Increased responsiveness in feeding behaviour of *Caenorhabditis elegans* after experimental coevolution with its microparasite *Bacillus thuringiensis*

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Immune responses, either constitutive or induced, are costly. An alternative defence strategy may be based on behavioural responses. For example, avoidance behaviour reduces contact with pathogens and thus the risk of infection as well as the requirement of immune system activation. Similarly, if pathogens are taken up orally, preferential feeding of pathogen-free food may be advantageous. Behavioural defences have been found in many animals, including the nematode *Caenorhabditis elegans*. We here tested nematodes from a laboratory based evolution experiment which had either coevolved with their microparasite *Bacillus thuringiensis* (BT) or evolved under control conditions. After 48 generations, coevolved populations were more sensitive to food conditions: in comparison with the controls, they reduced feeding activity in the presence of pathogenic BT strains while at the same time increasing it in the presence of non-pathogenic strains. We conclude that host–parasite coevolution can drive changes in the behavioural responsiveness to bacterial microbes, potentially leading to an increased defence against pathogens.

**Keywords**: pathogen avoidance; parasite–host coevolution; experimental evolution; *Caenorhabditis elegans*; *Bacillus thuringiensis*

1. INTRODUCTION

Parasites have detrimental effects on their hosts. Hosts should therefore evolve counter-mechanisms. One option is an elevated immune activity, either constitutively or upon pathogen contact [1,2]. Both cases have drawbacks: constitutive upregulation continuously requires energetic resources and is thus costly, especially without imminent parasite harm. Inducible responses, initiated by parasite contact, may be more economic but have the risk of parasite harm during the time-lag until activation of the full immune system. Yet another alternative is behavioural defence [1,3]. A special case of such defence is the increased responsiveness in feeding behaviour which may be advantageous if pathogens are taken up together with food. In this case, the feeding rate is increased under pathogen-free conditions, whereas it is decreased in the presence of pathogens. Although behavioural defence itself may also impose costs such as energy needed to express behaviours or limited food access, these costs may be outweighed by the twofold advantage of reduced infection-related damage and reduced energy requirements for immune system upregulation.

Behavioural defences against parasites are widespread among animals [3–5], including *Caenorhabditis elegans*. This nematode lives in microbe-rich habitats where it feeds on various micro-organisms but is also exposed to pathogens [6]. Under laboratory conditions, *C. elegans* can be specifically attracted by non-pathogenic bacteria [7–9] while it avoids various pathogenic strains [9–14]. Similarly, food uptake is reduced on pathogenic when compared with non-pathogenic bacteria [9–11,13].

We here test whether coevolution with pathogenic *Bacillus thuringiensis* (BT) strains, which infect *C. elegans* via oral uptake, selects for a change in feeding behaviour. We compared feeding rates of nematode populations that coevolved with pathogenic BT strains for about 48 generations with control populations that evolved in the absence of pathogenic strains under otherwise identical conditions [15].

2. MATERIAL AND METHODS

(a) **Study system**

The exact material and protocol for the evolution experiment is described elsewhere [15]. Importantly, to enhance evolutionary responses, we used mixtures of three different BT strains (NRRL B-18246, NRRL B-18247 and NRRL B-18679) in a 5 : 5 : 1 ratio and outcrossed populations of three different natural *C. elegans* isolates (MY8, MY15 and MY18; [16]). In the coevolution treatment, we selected for nematode resistance and parasite virulence. Control nematodes could adapt to the conditions of the evolution experiment including a non-evolving, non-pathogenic BT strain (DSM 350). Each treatment was replicated 20 times.

(b) **Feeding behaviour experiment**

The general experimental conditions were identical to those of the phenotypic assays described in the work of Schulte *et al.* [15]. All nematodes were taken from the replicate populations of the final host generation of the evolution experiment. For each behavioural test, 30 hermaphroditic L4 nematodes were placed on ‘worm balls’ (5 cm diameter [13]), which were either inoculated with a mixture of pathogenic BT strains identical to the initial mixture of the evolution experiment or with the non-pathogenic strain. We chose non-evolved pathogenic strains which were identical in all treatments to exclude pathogen adaptations to the host and thus enhance comparability across treatment groups. All worm balls additionally contained the standard laboratory nematode food *Escherichia coli* OP50 ad libitum to ensure that nematode behaviour was not influenced by absence of suitable food. After a 1 h acclimatization period (60.5 ± 5.8 min s.d.), we estimated the average feeding rate of five randomly chosen nematodes per worm ball by counting pharynx grinder movements within a 20 s period, which was previously identified as an informative time window for assessment of feeding responses to pathogens [10].

Four behavioural treatments were considered: coevolved hosts on pathogenic BT strains (*n* = 15 replicate populations from the evolution experiment), coevolved hosts on non-pathogenic BT strain (*n* = 5), control hosts on pathogenic BT strains (*n* = 19) and control hosts on non-pathogenic BT strain (*n* = 19). Replicate numbers were not 20 as in the study of Schulte *et al.* [15] since for some replicates the available worm numbers were insufficient to...
allow tests in both bacterial treatments. Moreover, one of the co-evolved populations was excluded from statistical analysis because worms did not feed at all under non-pathogenic conditions, which represents an artefact [10]. Each nematode was only tested once, i.e. a set of 30 randomly chosen worms of each replicate population was tested on pathogenic BT strains and a different set of 30 worms on non-pathogenic BT. Assessment of treatment groups was fully randomized.

