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Developmentally regulated volatiles geosmin and 2-methylisoborneol attract a soil arthropod to *Streptomyces* bacteria promoting spore dispersal

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Volatile compounds emitted by bacteria are often sensed by other organisms as odours, but their ecological roles are poorly understood[1,2]. Well-known examples are the soil-smelling terpenoids geosmin and 2-methylisoborneol (2-MIB)[3,4], which humans and various animals sense at extremely low concentrations[5,6]. The conservation of geosmin biosynthesis genes among virtually all species of *Streptomyces* bacteria (and genes for the biosynthesis of 2-MIB in about 50%)[7,8], suggests that the volatiles provide a selective advantage for these soil microbes. We show, in the present study, that these volatiles mediate interactions of apparent mutual benefit between streptomycetes and springtails (Collembola). In field experiments, springtails were attracted to odours emitted by *Streptomyces* colonies. Geosmin and 2-MIB in these odours induce electrophysiological responses in the antennae of the model springtail *Folsomia candida*, which is also attracted to both compounds. Moreover, the genes for geosmin and 2-MIB synthases are under the direct control of sporulation-specific transcription factors, constraining emission of the odorants to sporulating colonies. *F. candida* feeds on the *Streptomyces* colonies and disseminates spores both via faecal pellets and through adherence to its hydrophobic cuticle. The results indicate that geosmin and 2-MIB production is an integral part of the sporulation process, completing the *Streptomyces* life cycle by facilitating dispersal of spores by soil arthropods.

One of the most emblematic and abundant microbial volatile organic compounds (VOCs) is geosmin, which is familiar as a characteristic odour of soil[9]. Geosmin is a sesquiterpenoid produced by certain soil microbes, most notably streptomycetes and related actinobacteria, but also by some myxobacteria, cyanobacteria and filamentous fungi[8]. The enzyme geosmin synthase catalyses the cyclization of farnesyl diphosphate to germacradienol and germacrene D, and then converts germacradienol to geosmin[4]. The gene encoding geosmin synthase is conserved in virtually all sequenced *Streptomyces* genomes (see Supplementary Discussion)[7,8]. Streptomycetes are famous for their plethora of specialized metabolites, comprising an enormous range of chemical structures and biological activities, including most of the antibiotics in current clinical use[10]. Each *Streptomyces* isolate has the genetic capacity to produce dozens of specialized metabolites but any given compound is made by only a small percentage of strains. The ubiquity of geosmin production is therefore remarkable and suggests that it confers a selective advantage on the streptomycetes. In the present study, we investigated the biological role of geosmin and found that it is intimately connected to the developmental life cycle of these organisms. Streptomycetes grow vegetatively as mycelial networks that are entangled with the soil particles or other substrates on which they live. The mycelium can spread on a centimetre scale by a volatile (trimethylamine)-mediated, specialized exploratory mode of growth[11], but dispersal over longer distances occurs through single-celled dormant spores that are formed on specialized aerial hyphae emerging from the surface of colonies[12]. When encountering suitable conditions, spores germinate to give rise to new vegetative mycelium.

Many animals, including humans, can sense geosmin at very low levels, and we perceive it as an off-flavour, for example, in drinking water and foodstuffs[5]. *Drosophila melanogaster* dedicates an olfactory circuit to the sensing of geosmin, which induces a strong aversive behavioural response in the flies[6,13], and in *Aedes aegypti* mosquitoes it acts as an oviposition cue[14]. However, the advantage of geosmin to the producing microbes has remained unknown.

To investigate possible roles of geosmin and other *Streptomyces* VOCs in the context of soil ecosystems, we asked whether the smell of *Streptomyces* spp. might attract soil-dwelling arthropods. In a network of field traps baited with *Streptomyces coelicolor* colonies, we found significant attraction of springtails (Collembola) compared with control traps (Fig. 1a,c), whereas capture of insects and arachnids was not significantly affected by the *Streptomyces* colonies (see Extended Data Fig. 1a,b). Collembola are closely related to insects, but constitute a more basal branch of the Hexapoda (six-legged arthropods)[15]. The attraction of springtails to *S. coelicolor* was confirmed in a trapping experiment performed in a tropical greenhouse (Fig. 1b), and could finally be proven by a laboratory Y-tube bioassay with the model springtail *Folsomia candida* (Isotomidae) as the test organism (Fig. 1d,e). VOCs emitted by *S. coelicolor* were sufficient to attract *F. candida*.

Next, we recorded chemosensory responses in springtail antennae to identify the VOCs that are sensed by the animals. Odour samples of *S. coelicolor* that were analysed by gas chromatography (GC), combined with electrophysiological antennal detection (EAD),

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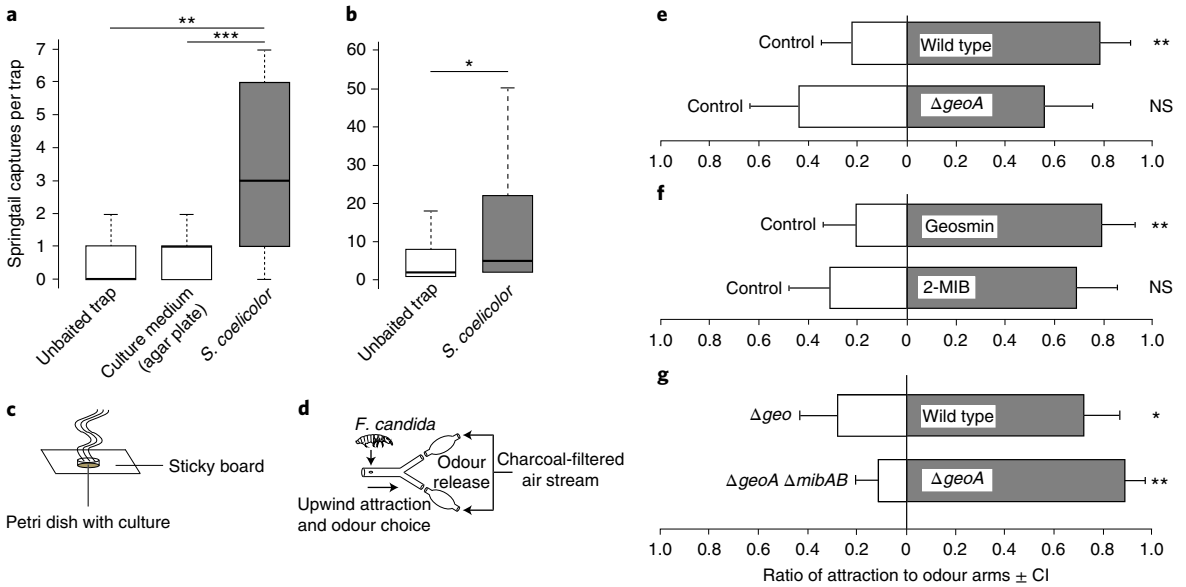

Fig. 1 | Attraction of springtails to *Streptomyces* headspace, geosmin or 2-MIB. a, Number of springtails captured with sticky traps baited with wild-type *S. coelicolor* strain M145, compared with control traps that were kept without bait or held a non-inoculated plate of culture medium ($n=19$) in a 1-d field experiment. **b**, Number of springtails captured with sticky traps baited with *S. coelicolor* strain M145 ($n=93$) compared with unbaited traps ($n=100$) in a glasshouse experiment over a period of 4 d. Traps baited with *S. coelicolor* attracted significantly more springtails in the field ($\chi^2_{2,57}=19.389$, $P<0.001$; Tukey's test, $^{**}P<0.005$; $^{***}P<0.001$) (**a**) as well as in the glasshouse ($\chi^2_{1,193}=6.049$, $^{*}P=0.014$) (**b**) than control traps. The boxplots show medians, and upper and lower quartiles, as well as the span of data points that are within 1.5× interquartile range. **c**, Schematic drawing of sticky trap with bait. **d**, Schematic drawing of Y-tube setup. **e**–**g**, Odour-mediated attraction of the springtail *F. candida* tested in a Y-tube. Headspace volatiles of *S. coelicolor* strain M145 (0.2 ng geosmin, 0.021 ng 2-MIB) induced significant attraction of *F. candida* ($^{**}P<0.001$) whereas the equivalent headspace of the geosmin-deficient mutant J3003 (*ΔgeoA*; 0.021 ng 2-MIB) was not attractive (NS: $P=0.69$) (**e**). Accordingly, geosmin (1 ng) alone was sufficient to attract *F. candida* ($^{**}P<0.001$) (**f**) and *F. candida* preferred *S. coelicolor* M145 to the *ΔgeoA* mutant when there was a choice between the two strains ($^{*}P=0.024$) (**g**). Attraction to 1 ng 2-MIB was not significant (NS: $P=0.061$) (**f**), but absence of 2-MIB reduced attraction ($^{**}P<0.001$) (**g**) when headspace of the double-mutant J2192 (*ΔgeoA ΔmibAB*) was tested against the equivalent headspace from J3003 (*ΔgeoA*, 3.15 ng 2-MIB, which is 150× the concentration compared with **e**). For each treatment, preference for one of the two arms of the Y-tube was examined by an exact binomial test. The bars show the ratios of attraction of springtails to the two arms of the Y-tube. Error bars show the 95% confidence intervals (CI). Equal volumes of the corresponding solvent (double-distilled heptane or hexane) were offered as controls in **e** and **f**.

