Bugs don't make worms kill

In the natural environment, *Caenorhabditis elegans* and closely related nematodes are thought to associate with invertebrate hosts for transport (phoresy) or for food (necromeny) (Kiontke and Sudhaus, 2006). Recently, Abebe et al. (Abebe et al., 2010) showed that by feeding *C. briggsae* (and other *Caenorhabditis* species) *Serratia marcescens* (*Serratia* sp. SCBI), they could turn a normally benign nematode into a lethal entomopathogen capable of infecting and killing wax moths (*Galleria mellonella*). This behavior is similar to entomopathogenic nematodes (EPNs) from the genera *Steinernema* and *Heterorhabditis*, which have formed a mutualistic symbiosis with the bacteria *Xenorhabdus* and *Photorhabdus*, respectively (Forst et al., 1997). These bacteria are released by nematodes upon penetration into the insect host and cause death within 24–48 hours as a result of bacterial toxin production (Ciche, 2007). The nematodes reproduce prolifically on the dead host and, once the food source is depleted, new infective juveniles are produced that then search for new hosts.

Can *Caenorhabditis* spp. become pathogenic insect killers similar to EPNs by feeding on *Serratia* sp. SCBI? Abebe et al. showed that *C. briggsae* fed *Serratia* sp. SCBI and then injected into insects caused toxicity (Abebe et al., 2010). *Serratia* sp. SCBI is extremely toxic to *G. mellonella* and causes 100% mortality when injected with 100 cells per insect. The authors state that the nematodes were "transformed to become entomopathogenic." However, both "nematodes and associated bacteria" were injected into *G. mellonella* and so *Serratia* sp. SCBI killed *G. mellonella* and the nematodes reproduced on the cadaver. In a second experiment, *Caenorhabditis* spp. were grown on *Serratia* sp. SCBI and exposed to *G. mellonella* for 7 days. Unfortunately, there was no surface sterilization procedure to eliminate bacteria from the nematode cuticle and therefore it is impossible to discount the possibility that residual transfer of *Serratia* sp. SCBI on the nematode epidermis or in the gut or bacterial proliferation in the prepared plate caused *G. mellonella* to die. The pathogenicity shown in Abebe et al. (Abebe et al., 2010) is simply the effect of *C. briggsae* inadvertently introducing *Serratia* sp. SCBI into and/or onto *G. mellonella*. In addition, *C. briggsae* and other *Caenorhabditis* species lack specific morphological adaptations that allow genuine symbiosis between nematodes and bacteria such as EPNs (Martens and Goodrich-Blair, 2005). Therefore, the authors have failed to demonstrate the pathogenicity of *Caenorhabditis* species and the symbiotic nature of this association.

All known EPNs can only reproduce on one bacterium, inside a parasitized host, and only infective stage juveniles, not adults, are found in soil. Abebe et al. tried to isolate adult *C. briggsae* from soil or rotting fruits to further show similarities to EPNs (Abebe et al., 2010). The sampling had a negative outcome and no adults were obtained, although the authors argue that this "clearly points towards the true entomopathogenic nature of the strain." We feel that this proves nothing about the life history of *C. briggsae*. It is rare to find adults from a range of phoretic and necromenic nematodes in soil; e.g. *C. elegans* and *Pristionchus pacificus* are generally only found as third-stage infective (dauer) juveniles and are not parasitic (Weller et al., 2010). It is thus overstating the case to say that *C. briggsae* or any other *Caenorhabditis* species are actually entomopathogenic nematodes.

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Response to 'Bugs don't make worms kill'

In their Correspondence article, Rae and Sommer raise a number of interesting issues concerning the findings we reported recently in *The Journal of Experimental Biology* (Abebe et al., 2010). Their main thesis that "the authors have failed to demonstrate the pathogenicity of *Caenorhabditis* species and the symbiotic nature of this association" is incorrect. We would like to restate that our *Caenorhabditis briggsae* strain, i.e. *C. briggsae* KT0001, was isolated from independent *Galleria mellonella* trap experiments from soils collected from three provinces, and in all cases we found the nematode with the bacteria *Serratia* sp. SCBI. Our subsequent experiments showed that the worms entered *G. mellonella*, reproduced successfully and emerged from the cadaver as infective dauer-stage juveniles. Very importantly, *C. briggsae* KT0001 was not able to kill *G. mellonella* in 24 hours when grown on *Escherichia coli*, and our results clearly showed that it was *Serratia* sp. SCBI and not the nematode that killed the insect, indicating

