Temperature effects on parasite prevalence in a natural hybrid complex

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Both host susceptibility and parasite infectivity commonly have a genetic basis, and can therefore be shaped by coevolution. However, these traits are often sensitive to environmental variation, resulting in genotype-by-environment interactions. We tested the influence of temperature on host–parasite genetic specificity in the Daphnia longispina hybrid complex, exposed to the protozoan parasite Caullerya mesnili. Infection rates were higher at low temperature. Furthermore, significant differences between host clones, but not between host taxa, and a host genotype-by-temperature interaction were observed.

Keywords: coevolution; Daphnia; genotype-by-environment interaction; host–parasite

2. MATERIAL AND METHODS

(a) System

Daphnia were isolated from Greifensee (Switzerland), in the summers of 2002–2004, except that clone 401 was isolated in winter, and B39 was hatched from a diapause egg. Based on species-specific allozymes [12], three clones per Daphnia galeata and D. longispina (nomenclature according to [16]) were selected (adequate for the detection of taxon differences; see [17]). Additionally, multi-locus-genotypes were determined using 15 unlabeled microsatellite markers [18].

(b) Experiment

Prior to the experiment, the parasite laboratory cultures as well as the six experimental Daphnia magna clones were kept for five generations under standardized conditions. They were fed chemostat-grown Scenedesmus obliquus daily (1 mg C l−1), and kept in 0.45 μm filtered lake water at 16°C on a 16 L : 8 D regime. Experimental neonates (less than 24 h) from the second or third clutch were randomly distributed among treatments. Thus, six genetically distinct clones (three of each two taxa) were infected with two parasite isolates at two temperatures (12°C and 20°C) and replicated eight times, leading to 192 experimental units.

On day 1, each jar was filled with 4 ml water and a single Daphnia. On day 2, heavily infected Daphnia were collected from laboratory cultures and homogenized in distilled water. These spore-suspensions were distributed among all jars (0.2 heavily infected Daphnia each). Daphnia galeata clone G68 was used as a negative control, receiving no extra treatment.

From the third day of the experiment on, all animals were checked daily for survival and reproduction. Dead animals were examined for Caullerya spore-clusters. Experimental manipulations were carried out at degree-days, the product of day and temperature [19]. Every 80 degree-days, water was increased by 20 ml until it reached 100 ml per jar. Subsequently, water was fully changed every 80 degree-days, or after a new clutch. The experiment was...
3. RESULTS

Both temperature and host clone (nested within taxon) significantly affected infection outcome. A higher proportion of Daphnia were infected at 12°C compared with the 20°C treatment (table 1 and figure 1). Further, there was a clone-by-temperature effect, with all clones but one showing higher infection levels at 12°C. The taxon and the parasite isolate effects and their interactions were not significant. Successful Caullerya infection resulted in host fecundity reduction by 97 per cent at 20°C and 77.4 per cent at 12°C. Moreover, host survival of Caullerya infected animals was more than 4.5 fold reduced compared with that of the uninfected individuals; both effects were significant (table 1). The genetic analyses showed a clustering pattern by taxon, with hybrids being genetically more diverse than parental clones (figure 2).

4. DISCUSSION

Temperature significantly affected Daphnia infection: a higher proportion of parasite-exposed Daphnia became infected at 12°C compared with the 20°C treatment.

Table 1. Results for the effect of temperature, Caullerya isolate, Daphnia taxon and clone, on the infection outcome and host life-history. (Non-significant interaction terms (numbers in italics, \( p > 0.05 \)) were removed.)

