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1 **Insufficient evidence of infection-induced phototactic behaviour in *Spodoptera exigua*: a**
2 **comment on van Houte *et al.* (2014)**

3

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10

11

12 Some of the most spectacular interactions between hosts and parasites occur when parasites
13 manipulate their hosts' behaviour. Acanthocephalan worms that infect gammarid shrimps induce
14 host behaviours which elevate predation vulnerability when they need to transmit to their final
15 vertebrate host [1]. *Ophiocordyceps* fungi similarly increase transmission by forcing ants to
16 clamp their jaws around leaves in elevated positions before killing them [2]. However, the
17 mechanisms underlying such manipulations remain relatively obscure.

18

19 A recent paper by van Houte *et al.* [3] claims to demonstrate that: 1) infection with the
20 baculovirus *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) causes *S. exigua*
21 larvae to die in an elevated position; and 2) this is achieved by the virus triggering a positive
22 phototactic response in its larval host. Their study is grounded in knowledge that baculoviruses
23 manipulate climbing behaviour in some lepidopteran species [4]. Here we argue van Houte *et*
24 *al.*'s study is flawed: the experimental design cannot test the authors' hypotheses and the data
25 presented are open to other interpretations that do not support the authors' conclusions.

26

27 **Death in elevated positions?**

28 First we consider van Houte *et al.*'s evidence that the virus SeMNPV induces death at an
29 elevated height. The authors placed infected larvae in jars and recorded larval height over

30 several days until all pupated or died of infection. Baculovirus infection caused substantial
31 mortality after 3-4 days. van Houte *et al.* show that larval height increased during an early
32 climbing phase and that mean height of infected larvae remained high until the end of the
33 experiment (their figure 1a). However, many of the larvae included in this data set had died of
34 infection. The authors repeatedly recorded the height of dead larvae, despite the fact these
35 larvae were clearly incapable of moving. We re-plotted their data excluding those that had
36 previously died (our figure 1a) and show the association between infection and climbing is
37 anything but clear cut.. We also note that ongoing larval death means that the true sample size
38 declined from 31 to 2 during van Houte *et al.*'s experiment. The observation that infected larvae
39 "die at elevated positions" could be adequately explained by two simple facts: (i) larvae naturally
40 climb; and (ii) viruses kill them, but not instantaneously.

41

42 **Is phototaxis in infected larvae caused by viral infection?**

43 Next we question the evidence the authors use to justify their conclusion that SeMNPV causes a
44 change in host behaviour by inducing phototaxis. van Houte *et al.* placed SeMNPV infected
45 larvae in three different light regimes: 'continuous dark', 'lit from above', and 'lit from below'. The
46 height of each larva at death was later recorded. The authors conclude that because larval
47 height at death differs strikingly between these lighting treatments, SeMNPV infection induces
48 phototaxis. This conclusion is undermined by the absence of suitable control experiments on
49 uninfected larvae. These controls are necessary to demonstrate that the response of infected
50 larvae to light regime change is caused by viral infection and does not also occur in uninfected
51 individuals. Whilst the authors did study uninfected larvae (which were mock-infected), they did
52 not subject them to the more informative 'lit from below' treatment (for reasons that were not
53 mentioned). We therefore only have measures of phototactic behaviour for both infection classes
54 for two of the lighting treatments ('lit from above' and 'continuous dark'). Unfortunately, even
55 these treatments are not comparable because the authors report completely different behavioural
56 metrics in the two infection classes (uninfected: 'height twice daily until pupation'; infected:
57 'height at death'). These metrics cannot be directly compared without information about the time
58 at which larvae died in the infected treatment.

59

60 The authors state that climbing in uninfected larvae is “not light-dependent”. This assertion is
61 crucial to their argument that the virus induces phototaxis. However, van Houte *et al.* restrict
62 their comparison of uninfected larvae in the light and dark treatment to two specific times at
63 which the larvae moulted. At other times climbing behaviour in uninfected larvae differs markedly
64 between the lighting regimes (our figure 1*b*). Indeed, the peak of climbing occurs 69 hours
65 earlier in the ‘dark’ than in the ‘lit from above’ treatment. By these metrics the climbing of
66 uninfected larvae *is* influenced by light. Therefore, it seems premature for the authors to
67 conclude that viral infection drives the observed phototaxis in the infected treatment.

