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Contrasting dynamics of cold resistance traits in field-fresh *Myrmica* ants during the active season

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ABSTRACT

As a result of acclimation populations of long-lived ectotherms should display lowered ability to counter cold stress in warmer periods of active season, and increased resistance in colder ones. We tested this proposition by investigating dynamics of cold resistance in *Myrmica* ants during most of the active season in two types of habitats. Resistance of ants to knock-down by cold and their rate of recovery after chill coma were expected to be lower in summer.

Cooled at a rate of 0.17 °C min^{-1}, the ants showed lower capability to resist knock-down in summer, and a significant lowering in knock-down temperature in response to colder weather both in spring and autumn as confirmed by linear regression against air temperatures. In a more eurytopic species *M. rubra* the responses were significantly faster in meadow than in forest habitats. However, times of recovery of the ants after 10 min at −3 °C did not change in parallel to air temperatures. Whereas *M. rubra* from forest habitats took less time to recover in early summer and early autumn, in their conspecifics from meadow habitats the contrary was the case. Regardless of habitat, recoveries tended to be faster in other investigated species, of which *M. ruginodis* (a forest stenotopic) recovered faster in early summer than later.

According to the knock-down data, in warmer months the ants are indeed less resistant to cold stress, whilst the recovery data do not always support the proposition. The contrasting seasonal dynamics of the two measures of low-temperature resistance in field-fresh *Myrmica* suggest that knock-down (chill coma onset) is a better index of thermal acclimation, whilst the rate of recovery from chill coma is more indicative of interspecific differences and, possibly, behavioural thermoregulation.

1. Introduction

In those ectotherms where active life spreads across several seasons, cold resistance (ability for continued activity at low temperature) may predictably change due to thermal acclimation; in field conditions this is referred to as acclimatization (Lagerspetz, 2006; Wilson and Franklin, 2002). The simplest prediction is that spring and autumn, as colder periods of active season, will result in higher cold resistance, possibly advantageous because it would allow extended activity in these periods. In insects, thermal resistance is often measured as the temperature at which knock-down occurs during chilling or as the time required to restore normal standing posture after a chilling treatment (Sinclair and Roberts, 2005). In colonies of *Myrmica* ants, whose adult stage lasts for a few years, lowering of these indices in the colder periods would extend the ability to perform foraging and other functions, thus benefiting the colonies.

Previous investigation of seasonality of knock-down temperature in *Myrmica* did not support the earlier stated proposition, but this outcome may have been caused by the effects of brief captive maintenance (Maysov and Kipyatkov, 2009). Recovery from chill coma in the ants has not been investigated to date. Another reason for revisiting the seasonality of cold resistance in *Myrmica* was the relatively fast cooling (~1 °C min^{-1}) used in the previous study. Cooling at rates faster than those naturally observed may result in substantial underestimation (Kelty and Lee, 1999) or overestimation (Terblanche et al., 2007) of cold resistance. When the resistances of different organisms are compared, outcomes may be reversed by changing the rate of cooling (Chown et al., 2009). We wanted to clarify the influence of the methodology in our earlier work by measuring the two indices of cold resistance on field-fresh ants cooled considerably slower.

To make this possible, in 2006 we modified previously employed thermogradient technique so that we could determine the indices simultaneously on samples from several ant colonies. In this article the measurements are presented and examined for indication of poorer cold resistance in summer *Myrmica*. We then put the same question in a more specific form: can the change observed in

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the knock-down temperature or chill coma recovery time of the ants be explained by the macroclimatic temperature observed in the studied area?

2. Materials and methods

2.1. Insects and habitats

Species of the Holarctic ant genus *Myrmica* are diverse (Jansen et al., 2010) and characterized by potentially perpetual polygynous colonies with brood development prolonged across years, which means that worker females start as eggs throughout summer, but those starting late develop into adults only after overwintering as final-instar larvae (Kipyatkov, 2001). In the North-West Russia, adults emerge in July–August, those from the wintered larvae emerging first (Kipyatkov and Lopatina, 1997). With the adult lifetime of queens averaging to 1.5 years (Seppä, 1994), and that of worker caste as long as two years (Brian, 1972), a mature colony typically contains workers of different lineages, generations, as well as ages.

