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1 **Temperature responses in a subarctic springtail from two geothermally**  
2 **warmed habitats**

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



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48 **Key words:** development, geothermal habitat, locomotion, reaction norm, plasticity, thermal  
49 adaptation

50

## 51 **1. Introduction**

52 There is an overwhelming array of documented biotic responses to global climate change, and  
53 yet relatively few studies have been designed to test whether the observed response is evolved  
54 or based on pre-existing plasticity and still fewer did find evolutionary modifications  
55 (Bradshaw and Holzapfel 2008; Franks et al. 2014; Van Petegem et al. 2016; Ramakers et al.  
56 2019). Most climate-driven changes in living organisms seem to have occurred due to  
57 phenotypic plasticity, i.e., represent physiological, ecological, behavioral and other direct  
58 responses to environmental change (Gienapp et al. 2008).

59 Some manifestations of phenotypic plasticity are non-adaptive in the sense that these do not  
60 arise directly from natural selection. For example, the organism's constitutional compounds  
61 and biochemical machinery are temperature-sensitive by their physical nature (Schlichting  
62 and Pigliucci 1998; de Jong and van der Have 2009). More subtle differences in temperature  
63 responses, including magnitude (i.e., lower and upper thresholds) and degree of plasticity  
64 (i.e., slope of the reaction norm), often represent adaptations to particular thermal  
65 environments (Schlichting and Pigliucci 1998; Angilletta 2009). Adaptive plasticity mirrors  
66 environmental heterogeneity experienced by previous generations in the evolutionary past and  
67 thereby may help to mitigate, at least partly, the effects of future climatic transformation  
68 (Nussey et al. 2005; Chown et al. 2007; Gienapp et al. 2008). However, plasticity may as well  
69 become maladaptive in a novel environment where conditions fall outside the historically  
70 experienced range: in such a case, selection would favor a shallower reaction norm  
71 (Ghalambor et al. 2007).



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

81 A workaround approach, based on ‘space-for-time substitution’ (Franks et al. 2014), enables  
82 one to infer future effects of climatic change from spatial patterns of trait variation. For  
83 example, populations and communities can be compared in situ along an experimentally  
84 manipulated (Hodkinson et al. 1998; Holmstrup et al. 2017) or naturally occurring  
85 (O’Gorman et al. 2015) environmental gradient. However, as hereditary and plastic effects are  
86 indistinguishable in the field, detecting an evolutionary change in such cases requires  
87 bioinformatic scrutiny (Bataillon et al. 2016). Alternatively, various ecophysiological traits  
88 (rates of growth and locomotion, critical temperatures, etc.) can be measured under  
89 controlled, common-garden conditions in populations originating from cool and warm  
90 habitats (Walther et al. 2010; Gaitán-Espitia et al. 2014; Van et al. 2014; Van Petegem et al.  
91 2016). The results, however, are often disparate and likely depend, among other confounding  
92 factors, on the biology of the species and on the trait studied. In some cases, there is no  
93 latitudinal cline in spite of significant inter-population trait variation across the studied sites  
94 (Miles, 1994; Liefting et al. 2009; Gardner et al. 2011; Sengupta et al. 2016, 2017),  
95 suggesting the presence of biogeographic or fine-scale environmental differences, as well as



96 possibly indicating a high degree of genetic drift. Furthermore, temperature per se may not be  
97 as important as its seasonal variation: in fact, studies conducted at several experimental  
98 regimens often show that the degree of trait plasticity is greater in more variable climates  
99 (Gaitán-Espitia et al. 2014; Kosmala et al. 2018).

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

106 In this study, we exploit local-scale habitat warming to test for associated changes in the  
107 thermal phenotype of a soil-dwelling arthropod. In 2008, an earthquake in Iceland dislocated  
108 hot groundwater in the bedrock to a previously non-geothermal area. As a result, both mean  
109 and maximum temperatures in the soil increased without a substantial change in key soil  
110 chemical parameters (Sigurdsson et al. 2016). About 2.5 km to the northwest of the impact  
111 site, there is another geothermal hot spot that has been known at least since the early 1960s  
112 (O’Gorman et al. 2015). Both localities represent a unique natural experiment with soil  
113 warming and so have become important field test sites for climate change research under the  
114 ForHot project ([www.forhot.is](http://www.forhot.is)). One of the biodiversity surveys, which focused on the  
115 springtail community (Holmstrup et al. 2018), revealed a soil-dwelling species that was  
116 abundant along the geothermal gradients. As this springtail seemed to have successfully  
117 coped with local warming, we carried out common-garden experiments with individuals  
118 originating from unwarmed and warmed plots in order to detect clinal variation in cold  
119 tolerance and in thermal reaction norms for development and walking speed. We expected to



