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Temperature responses in a subarctic springtail from two geothermally warmed habitats

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ABSTRACT

Common-garden experiments with populations sampled along natural thermal gradients help to reveal local adaptation, disentangle environmental and genetic effects, and ultimately predict, by analogy, future biotic responses to climate change. In this regard, geothermal habitats are useful model systems as they exhibit dramatic changes in soil temperature. The springtail *Protaphorura pseudovanderdrifti* has apparently coped with such local geothermal warming in Iceland, as this species occurs along a more than half-century-old geothermal gradient in a grassland and persists along a newly emerged temperature gradient in a previously non-geothermal planted spruce forest. We measured thermal reaction norms for development and walking speed and acute cold shock tolerance of *P. pseudovanderdrifti* originating from the grassland and forest geothermal gradients. Temperature-dependent juvenile development showed little variation among subpopulations from the recently warmed forest, probably due to insufficient evolutionary time, but springtails from the warmed grassland plots had significantly steeper reaction norms than their counterparts from the corresponding unwarmed plot. In contrast, cold tolerance and locomotory activity showed no conclusive clinal pattern despite significant within-habitat variation. There appeared to be significant differences between habitats, as springtails from the forest had more temperature-sensitive developmental rate and locomotory activity, walked faster, and exhibited more variable cold tolerance than grassland springtails did. The planting of a forest, therefore, seems to have exerted a stronger effect on the thermal phenotype of *P. pseudovanderdrifti* than the emergence of a geothermal gradient. Thus, habitat properties may be no less important in shaping thermal reaction norms than the mean temperature. These local-scale findings suggest that, in addition to warming per se, global transformation of communities may drive the evolution of thermal phenotypes to an extent comparable with the effect of rising environmental temperature.

1. Introduction

There is an overwhelming array of documented biotic responses to global climate change, and yet relatively few studies have been designed to test whether the observed response is evolved or based on pre-existing plasticity and still fewer did find evolutionary modifications (Bradshaw and Holzapfel, 2008; Franks et al., 2014; Van Petegem et al., 2016; Ramakers et al., 2019). Most climate-driven changes in living organisms seem to have occurred due to phenotypic plasticity, i.e.,

represent physiological, ecological, behavioral and other direct responses to environmental change (Gienapp et al., 2008).

Some manifestations of phenotypic plasticity are non-adaptive in the sense that these do not arise directly from natural selection. For example, the organism's constitutional compounds and biochemical machinery are temperature-sensitive by their physical nature (Schlichting and Pigliucci, 1998; de Jong and van der Have, 2009). More subtle differences in temperature responses, including magnitude (i.e., lower and upper thresholds) and degree of plasticity (i.e., slope of

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the reaction norm), often represent adaptations to particular thermal environments (Schlichting and Pigliucci, 1998; Angilletta, 2009). Adaptive plasticity mirrors environmental heterogeneity experienced by previous generations in the evolutionary past and thereby may help to mitigate, at least partly, the effects of future climatic transformation (Nussey et al., 2005; Chown et al., 2007; Gienapp et al., 2008). However, plasticity may as well become maladaptive in a novel environment where conditions fall outside the historically experienced range: in such a case, selection would favor a shallower reaction norm (Ghalambor et al., 2007).

Plasticity can also evolve in response to anthropogenic disturbance, including climate change (Nussey et al., 2005; Crispo et al., 2010): e.g., both the elevation and slope of the reaction norm for egg hatch in a geometrid moth have rapidly evolved under global warming to reduce the mismatch with host-plant phenology (van Asch et al., 2012). Nevertheless, evidence supporting current climate change-driven evolution, as opposed to purely plastic responses, remains scarce (Merilä, 2012; Vázquez et al., 2017). Often, research is started after phenotypic changes have already happened, which makes it challenging to partition the observed phenotypic variation into environmental and genetic components because the initial condition of the system studied is not known.

A workaround approach, based on 'space-for-time substitution' (Franks et al., 2014), enables one to infer future effects of climatic change from spatial patterns of trait variation. For example, populations and communities can be compared in situ along an experimentally manipulated (Hodkinson et al., 1998; Holmstrup et al., 2017) or naturally occurring (O'Gorman et al., 2015) environmental gradient. However, as hereditary and plastic effects are indistinguishable in the field, detecting an evolutionary change in such cases requires bioinformatic scrutiny (Bataillon et al., 2016). Alternatively, various eco-physiological traits (rates of growth and locomotion, critical temperatures, etc.) can be measured under controlled, common-garden conditions in populations originating from cool and warm habitats (Walther et al., 2010; Gaitán-Espitia et al., 2014; Van et al., 2014; Van Petegem et al., 2016). The results, however, are often disparate and likely depend, among other confounding factors, on the biology of the species and on the trait studied. In some cases, there is no latitudinal cline in spite of significant inter-populational trait variation across the studied sites (Miles, 1994; Liefting et al., 2009; Gardner et al., 2011; Sengupta et al., 2016, 2017), suggesting the presence of biogeographic or fine-scale environmental differences, as well as possibly indicating a high degree of genetic drift. Furthermore, temperature per se may not be as important as its seasonal variation: in fact, studies conducted at several experimental regimens often show that the degree of trait plasticity is greater in more variable climates (Gaitán-Espitia et al., 2014; Kosmala et al., 2018).

Thus, thermal phenotypes of ectotherms are not always related to environmental temperature (or its proxy, such as latitude or altitude) in a simple and predictable manner. It would be intriguing to examine clinal variation in temperature-dependent traits in a species from several locations that are as uniform as possible in all respects other than temperature. Such uniformity may be found in geothermal systems where temperature gradients emerge quickly and over short distances.