The study focused on two species (*M. rubra* and *M. ruginodis*) most commonly encountered in the vicinity of Petergof (59° 53′ N, 29° 54′ E), but occasionally included those less readily found (*M. scabrinodis* and *M. rugulosa*). In the specified area only *M. rubra* displays certain versatility in the habitats occupied, while the other species are quite stenotopic: colonies of *M. ruginodis* inhabit forests, whereas those of *M. scabrinodis* and *M. rugulosa* inhabit meadow-like grassy habitats.

2.2. Collection and maintenance for captivity trials

We established a wandering route through the area that surrounded our base (the Laboratory of Entomology in Petergof): a forested territory of about 1.5 km^2, void of immediate industrial and traffic influences. Along the route several patches of forest and meadow vegetation were selected, more than 200 m apart, where colonies were collected several times during the study period (May–September). The two common species were abundant enough to allow a different colony from a patch each time. To avoid exhausting the same colonies of the less abundant species, we collected *M. scabrinodis* twice and *M. rugulosa* only once. The collected colony fractions (250 worker ants with brood and a queen) were released as autonomous colonies after cold resistance estimation, unless they were destined for captivity trials. The first such trial imitated the conditions of transportation from a distant locality in our previous study: fractions were kept unfed for 3–4 days in 50 ml plastic vials (with a netted hole and a moistened sponge), outdoors under an artificial shelter protected from direct sunlight by dense vegetation. The second trial tested the effect of laboratory conditions similar to those used in 2005: fractions were kept for 2 months in formicaries at 22 °C under 22:2 light:dark cycle, with a food renewal every fourth day. At the end of both trials thermal resistance was re-measured and the fractions were released.

2.3. Equipment and procedure

We followed the earlier-described experimental protocol (Maysov and Kipyatkov, 2009) after improving it as detailed below. The thermogradient device (essentially a styrofoam-insulated aluminium plate cooled from one end) had a wider surface (1200 × 300 mm, covered by 4-mm glass) available for placing well containers of higher capacity (duralumin bars 122 × 25 × 7 mm with 36 small wells) loaded with ants. The wells (arranged as 12 triplet sets) were located between two horizontal plugholes, which were drilled (parallel to the triplets) in one of longer sides and used for plugging thermoprobes. Wires connected the plugged probes to thermometers (Digi-thermo, Brannan, UK, 0.1 °C, one measurement per second). By gentle pulling of the wires (passing through tiny grooves in the styrofoam under the glass cover) and thus moving a container along the thermogradient, experimenter could achieve smooth and nearly synchronous cooling on both container ends (± 0.1 °C) without a need to open the cover during the process. Loaded containers themselves were covered by a 1-mm glass slightly exceeding their width and bearing rubber pieces on hanging edges to prevent sliding.

Simultaneously measured species were evenly distributed along a container, each time with randomly pre-defined relative positions (triplets of wells) for conspecific colonies. Sampling from the collected colony fractions was random, with the following exception. In July, when adult emergence starts, we avoided newly emerged worker ants (calves) distinguished by their light-coloured cuticle sharply contrasting with black oculi. Calves are dependent on mature workers, and in pilot tests they showed knock-down temperatures much higher than 5 °C, from which we started all measurements in the present study. The next trial was delayed until early September, by which time already indistinguishable new adults can perform social functions.

We chose to cool the insects by 0.5 °C per 3 min (=0.17 °C min^{-1}), because the cooling rate was convenient to maintain manually. It allowed a measurement session to be completed in about 1.5 h (~20 min for loading, ~50 min for cooling from 5 to −3 °C, up to 20 min for recovery); therefore, two or three sessions per habitat could be made on the day of collection to increase replication (6–9 ants per colony).

To follow the condition of 36 ants at once, instead of direct observation a container was automatically photographed by a digital camera (Olympus SP350, one shot per minute) mounted downward on a table tripod (Hakuba 7500). Experimenter had to move the tripod on the glass cover and to record the temperature from both thermometers. The latter task was facilitated by a laptop (synchronized with the camera) with a freeware Hotkeys2000, which allowed current time and date to be saved in an Excel workbook with two spreadsheets, each with a list of temperatures from 5 to −3 °C over 0.5°C intervals.