revealed that geosmin, its biosynthetic intermediate germacradienol and the shunt product germacrene D induce sensory responses in *F. candida* (Fig. 2). In addition, the monoterpene 2-MIB also elicits a response. Similar to geosmin, 2-MIB has an earthy smell, and the genes for 2-MIB biosynthesis are found in approximately half of sequenced *Streptomyces* genomes[7,8]. The sensory detection of geosmin and 2-MIB was further confirmed through electroantennography (EAG) using authentic compounds, which induced dose-dependent responses at 10 and 100 ng (see Extended Data Fig. 1c). In Y-tube assays, the springtails showed clear attraction behaviour to geosmin at a dose of 1 ng (Fig. 1f). Furthermore, there was significant attraction towards headspace samples from wild-type *S. coelicolor* (diluted to contain 200 pg geosmin) but not to headspace samples from a congenic *geoA* mutant (Fig. 1e), and the springtails clearly preferred the wild-type over the *geoA* mutant in a choice assay (Fig. 1g). The 2-MIB was less active than geosmin and showed no statistically significant attraction when tested in isolation at a dose of 1 ng (Fig. 1f), or at the low dose of 2-MIB present in the diluted extracts of the *geoA* single mutant used in Fig. 1e (21 pg; see Extended Data Fig. 2). However, given the choice between a concentration of headspace extract from the geosmin mutant containing 3.2 ng 2-MIB, and a similar extract from the double mutant lacking both geosmin and 2-MIB (see Extended Data Figs. 1d–f and 2), *F. candida* preferred the mutant-emitting 2-MIB (Fig. 1g), clarifying that both earthy odorants are behaviourally active and serve as attractants for the springtail.

F. candida is mostly known to feed on fungi, but it also ingests and digests bacteria, and springtails have been reported to sense volatile chemical signals for localization of food sources[16,17]. In the present study, it was obvious that the springtails were feeding on sporulating colonies of *S. coelicolor* (see Supplementary Video 1), and dark-grey bacterial biomass was clearly visible in the intestines of the largely transparent *F. candida* (see Extended Data Fig. 3a). In addition, *S. coelicolor* colonies placed in soil microcosms were grazed on by *F. candida* individuals until most of the colony was consumed (see Extended Data Fig. 3b). Furthermore, although the prolific specialized metabolism of streptomycetes makes them toxic to some invertebrates[6,18,19], providing *S. coelicolor* as a sole food source had positive effects on the moulting and oviposition of *F. candida* springtails, and no negative effects on survival (see Extended Data Fig. 4). Thus, the ability to sense geosmin and 2-MIB guides springtails to the bacteria as a source of food.

To address the possible significance of the VOCs to the bacteria, we investigated expression of geosmin and 2-MIB biosynthetic genes during the *Streptomyces* developmental life cycle. These studies were conducted with the alternative model species *S. venezuelae*, which is superior to *S. coelicolor* for the analysis of developmental regulation[12]. In time-resolved global transcriptome analyses, the biosynthetic genes for both geosmin (*geoA*[4]) and 2-MIB (*mibA* and *mibB*, encoding a monoterpene cyclase and a methyl transferase, respectively, which together convert geranyl diphosphate into 2-MIB[3]) were found to be developmentally upregulated in wild-type

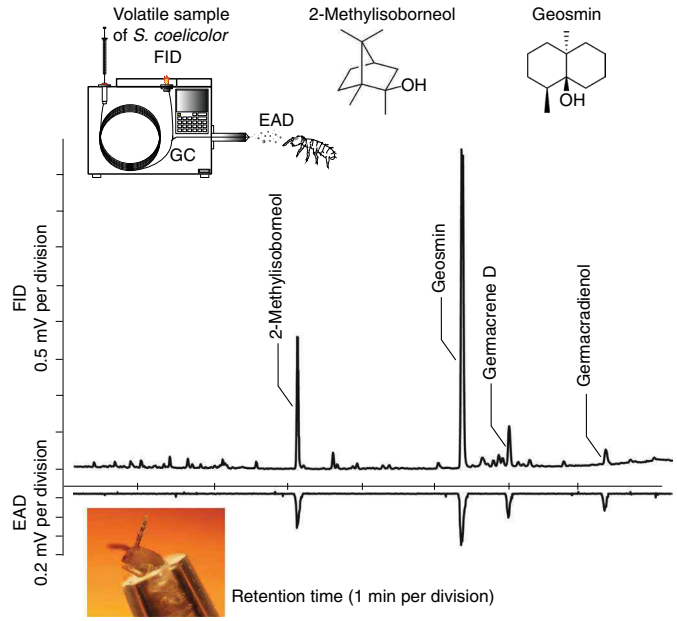

Fig. 2 | Antennal responses to *Streptomyces* headspace components in *F. candida*. Gas chromatogram of sampled *S. coelicolor* volatiles (upper trace) and electroantennogram (lower trace) showing the mean responses ($n = 6$) of *F. candida* springtail antennae towards the volatiles eluting from a HP-5 column of a GC. The sketch illustrates the GC with FID and coupled EAD. The photographic insert shows an immobilized female of *F. candida* fixed into a glass capillary with a precise head dissection for antennal recording.

S. venezuelae around the time that sporulation is initiated (Fig. 3a). Furthermore, expression of both *geoA* and *mibA–mibB* depended on the regulatory gene *bldM*, encoding a response regulator transcription factor required for development of aerial hyphae and spores (Fig. 3a)[20]. Unexpectedly, the *mibA–mibB* genes were found to form an operon with *eshA* (encoding a putative cyclic nucleotide-binding protein of unclear function[21]), which has the same pattern of expression as *mibA* and *mibB* (Fig. 3a). Chromatin immunoprecipitation sequencing (ChIP–seq) analysis showed that BldM directly regulates the promoter of the *eshA–mibA–mibB* operon (Fig. 3b). Gas chromatography–mass spectrometry (GC–MS) analysis of collected headspace samples showed that production of geosmin and 2-MIB is essentially absent from *an S. venezuelae bldM* mutant (see Extended Data Fig. 5).

There was no BldM ChIP–seq signal upstream of *geoA* (Fig. 3b), suggesting that BldM does not regulate *geoA* directly. A survey of other key sporulation regulators revealed that *geoA* also failed to be developmentally upregulated in *a whiH* mutant (Fig. 3a). WhiH is a transcription factor that is required for proper septation of aerial hyphae during spore formation[22]. ChIP–seq analysis showed that WhiH directly regulates the *geoA* promoter (Fig. 3c), and emission of geosmin was strongly reduced in the *whiH* mutant compared with the wild-type (see Extended Data Fig. 5). Previous studies in *S. coelicolor* showed that *whiH* itself is regulated by another developmental regulator, the RNA polymerase sigma factor encoded by *whiG*[23]. However, expression of *whiH* in *S. venezuelae* is only partially dependent on *whiG*[24]. This observation probably explains why *geoA* is expressed in the *whiG* mutant, although apparently with slight delay compared with the wild-type (Fig. 3a). In summary, the production of both earthy odorants is directly coupled to spore formation via transcriptional control of the *geoA* and *eshA–mibA–mibB* loci by key sporulation regulators. These insights provide a mechanistic explanation for the previously reported correlation between geosmin production and sporulation in *Streptomyces* spp.[25,26].