In this study, we exploit local-scale habitat warming to test for associated changes in the thermal phenotype of a soil-dwelling arthropod. In 2008, an earthquake in Iceland dislocated hot groundwater in the bedrock to a previously non-geothermal area. As a result, both mean and maximum temperatures in the soil increased without a substantial change in key soil chemical parameters (Sigurdsson et al., 2016). About 2.5 km to the northwest of the impact site, there is another geothermal hot spot that has been known at least since the early 1960s (O'Gorman et al., 2015). Both localities represent a unique natural experiment with soil warming and so have become important field test sites for climate change research under the ForHot project (www.forhot.is). One of the biodiversity surveys, which focused on the springtail community

(Holmstrup et al., 2018), revealed a soil-dwelling species that was abundant along the geothermal gradients. As this springtail seemed to have successfully coped with local warming, we carried out common-garden experiments with individuals originating from unwarmed and warmed plots in order to detect clinal variation in cold tolerance and in thermal reaction norms for development and walking speed. We expected to find differences in both the trait values and their plasticity along the natural thermal gradient, especially in the grassland subpopulations that had experienced warming for a longer time. The presence of such clinal variation in cold tolerance, development, and/or locomotion would indicate that thermal phenotypes of soil arthropods can rapidly evolve in response to warming.

2. Materials and methods

2.1. Collection sites

The springtail *Protaphorura pseudovanderdrifti* (Gisin, 1957) (Collembola: Onychiuridae) was collected in September 2014 at two ForHot sites in southern Iceland (64.0 °N, 21.2 °W), hereinafter referred to as habitats. The first habitat was a planted Sitka spruce (*Picea sitchensis*) forest that had been warmed since a large earthquake in 2008, and the second habitat was an unmanaged *Agrostis capillaris* dominated grassland 2.5 km away that had been warmed geothermally for at least half a century. Each habitat was divided into plots labeled from A to E in the order of increasing soil temperature (approximately +0, 1, 2, 3, and 5 °C warming; see below). In the grassland, the plots measured 2 × 2 m, whereas those in the forest were set up in between trees and had a size of 1 × 1 m due to less space being available. A detailed description of both habitats, denoted respectively FN (forest, new warming) and GO (grassland, old warming), as well as of the plots within them, is provided by Sigurdsson et al. (2016), including a map of the area. Soil temperature in the plots was recorded with HOBO TidbiT v2 Water Temperature Data Loggers (Onset Computer Corporation, USA) on an hourly basis at 10 cm depth from 8-May-2013 until the sampling of springtails on 11-Sep-2014. Soil temperature at this depth only negligibly differed from that 5 cm below the surface (Sigurdsson et al., 2016) and reliably approximated the thermal conditions in the soil layer where *P. pseudovanderdrifti* was typically found. Based on the plot-specific temperature data we calculated overall mean temperature (mean of all hourly measurements during the period), absolute maximum, absolute minimum, overall temperature amplitude and mean diurnal temperature amplitude. Distance between plots was at least 10 m. Samples were only taken from plots A, B, D, and E, with five replicates per plot. Distance between replicates varied from several meters to several hundreds of meters and was generally greater than that between plots with different warming levels.

Soil cores (diameter 6 cm; depth 5 cm), one per plot, were sampled in order to isolate individual species and establish laboratory cultures for experiments. Springtails were extracted over one week in a high gradient extraction apparatus (MacFadyen type) where the temperature in the upper compartment increased stepwise from 25 to 50 °C over seven days while the temperature in the lower compartment remained constant at 5 °C. Springtails were collected in beakers with a bottom covered with a layer of moistened plaster of Paris and subsequently sorted. The high-gradient extraction method used in our laboratory has been thoroughly tested and always shows a better than 90% extraction efficiency of various springtail species including *Protaphorura* spp. (Petersen, 1978; Holmstrup and Krogh, 2001; Sjørnsen and Holmstrup, 2004). Any bias towards heat-tolerant specimens (or the opposite) when establishing the laboratory cultures seems therefore unlikely.

Cultures of *P. pseudovanderdrifti* were established in the laboratory in Silkeborg, Denmark, using the same method as for other euedaphic springtails (Krogh, 1995). Cultures originating from different plots are referred to as subpopulations in the text. These were maintained in separate Petri dishes (diameter 9 cm; height 1 cm) that were half-filled

with plaster-charcoal substrate (8:1 w/w). The plaster-charcoal was kept moist with tap water. Springtails were fed *ad libitum* with yeast granules and kept in a walk-in environmental chamber at a constant temperature of 15 °C and a 12L:12D photoperiod for approximately 4–8 generations before the start of the experiments. The species identity was first determined according to Fjellberg (1998) and later confirmed by examining the COI barcode region; detailed description of DNA isolation, PCR, and sequencing is provided in the online Appendix. Only a subset of the soil samples contained enough *P. pseudovanderdrifti* to establish laboratory cultures and not all of these subpopulations persisted or proliferated. In the end, we had a limited number of subpopulations to include in the present study.

2.2. Temperature-dependent development

The experiment was carried out in the autumn of 2016. Eggs were removed from laboratory cultures with a moist small paintbrush and placed in separate Petri dishes where they were monitored daily. On the day of hatching, each juvenile was in the same manner transferred into one of 24 wells of a Nunclon™ multidish Petri dish (Nunc A/S, Roskilde, Denmark) with a layer of moistened plaster-charcoal substrate on the bottom and supplied with a small amount of dried baker's yeast and flake fish food. To avoid pseudoreplication issues, multidishes harbored randomly picked juveniles from different habitats and plots. Newly hatched, individually kept juveniles in multidishes were apportioned among four experimental temperatures (10, 15, 20, and 25 °C) in walk-in climate rooms. The actual temperature in the climate rooms was logged at 10 min intervals using TinyTag temperature data loggers accurate to ± 0.1 °C (Gemini Data loggers, Chichester, UK), and placed near the multidishes. The multidishes were checked daily in order to maintain high moisture, remove excessive mold from the food, and record juvenile molts. Due to time limits, we were only able to track springtail development until the second molt. The amount of food given at the beginning of the experiment was usually sufficient for a single juvenile to develop over the studied period; otherwise, food was replenished as soon as necessary. Developmental rate was calculated for each individual as the inverse combined duration of the two first instars (days^{-1}).