At −3 °C a container was held for 10 min and then taken out, its content was immediately placed into another container (kept at 20 °C), and automatic photographing resumed to measure recovery times. After this, the ants were frozen for the measurement of size (width of head).

Later, for each measurement session we constructed a graph with time–temperature curves for both container ends. Then, if an ant in a photo appeared to have entered chill coma, the timing of the photo was converted to knock-down temperature by reading the graph. If the coma time corresponded to asynchronous curves, we took intermediate temperature values proportional to the position of the ant between the probes. Chill coma was detected from either a clearly seen knock-down or from signs of inability of an ant to move (indicated by several legs stretched in air, curved body or heavy tilt). Recovery was defined as a complete return of the insect to firm standing position, usually followed by walking in the well. It was counted in minutes, directly from the timing of the photo.

For technical reasons the collection of recovery and size data started with a lag (resulting in missing observations for early trials).

2.4. Statistical analysis

Sets of conspecific data were selected for the analysis of variance (ANOVA) to test the impact of captivity, habitat and season.

Table 1
Summary of AN(C)OVA results for knock-down temperatures, significant effects boldfaced ($p < .05$). Bracketed "n.s." indicates non-significant interactions removed from final models to standardize variance. For the same purpose colony means were used in the vials test for *M. rubra* (hence repeated-measure design). Italics: significance confirmed by Dunnett's *C* (see Section 2.4 for details).

		Habitat (*h*)	Date (*d*)	Colony (*c*)	*hd*	*dc*	Size
Habitats							
M. rubra	*F*	.08	**11.96**	**3.81**	**1.60**		
	df	1, 33	2, 33	2, 33	30, 252		
Vials							
M. rubra	*F*	.82	**92.20**	2.17	**28.47**		
	df	1, 20	2, 15	16, 15	1, 15		
M. ruginodis	*F*		**14.96**	1.48		.80	
	df		1, 5	5, 5		5, 60	
Laboratory							
M. rubra	*F*		**4.21**	1.89		(n.s.)	.0003
	df		1, 100	5, 100			1, 100
M. scabrinodis	*F*		**13.09**	.71		(n.s.)	.03
	df		1, 100	5, 100			1, 100
M. rugulosa	*F*		**10.40**	.27		(n.s.)	.01
	df		1, 82	5, 82			1, 82
Seasons							
M. rubra (forests)	*F*		***5.97***	1.68			.23
	df		2, 20	19, 79			1, 79
(meadows)	*F*		**5.15**	1.01			.32
	df		1, 8	10, 95			1, 95
M. ruginodis	*F*		**45.29**	.55			**10.22**
	df		1, 26	10, 59			1, 59
M. scabrinodis	*F*		**19.81**	**2.10**			1.02
	df		1, 11	10, 95			1, 95

For knock-down temperature, the selected sets were the habitats (*M. rubra*: three earliest dates), the vials, the laboratory and the seasonal dynamics (Table 1). In the ANOVAs, the only random-effect factor was the colony. Hence, most captivity trials (the vials test for *M. ruginodis* and all tests of laboratory effect) had mixed-model two-way design, with factors of date and colony interacting. The vials test and the habitats test for *M. rubra* combined the two-way fixed-effect design (habitat interacting with date) with nesting (colony nested, respectively, within habitat and within habitat–date interaction). The design of seasonal dynamics tests was nested (colony within dates), and subsets of the data with size measurements available were subjected to the analysis of covariance (ANCOVA): same design plus head width as a covariate (Sokal and Rohlf, 1995).

Analysed datasets were checked for normality (Shapiro–Wilk's test) and for variance homogeneity (Levene's test). If only normality was violated, we proceeded with the ANOVA, relying on its robustness to deviations from distributional assumptions (Quinn and Keough, 2002; Zar, 1999). In cases of irreparable heterogeneity of variances, outcomes of nested ANOVA were verified by post-hoc multiple comparisons unaffected by the heterogeneity (Dunnett's *C* criterion). Otherwise the problem was solved either by data transformations, or by removal of an interaction term proved to be non-significant (reduced model) or by analysis of colony means. In captivity trials, the colony means required repeated-measure design, i.e. without colony–date interaction. Individual observations were preferred for testing the effect of size in the ANCOVA; in case of significance, it was verified on colony means. Covariation slopes for different dates were tested for homogeneity by an interaction term of the factor (date) and the covariate (head width), temporally introduced in the model.