The geosmin- and 2-MIB-mediated attraction of springtails and the direct coupling of these VOCs to spore formation suggested that springtails might act as vectors for spore dispersal. Springtails are characterized by anti-adhesive skin patterns and a cuticle covered with hydrophobic layers of wax, leading to a poor adherence of most bacteria to the animal's surface[27,28]. However, we found that 10^4–10^5 spores could be washed off from the surface of *F. candida* individuals that had been exposed to sporulating *S. coelicolor* colonies, and scanning electron microscopy (SEM) showed that spores adhered to hair-like setae on the *Folsomia* body (seen as short chains of spores in Fig. 4a). Our findings are in agreement with previously reported adherence of *S. griseus* spores to the hydrophobic cuticles of springtails and mites[29]. It is probable that the adherence to the unwettable cuticle of the springtails is mediated by the extremely hydrophobic sheath that covers *Streptomyces* spores[12]. To compare adherence of spores and vegetative hyphae, we allowed springtails to feed for 3 d on either *S. coelicolor* wild-type strain M145 or the congenic, non-sporulating, *whiG* mutant J2400 (a developmental mutant producing only non-sporulating aerial hyphae in addition to the vegetative mycelium). A substantial number of colony-forming units (CFUs) adhering to the body surface could be washed off from springtails having been exposed to the sporulating strain, although almost no CFUs in the form of hyphae adhered to or could be washed off from springtails exposed to the non-sporulating strain (see Extended Data Fig. 6a).

In addition to transport of spores on the body surface, the feeding behaviour of the animals offers a second possible route for spore dispersal through defecation. To test this possibility, we took springtails that had been offered *S. coelicolor* biomass as food, washed off surface-adhered spores and then collected the faecal pellets that they released; 70.8% ($n = 24$) of the animals released faecal pellets containing viable *Streptomyces* spores that gave rise to colonies when plated on agar media, confirming that spores were being ingested by the animals and could survive passage through their guts. Springtails that had been fed with the non-sporulating *whiG* strain (containing only vegetative and aerial hyphae but no spores) and thereafter washed, released very few CFUs by defecation, while the sporulating strain gave over 100-fold more CFUs under these conditions (see Extended Data Fig. 6b). Overall, the results show that *Streptomyces* spores are much more efficiently dispersed by *F. candida* than non-sporulating hyphae, and that dispersal can occur via two different routes: by adherence to the surface of the cuticle and by passage through the gastrointestinal tract.

Finally, using Petri dish bioassays, it was confirmed that spore dispersal by springtails is influenced by the VOCs. We placed the same number of viable spores of wild-type *S. coelicolor* and the geosmin- and 2-MIB-deficient double mutant on agar plates, and compared the extent to which they were dispersed by *F. candida*. The number of spores dispersed and the average distance of their dispersal were positively affected by production of the volatiles in wild-type *S. coelicolor* (Fig. 4b,c). We also complemented the double-mutant strain J2192 *in trans* with *geoA* and *mibA–mibB* carried on an integrated plasmid (pIJ10646) and compared it with the same mutant carrying only the empty vector pIJ10770. Complementation restored production of geosmin and 2-MIB and enhanced *F. candida*-mediated dispersal of spores from developed colonies (see Extended Data Fig. 6c,d).

In summary, we found in laboratory experiments that the model species *F. candida* senses both geosmin and 2-MIB, and that attraction to the odours mediates spore dispersal for *S. coelicolor* and guides springtails to a source of food. Collembola are dietary generalists that feed on a wide range of microorganisms and even plant material. Numerous studies have shown that microorganisms differ in their quality as food resources for springtails, and springtails discriminate when offered different microbial species as food[30,31]. Two genome sequences show that collembolans have a broad repertoire