120 find differences in both the trait values and their plasticity along the natural thermal gradient,  
121 especially in the grassland subpopulations that had experienced warming for a longer time.  
122 The presence of such clinal variation in cold tolerance, development, and/or locomotion  
123 would indicate that thermal phenotypes of soil arthropods can rapidly evolve in response to  
124 warming.

125

## 126 2. Materials and methods

### 127 2.1. Collection sites

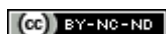
128 The springtail *Protaphorura pseudovanderdrifti* (Gisin, 1957) (Collembola: Onychiuridae)  
129 was collected in September 2014 at two ForHot sites in southern Iceland (64.0°N, 21.2°W),  
130 hereinafter referred to as habitats. The first habitat was a planted Sitka spruce (*Picea*  
131 *sitchensis*) forest that had been warmed since a large earthquake in 2008, and the second  
132 habitat was an unmanaged *Agrostis capillaris* dominated grassland 2.5 km away that had been  
133 warmed geothermally for at least half a century. Each habitat was divided into plots labeled  
134 from A to E in the order of increasing soil temperature (approximately +0, 1, 2, 3, and 5 °C  
135 warming; see below). In the grassland, the plots measured 2×2 m, whereas those in the forest  
136 were set up **in between trees and had a size of 1×1 m due to less space being available**. A  
137 detailed description of both habitats, denoted respectively FN (forest, new warming) and GO  
138 (grassland, old warming), as well as of the plots within them, is provided by Sigurdsson et al.  
139 (2016), **including a map of the area**. Soil temperature in the plots was recorded with HOBO  
140 TidbiT v2 Water Temperature Data Loggers (Onset Computer Corporation, USA) on an  
141 hourly basis at 10 cm depth from 8-May-2013 until sampling of springtails on 11-Sep-2014.  
142 **Vertical soil temperature profiles of the plots reported in Sigurdsson et al. (2016) shows that**  
143 **temperature differences between 5 and 10 cm depth are small**. Based on the plot-specific



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144 temperature data we calculated overall mean temperature (mean of all hourly measurements  
145 during the period), absolute maximum, absolute minimum, overall temperature amplitude and  
146 mean diurnal temperature amplitude. Distance between plots was at least 10 m. Samples were  
147 only taken from plots A, B, D, and E, with five replicates per plot. Distance between  
148 replicates varied from several meters to several hundreds of meters and was generally greater  
149 than that between plots with different warming levels.

150 Soil cores (diameter 6 cm; depth 5 cm), **one per plot**, were sampled in order to isolate  
151 individual species and establish laboratory cultures for experiments. Springtails were  
152 extracted over one week in a high gradient extraction apparatus (MacFadyen type) where the  
153 temperature in the upper compartment increased stepwise from 25 to 50 °C over seven days  
154 while the temperature in the lower compartment remained constant at 5 °C. Springtails were  
155 collected in beakers with a bottom covered with a layer of moistened plaster of Paris and  
156 subsequently sorted. **The extraction efficiency of the high-gradient extraction method used in  
157 our laboratory has been thoroughly tested and always shows a better than 90% extraction  
158 efficiency of various springtail species including *Protaphorura spp.* (Petersen 1978; Sjørnsen  
159 and Holmstrup 2004; Holmstrup and Krogh 2001). The risk of a bias towards heat-tolerant  
160 specimens (or the opposite) used for establishment of laboratory cultures seems therefore  
161 unlikely.** Cultures of *P. pseudovanderdrifti* were established in the laboratory in Silkeborg,  
162 Denmark, using the same method as for other euedaphic springtails (Krogh 1995). Cultures  
163 originating from different plots are referred to as subpopulations in the text. These were  
164 maintained in separate Petri dishes (diameter 9 cm; height 1 cm) that were half-filled with  
165 plaster-charcoal substrate (8:1 w/w). The plaster-charcoal was kept moist with tap water.  
166 Springtails were fed *ad libitum* with yeast granules and kept in a walk-in environmental  
167 chamber at a constant temperature of 15 °C and a 12L:12D photoperiod for approximately 4–