Analysis of recovery times lacked the habitat test due to missing data; otherwise tests were conducted in the same way as for knock-down temperature (Table 2). An outlier threefold greater than conspecific values was found in *M. rugulosa* and excluded from the analysed dataset.

To test the connection with macroclimatic temperature, regressions were performed against it for colony means of those traits, which were measured at least five times per species per habitat. Air temperature values (2 m above the ground, over 6 h intervals) at the nearest weather station located similarly at the Gulf of Finland (Ozerki, 60° 12′ N, 29° 01′ E) were obtained from an archive at http://rp5.ru (accessed on 08.12.2006). Linear regressions were repeated on temperature averages spanning 1–4 preceding days, with Bonferroni corrections for the four similar tests (within a species/habitat/trait). Standardized residuals from each model were plotted against leverage indices (to detect outliers/influential points) and against standardized predicted values (to check for linearity/variance homogeneity; Sokal and Rohlf, 1995). For each of the spans, habitat differences between regression slopes in *M. rubra* were tested by F-ratio-based method of multiple slope comparisons (Zar, 1999).

All the analyses were performed with SPSS 12.0 RU.

3. Results

Both abundant species displayed similar dynamics of knock-down resistance across the study period. Specifically, knock-down temperature fluctuated between 0 and −1 °C in late spring and early summer, then rose to 2 °C in mid-summer and decreased to 1 °C in early autumn. Captive ants were less resistant than their kin measured field-fresh. The meadow species demonstrated temporal shifts of resistance largely parallel to corresponding parts of the dynamics outlined above (Fig. 1). Analyses always showed significance of temporal changes in knock-down temperature (Table 1); in *M. rubra* the rate of the changes was significantly affected by habitat: in the field it was faster in meadow colonies, whilst in their forest counterparts it was faster in vials.

Recovery time trajectories were more diverse (Fig. 2). Residents of forests and meadows in *M. rubra* recovered at significantly different rates (Table 2) and showed a remarkable switch from initially faster recovery in forest population to the opposite

Table 2
Summary of AN(C)OVA results for recovery times, significant effects boldfaced ($p < .05$). Bracketed "n.s." indicates a non-significant covariate removed from final model to include dates for which data on size were missing. Colony means were used in the vials tests to standardize variance (hence repeated-measure design). Italics: significance confirmed by Dunnett's *C* (see Section 2.4 for details).

		Habitat (*h*)	Date (*d*)	Colony (*c*)	*hd*	*dc*	Size
Vials							
M. rubra	*F*	**8.29**	**16.29**	**4.68**	**21.84**		
	df	1, 17	2, 15	16, 15	1, 15		
M. ruginodis	*F*		**8.18**	.22			
	df		1, 5	5, 5			
Laboratory							
M. rubra	*F*		**10.50**	2.39		**3.31**	.002
	df		1, 4	5, 5		5, 94	1, 94
M. scabrinodis	*F*		1.62	**8.30**		.29	1.72
	df		1, 9	5, 8		5, 95	1, 95
M. rugulosa	*F*		.18	3.37		.83	2.56
	df		1, 3	5, 3		3, 78	1, 78
Seasons							
M. rubra (forests)	*F*		***8.79***	**2.17**			0.13
	df		2, 19	19, 79			1, 79
(meadows)	*F*		***7.34***	1.39			.33
	df		1, 9	10, 95			1, 95
M. ruginodis	*F*		***19.15***	.88			(n.s.)
	df		2, 18	15, 90			
M. scabrinodis	*F*		0.82	1.34			1.37
	df		1, 13	10, 94			1, 94