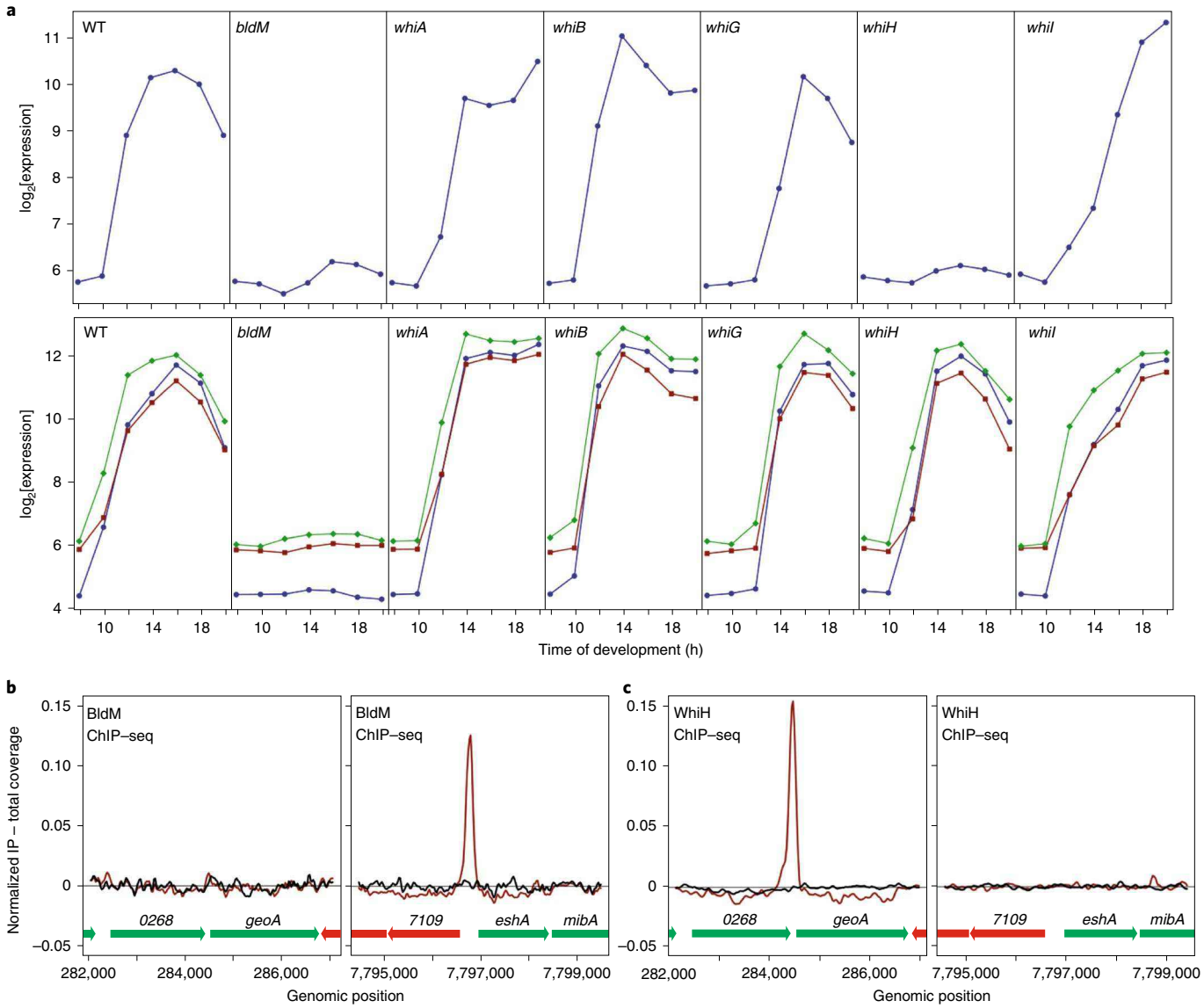

Fig. 3 | Developmental regulation of geosmin and 2-MIB biosynthetic genes. a, Microarray transcriptional profiling data for *geoA*, encoding geosmin synthase (upper panels) and the three co-transcribed genes *eshA*, *mibA* and *mibB* (*eshA* in blue, *mibA* in red and *mibB* in green) (lower panels; the latter two genes encode the 2-MIB biosynthetic enzymes), during submerged sporulation in wild-type (WT) *S. venezuelae* and congenic mutants lacking the key regulators of sporulation *bldM* (strain SV13), *whiA* (strain SV11), *whiB* (strain SV7), *whiG* (strain SV6), *whiH* (strain SV8) and *whiI* (strain SV10). In each panel, the *x* axis indicates the age of the culture in hours, and the *y* axis indicates the per-gene normalized transcript abundance (log_2), based on three independent cultures. For the wild type, 10–14 h corresponds to vegetative growth, 14–16 h to the onset of sporulation (fragmentation) and 16 h onwards to sporulation. **b**,**c**, ChIP-seq data show differences in normalized local enrichment of sequence reads between immunoprecipitated DNA (IP) and total DNA, plotted against genome position. BldM binding to the 2-MIB biosynthetic locus (right panel). Anti-BldM polyclonal ChIP-seq data for WT *S. venezuelae* are shown in brown and anti-BldM polyclonal ChIP-seq data for the Δ*bldM* control strain are shown in black. The equivalent data for the *geoA* locus are shown as a negative control (left panel) (**b**). WhiH binding to the *geoA* locus (left panel). Anti-FLAG ChIP-seq data for the strain expressing a functional, C-terminally 3xFLAG-tagged WhiH are shown in brown and anti-FLAG ChIP-seq data for the control strain (WT *S. venezuelae*) are shown in black (**c**). The equivalent data for the *eshA*–*mibA*–*mibB* locus are shown as a negative control (right panel). Genes running left to right are shown in green and those running right to left in red.

of enzymes for the degradation of cell wall material, including the peptidoglycan of bacteria[32,33]. Certain microbes probably use structural and chemical defence mechanisms to defend against grazing, for example toxic specialized metabolites[19,34,35]. Streptomycetes and other geosmin-emitting microbes often produce such metabolites that are toxic to invertebrates[10,18,36]. A recent study showed that *Caenorhabditis elegans* avoids toxin-producing streptomycetes by sensing and escaping from small molecules released by these bacteria[19]. Furthermore, the strong aversion of *Drosophila* flies towards geosmin was speculated to help them avoid contaminated and potentially poisonous food sources[6]. It is therefore interesting that the *F. candida* springtails were not negatively affected when feeding on *S. coelicolor* as the only source of food. Springtails are adapted to exposure to various toxic organic substances and xenobiotics in the soil, and their genomes contain expanded gene families implicated in detoxification mechanisms[32,33], presumably giving

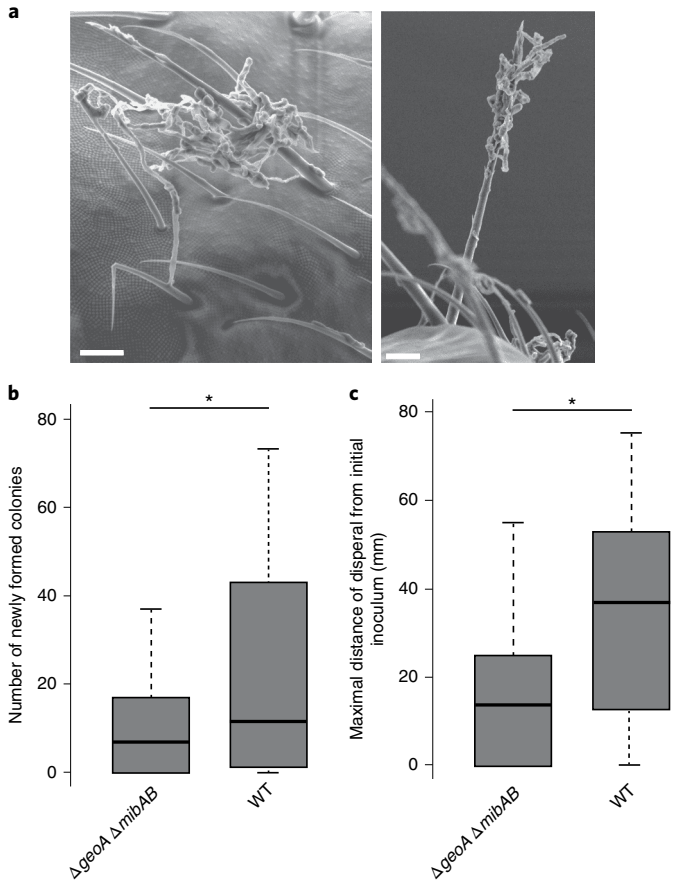

Fig. 4 | Dispersal of *S. coelicolor* spores mediated by springtails. **a**, Adherence of short chains of spores to setae on springtails that had been exposed to sporulating *S. coelicolor* culture, visualized by SEM. Representative results from two independent experiments are shown. Scale bars, 5 µm. **b**,**c**, Effect of geosmin and 2-MIB on springtail-mediated spore dispersal from *S. coelicolor* colonies. The presence of geosmin and 2-MIB in *S. coelicolor* strain M145 (WT; $n = 24$) colonies resulted in higher numbers of newly formed colonies due to springtail dispersal ($\chi^2_{1,49} = 4.872$, $^*P = 0.0273$) (**b**) and greater maximal distance ($\chi^2_{1,49} = 6.467$, $^*P = 0.011$) (**c**) of dispersal from the initial inoculum, in comparison to double-mutant colonies (Δ*geoA* Δ*mibAB; n* = 25), in an assay on agar plates. The boxplots show medians, and upper and lower quartiles, as well as the span of data points that are within 1.5× interquartile range.

them the capacity to tolerate specialized metabolites produced by streptomycetes. Interestingly, exposure to *Streptomyces* spp. also stimulated ecdysis (see Extended Data Fig. 4b), which might be seen as a growth response to the availability of food, or act as a detoxification mechanism[37]. It seems probable that such mechanisms allow springtails to feed on *Streptomyces* spp., a resource that can be toxic to other organisms, such as nematodes[19]. In addition, apart from being food, *Streptomyces* spp., as producers of antibiotics, could play a role in protecting springtails from pathogens, comparable to the relationship described between *Streptomyces* spp. and soil-dwelling beewolf larvae[38].

The *Streptomyces* genus is of an early origin[39], and the broad distribution of geosmin and 2-MIB production among streptomycetes indicates that both traits are ancestral. Interestingly, although *Drosophila melanogaster* uses an olfactory receptor to detect geosmin, Collembola, which separated from insects over 450 million years ago[15], use a different chemoreceptor, which must be of earlier type because olfactory receptors, and their associated co-receptor Orco, evolved relatively late in insect evolution and are not found in Collembola (see Supplementary Discussion) and other basal hexapods[40,41]. Overall, the VOC-mediated interaction between *Streptomycetes* spp. and springtails that we describe in the present study is likely to be ancient.

Our laboratory experiments suggest that streptomycetes benefit from emitting geosmin and 2-MIB as part of their developmental programme because these volatile scents guide springtails to sporulating microcolonies, and the animals serve as vectors for spore dispersal. The top layers of soil, where both *Streptomyces* spp. and springtails are abundant, form a heterogeneous and highly structured matrix, which may impede long-distance spore transport by water and wind. Vectoring by soil arthropods should provide a beneficial mode of dispersal in such below-ground environments, and contribute to shaping the microbial communities therein[42]. Undoubtedly, chemical information in the soil ecosystem is not limited to geosmin and 2-MIB, and other volatiles that may act as either attractants or repellents, such as oxylipins, need to be considered for a comprehensive understanding of chemical ecological and specific trophic interactions between soil organisms[43]. Thus, the relevance of the *Streptomyces* spp.-produced geosmin and 2-MIB in the interactions between the bacteria and springtails should be further tested in a community context.