<table>
<thead>
<tr>
<th></th>
<th>d.f.</th>
<th>( F ) or ( \chi^2 )</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>infection outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>temperature</td>
<td>1</td>
<td>6.41</td>
<td>0.011</td>
</tr>
<tr>
<td>parasite</td>
<td>1</td>
<td>0.06</td>
<td>0.815</td>
</tr>
<tr>
<td>taxon</td>
<td>1</td>
<td>1.06</td>
<td>0.303</td>
</tr>
<tr>
<td>clone (taxon)</td>
<td>4</td>
<td>10.50</td>
<td>0.033</td>
</tr>
<tr>
<td>temperature ( \times ) clone (taxon)</td>
<td>4</td>
<td>9.56</td>
<td>0.049</td>
</tr>
<tr>
<td>host fecundity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>temperature</td>
<td>1</td>
<td>86.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>parasite</td>
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<td>0.877</td>
</tr>
<tr>
<td>clone</td>
<td>5</td>
<td>7.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>infection</td>
<td>1</td>
<td>9.84</td>
<td>0.002</td>
</tr>
<tr>
<td>temperature ( \times ) clone</td>
<td>5</td>
<td>12.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>host survival</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<tr>
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<td>0.006</td>
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<tr>
<td>infection</td>
<td>1</td>
<td>33.04</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

This strong effect of temperature is consistent with previous studies [22]. There was also genotype-by-environment effect: contrary to all other clones, clone B39 was more susceptible at 20°C. This raises the possibility that host genotypes will differentially suffer the effects of parasitism depending on how temperature fluctuates. Such effects can lead to the maintenance of genetic polymorphisms within and between populations [23]. Since clone B39 was the only clone hatched from a sediment core, one could argue that it never underwent natural selection and thus shows this different infection pattern. However, its fitness (measured as size of first clutch) is not different from other clones (ANOVA: 20°C – \( F_{1,67} = 0.48, p = 0.49 \); 12°C – \( F_{1,33} = 0.28, p = 0.60 \); C. N. Schoebel 2010, unpublished data). Moreover, clone B39 does not diverge from the range of other clones with respect to their genetic signature (figure 2). Furthermore, no difference between susceptibilities was found between hatched versus planktonic host for another Daphnia-microparasite system [24].
Interestingly, the direction of the temperature effect is opposite to all previous studies on *D. magna* and its bacterial parasite *Pasteuria*, where infection peaked at higher temperatures (e.g. [19]). In contrast to the *D. longispina* complex living in permanent lakes, *D. magna* inhabits temporary ponds of small size and rarely overwinters. Moreover, *Pasteuria* has its peak prevalence in summer [25], whereas *Caullerya* in winter [12]. Thus, *Caullerya*’s higher infection success in the cold may reflect the parasite’s thermal optimum. Alternatively, *Daphnia* immune functions might be less responsive at colder temperatures. In the *Daphnia dentifera* system inhabiting North American permanent lakes, the onset of epidemics caused by a yeast (*Metschnikowia*) also coincides with late-summer cooling of the lake [26]. This is owing to physical processes in a lake: decreasing temperature induces vertical water mixing, and consequently re-suspending parasite spores in the water column.

We did not detect a host taxon effect for the infection outcome, while there were differences among clones. Yet, microsatellite markers indicated greater genetic variation between, rather than within taxa. This contrast between variation at the phenotypic and at the genotypic level is consistent with another study on this hybrid complex [17].

We tested, but found no evidence for *Caullerya* adaptation to a particular host clone over approximately 50 host generations, even though rapid parasite adaptation was suggested by field data [12]. Conversely, parasite adaptation was found within five generations in the *D. magna-Pasteuria* system [27]. This difference is peculiar given that the *Pasteuria* study started with a single strain, whereas we used a diverse mixture of *Caullerya* spores, suggesting greater evolutionary potential. Nonetheless, this failure of the parasite to adapt to different host genotypes is consistent with findings for another *Daphnia*-parasite system [24].

In summary, the significant effect of temperature and the genotype-by-temperature interaction (with one clone being more susceptible at higher, whereas all others at lower temperature) demonstrate that the interaction of heterogeneous environments and infection has the potential to influence host population dynamics.

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20 Firth, D. 1993 Bias reduction of maximum likelihood estimates. *Biometrics* 80, 27.


22 Blanford, S., Thomas, M. B., Pugh, C. & Pell, J. K. 2003 Temperature checks the Red Queen? Resistance and viru-