2.3. Temperature-dependent locomotion

The path length traveled by adult *P. pseudovanderdrifti* was recorded in five climate-controlled rooms with temperature set to 5, 10, 16, 22, and 27 °C, respectively. The actual temperature of the Petri dish on which the walking speed was measured was logged at 1 min intervals using TinyTag temperature data loggers accurate to ± 0.1 °C (Gemini Data loggers, Chichester, UK). Groups of 7–14 individuals (three groups per experimental temperature) were placed in a Petri dish (diameter 5.4 cm) with a water-saturated plaster of Paris bottom. Locomotory activity of each individual animal was then tracked during 60 s using a Pentax TV lens (model C1614-M) mounted on an Imagine Source DMK 23U274 interface (Chennai, India) coupled to a computer. Each recorded video sequence was analyzed using BIORAS software (BIORAS, Kvistgaard, Denmark) that provided the total path length travelled by each individual, mean and maximum walking speed during the measuring sequence, and the length of each individual, which allowed calculation of size-normalized walking speed.

2.4. Cold tolerance

Acute cold shock tolerance of *P. pseudovanderdrifti* was defined as survival rate following exposure to five different temperatures (–6, –7, –8, –9 and –10 °C). Springtails were transferred with the aid of an aspirator to 2 mL Eppendorf vials (approx. 15 specimens in each vial and five replicates at each temperature) and then exposed to a cold shock temperature for 2 h in a temperature-controlled bath with a

precision of ± 0.05 °C (Lauda Eco RE 1050, VWR Bie and Berntsen A/S, Herlev, Denmark). After the cold shock treatment, springtails were allowed to recover on moistened plaster of Paris/charcoal Petri dishes at 20 ± 1 °C for 24 h before their survival was assessed as the ability to walk with the same speed and coordination as control animals when gently stimulated with a fine paint brush.

2.5. Statistical analyses

Statistical analyses were carried out in R version 3.5.2 with RStudio (RStudio Team, 2016; R Core Team, 2018). In all analyses, experimental temperature was treated as a continuous independent variable. Variation across the warming levels in developmental rate, locomotory activity, and cold tolerance was first analyzed using linear and generalized linear mixed models implemented in R packages nlme (Pinheiro et al., 2018) and lme4 (Bates et al., 2015). Warming level replicates were treated as random intercepts and were allowed to have different slopes. Maximum likelihood models with and without random effects were compared based on AIC difference.

Temperature-dependent rates of development and locomotion were heteroscedastic and were thus analyzed using nlme as this package allowed to specify a different variance per temperature level. When the generalized least-squares (GLS) method was used, models were fitted by restricted maximum likelihood and significance of the effects was determined with *F*-tests based on type I (sequential) sum of squares. Model assumptions of homoscedasticity, linearity, and normality of residuals were verified by inspection of raw and standardized residuals plots. In addition, the responses of developmental rate and walking speed to experimental temperature were approximated by means of linear regression equations. Generalized least squares models were re-run for each subpopulation with experimental temperature as the single explanatory variable and the slopes of these reaction norms were calculated with their standard errors. Cold tolerance data, where survivors were encoded as 1 and perished individuals as 0, were first analyzed using the glmer() function in lme4 with a logit link and binomial error structure. The effect of habitat (forest vs. grassland) on the traits studied was tested similarly, using an appropriate mixed model where the random-intercepts structure consisted of subpopulations nested within warming levels. Negative temperature resulting in 50% mortality (LT50) was calculated separately for each subpopulation from a logistic regression fitted to the binomial data.

3. Results

3.1. Temperature dynamics and gradients in the habitats

Regardless of geothermal perturbation, the forest soil was cooler than the grassland soil and was characterized by dampened temperature fluctuations (Fig. 1). Mean daily temperature range was 0.5 and 0.7 °C in the two habitats, respectively. The extreme (minimum and maximum) values recorded during the study period spanned a range of 13.4 °C in the forest and 16.5 °C in the grassland soil. Geothermal warming somewhat increased the magnitude of fluctuations, but its overall effect was additive and the entire temperature profile shifted up (Fig. 1). In the forest, five unwarmed replicates (plot A) had a mean annual soil temperature (\pm SD) of 6.0 ± 0.15 °C. Using this temperature as a reference, plots B, D, and E had corresponding mean warming levels of +0.9, +3.0, and +6.5 °C (data from the plot E were subsequently discarded, see below). In the grassland, mean (\pm SD) unwarmed soil temperature across five replicates was 7.0 ± 0.75 °C, while the plots B, D, and E were on average +0.3, +2.9, and +5.2 °C warmer, respectively. For convenience, hereafter all experimental subpopulations of *P. pseudovanderdrifti* are referred to by mean warming levels they had experienced in the corresponding plots.