Methods

Bacterial strains, plasmids, oligonucleotides and growth media. Strains, plasmids and oligonucleotides used in the present study are described in Supplementary Table 1. *Escherichia coli* strain DH5α was used for plasmid and cosmid propagation. *E. coli* strain BW25113 containing a λRed plasmid, pIJ790, was used to create disrupted cosmids[44]. Cosmids and plasmids were conjugated from the *dam dcm hsdS E. coli* strain ET12567 containing pUZ8002, as described previously[45–47]. Strains of *S. coelicolor* were cultivated on soya flour mannitol agar medium (SFM) and spores were prepared, as described previously[47]. Strains of *S. venezuelae* were cultivated on maltose yeast extract medium, as described by Bush et al.[48].

Construction of *S. coelicolor* and *S. venezuelae* mutants. Using 'Redirect' PCR targeting[45,46], an *S. venezuelae whiH* mutant was generated in which the *whiH* coding sequence was replaced with an apramycin resistance (*apr*) cassette. Cosmid 1D05 was introduced into *E. coli* BW25113 containing pIJ790, and the *whiH* gene (*vnz27205*) was replaced with the *apr-oriT* cassette amplified from pIJ773 using the primer pairs *whiH*_DEL_F and *whiH*_DEL_R. The resulting construct was introduced into wild-type *S. venezuelae* by conjugation and null mutant derivatives, generated by double crossing-over, were identified by their apramycin-resistant, kanamycin-sensitive and morphological phenotypes. A representative *whiH* null mutant was designated SV8.

The *S. coelicolor* Δ(*mibA–mibB*)*::apr* mutation was generated via the same approach, using λRed-mediated recombination to modify the 6D11 cosmid that carries the *mibA* and *mibB* genes (*sco7700-7701*). The *mibA* and *mibB* genes were replaced with a single *apr-oriT* cassette amplified from pIJ773 using the primer pairs *mib*_DEL_F and *mib*_DEL_R. The resulting mutant allele was introduced into the chromosome of the unmarked Δ*geoA* mutant of *S. coelicolor* (J3003)[45], and a representative Δ*geoA* Δ(*mibA–mibB*)*::apr* double mutant was designated J2192.

For *in trans* complementation of *geoA* and *mibAB* mutations, the *eshA–mibA–mibB* region was amplified from *S. coelicolor* genomic DNA with primers mib_F and mib_R and cloned between the HindIII and AvrII sites in the integrating vector pIJ10770; the *geoA* gene was amplified with primers geoA_F and geoA_R and cloned in the EcoRV site of the same plasmid, resulting in plasmid pIJ10646.

Trap networks in the field. Sticky traps were used to test the attraction of soil arthropods to live cultures of *S. coelicolor* (strain M145, grown on SFM agar) at two woodland sites in Alnarp, Sweden (55°39'38.0" N 13°04'36.8" E). Both sites were divided into five plots and each plot received all treatments (sticky traps baited with *S. coelicolor* or controls). Cell cultures of *S. coelicolor* were prepared by streaking approximately 7×10^4 CFUs on to Petri dishes (3.5-cm diameter) with SFM agar. Plates were incubated in darkness at 27 °C for 8 d before use in the traps. As control treatments, traps baited with the substrate only (SFM agar) and unbaited traps were used. Petri dishes of the bacterial cultures or controls were uncovered and placed in the centre of white sticky traps (glue boards of $16 \times 9\,cm^2$, Silvandersson) on to the ground of the study sites. A random number generator was used to decide on the location of the treatments in the plots. After 24 h, the traps were collected and the number of captured insects, springtails and arachnids was counted. The experiment was repeated once more on the next day (resulting in 20 traps per treatment in total).

The attraction of springtails to cultures of *S. coelicolor* was also evaluated in a glasshouse with beds containing tropical plants, at the Swedish University of Agricultural Sciences, Alnarp. The same type of traps was used as described for the field experiment, with unbaited traps as the control. The glasshouse was subdivided in five plots, with each plot receiving five replicates of bacterial baited trap and control. The traps were collected 4 d after their placement and the number of springtail captures was recorded. The experiment was repeated four times on consecutive dates, resulting in 100 traps per treatment in total.

Generalized linear mixed models were used to analyse the effect of trap bait on the captures of springtails, insects and arachnids from the field experiment. A negative binomial distribution was used to correct for overdispersion of data when initially analysed with Poisson's distribution[49]. Models included treatment as the explanatory variable (*S. coelicolor*, agar substrate and unbaited traps). The site and date of collection were included as random factors. Similarly, we also analysed the data from the glasshouse experiment using generalized linear mixed models, with negative binomial distribution. Treatment was included as the explanatory variable (*S. coelicolor* and unbaited traps), whereas plot and date were added to the models as random factors. The significance of the explanatory variable was tested with type II Wald χ^2 statistics. Bonferroni's correction was applied when pairwise comparisons were performed. All analyses were carried out in R (v.3.3.3; R Foundation for Statistical Computing) with the packages lme4, MASS and multcomp.

Collembola. We established rearing of the springtail *F. candida* Willem (Terra-Jungle). The identity of the species was confirmed by sequencing of the cytochrome *c* oxidase subunit 1 showing 99% sequence similarity with *F. candida* in a BLAST search on the National Center for Biotechnology Information database. A few experiments (tests involving non-sporulating *whiG* strain or complemented double-mutant strain J2192) were performed with a different colony of *F. candida* originating from a laboratory culture at Aarhus University, Denmark[50]. The rearing was maintained in darkness at 20 °C, on a mixture of gypsum plaster and charcoal (Sigma) (9:1, v:v), kept in sealed Petri dishes. Once a week a pinch of semi-artificial springtail food (Terra-Jungle) was added to the dishes, while distilled water was added every second day to retain moist. Preliminary experiments indicated that *F. candida* was behaviourally more active when previously kept in a soil substrate. Springtails used for behavioural assays were therefore kept on commercial soil substrate (Kronmull, Weibull Trädgård AB) in high Petri dishes (6.5-cm high and 12-cm diameter) and transferred to gypsum–charcoal plaster without food 24 h before testing.

Volatile collections. Open plates of *S. coelicolor* cultures were individually enclosed in 500-ml PET cooking bags (Toppits). A stream of charcoal-filtered air (50 ml min^{-1}) was pulled over the headspace of the bacterial cultures, leaving the bag through an air filter adsorbing the emitted bacterial volatile compounds. Filters were made of glass tubes (40-mm length, 0.3-mm inner diameter (ID)) containing 35 mg Porapak Q (80/100 mesh, Altech) held between glass wool plugs. Before sampling, filters were rinsed with 2 ml redistilled methanol and 2 ml redistilled heptane. Volatiles were collected for 23 h at 22 ± 2 °C and then eluted from the filters with 0.8 ml redistilled heptane. For normalization of quantitative chemical analyses, the bacterial biomass, grown on agar medium covered with a cellophane membrane, was scraped off from the agar medium and the dry weight determined after drying overnight at 80 °C. Moreover, heptyl acetate was added as an internal standard for the quantification of metabolites produced by mutants, but not applied to material tested in the bioassays.

Chemical analysis. Headspace collections were analysed by coupled GC–MS (6890 GC and 5975 MS; Agilent Technologies), operated in the electron impact ionization mode at 70 eV. The GC was equipped with a HP-5MS (Agilent Technologies) fused silica capillary column (60 m × 0.25 mm^2; d_f = 0.25 µm). Helium was used as the carrier gas at an average linear flow rate of 35 cm s^{-1}. Of each sample, 2 µl was injected (splitless mode, 30 s, injector temperature 225 °C). The GC oven temperature was programmed from 50 °C (2-min hold) at 8 °C min^{-1} to 230 °C (10-min hold). The transfer line between the GC and MS was programmed to hold at 150 °C and to track in synchrony with the GC oven above that temperature. Compounds were tentatively identified by matching their mass spectra with those in the MS Libraries (NIST 11, Wiley) using the software ChemStation (MSD ChemStation D.01.02.16 Agilent Technologies), and further verified by co-injection of reference compounds (except germacradienol) and comparison against published Kovats retention index values and mass spectra[51–54].