168 8 generations before the start of the experiments. The species identity was first determined  
169 according to Fjellberg (1998) and later confirmed by examining the COI barcode region;  
170 detailed description of DNA isolation, PCR, and sequencing is provided in the online  
171 Appendix. *Only a subset of the soil samples contained enough *P. pseudovanderdrifti* to*  
172 *establish laboratory cultures and not all of these subpopulations persisted or proliferated. In*  
173 *the end, we had a limited number of sub-populations to include in the present study.*

174

## 175 *2.2. Temperature-dependent development*

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



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192 calculated for each individual as the inverse combined duration of the two first instars (days<sup>-1</sup>).  
193

194

### 195 2.3. Temperature-dependent locomotion

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

208

### 209 2.4. Cold tolerance

210 Acute cold shock tolerance of *P. pseudovanderdrifti* was defined as survival rate following  
211 exposure to five different temperatures (-6, -7, -8, -9 and -10°C). Springtails were transferred  
212 with the aid of an aspirator to 2 mL Eppendorf vials (approx. 15 specimens in each vial and  
213 five replicates at each temperature) and then exposed to a cold shock temperature for 2 hours  
214 in a temperature-controlled bath with a precision of  $\pm 0.05$ °C (Lauda Eco RE 1050, VWR Bie



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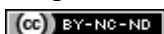
215 and Berntsen A/S, Herlev, Denmark). After the cold shock treatment, springtails were allowed  
216 to recover on moistened plaster of Paris/charcoal Petri dishes at  $20\pm 1^\circ\text{C}$  for 24 h before their  
217 survival was assessed as the ability to walk **with the same speed and coordination as control**  
218 **animals when gently stimulated with a fine paint brush.**

219

## 220 *2.5. Statistical analyses*

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

229 Temperature-dependent rates of development and locomotion were heteroscedastic and were  
230 thus analyzed using nlme as this package allowed to specify a different variance per  
231 temperature level. When the generalized least-squares (GLS) method was used, models were  
232 fitted by restricted maximum likelihood and significance of the effects was determined with  
233 *F*-tests based on type I (sequential) sum of squares. Model assumptions of homoscedasticity,  
234 linearity, and normality of residuals were verified by inspection of raw and standardized  
235 residuals plots. In addition, the responses of developmental rate and walking speed to  
236 experimental temperature were approximated by means of linear regression equations.  
237 Generalized least squares models were re-run for each subpopulation with experimental  
238 temperature as the single explanatory variable and the slopes of these reaction norms were



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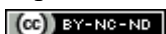
239 calculated with their standard errors. Cold tolerance data, where survivors were encoded as 1  
240 and perished individuals as 0, were first analyzed using the `glmer()` function in `lme4` with a  
241 logit link and binomial error structure. The effect of habitat (forest vs. grassland) on the traits  
242 studied was tested similarly, using an appropriate mixed model where the random-intercepts  
243 structure consisted of subpopulations nested within warming levels. Negative temperature  
244 resulting in 50% mortality (LT50) was calculated separately for each subpopulation from a  
245 logistic regression fitted to the binomial data.

246

### 247 **3. Results**

#### 248 *3.1. Temperature dynamics and gradients in the habitats*

249 Regardless of geothermal perturbation, the forest soil was cooler than the grassland soil and  
250 was characterized by dampened temperature fluctuations (Fig. 1). Mean daily temperature  
251 range was 0.5 and 0.7 °C in the two habitats, respectively. The extreme (minimum and  
252 maximum) values recorded during the study period spanned a range of 13.4 °C in the forest  
253 and 16.5 °C in the grassland soil. Geothermal warming somewhat increased the magnitude of  
254 fluctuations, but its overall effect was additive and the entire temperature profile shifted up  
255 (Fig. 1). In the forest, five unwarmed replicates (plot A) had a mean annual soil temperature  
256 ( $\pm$ SD) of  $6.0\pm 0.15$  °C. Using this temperature as a reference, plots B, D, and E had  
257 corresponding mean warming levels of +0.9, +3.0, and +6.5 °C (data from the plot E were  
258 subsequently discarded, see below). In the grassland, mean ( $\pm$ SD) unwarmed soil temperature  
259 across five replicates was  $7.0\pm 0.75$  °C, while the plots B, D, and E were on average +0.3,  
260 +2.9, and +5.2 °C warmer, respectively. For convenience, hereafter all experimental  
261 subpopulations of *P. pseudovanderdrifti* are referred to by mean warming levels they had  
262 experienced in the corresponding plots.