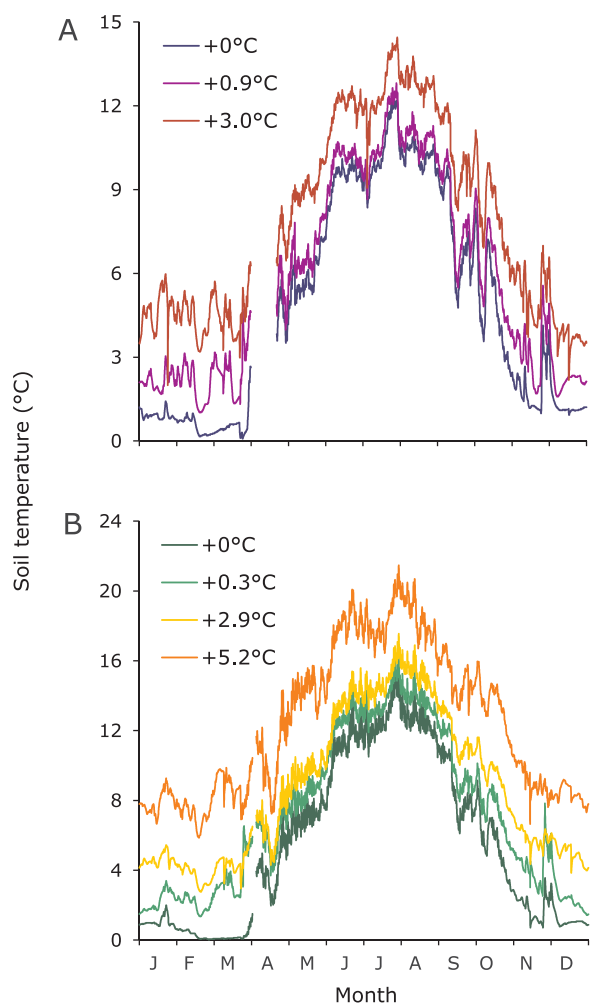


Fig. 1. Annual temperature cycles in the geothermally warmed (A) forest and (B) grassland habitats of *P. pseudovanderdrifti*. Hourly data were collected from 8-May-2013 to 11-Sep-2014, temperatures for overlapping dates averaged. Gaps in the graphs are due to missing data. Temperature values in the legend refer to mean warming levels relative to the ambient conditions.

3.2. Temperature-dependent juvenile development

Of the 12 subpopulations that gave rise to stable laboratory cultures, one, according to DNA barcoding results, turned out to be a different species, *P. cancellata* (Gisin, 1956). Unfortunately, that was the only available subpopulation from the warmest plot in the forest (+6.5 °C). Also, only eight out of the remaining 11 subpopulations had produced a sufficient number of eggs by the beginning of the experiment. Thus, our data on temperature-dependent juvenile development were limited to five forest subpopulations (two replicates for +0, one for +0.9, and two for +3.0 °C) and three from the grassland (+0, +2.9, and +5.2 °C). Juvenile survival rates during each of the studied instars ranged from 92 to 100% with no consistent differences across experimental temperature regimens and subpopulations (Table 1).

For the forest subpopulations, a fixed-effects model was the least informative ($\Delta\text{AIC} = 18$) but a model with random slopes and intercepts turned out to be overfitted; thus, a random-intercepts model was chosen. There was no random effects structure in the grassland data, and so a general least-squares model was fitted. As expected for an ectothermic organism, juvenile development of *P. pseudovanderdrifti* was strongly influenced by rearing temperature (Table 1; Fig. 1A and B): log-likelihood ratio test $\chi^2 = 853.3$, $P < 0.0001$ (forest), $F_{1,376} = 5744$, $P < 0.0001$ (grassland). Developmental rates in forest springtails were not affected by either warming level ($\chi^2 = 0.9$, $P =$

0.4) or its interaction with rearing temperature ($\chi^2 = 3.5$, $P = 0.06$). Similarly, the effect of warming level on juvenile development was non-significant in grassland springtails ($F_{1, 376} = 0.04$, $P = 0.8$), but the interaction was significant ($F_{1, 376} = 23.2$, $P < 0.0001$). The linear mixed model showed a non-significant effect of habitat (forest vs. grassland) on developmental rate (log-likelihood ratio test $\chi^2 = 1.6$, $P = 0.2$) but a significant habitat by rearing temperature interaction ($\chi^2 = 5.5$, $P = 0.02$). In terms of reaction norms, these two significant interactions meant that the slopes of the rate-temperature relationship were significantly steeper in warmed than in intact grassland subpopulations and overall steeper in the subpopulations from the forest than in those from the grassland (Fig. 3A).

3.3. Temperature-dependent locomotion

Eleven subpopulations (five from the forest and six from the grassland) were used in the experiment. The results of analyses were essentially the same for mean and maximum walking speed, and body length normalization did not affect the conclusions either. Thus, only data for mean walking speed are presented. Fixed-effects models had a substantially poorer support: $\Delta\text{AIC} = 18$ for the forest and $\Delta\text{AIC} = 32$ for the grassland subpopulations, relative to the models with random intercepts and slopes. Experimental temperature had a significant effect on adult walking speed (forest: log-likelihood ratio test $\chi^2 = 15.0$, $P = 0.0001$; grassland: $\chi^2 = 18.3$, $P < 0.0001$). By contrast, warming level had no effect on walking speed (forest: $\chi^2 = 0.8$, $P = 0.4$; grassland: $\chi^2 = 0.7$, $P = 0.4$) and neither did its interaction with experimental temperature (forest: $\chi^2 = 0.3$, $P = 0.6$; grassland: $\chi^2 = 0.1$, $P = 0.7$). The main effect of habitat was significant (forest vs. grassland: $\chi^2 = 8.5$, $P = 0.004$), and so was the interaction of habitat with experimental temperature ($\chi^2 = 6.2$, $P = 0.01$). In general, forest springtails moved faster than their grassland counterparts and their walking speed increased somewhat steeper with experimental temperature (Figs. 2C, D and 3 B).