EAG. Coupled GC–EAD analysis was performed to identify the key bacterial odour components that elicit antennal responses in *F. candida*. Agilent 6890N gas chromatograph (Agilent Technologies), equipped with a HP-5 capillary column (30 m × 0.32 mm^2; d_f = 0.25 µm; J&W Scientific), was used with on-column injection mode. The oven temperature was programmed as follows: 50 °C for 1 min, then 15 °C min^{-1} up to 230 °C and 5 min isothermal. Helium was the carrier gas at constant flow rate of 45 cm s^{-1}. The GC effluent was split equally (Gerstel Graphpack 3D/2 crosspiece) in half, allowing simultaneous signal detection at a flame ionization detector (FID) (280 °C) and a heated EAD port (220 °C) (transfer line, Syntech). At the EAD port, the capillary effluent was delivered to the antennal preparation in a stream of charcoal-filtered and humidified air (1 l min^{-1}) in a glass tube (150 × 8 mm^2). The head of a randomly selected adult female *F. candida* was excised and inserted into a pulled glass capillary (ID 1.17 mm, Syntech) filled with Ringer solution[55] and attached to a reference silver/silver chloride electrode held in a micromanipulator (MP-15, Syntech). The antennal signal was amplified 10×, converted to a digital signal by a high-input impedance DC amplifier interface (IDAC-232, Syntech) and recorded simultaneously with the FID signal using GC–EAD software (GC–EAD 2000, v.1.2.3, Syntech). For every recording a new antennal preparation was used and 2 µl *S. coelicolor* strain M145 volatile collection extract was injected into the GC. In total six GC–EAD recordings were averaged to analyse for consistent antennal responses. The GC–EAD active compounds were also compared with authentic standards. The quantity of each compound was calculated on the basis of the peak area and calibrated by comparison with decyl acetate as the internal standard.

Antennal responses of geosmin and 2-MIB from bacterial headspace collections were further verified by EAG, puffing nanogram amounts of authentic compounds on to the antennae. Geosmin and 2-MIB were applied at 10 and 100 ng in *n*-hexane on a filter paper disc (12.7 mm diameter; Schleicher & Schnell GmbH), which was then placed into a Pasteur pipette. The blank (empty filter paper), solvent blank (filter with *n*-hexane) and test compound stimuli were randomized and tested in eight replicates on female antennae. The stimulation time was 0.5 s followed by a 1-min recovery period. For the analysis, the same instrument, odour delivery system and mounting technique were used as described in the previous paragraph. EAG amplitudes to the tested compounds were log-transformed, to meet the assumptions for parametric tests and analysed using linear mixed-effect models. Models included stimulus as the explanatory variable, although antenna was added as a random factor. Tukey's post-hoc test was used to perform pairwise comparisons within the different stimuli, with Bonferroni's correction. Wald's tests were carried out to test the significance of the explanatory variable, whereas all statistical procedures were conducted using the R packages lme4, MASS, car and multcomp.

Y-tube olfactometer assay. Before testing, springtails were starved for 24 h in new Petri dishes lined with the mixture of gypsum plaster and charcoal (9:1, v:v), moistened with distilled water. Springtails were tested for attraction to volatile collections of different *S. coelicolor* strains, geosmin and 2-MIB in a Y-shaped olfactometer (see schematic drawing in Fig. 1d) made of glass tubing (8-mm ID) designed on the basis of a similar type of olfactometer described by Bengtsson et al.[56]. The olfactometer had a 30-mm-long base and 40-mm-long arms (at an angle of 45°). Each side arm was connected to a glass cylinder (60-mm long, 20-mm ID), which served as an odour-release compartment. Both arms were connected to a charcoal filter. The base of the Y-tube was connected with Teflon tubing to a flow metre and then to a suction pump generating a flow of filtered air (12 ml min^{-1}) through the two arms, with the odour-release compartments to the base. A piece of gauze was interjected between the tip of the base and the Teflon tubing to prevent animals from escaping. On the top, at a distance of 1.5 cm from the base end, the olfactometer had a hole of 3-mm ID through which springtails were introduced. After introducing a single springtail, the hole was closed with a Teflon plug. The position of the springtail was recorded for 10 min or until the springtail entered into a side arm, which was counted as a response. Assays were performed in a climate chamber at 25 °C and 60 ± 5% relative humidity, under diffuse dim light (5 lux). All headspace samples and geosmin were diluted in redistilled heptane, whereas 2-MIB was diluted in redistilled hexane. Headspace samples were diluted to certain concentrations of geosmin, 2-MIB or chalcogran (used to equilibrate samples of the *ΔgeoA ΔmibAB* mutant) and tested at the doses shown in Extended Data Fig. 2. Of the odour sample or solvent (heptane or hexane) as a control, 5 µl was loaded on to filter papers (1 cm^2) and inserted into the odour-release chambers. Each day the arms delivering the odour samples or control were switched and each olfactometer was used for only 60 min (approximately five to seven replicates). The total number of replicates for each test is given in Extended Data Fig. 2. Odour attraction of springtails was analysed by an exact binomial test for the hypothesis that the true probability of success equals 0.5.

Performance tests of *F. candida*. Survival was measured for individual *F. candida* adults, which were kept for 10 d in glass tubes (1.6 × 10 cm^2) sealed with metal lids (Kapsenberg caps) containing 1-week-old cultures of *S. coelicolor* strain M145 completely covering the SFM agar medium (inoculation with 6.6×10^7 CFUs; *n* = 31). All springtails were kept without food for 24 h before experimentation. A control group was tested in glass tubes with pure SFM agar medium (*n* = 23). To reduce the development of microbial contamination in the control treatment, the springtails were transferred every third day to glass tubes with fresh SFM medium. Preliminary experimentation revealed no mortality of springtails due to the transfer process. In addition to survival, the time of ecdysis, the time of oviposition and the total number of eggs produced per adult were also recorded and analysed. Average survival, oviposition and ecdysis times were calculated and the treatment versus control was compared using the Kaplan–Meier survival analysis[57]. The average numbers of eggs was compared using likelihood ratio tests produced by general linear model procedures, with a negative binomial distribution, for a level

of significance $\alpha = 0.05$, whereas all statistical procedures were conducted using the R packages MASS, car and survival.

RNA isolation and DNA microarray analysis. RNA isolation and microarray transcriptional profiling of the *S. venezuelae* developmental RNA time courses were performed as described previously[58,59]. The resulting data were processed as described by Bush et al.[48].

ChIP, library construction, sequencing and ChIP–seq data analysis. For WhiH, wild-type *S. venezuelae* (American Type Culture Collection, strain B-65442) and its derivative SV8-pIJ6793 (*whiH::apr attBΦBT1::whiH-3xFLAG*) were grown in maltose yeast extract liquid sporulation medium and ChIP was conducted using anti-FLAG M2 gel suspension (Sigma-Aldrich, catalogue no. A2220), as described previously[48]. For BldM, wild-type *S. venezuelae* and its congenic *bldM* null mutant (SV13) were grown in the same way, and ChIP was conducted using an anti-BldM polyclonal antibody, as described previously[20]. Library construction and sequencing were performed as described previously[48] by the Earlham Institute, Norwich Research Park Norwich, UK. The ChIP–seq data were analysed as described by Bush et al.[48].

Spore vectoring, feeding and dispersal assays. We investigated the possible mode of spore dispersal by *F. candida*. First, we studied whether springtails transmit spores via their outer body. Two groups of *F. candida* adults, kept without food for 24 h before experimentation, were exposed for 1 h at 25 °C to sporulating cultures of *S. coelicolor* on SFM substrate. Individuals of the first group were used for SEM. The animals were immersed in acetone for 15 min and then left to air dry. The dried samples were carefully glued on to SEM stubs, and sputter coated with gold (Cesington 108 auto, 45 s, 20 mA). The preparations were examined using a scanning electron microscope (Hitachi SU3500) at 5 kV. Individuals of the second group (ten replicates) were transferred singly to 50 µl 0.05% Tween-20 and vortexed gently for 30 s to obtain suspensions of spores that were washed off the springtail bodies[60]. The suspensions were diluted, plated on SFM substrate and incubated to estimate the number of spores (CFUs) that were attached to the body surface of the springtails.