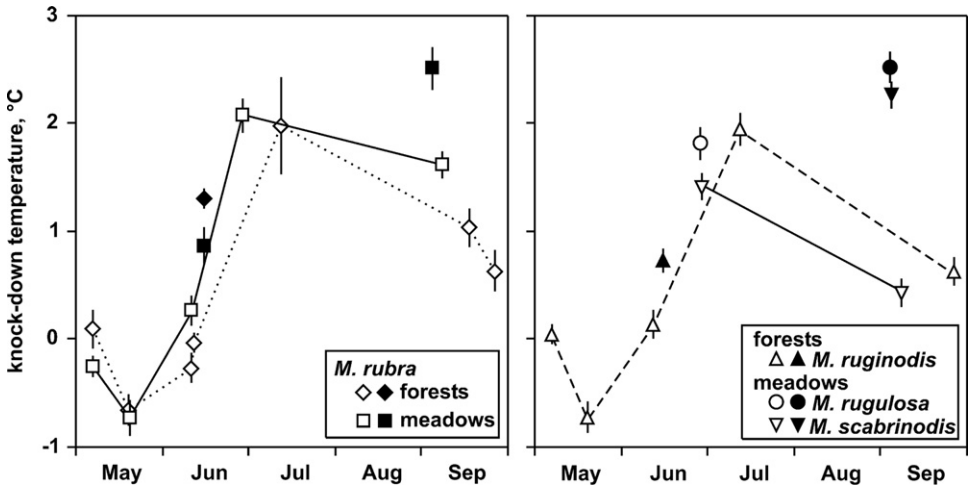

Fig. 1. Chill coma temperatures of *Myrmica* (means ± standard errors, $n=6$ colonies, except fresh *M. rugulosa*: $n=4$ colonies). Open markers—field-fresh, solid markers—captive: kept in vials (June) or in laboratory (September). A solid marker is for the same colonies that the nearest earlier open marker of the same shape represents, except *M. rubra* of forests: two nearest earlier markers (11–12 June).

situation later in season. In vials the rates converged: forest colonies quickly slowed recovery to the level of meadow ones. In *M. ruginodis* recovery time tended to be shorter, but, unlike *M. rubra*, it changed in early summer only. The meadow species recovered even faster than the above two; however, they gave no indication of a temporal change in the trait.

A significant positive covariation of knock-down temperature and head width was observed in *M. ruginodis* (confirmed by ANCOVA on colony means: $F_{1,9}=8.83$, $p=.016$; common slope: $2.21 \pm .94$).

Air temperature explained 41–91% of variation in resistance to knock-down, averages spanning one preceding day always being the weakest predictors. In each span the regression slope for meadows in *M. rubra* was steeper than for forests (significantly, except for the one-day span). About 23% of variation in recovery times of forest *M. rubra* was explained by temperature averages spanning three days (Table 3).

4. Discussion

4.1. Cold resistance measured by knock-down temperature

The dependence of cold knock-down on macroclimatic temperature suggests this is an acclimation response, provided that no other factors were interfering. One such factor may be ontogeny or age (Bowler and Terblanche, 2008). Temperature resistance markedly changes with age in other insects (Meats, 1973; Nyamukondiwa and Terblanche, 2009). In *Myrmica*, calves were indeed less resistant to cold knock-down, beyond our measurement scale (see Section 2.3), but mature ants of various ages (see Section 2.1) had equal sampling probability in the present study. Another possible factor is feeding, which improves the resistance (e.g. Nyamukondiwa and Terblanche, 2009), but naturally fed ants lowered their resistance in early summer (together with unfed ones in vials) at the time when food availability