3.4. Cold tolerance

The same eleven subpopulations used in the locomotion experiments were also tested for tolerance of acute cold shock. Warming level replicate was retained in the models as random intercept because its exclusion led to high ΔAIC values (> 5). Although there seemed to be considerable variation in response slopes within warming levels as well (Fig. 4), these introduced overfitting, and so the random-slopes term was dropped. Survival of forest springtails significantly depended on the experimental temperature (log-likelihood ratio test $\chi^2 = 463.1$, $P < 0.0001$) but the warming level ($\chi^2 = 0.01$, $P = 0.9$) and its interaction with experimental temperature ($\chi^2 = 0.3$, $P = 0.6$) had no effect. In springtails from the grassland, survival was also significantly influenced by temperature ($\chi^2 = 371.8$, $P < 0.0001$) but not by warming level or its interaction with temperature ($\chi^2 = 0.4$, $P > 0.5$ in both cases). However, survival at negative temperatures was significantly different between springtails originating from the two habitats ($\chi^2 = 14.6$, $P = 0.0001$), and the habitat by temperature interaction was significant as well ($\chi^2 = 8.6$, $P = 0.003$). Although LT50 values (Fig. 4) were very similar in all subpopulations, averaging at -8.9 °C, forest *P. pseudovanderdrifti* showed a less abrupt fall in survivorship between -8 and -10 °C than springtails from the grassland did (Fig. 4).

4. Discussion

Newly emerged gradients of soil temperature in geothermal areas are convenient models for studying the possibility of rapid adaptation to warming (O'Gorman et al., 2015). At the same time, the serendipitous nature of these unique systems, such as those arising in the aftermath of an earthquake, prevents proper replication of study sites,

Table 1Common -garden temperature-dependent development and survival in subpopulations of *P. pseudovanderdrifti* originating from two habitats with natural thermal gradient.

Warming level, °C	Experimental temperature, °C	1st instar duration, d (mean ± SD)	Final N	Survival rate, %	2nd instar duration, d (mean ± SD)	Final N	Survival rate, %
Forest habitat							
+0	9.6	14.0 ± 1.24	28	93.3	15.5 ± 2.93	28	100
	14.6	8.4 ± 1.11	42	97.7	9.5 ± 1.63	41	97.7
	20.0	5.5 ± 0.60	31	96.9	6.7 ± 1.32	30	96.8
+0	25.0	4.4 ± 0.67	35	100	5.3 ± 0.85	33	94.3
	9.6	16.1 ± 2.22	34	97.1	15.5 ± 2.67	34	100
	14.6	9.4 ± 1.16	39	100	9.3 ± 1.56	39	100
+0.9	20.0	6.5 ± 0.80	39	100	6.7 ± 1.30	39	100
	25.0	5.2 ± 0.72	54	96.8	5.8 ± 1.10	51	94.6
	9.6	15.2 ± 1.72	30	94.1	15.8 ± 2.62	30	100
+3.0	14.6	9.4 ± 1.16	26	100	9.8 ± 1.32	25	96.2
	20.0	6.1 ± 0.88	30	97.2	6.9 ± 0.87	30	100
	25.0	5.5 ± 0.62	36	100	5.4 ± 1.39	35	97.3
+3.0	9.6	15.5 ± 1.72	37	97.4	15.8 ± 2.33	37	100
	14.6	9.2 ± 1.05	34	97.4	8.6 ± 1.65	33	97.2
	20.0	6.7 ± 0.72	38	100	6.9 ± 1.38	38	100
+3.0	25.0	5.2 ± 0.71	29	100	5.4 ± 1.26	29	100
	9.6	15.3 ± 1.62	36	97.3	15.7 ± 2.80	36	100
	14.6	8.9 ± 0.79	38	100	9.6 ± 1.25	37	97.4
+3.0	20.0	6.4 ± 0.70	31	100	7.1 ± 1.30	30	96.7
	25.0	5.4 ± 0.71	25	93.3	5.4 ± 1.54	23	92.3
	Grassland habitat						
+0	9.6	15.4 ± 1.75	35	100	15.6 ± 2.32	35	100
	14.6	9.1 ± 1.02	30	100	9.2 ± 1.15	29	96.7
	20.0	6.7 ± 0.87	28	96.8	7.3 ± 1.09	28	100
+2.9	25.0	5.5 ± 0.65	35	100	6.1 ± 0.81	34	97.4
	9.6	15.6 ± 1.93	37	97.4	15.5 ± 2.16	37	100
	14.6	9.0 ± 1.13	33	100	8.8 ± 1.50	33	100
+5.2	20.0	6.5 ± 0.62	28	100	6.9 ± 0.95	28	100
	25.0	5.5 ± 0.78	31	100	5.5 ± 1.57	31	100
	9.6	16.7 ± 1.81	32	100	15.2 ± 2.33	32	100
+5.2	14.6	9.6 ± 1.08	32	100	8.8 ± 1.52	30	93.8
	20.0	6.5 ± 0.98	29	97.0	6.8 ± 1.14	29	100
	25.0	5.3 ± 0.89	34	97.5	5.9 ± 1.46	32	94.7

and caution is warranted when discussing the generality of the observed biological responses to these local and rapid environmental changes.

In this study, we tested whether the springtail *P. pseudovanderdrifti* shows hereditary phenotypic differences along a more than half-century-old temperature gradient in a grassland and a newly emerged temperature gradient in a spruce forest. In particular, we measured temperature-dependent juvenile development and locomotion as well as cold tolerance in springtails originating from different parts of these two gradients under a common-garden experimental design.