Next, we tested whether springtails disperse viable spores through their faeces. *S. coelicolor* cultures were grown on SFM medium covered with cellophane membranes[47], to provide springtails with sporulating *S. coelicolor* biomass free from remnants of nutrient agar. From sporulated *S. coelicolor* cultures (8 d of incubation at 27 °C), the bacterial biomass was scraped off the cellophane surface using an inoculation loop. Groups of *F. candida* adults previously kept without food for 24 h were transferred on to a Petri dish lined with plaster, and provided with a wad of *S. coelicolor* biomass corresponding to a quarter of the culture from a cellophane disc (providing biomass to feed on freely). Springtails were left to feed on the bacteria for 48 h. As *F. candida* is whitish and to a large extent transparent, the feeding activity could be confirmed by a colour change of their intestines to the dark grey of the bacterial biomass (see Extended Data Fig. 3a). After the feeding period, single springtails were transferred to 1.5-ml Eppendorf tubes containing 50 µl 0.05% Tween-20 and vortexed gently for 30 s to remove spores from their cuticle[60]. No significant number of spores was detected after repeated Tween-20 washings, showing that the washing procedure was efficient. The springtails were then transferred individually into Petri dishes (3.5-cm diameter) lined with SFM agar and incubated for 3 h at 20 °C in darkness. Subsequently faecal pellets produced by each individual were collected and smeared on to new SFM agar plate. After an incubation period of 8 d at 27 °C, colonies of *S. coelicolor* deriving from the faecal pellets were recorded.

To compare dispersal of a sporulating with that of a non-sporulating strain, *S. coelicolor* strain M145 and the congenic *whiG* mutant strain J2400 were cultivated on SFM medium for 10 d. Both strains had formed a dense lawn of mycelia, but only strain M145 had formed spores, whereas strain J2400 produced only vegetative and aerial hyphae. A $2 \times 2\,cm^2$ piece of the mycelial lawn of each strain was scraped off and used to feed 12 springtails (starved for 24 h) for 3 d in a plaster-lined Petri dish. Each springtail was then washed in 0.05% Tween-20 as described earlier. The washing liquid was plated on SFM medium to determine the number of CFUs washed off per animal. The washed springtails were then kept individually on mannitol minimal medium agar for 20 h to allow time for defecation. Springtails were then removed and the number of excreted CFUs per plate that formed on incubation at 30 °C was determined. Numbers of CFUs originating from treatment with sporulating and non-sporulating strains were compared using the Mann–Whitney test.

Furthermore, we monitored grazing by *F. candida* on *S. coelicolor* in soil microcosms. *S. coelicolor* strain J2192/pIJ10646 was grown to a sporulating lawn on mannitol minimal medium, and agar plugs with bacterial lawn on surface were excised and placed in 18 g autoclaved potting soil (Weibulls) in plastic cups (9-cm diameter). Ten *F. candida* individuals were added to the soil of one set of cups, and no springtails to the other. Appearance of the bacterial lawn was monitored and photographed after 6 d of incubation at room temperature.

Finally, we monitored the effect of geosmin and 2-MIB emitted by *S. coelicolor* bacteria on spore dispersal by springtails. A droplet (5 µl) of spore suspension (5×10^8 CFU ml^{-1}) of either *S. coelicolor* M145 (wild-type) or J2192 (*ΔgeoA ΔmibAB*) was added at a distance of 3.5 cm from the centre on to a Petri dish (9-cm diameter) containing SFM agar. The droplet was left to soak into the agar for 30 min, to form a round film of spores on the surface of the SFM agar. Three *F. candida* adults that were kept without food for 24 h were transferred to the centre of the dish, and left to move freely for 30 min in the dark at room temperature. Springtails were then removed and the plates were incubated at 27 °C for 5 d. Each treatment was replicated 25 times and, after the incubation period, the number of newly formed colonies (not contacting the area of the initial 5-µl inoculum) and the distance between inoculum and the farthest newly formed colony were measured. For the comparison between different treatments, general linear model procedures were used to produce the analysis of deviance for both the number of newly formed colonies and the distance, with a normal distribution for distance data and quasi-Poisson's distribution to correct for overdispersion of the number of newly formed colonies. Data corresponding to the distance were initially analysed using Bartlett's test to check for homogeneity of variances, whereas normality of the residuals and the Q–Q plots were checked through visual inspection.

To determine whether *in trans* complementation with *geoA* and *mibA–mibB* improved the ability of the double-mutant strain J2192 to be dispersed by springtails in agar plate assays, strain J2192/pIJ10770 (carrying an integrated empty vector) and strain J2192/pIJ10646 (with the same vector-carrying *geoA* and *eshA–mibA–mibB* genes) were inoculated with the same number of spores (5×10^3) in a 10-µl drop on mannitol minimal medium agar plates, and incubated to allow formation of a sporulated patch in the middle of the plate. The two strains were found to produce similar amounts of viable spores in such patches, and harvested spores of the strains germinated with similar efficiency ($77 \pm 20\%$ and $74 \pm 17\%$ viability, respectively). To each plate two starved springtails were added and allowed to roam for 2 h, before they were removed. Plates were incubated for 8 d to allow new colonies to develop, and the number of dispersed colonies per plate were determined and compared using the Mann–Whitney test.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The transcriptional profiling data from Affymetrix arrays and the ChIP–seq data in Fig. 3 have been deposited at the ArrayExpress Archive of Functional Genomics Data (http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-5853 for the *whiH* mutant array data, E-MTAB-2716 for the *bldM* mutant array data, E-MTAB-6702 for the WhiH ChIP–seq data and E-MTAB-2698 for the BldM ChIP–seq data). Source data for Figs. 1a,b,e,f,g and 4b,c and Extended Data Figs. 1a,b,c, 4, 5 and 6a,b,c are included in this letter and its Supplementary Information files. Other data that support the findings of the present study are available from the corresponding authors upon reasonable request.

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Author contributions

M.J. Buttner, P.G.B. and K.F. designed the research. V.V. performed the field trap experiments and statistical analyses. V.V. and P.G.B. did the work involving springtails

and analyses of volatiles; together with E.B. and K.F. they performed the spore dispersal assays. B.P.M. performed the GC–EAD experiments. M.J. Bibb and M.M.A.-B. performed the time-resolved transcriptional profiling, M.J. Bibb, M.J. Bush and M.M.A.-B. performed the ChIP–seq experiments. G.C. analysed the ChIP–seq data. M.J. Bibb and M.J. Bush constructed mutants and plasmids. E.B. and K.F. identified WhiH as a regulator of *geoA*. L.S. and G.L.C. performed initial GC–MS analyses of the *Streptomyces* mutants. P.G.B. and K.F. wrote the manuscript together with V.V. and M.J. Buttner. All the authors discussed the results and commented on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Supplementary information is available for this paper at https://doi.org/10.1038/s41564-020-0697-x.