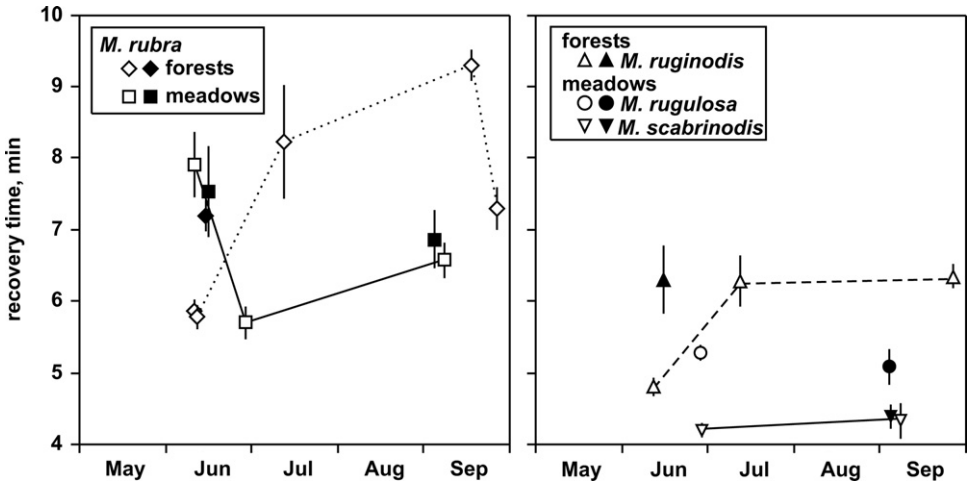

Fig. 2. Seasonal and captivity-induced changes in chill coma recovery time in *Myrmica* (means ± standard errors, $n=6$ colonies, except fresh *M. rugulosa*: $n=4$ colonies). Notation— as in the previous figure.

Table 3
Summary of regressions of cold resistance traits on air temperature averages spanning 1–4 preceding days. Significance of differences from zero for slopes after Bonferroni adjustment: $^{*}p<.00125$ and $^{***}p<.00025$.

		Days	Intercept	Slope	r^2	t	df
Knock-down	*M. rubra* (forests)	1	−1.905 ± .443	.173 ± .031	.407	5.495***	1, 44
		2	−2.347 ± .373	.208 ± .027	.578	7.756***	
		3	−2.325 ± .351	.207 ± .025	.605	8.201***	
		4	−1.800 ± .340	.175 ± .025	.523	6.939***	
	(meadows)	1	−2.836 ± .598	.264 ± .045	.555	5.905***	1, 28
		2	−3.914 ± .550	.355 ± .042	.715	8.372***	
		3	−4.339 ± .445	.394 ± .035	.821	11.320***	
		4	−4.116 ± .280	.379 ± .022	.914	17.230***	
	M. ruginodis	1	−2.054 ± .386	.174 ± .026	.612	6.646***	1, 28
		2	−2.398 ± .274	.203 ± .019	.803	10.680***	
		3	−2.257 ± .277	.196 ± .019	.784	10.070***	
		4	−1.957 ± .200	.177 ± .014	.849	12.562***	
Recovery	*M. rubra* (forests)	1	6.054 ± 1.345	.102 ± .091	.038	1.122	1, 32
		2	4.946 ± 1.147	.180 ± .078	.144	2.318	
		3	4.362 ± 1.067	.219 ± .071	.227	3.061*	
		4	6.115 ± 1.038	.101 ± .071	.059	1.417	

and dietary breadth must increase. Over the period of adult emergence only laboratory-cultivated ants lowered knock-down resistance. Given their improved nutritional status (unlike in the first feeding, colonies always left food remnants during maintenance), the divergence from field-fresh kin or conspecifics provides further evidence that neither age nor nutrition was a likely driver of the changes observed in cold knock-down.

The fact that longer-term averages of air temperatures produced better regression results for knock-down temperature in *Myrmica* confirms that we are dealing with a slow form of acclimation (Bowler, 2005), i.e. a response to conditions including those immediately preceding the trials and those observed earlier. Higher temperature dependence (and percent variation explained) of the knock-down resistance in open habitats is of no surprise, because the temperatures of air above an open surface were used for the regressions.

4.2. Cold resistance measured by recovery time

Although forest *Myrmica* did lend some support to the idea of slower recovery in summer, obviously temperature acclimation played only a minor role in changing this trait. Furthermore, with the recovery dynamics in meadow *M. rubra*, any single-factor explanation of the changes becomes unlikely. In fact, the observations may suggest behavioural thermoregulation: the meadow colonies concentrate in surface chambers of nests in colder periods (cf. Brian, 1972) and hide deeper in the nests during the warmer ones. While collecting meadow *Myrmica*, we observed that the top chambers in their nests (soil hillocks built over grass tussocks or stems of herbs) did appear empty in the hottest hours of sunny days. However if such thermoregulation does affect recovery, this explanation is also 'acclimation' in essence and thus presents a problem: how knock-down resistance, measured from the same individuals, remains unaffected? For the behavioural hypothesis the main difficulty is to explain the marked among-habitat difference in a less temperature-sensitive trait (recovery) and practically no such difference in a trait is strongly affected by thermal acclimation (knock-down, Table 3). Therefore, it must be additionally assumed that the two traits are differently affected by the daily period of maximal temperatures.