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263

### 264 3.2. Temperature-dependent juvenile development

265 Of the 12 subpopulations that gave rise to stable laboratory cultures, one, according to DNA  
266 barcoding results, turned out to be a different species, *P. cancellata* (Gisin, 1956).  
267 Unfortunately, that was the only available subpopulation from the warmest plot in the forest  
268 (+6.5 °C). Also, only eight out of the remaining 11 subpopulations had produced a sufficient  
269 number of eggs by the beginning of the experiment. Thus, our data on temperature-dependent  
270 juvenile development were limited to five forest subpopulations (two replicates for +0, one  
271 for +0.9, and two for +3.0 °C) and three from the grassland (+0, +2.9, and +5.2 °C). Juvenile  
272 survival rates during each of the studied instars ranged from 92 to 100% with no consistent  
273 differences **between** experimental temperatures and **between** subpopulations (Table).

274 For the forest subpopulations, a fixed-effects model was the least informative ( $\Delta\text{AIC} = 18$ )  
275 but a model with random slopes and intercepts turned out to be overfitted; thus, a random-  
276 intercepts model was chosen. There was no random effects structure in the grassland data, and  
277 so a general least-squares model was fitted. As expected for an ectothermic organism,  
278 juvenile development of *P. pseudovanderdrifti* was strongly influenced by rearing  
279 temperature (Table; Figs 1A and 1B): log-likelihood ratio test  $\chi^2 = 853.3$ ,  $P < 0.0001$  (forest),  
280  $F_{1,376} = 5744$ ,  $P < 0.0001$  (grassland). Developmental rates in forest springtails were not  
281 affected by either warming level ( $\chi^2 = 0.9$ ,  $P = 0.4$ ) or its interaction with rearing temperature  
282 ( $\chi^2 = 3.5$ ,  $P = 0.06$ ). Similarly, the effect of warming level on juvenile development was non-  
283 significant in grassland springtails ( $F_{1,376} = 0.04$ ,  $P = 0.8$ ), but the interaction was significant  
284 ( $F_{1,376} = 23.2$ ,  $P < 0.0001$ ). A linear mixed model showed a non-significant effect of habitat  
285 (forest vs. grassland) on developmental rate (log-likelihood ratio test  $\chi^2 = 1.6$ ,  $P = 0.2$ ) but a  
286 significant habitat by rearing temperature interaction ( $\chi^2 = 5.5$ ,  $P = 0.02$ ). In terms of reaction



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287 norms, these two significant interactions meant that the slopes of the rate-temperature  
288 relationship were significantly steeper in warmed than in intact grassland subpopulations and  
289 overall steeper in the subpopulations from the forest than in those from the grassland (Fig.  
290 3A).

291

### 292 3.3. Temperature-dependent locomotion

293 Eleven subpopulations (five from the forest and six from the grassland) were used in the  
294 experiment. The results of analyses were essentially the same for mean and maximum  
295 walking speed, and body length normalization did not affect the conclusions either. Thus,  
296 only data for mean walking speed are presented. Fixed-effects models had a substantially  
297 poorer support:  $\Delta\text{AIC} = 18$  for the forest and  $\Delta\text{AIC} = 32$  for the grassland subpopulations,  
298 relative to the models with random intercepts and slopes. Experimental temperature had a  
299 significant effect on adult walking speed (forest: log-likelihood ratio test  $\chi^2 = 15.0$ ,  $P =$   
300  $0.0001$ ; grassland:  $\chi^2 = 18.3$ ,  $P < 0.0001$ ). By contrast, warming level had no effect on  
301 walking speed (forest:  $\chi^2 = 0.8$ ,  $P = 0.4$ ; grassland:  $\chi^2 = 0.7$ ,  $P = 0.4$ ) and neither did its  
302 interaction with experimental temperature (forest:  $\chi^2 = 0.3$ ,  $P = 0.6$ ; grassland:  $\chi^2 = 0.1$ ,  $P =$   
303  $0.7$ ). The main effect of habitat was significant (forest vs. grassland:  $\chi^2 = 8.5$ ,  $P = 0.004$ ), and  
304 so was the interaction of habitat with experimental temperature ( $\chi^2 = 6.2$ ,  $P = 0.01$ ). In  
305 general, forest springtails moved faster than their grassland counterparts and their walking  
306 speed increased somewhat steeper with experimental temperature (Figs. 2C, D and 3B).