There are two principal findings of our study. First, the traits measured show almost no sign of local adaptation to geothermal warming: within each habitat, there are no significant differences across subpopulations except for significantly steeper reaction norms for juvenile developmental rate in springtails from the warmed grassland plots (Figs. 2B and 3 A). Second, springtails from the forest habitat have, on average, steeper reaction norms for temperature-dependent development and walking speed as compared with their grassland counterparts (Fig. 3) but, in contrast, their survival at negative temperatures declines less steeply (Fig. 4), which could indicate a higher genetic variability for cold shock tolerance. This is yet another evidence that arthropods often have the flexibility to cope with varying environmental conditions (e.g., Gilbert, 1988).

Subpopulations of *P. pseudovanderdrifti* that inhabit warmed parts of the grassland develop slightly faster at high temperatures than their conspecifics from intact (unwarmed) plots (Fig. 2B). Although the difference is small, it is remarkably similar, both in character and degree, to latitudinal and altitudinal variation shown by other insect species over hundreds of kilometers (Bateman, 1967; Fujiyama and Harada, 1996; Liefing et al., 2009; Kutcherov et al., 2015), including the soil-

dwelling springtail *Folsomia candida* (Stam et al., 1996). A taxonomically and conceptually distant but essentially similar example has recently come from phytoplankton studies: modern-day dinoflagellate strains, which experience seawater warming, show slightly faster growth rates, also at higher temperatures only, than strains revived from century-old sediments (Hinners et al., 2017). In all these cases, organisms from warmer thermal environments develop and/or grow slightly faster at higher temperatures relative to their colder-climate counterparts and reaction norms intersect around the lowest used experimental temperatures. However, while this intraspecific pattern recurs ubiquitously, it is by no means universal (Stam, 1997; Kipyatkov and Lopatina, 2002; Walther et al., 2010; Lopatina et al., 2012; Sengupta et al., 2016, 2017).

Differences in developmental rate along the grassland gradient are very small, but so they are in all of the cited cases in the previous paragraph. Repeatability of the same pattern of interpopulation variation in different species argues against a game of chance or experimental artifact. Presumably, natural selection in all these cases, and in *P. pseudovanderdrifti* from the grassland, too, has acted not only and not so much upon the rate of growth or development per se as on other temperature-dependent biological rates. So, the resultant slight relative change in growth or developmental rate at high temperatures may be a byproduct of more profound thermal adaptation of general biochemical processes or correlated life-history traits (Liefing et al., 2015). The absence of clinal phenotypic variation in the recently warmed forest subpopulations of *P. pseudovanderdrifti* may indicate that several years of warming are insufficient for such evolution to produce a detectable result. However, it is not possible under our experimental design to separate the factors of time and habitat.