68

69 **Alternative interpretations of the data and suggestions for future studies**

70 We would like to be more constructive than simply to point out problems in van Houte *et al.*'s
71 paper. Their data are compatible with a different hypothesis that does not require viral
72 manipulation. SeMNPV may simply induce larval death during a peak in natural climbing
73 behaviour, meaning that larvae die in elevated positions. This could potentially represent an
74 interesting example of optimally timed host-killing by a pathogen [5] (but further experiments are
75 necessary to properly test this). Whilst virally-induced host climbing has been demonstrated in
76 another system [6], ‘tree top disease phenomena’ have been reported in numerous host species.
77 In each of these cases it is important to determine whether this phenomenon results from viral
78 manipulation of climbing behaviour or optimally timed larval killing – or both. To show that host
79 behavioural changes actually result from parasite manipulation, future studies should endeavour
80 to rule out plausible alternative explanations, including changes resulting from morbidity
81 associated with infection, or adaptive host responses to parasitism [7]. Demonstration that the
82 behaviour of infected hosts changes at a specific time which favours the parasite’s own fitness
83 can provide definitive evidence of behavioural manipulation [8].

84

85 We do not doubt that behavioural manipulation of lepidopteran larvae by baculoviruses occurs in
86 some host-virus systems, potentially including this one. Unfortunately, van Houte *et al.*'s
87 experiments lack sufficient comparable controls and cannot rule out possible alternative

88 explanations. It is our opinion that the data presented provide insufficient evidence to support
89 van Houte et al.'s conclusions.

90

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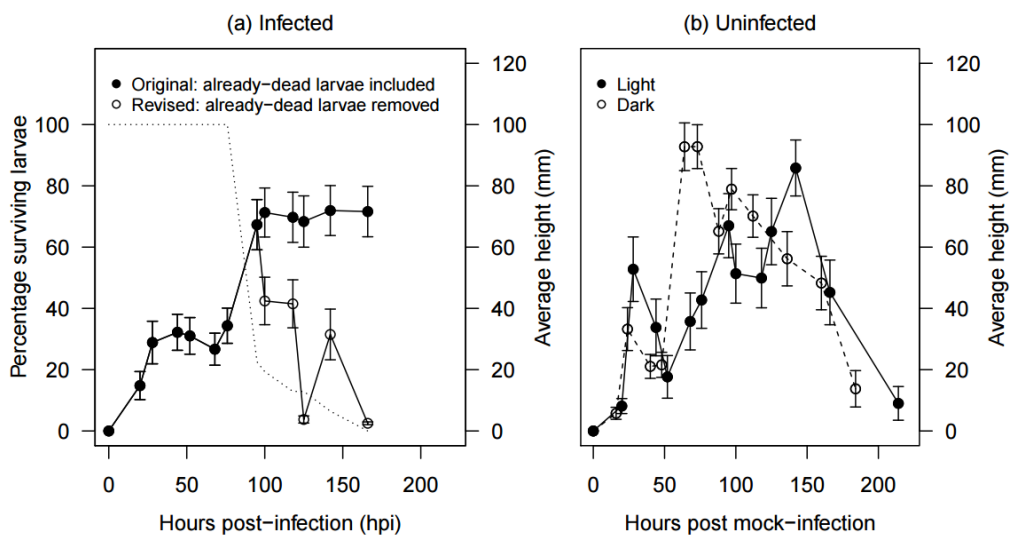
122

123 **Figure Legend**

124

125 **Figure 1.** Mean height of baculovirus-infected and healthy larvae. (a) Height of larvae following
126 exposure to the baculovirus. Closed circles: original data. Open circles: data where larvae were
127 excluded after the first point at which they are found to be dead (and therefore lose the ability to
128 move). The dotted line represents % survival (left hand axis). (b) Height of uninfected larvae in
129 light (12 L: 10 D) and dark (0 L: 24 D) treatments (closed and open circles, respectively) (data
130 unchanged from van Houte *et al.*). Error bars show SE.

131



132