that the *Caenorhabditis–Serratia* sp. SCBI association was critical in producing insect mortality; *G. mellonella* mortality when topically exposed to *Serratia* sp. SCBI was not significantly different from similar exposure to *E. coli* OP50 or mortality in the absence of any manipulation. These results are similar to those reported in the literature for *Heterorhabditis* and *Steinernema* (Gouge and Snyder, 2006). So although we know little about the exact nature of the association or how common it is in nature, we clearly demonstrate the ability of this association to hasten the death of an insect.

The umbrella term 'symbiosis' represents a diverse array of biological associations between organisms. At this time we don't have data to support speculation on the kind of association that exists between *C. briggsae* KT0001 and *Serratia* sp. SCBI. We fully agree with Rae and Sommer that *Caenorhabditis* lacks specific morphological adaptations that support symbiosis between some

nematodes and bacteria. However, similar to *Caenorhabditis*, the well-recognized entomopathogenic nematode (EPN), *Heterorhabditis* also lack any morphological adaptations; instead, the bacterial associates of *Heterorhabditis* colonize the entire intestine (Ciche and Ensign, 2003). Phylogenetically, *Caenorhabditis* are closer to *Heterorhabditis* (Kiontke and Fitch, 2005) than to *Steinernema*. As a result, it is not surprising that *Caenorhabditis* lack the adaptation that *Steirnernema* has to host its bacterial associate. In terms of maintaining the association, we don't have evidence to exclude any mechanism, including invasion of the intestinal cells by the bacteria, similar to what is reported for *Heterorhabditis* (Ciche and Ensign, 2003). Félix and Braendle reported "intestinal colonization by live bacteria" for *C. elegans* (Félix and Braendle, 2010). Consequently, the current lack of evidence regarding the mechanism of maintenance of the association between the nematode and bacteria does not prove the absence of such a mechanism. However, as is obvious from the comments of Rae and Sommer, these are very interesting questions that will be crucial to understanding the evolution of such associations.

The entry of the bacteria and nematode into the insect varies among well-characterized EPNs. In *Steinernema*, infective juveniles enter the insect body through natural opening such as the mouth, spiracles and anus; in *Heterorhabditis*, infective juveniles enter the hemocoel directly through the insect's integument (Wang and Gaugler, 1998). Félix and Braendle pointed out that *C. elegans*' associations with insects could be "phoretic, necromenic and possibly commensal or parasitic" (Félix and Braendle, 2010). From this, it is plausible to conclude that *Caenorhabditis* species may enter the body cavities of invertebrates as a common feature of their life cycles.

Rae and Sommers also state that "all known EPNs can only reproduce on one bacterium." This generalization may not hold true on closer inspection. Gouge and Snyder provided an extensive list of bacterial species reported from *Heterorhabditis* and *Sterinernema* in addition to their well-documented bacterial associates (Gouge and Snyder, 2006). Often those associated bacteria, other than *Xenorhabdus* spp. and *Photorhabdus* spp., were isolated from surface-sterilized worms (e.g. Babic et al., 2000). Many gram-negative bacteria were also shown to support reproduction of steinernematids (Boemare et al., 1983; Ehlers et al., 1990). There is extensive evidence that *Caenorhabditis* indeed establish phoretic or necromenic association with insects (Kiontke and Sudhaus, 2006). Some have argued that such associations might have produced entomopathogenic associations (Sudhaus, 1993).

So although we agree with Rae and Sommer that we have not characterized the nature of the association between this strain of *Serratia* and *Caenorhabditis*, we have demonstrated that in some cases they are capable of acting as an entomopathogenic complex. We fully recognize that the *Caenorhabditis–Serratia* association is not as well characterized as the archetypical EPNs. However, we look forward to the possibility that this finding may shed light on the life history of one of our premiere model organisms in science as, although great strides are being made toward finding and describing natural populations of *Caenorhabditis* (Barrière and Félix, 2005), we remain in the almost dark about the role of this nematode and its relatives in nature (Félix and Braendle, 2010).

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