Given the small distances between study plots, it may seem

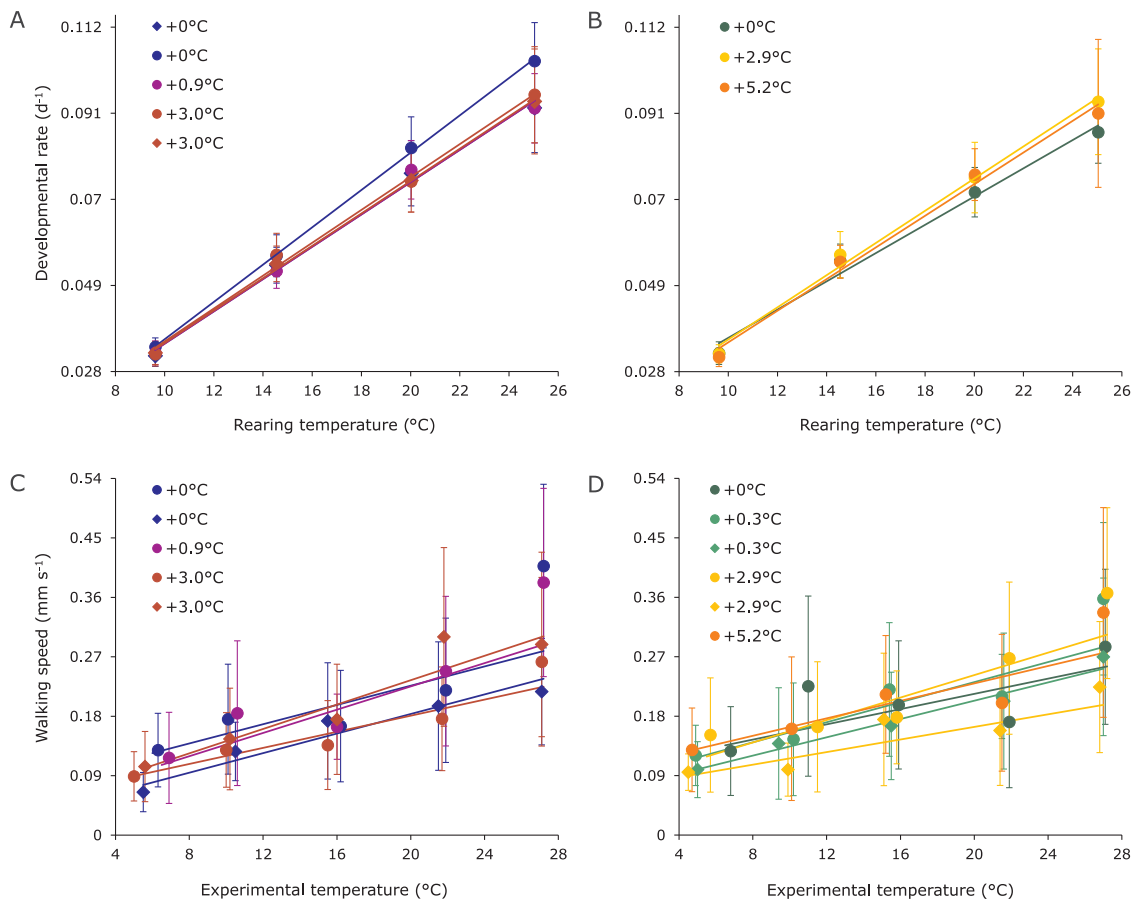


Fig. 2. Common-garden (A, B) juvenile developmental rates during the 1st + 2nd instar and (C, D) adult walking speed in *P. pseudovanderdrifti* originating from the natural geothermal gradients (A, C) in the forest and (B, D) in the grassland. Temperature values in the legend refer to mean warming levels at sampling plots relative to the ambient conditions. Symbols with vertical bars refer to means \pm SDs and are shown for illustration purposes. Regression lines are plotted using the generalized least squares method.

reasonable that gene flow along the geothermal gradients might have hampered local adaptation. Unfortunately, COI barcodes lack resolution to describe the genetic structure of the subpopulations studied. However, active dispersal in soil-dwelling species such as *P. pseudovanderdrifti* occurs on a scale as low as few decimeters annually (Bengtsson et al., 1994). Since the plots from which laboratory cultures originated were 5–10 m apart (Sigurdsson et al., 2016), active migration has likely not been of importance for the gene exchange between the various subpopulations that we used for our studies.

A more serious limitation of our study is that we may have used a species that was likely a thermal generalist resistant to climate change, since it was abundant in both habitats and along both natural temperature gradients. Consistent with these characteristics, *P. pseudovanderdrifti* shows a nearly 100% survival rate in the laboratory conditions between 10 and 25 °C (Table 1), tolerates occasional heating to 30 °C in the field (Holmstrup et al., 2018), and withstands very low temperatures for long periods (Holmstrup, 2018). Nevertheless, an earlier field survey (Holmstrup et al., 2018; M. Holmstrup unpublished) has shown that warming clearly reduced the abundance of this species in the field suggesting that local populations were under selective pressure at elevated temperatures. Still, springtail species with narrower thermal tolerance ranges might be even more sensitive to warming (Janion-Scheepers et al., 2018). It may also be argued that culturing *P. pseudovanderdrifti* under laboratory conditions could have led to genetic changes in their temperature responses. This is a global problem in common garden studies, however, a recent study by MacLean et al. (2018) shows that arthropod thermal phenotypes generally persist for many generations. We reared our springtail cultures under

favorable, non-stressful conditions, and believe that no significant genotypic changes could accumulate over as few as 4–8 generations.

We only took into account three selected traits, which are among the most frequently studied components of the organism's thermal phenotype, and measured development time for the first two instars only. It is possible that clinal differences may accrue during later instars and/or may be discovered in other traits such as growth rate. Perhaps a completely different experimental approach, such as measuring responses to artificial selection in different thermal environments (Kingsolver et al., 2007), would be a more powerful tool to explore thermal adaptation and to separate genetic from environmental effects.

The near absence of clinal variation along the natural warming gradients is in stark contrast to significant variation found between warming level replicates and between the two habitats (Figs. 2–4). Even within the same level of warming, subpopulations differ in temperature-dependent development and locomotion and in acute cold shock tolerance, and this variation is comparable in magnitude to that seen along the whole temperature gradient. Thus, there exists material for evolutionary change (i.e., genetic variation) in this species and thermal reaction norms seem to evolve, even though not in response to mean soil temperature. In fact, forest and grassland springtails significantly differ from each other in all the three traits studied. These differences between habitats are observed not only in the mean trait values but also in the degree of thermal plasticity and have arisen relatively quickly: according to a personal communication cited in Sigurdsson et al. (2016), the forest was planted in 1966–1967. From a historical perspective, the emergence of a forest seems to have exerted a stronger effect on the thermal phenotype of *P. pseudovanderdrifti* than the

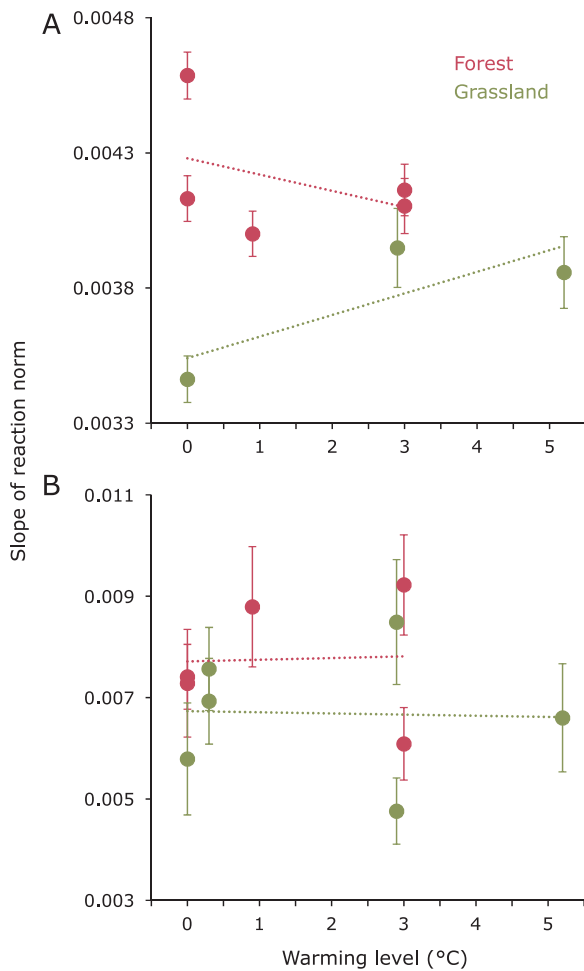


Fig. 3. Thermal plasticity of *P. pseudovanderdrifti* originating from forest and grassland geothermal gradients. Degree of plasticity is expressed as the slope of the reaction norm for a given temperature-dependent trait: juvenile developmental rate (A) and adult walking speed (B). Vertical bars denote SE values of the slope.

emergence of a geothermal gradient. In the case of developmental rate, the patterns of within- and between-habitat variation are even conflicting: although the slope of reaction norms increases with environmental temperature in the grassland, it is still higher in the forest, where the soil is in fact cooler (Fig. 3A).