Certain thermal physiology traits have been shown to be uncoupled in insects, although examples mainly concern uncoupling of thermal resistance and tolerance (survival at thermal extremes). For instance, in *Drosophila* spp., positive correlations across species, e.g. between knock-down resistance and survival in

the cold (Hori and Kimura, 1998) and heat (Berrigan and Hoffmann, 1998; Berrigan, 2000), or across distant populations, e.g. between chill coma recovery rate and cold survival (Hoffmann et al., 2002), may simply be a result of simultaneous selection of the traits, not of their physiological coupling (Berrigan, 2000; Hoffmann et al., 2002). As laboratory experiments have demonstrated, selection for heat knock-down resistance may not improve heat survival (Hoffmann et al., 1997; see also Bubliy and Loeschcke (2005) for a contradictory result). Selection for cold-stress recovery may improve cold survival (Anderson et al., 2005; Mori and Kimura, 2008), but it may not work the other way round (MacMillan et al., 2009). The evidence, though, comes from comparisons across 'populations' (replicated laboratory cultures) that were reproductively isolated. In the present study we found little or no correspondence between trajectories of different indices of cold resistance across seasons within two subpopulations in no way isolated from each other. Thus, seasonal dynamics may be used for testing connectedness of thermal resistance traits (with each other or with a climatic factor) in organisms for which selection or acclimation experiments would be impractical or totally impossible.

Finally, the notable interspecific differences of ants by the recovery time show that it is more dependent on species-specific physiological abilities. In forest *Myrmica* the differences correspond to preferred within-forest niches (shaded patches by *M. ruginodis* and insolated by *M. rubra*), but their meadow congeners recover from chill coma consistently faster even after 2 months in laboratory. Taking into account that *M. scabrinodis* also indicated a little higher cold knock-down resistance than the more abundant forest congeners, such a result superficially resembles one obtained for two species of *Drosophila*, in which heat knock-down resistance and survival responded to heat acclimation differently, indicating correlation across species, but not across treatments (Berrigan and Hoffmann, 1998).

4.3. Comparing to previous estimates: effects of captivity and cooling rate

Temperate or boreal ectotherms cycle through low and high cold hardiness (ability to survive prolonged cold exposure) in active and wintering seasons, respectively (e.g. Block, 1990; Vernon and Vannier, 2002), and ants are not an exception from this rule (Berman et al., 2007; Leirikh, 1989). High cold hardiness and diapausing state are often physiologically associated (Denlinger, 1991); supposedly, knock-down resistance may also rise with diapause, which begins in *Myrmica* colonies by the end of August in the studied area (Kipyatkov and Lopatina, 1997). Combined with our earlier estimates of knock-down temperature in the ants (Maysov and Kipyatkov, 2009), this work demonstrates that a brief captivity (2–5 days in the previous study) can conceal the moderate autumn decrease in this parameter, indicating its lack of connection with diapause. On the other hand, the knock-down temperatures, measured on field-fresh ants at a cooling rate fivefold lower than previously used, varied temporally only one degree wider than the earlier estimates, perhaps merely due to extension of the study period to late spring. Other researchers (Chown et al., 2009; Kelty and Lee, 1999; Terblanche et al., 2007) found a change of several degrees in critical thermal minima for muscle coordination in flies or ants with fivefold or just twofold reduction in cooling rate.

4.4. Conclusions

The results demonstrate that seasonal dynamics of cold resistance traits in field conditions may clarify which of the traits is stronger linked to macroclimatic temperature (the ultimate determinant of thermal environment), thus revealing 'independent acclimation processes' within single *Myrmica* species. The suggested hypothesis of behavioural thermoregulation (and its corollary that the traits are differently affected by the daily period of maximal temperatures) requires further research in connection with microclimatic temperature in the habitats. Furthermore, the results warn against relying on only one selected measure of cold resistance when studying it in ectotherms in the field.

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