### Table 1. Linear mixed model testing for the fixed effects of host selection regime (coevolution or control), bacteria type (non-pathogen or pathogen), their interaction and replicate nested within host selection regime as a random factor to account for the paired experimental set-up.

<table>
<thead>
<tr>
<th></th>
<th>d.f.</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>constant term</td>
<td>1</td>
<td>361.529</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>host-selection regime × bacteria</td>
<td>1</td>
<td>7.706</td>
<td>0.009</td>
</tr>
<tr>
<td>host-selection regime</td>
<td>1</td>
<td>0</td>
<td>0.986</td>
</tr>
<tr>
<td>bacteria</td>
<td>1</td>
<td>148.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>replicate (host-selection regime) (random)</td>
<td>32</td>
<td>1.981</td>
<td>0.029</td>
</tr>
</tbody>
</table>

(c) **Statistics**

Statistical analysis was performed with the program PASW STATISTICS v. 18.0.2. The observed variation was assessed with a mixed linear model including host-selection regime (coevolution versus control) and bacteria (pathogenic versus non-pathogenic) and their interaction as fixed factors and replicate within host-selection regime as random nested factor to account for the paired set-up as each population was tested on both non-pathogenic and pathogenic BT strains. Data were transformed \((\phi^{1/3})\) to fit model assumptions.

### 3. RESULTS

Nematodes fed significantly less on pathogenic than non-pathogenic BT (significant effect of factor ‘bacteria’; table 1 and figure 1), whereas the evolution treatment did not lead to a general difference in feeding rate (insignificant effect of factor ‘host-selection regime’; table 1). Importantly, however, coevolved nematodes showed reduced feeding on pathogenic but increased feeding on non-pathogenic BT strains when compared with the control (significant interaction term, table 1; see also crossing lines in figure 1), indicating a significantly increased responsiveness in feeding behaviour. Finally, the replicate populations within treatments also showed significant variation within the evolution treatments (table 1).

### 4. DISCUSSION

The nematode *C. elegans* encounters a diverse microbial community in its natural habitat [6]. Therefore, it would be beneficial to evolve behavioural responses towards both food and pathogens [10,13,14,17–19]. One of these behaviours concerns feeding. In fact, *C. elegans* was previously shown to reduce feeding in the presence of pathogenic rather than in non-pathogenic bacteria of the same species [9–11,13]. Our study confirms the high specificity of the response, as worms fed significantly less in the presence of pathogenic BT strains than in non-pathogenic BT strains—irrespective of the origin of the nematodes. Our analysis also revealed a significant effect of replicate within host-selection regime. This effect is probably a consequence of independent evolutionary trajectories in the replicate populations within the same evolution treatment, as already shown for these populations previously [15,20].

Furthermore, we demonstrate a higher responsiveness in feeding behaviour of the coevolved worms as indicated by the significant interaction term. This higher responsiveness should increase protection from infection while ensuring uptake of suitable microbes as food, because it allows the worms to take up more beneficial microbes in pathogen-free environments and, at the same time, fewer pathogenic bacteria in pathogen-containing environments. Moreover, this behaviour could reduce infection rates and could thus reduce upregulation of immune responses, possibly freeing resources that can then be used for increased offspring production. Therefore, selection during host–parasite coevolution is indeed expected to favour the observed increased responsiveness. Interestingly, our previous work demonstrated that relative to control nematodes, the coevolved worms were more resistant to pathogenic BT strains and, at the same time, had lower population growth rates on the non-pathogenic strain [15], indicating a cost of the evolved increase in defence. The previous and current results suggest that coevolution with parasites leads to changes in two different defence traits: immunity and the increased responsiveness in feeding behaviour. Moreover, they also suggest that the higher feeding activity under pathogen-free conditions cannot compensate for the evolutionary cost of increased resistance, because fitness is still lower for the coevolved worms in the absence of pathogenic BT strains [15].

The simultaneous investment in both types of defences may be explained by the biology of this nematode. Although *C. elegans* is known to avoid pathogenic patches and choose benign ones [7–12,14], it seems unlikely that feeding itself is selective, i.e. that *C. elegans* can specifically ingest only the non-pathogenic bacterial cells [21]. Instead, it appears that they rather reduce food uptake in environments containing pathogens and then search for pathogen-free food patches. In spite of the reduced ingestion, the worms are still
exposed to pathogens until they leave the pathogen-containing patch. Therefore, selection may favour simultaneous expression of both types of defences—at least to some extent. Interestingly, the insulin-like signalling cascade influences both defence types, apparently as part of a general stress response [11,22]. Similarly, serotonin and G protein-dependent signalling may also link the two defences [17].

To our knowledge, this is the first study which addresses behavioural responses after experimental host–parasite coevolution. We previously demonstrated multiple phenotypic and genetic changes for exactly the same coevolved host populations [15,20]. The increased responsiveness in feeding behaviour adds another trait of relevance for the interaction with parasites that is directly influenced by coevolutionary adaptations.

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