The spruce forest, besides being cooler than the grassland, massively buffers seasonal and diel variation of temperature (Fig. 1). Such differences in surface and soil temperature variation between dense coniferous forests, sparser forests, and treeless habitats are well known (Oliver et al., 1987). There is a body of theory regarding possible evolutionary consequences of temporal heterogeneity for the degree of plasticity (thoroughly reviewed in Angilletta, 2009), although these models mostly deal with maximum performance and thermal limits and not the slope of reaction norms.

In principle, within-generation temporal heterogeneity in temperature should select for canalization of adaptive traits and thus for flatter reaction norms (Liefting et al., 2009). Indeed, grassland subpopulations of *P. pseudovanderdrifti* have flatter thermal reaction norms for development and walking speed than forest subpopulations. This pattern agrees with previous findings for the surface-dwelling springtail *Orchesella cincta* whose growth rates are also more temperature-sensitive (i.e., reaction norms are steeper) in the more stable forest than in the more variable heathland (Liefting and Ellers, 2008). Contrary to the theoretical expectation, thermal reaction norms often have shallower slopes in populations from less variable climates (Hassall et al., 2005;

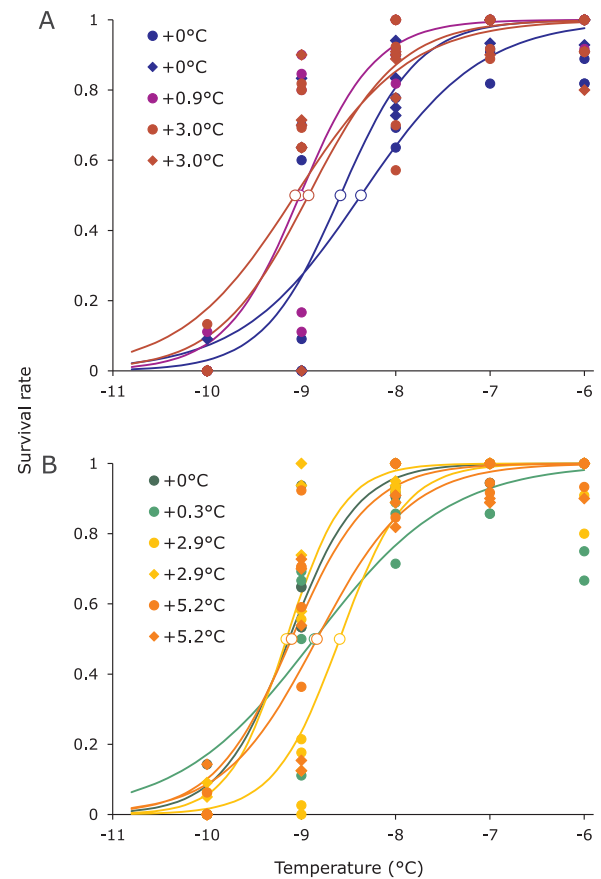


Fig. 4. Survival rates of adult *P. pseudovanderdrifti* originating from (A) forest and (B) grassland geothermal gradients after 2-h exposure to subzero temperatures. Temperature values in the legend refer to mean warming levels at sampling plots relative to the ambient conditions. Survivorship data (filled symbols) are approximated with maximum likelihood logistic regression curves; hollow symbols denote calculated lethal temperatures that result in 50% mortality (LT50).

Molina-Montenegro and Naya, 2012; Gaitán-Espitia et al., 2014; Kosmala et al., 2018). In the soil-dwelling springtail *Folsomia quadrioculata* studied from High Arctic to Denmark, forest populations exhibit lower thermal plasticity of juvenile development time than populations from the more heterogeneous grass field and tundra (Sengupta et al., 2017). The problem is further complicated when additional traits are taken into account, as these may have opposite patterns of thermal plasticity (Liefting et al., 2009; Sengupta et al., 2017).

Of course, forests differ from grasslands in a multitude of aspects, only one of which is the degree of thermal heterogeneity. Our conclusions regarding the between-habitat differences are by necessity tentative because the experiment mostly focused on the clinal variation within each habitat and was designed accordingly. Ideally, plots representing combinations of thermal conditions and habitat type should be replicated and randomly distributed in the area, but this is rarely possible in natural settings for logistic and economic reasons, although examples do exist (Schindler, 1974). Suffice to say, organismal thermal plasticity does evolve even at fine spatial scales, but the intricate effects of macro- and microclimate, as well as other possible factors and – last but not least – genetic drift (Crispo et al., 2010) are difficult to disentangle, especially when the differences are minuscule. Given this uncertainty, prediction of the future pathways of phenotypic evolution driven by climate change seems to be an extremely challenging task. While mean temperature is the most significant factor determining performance in ectotherms, thermal heterogeneity and the plethora of other ecological factors are also important, and so habitat type may

drive the evolution of thermal phenotypes to an extent comparable with the effect of environmental temperature.

Declaration of Competing Interest

None. The funding sources had no involvement in the study design, data analysis, and interpretation of results.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.pedobi.2019.150606>.

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