307

### 308 3.4. Cold tolerance



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309 The same eleven subpopulations used in the locomotion experiments were also tested for  
310 tolerance of acute cold shock. Warming level replicate was retained in the models as random  
311 intercept because its exclusion led to high  $\Delta$ AIC values ( $> 5$ ). Although there seemed to be  
312 considerable variation in response slopes within warming levels as well (Fig. 4), these  
313 introduced overfitting, and so the random-slopes term was dropped. Survival of forest  
314 springtails significantly depended on the experimental temperature (log-likelihood ratio test  $\chi^2$   
315 = 463.1,  $P < 0.0001$ ) but the warming level ( $\chi^2 = 0.01$ ,  $P = 0.9$ ) and its interaction with  
316 experimental temperature ( $\chi^2 = 0.3$ ,  $P = 0.6$ ) had no effect. In springtails from the grassland,  
317 survival was also significantly influenced by temperature ( $\chi^2 = 371.8$ ,  $P < 0.0001$ ) but not by  
318 warming level or its interaction with temperature ( $\chi^2 = 0.4$ ,  $P > 0.5$  in both cases). However,  
319 survival at negative temperatures was significantly different between springtails originating  
320 from the two habitats ( $\chi^2 = 14.6$ ,  $P = 0.0001$ ), and the habitat by temperature interaction was  
321 significant as well ( $\chi^2 = 8.6$ ,  $P = 0.003$ ). Although LT50 values (Fig. 4) were very similar in  
322 all subpopulations, averaging at  $-8.9^\circ\text{C}$ , forest *P. pseudovanderdrifti* showed a less abrupt fall  
323 in survivorship between  $-8$  and  $-10^\circ\text{C}$  than springtails from the grassland did (Fig. 4).

324

#### 325 **4. Discussion**

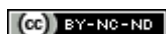
326 **Newly sprung** gradients of soil temperature in geothermal areas are convenient models for  
327 studying the possibility of rapid adaptation to warming. We tested whether the springtail *P.*  
328 *pseudovanderdrifti* shows hereditary phenotypic differences along a more than half-century-  
329 old temperature gradient in a grassland and a newly emerged temperature gradient in a spruce  
330 forest. In particular, we measured temperature-dependent juvenile development and  
331 locomotion as well as cold tolerance in springtails originating from different parts of these  
332 two gradients under a common-garden experimental design.



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333 There are two principal findings of our study. First, the traits measured show almost no sign  
334 of local adaptation to geothermal warming: within each habitat, there are no significant  
335 differences **between** subpopulations except for significantly steeper reaction norms for  
336 juvenile developmental rate in springtails from the warmed grassland plots (Figs. 2B and 3A).  
337 Second, springtails from the forest habitat have, on average, steeper reaction norms for  
338 temperature-dependent development and walking speed as compared with their grassland  
339 counterparts (Fig. 3) but, in contrast, their survival at negative temperatures declines less  
340 steeply (Fig. 4), which could indicate a higher genetic variability for cold shock tolerance.  
341 **This is not necessarily new, but shows that arthropods often have the flexibility to cope with**  
342 **varying environmental conditions (e.g. Gilbert, N., 1988. Control of fecundity in *Pieris rapae*.**  
343 **5. Comparisons between populations. *Journal of Animal Ecology*. 57, 395-410).**

344 Subpopulations of *P. pseudovanderdrifti* that inhabit warmed parts of the grassland develop  
345 slightly faster at high temperatures than their conspecifics from intact (unwarmed) plots (Fig.  
346 2B). Although the difference is small, it is remarkably similar to latitudinal and altitudinal  
347 variation shown by other insect species over hundreds of kilometers (Bateman 1967;  
348 Fujiyama and Harada 1996; Liefing et al. 2009; Kutcherov et al. 2015), including the soil-  
349 dwelling springtail *Folsomia candida* (Stam et al. 1996). A taxonomically and conceptually  
350 distant but essentially similar example has recently come from phytoplankton studies:  
351 modern-day dinoflagellate strains, which experience seawater warming, show slightly faster  
352 growth rates, also at higher temperatures only, than strains revived from century-old  
353 sediments (Hinners et al. 2017). In all these cases, organisms from warmer thermal  
354 environments develop and/or grow slightly faster at higher temperatures relative to their  
355 colder-climate counterparts and reaction norms intersect around the lowest used experimental  
356 temperatures. However, while this intraspecific pattern recurs ubiquitously, it is by no means





