische Fragestellungen verwendet werden, die aber in der einschlägigen Literatur oft nur in wenigen Zeilen abgehandelt werden. Im allgemeinen sind die morphologischen Beschreibungen kurz gehalten, und die Systematik wird nur insoweit aufgeführt, daß eine grobe Orientierung im zoologischen System möglich ist.

Auffallend sind die sehr gut gelungenen graphischen Darstellungen der Lebenszyklen der verschiedenen Parasiten, wobei besonderer Wert auf die Beziehung Parasit - Umwelt gelegt wurde. Besonders herausgestellt wurde auch die Tatsache, daß einige Parasitenarten, so z.B *Trypanosoma cruzi*, *Leishmania major* und *Entamoeba histolytica* als Modellorganismen für die Erforschung grundlegender biologischer Vorgänge wie Mechanismen der Enzymregulation und der Enzymkinetik dienen. Untersuchungen an *T brucei brucei* zum Austausch genetischen Materials haben zum Verständnis der sexuellen Differenzierung erheblich beigetragen. Ausführlich wird auch die Immunologie parasitenbedingter Erkrankungen besprochen.

Zur weiteren Orientierung des Lesers ist jeweils zu Ende eines Kapitels ein umfangreiches Literaturverzeichnis angeführt, welches insbesondere neuere Arbeiten berücksichtigt. Im letzten Kapitel werden die wichtigsten Verfahren zur in vivo- und in vitro-Kultur von ein- und mehrzelligen Parasiten vorgestellt.

Insgesamt betrachtet, stellt „Introduction to Animal Parasitology" eine gelungene Synthese dar zwischen den Aspekten der klassischen Parasitenforschung und den neuen Erkenntnissen der Ultrastrukturforschung, der Genetik, der Molekularbiologie und der Immunologie. Das Buch ist jedem zu empfehlen, der eine umfassende Einführung in die moderne Parasitologie wünscht.

Günter Schmahl (Ruhr-Universität Bochum)