357 universal (Stam 1997; Kipyatkov and Lopatina 2002; Walther et al. 2010; Lopatina et al.  
358 2012; Sengupta et al. 2016, 2017).

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

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

379 A more serious limitation of our study is that we may have used a species that was likely a  
380 thermal generalist resistant to climate change, since it was abundant in both habitats and along



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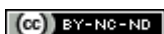
381 both natural temperature gradients. Consistent with these characteristics, *P.*  
382 *pseudovanderdrifti* shows a nearly 100% survival rate in the laboratory conditions between 10  
383 and 25 °C (Table), tolerates occasional heating to 30 °C in the field (Holmstrup et al. 2018),  
384 and withstands very low temperatures for long periods (Holmstrup 2018). We attempted to  
385 establish laboratory cultures of other abundant and generalist species (e.g. *Isotomiella minor*  
386 and *Parisotoma notabilis*), but without success. The choice of *P. pseudovanderdrifti* was  
387 therefore also made for practical reasons, but also because we saw from field surveys that  
388 warming was indeed reducing local populations of our focus species (Holmstrup et al. 2018;  
389 M. Holmstrup unpublished) suggesting that local populations were under selective pressure at  
390 elevated temperatures. It could be argued, however, that experimenting with a specialist  
391 species would have potentially forced the experiment to show a strong negative effect of  
392 warming as specialist species might be more sensitive to environmental change (Janion-  
393 Scheepers, C., Phillips, L., Sgrò, C.M., Duffy, G.A., Hallas, R., Chown, S.L., 2018. Basal  
394 resistance enhances warming tolerance of alien over indigenous species across latitude.  
395 Proceedings of the National Academy of Science USA. 115, 145-150). Further, it could also  
396 be argued that culturing *P. pseudovanderdrifti* for three generations under laboratory  
397 conditions would generate genetic changes with regard to thermal reaction norms. This is a  
398 global problem in common garden studies, however, a recent study by MacLean et al. (2018)  
399 shows that thermal reaction norms of arthropods often persist for many generations. We  
400 reared our springtail cultures under favorable, non-stressful conditions, we suggest that  
401 significant genotypic changes have not emerged after only three generations.

402 We only took into account three selected traits, which are among the most frequently studied  
403 components of the organism's thermal phenotype, and measured development time for the  
404 first two instars only. It is possible that clinal differences may accrue during later instars



405 and/or may be discovered in other traits such as growth rate. Perhaps a completely different  
406 experimental approach, such as measuring responses to artificial selection in different thermal  
407 environments (Kingsolver et al., 2007), would be a more powerful tool to explore thermal  
408 adaptation and to separate genetic from environmental effects.

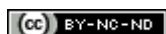
409 The near absence of clinal variation between subpopulations is in stark contrast to significant  
410 variation found between warming level replicates and between the two habitats (Figs. 2–4).  
411 Even within the same level of warming, subpopulations differ in temperature-dependent  
412 development and locomotion and in acute cold shock tolerance, and this variation is  
413 comparable in magnitude to that seen along the whole temperature gradient. Thus, there exists  
414 material for evolutionary change (i.e., genetic variation) in this species and thermal reaction  
415 norms seem to evolve, even though not in response to mean soil temperature. In fact, forest  
416 and grassland springtails significantly differ from each other in all the three traits studied.  
417 These differences between habitats are observed not only in the mean trait values but also in  
418 the degree of thermal plasticity and have arisen relatively quickly: according to a personal  
419 communication cited in Sigurdsson et al. (2016), the forest was planted in 1966–1967. From a  
420 historical perspective, the emergence of a forest seems to have exerted a stronger effect on the  
421 thermal phenotype of *P. pseudovanderdrifti* than the emergence of a geothermal gradient. In  
422 the case of developmental rate, the patterns of within- and between-habitat variation are even  
423 conflicting: although the slope of reaction norms increases with environmental temperature in  
424 the grassland, it is still higher in the forest, where the soil is in fact cooler (Fig. 3A). Although  
425 these observations are interesting and possibly representing genetic differences, recall that the  
426 two habitat types were not replicated in a complete randomized design, which limits the  
427 conclusions we can draw. Ideally, plots representing combinations of thermal conditions and  
428 habitat type (grassland versus spruce forest) should be replicated and randomly distributed in



429 the area, however, this is rarely possible in natural settings for logistic and economic reasons  
430 although examples do exist (e.g. Schindler, D.W., 1974. Eutrophication and recovery in  
431 experimental lakes - implications for lake management. *Science*. 184, 897-899).

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

439 In principle, within-generation temporal heterogeneity in temperature should select for  
440 canalization of adaptive traits and thus for flatter reaction norms (Liefting et al. 2009). Indeed,  
441 grassland subpopulations of *P. pseudovanderdrifti* have flatter thermal reaction norms for  
442 development and walking speed than forest subpopulations. This pattern agrees with previous  
443 findings for the surface-dwelling springtail *Orchesella cincta* whose growth rates are also  
444 more temperature-sensitive (i.e., reaction norms are steeper) in the more stable forest than in  
445 the more variable heathland (Liefting and Ellers 2008). Contrary to the theoretical  
446 expectation, thermal reaction norms often have shallower slopes in populations from less  
447 variable climates (Hassall et al. 2005; Molina-Montenegro and Naya 2012; Gaitán-Espitia et  
448 al. 2014; Kosmala et al. 2018). In the soil-dwelling springtail *Folsomia quadriculata* studied  
449 from High Arctic to Denmark, forest populations exhibit lower thermal plasticity of juvenile  
450 development time than populations from the more heterogeneous grass field and tundra  
451 (Sengupta et al. 2017). The problem is further complicated when additional traits are taken



452 into account, as these may have opposite patterns of thermal plasticity (Liefing et al. 2009;  
453 Sengupta et al. 2017).

454 Of course, forests differ from grasslands in a multitude of aspects, only one of which is the  
455 degree of thermal heterogeneity. Suffice to say, organismal thermal plasticity does evolve  
456 even at fine spatial scales, but the intricate effects of macro- and microclimate, as well as  
457 other possible factors and – last but not least – genetic drift (Crispo et al. 2010) are difficult to  
458 disentangle, especially when the differences are minuscule. Given this uncertainty, prediction  
459 of the future pathways of phenotypic evolution driven by climate change seems to be an  
460 extremely challenging task. While mean temperature is the most significant factor  
461 determining performance in ectotherms, thermal heterogeneity and the plethora of other  
462 ecological factors are also important, and so habitat type may drive the evolution of thermal  
463 phenotypes to an extent comparable with the effect of environmental temperature.

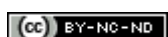
464

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473

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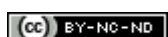
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642 **Figure captions**

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644 **Fig. 1** Annual temperature cycles in the geothermally warmed (A) forest and (B) grassland  
645 habitats of *P. pseudovanderdrifti*. Hourly data were collected from 8-May-2013 to 11-Sep-  
646 2014, temperatures for overlapping dates averaged. Gaps in the graphs are due to missing  
647 data. Temperature values in the legend refer to mean warming levels relative to the ambient  
648 conditions

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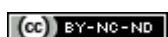
650 **Fig. 2** Common-garden (A, B) juvenile developmental rates during the 1st + 2nd instar and  
651 (C, D) adult walking speed in *P. pseudovanderdrifti* originating from the natural geothermal  
652 gradients (A, C) in the forest and (B, D) in the grassland. Temperature values in the legend  
653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

656

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

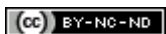
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662 **Fig. 4** Survival rates of adult *P. pseudovanderdrifti* originating from (A) forest and (B)  
663 grassland geothermal gradients after 2-h exposure to subzero temperatures. Temperature  
664 values in the legend refer to mean warming levels at sampling plots relative to the ambient  
665 conditions. Survivorship data (filled symbols) are approximated with maximum likelihood



666 logistic regression curves; hollow symbols denote calculated lethal temperatures that result in  
667 50% mortality (LT50).

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