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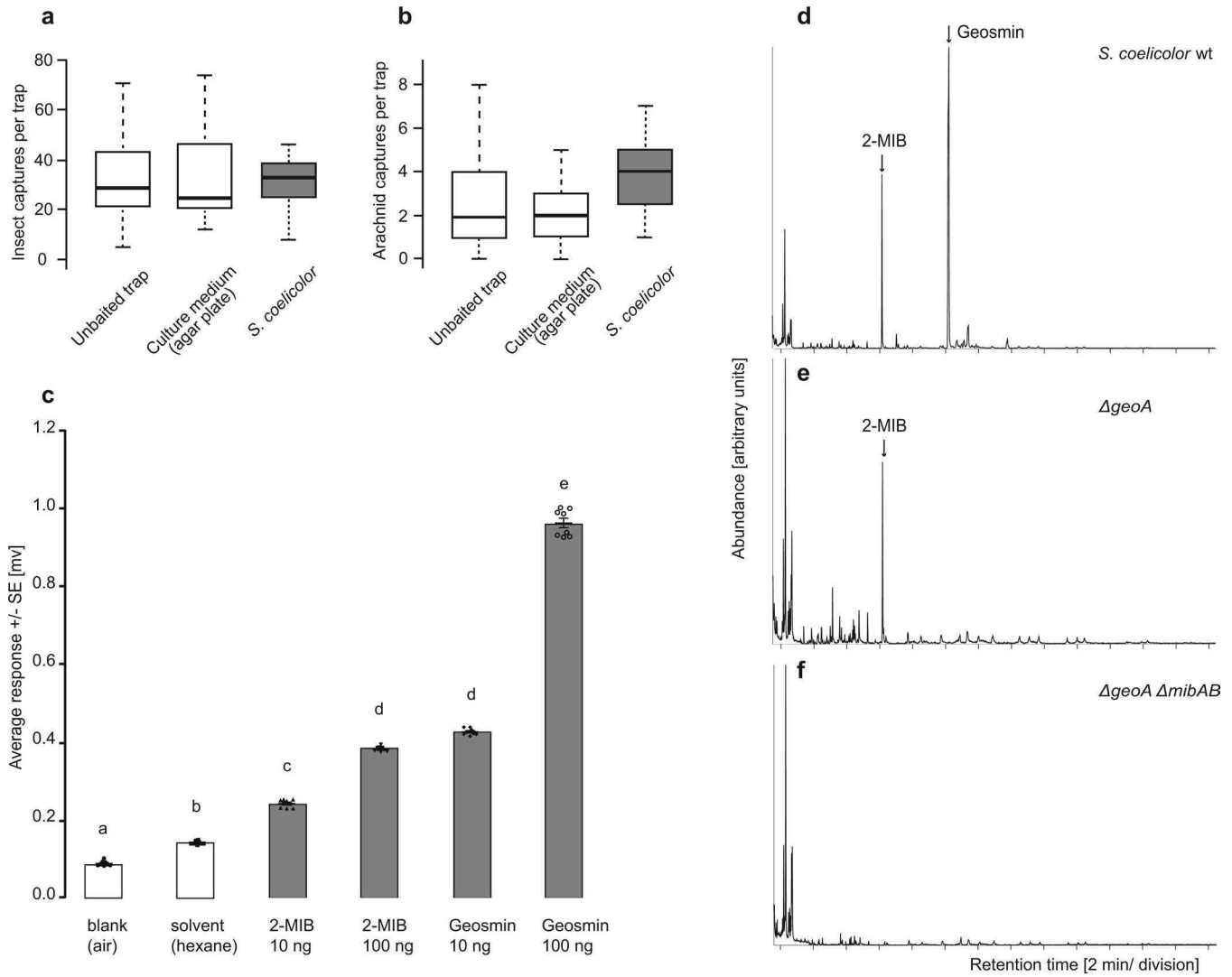


Extended Data Fig. 1 | Effects and production of volatiles from *Streptomyces coelicolor*. Captures of insects (**a**) and arachnids (**b**) on the ground of a field site using sticky traps baited with *Streptomyces coelicolor* cultured on agar medium, compared to control traps that were kept without bait or held a fresh, non-inoculated plate of agar culture medium (n=19). The boxplots show the medians, lower- and upper quartiles as well as the span of data points that are within 1.5 times the interquartile range. Electroantennographic responses (**c**) towards 2-MIB or geosmin puffed into an air stream passing over antennae of the springtails *Folsomia candida*. The average responses to all tested stimuli (n=8 per stimulus) significantly differed from the antennal response to charcoal filtered air. Statistical difference is labelled by different letters above the columns; error bars show the standard errors ($X^2_{5,48}$ =629.54, P<0.001). GC-MS chromatograms (**d**) of *S. coelicolor* wild type and two biosynthetic mutants show that geosmin or geosmin and 2-MIB, respectively, are missing from the *geoA* and *geoA mibAB* mutants (strains J3003 and J2192, respectively). The headspace collection for the specific strains was repeated three times with similar results.

Absolute amounts (ng) of geosmin and 2-methylisoborneol (2-MIB) tested as individual compounds or as components of *Streptomyces* headspace in the Y-tube choice assay.

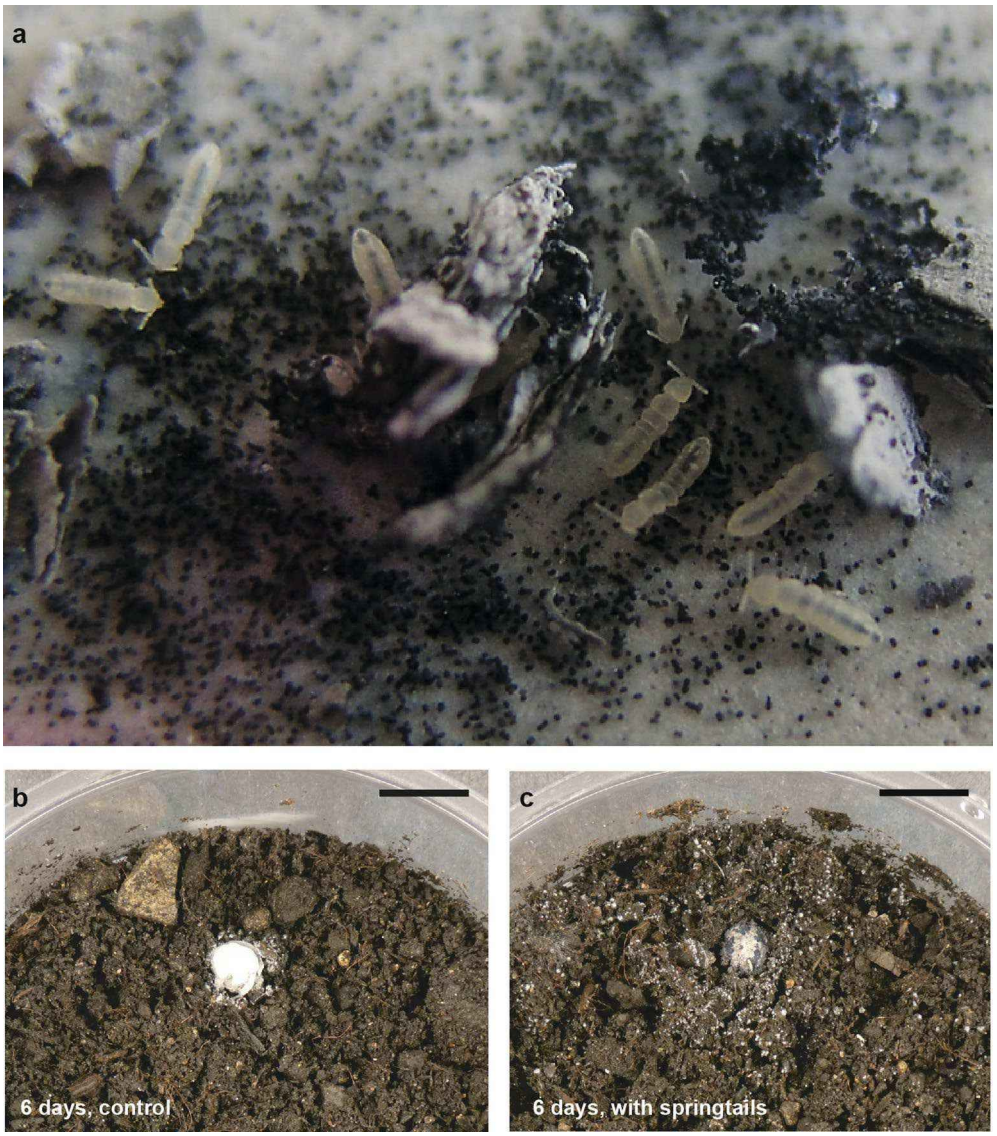
Stimulation	Stimulus tested (bold) or compound used for equilibration of headspace (ng)			Number of tested animals	Number of responding animals (%)[†]
	Geosmin	2-MIB	Chalcogran*		
Tests with one stimulus (sample *vs* control)					
geosmin	**1**	-	-	55	34 (62%)
2-MIB	-	**1**	-	53	29 (55%)
Headspace wild-type[‡]	**0.2**	**0.021**	n.d.	66	41 (62%)
Headspace *ΔgeoA*[‡]	-	0.021	n.d.	74	25 (34%)
Control	-	-	-	70	31 (44%)
Tests with two stimuli (sample 1 *vs* sample 2)					
Headspace wild-type *vs* *ΔgeoA*[‡]	**0.2** *vs* 0	0.007 *vs* 0.007	n.d.	58	29 (50%)
Headspace *ΔgeoA vs ΔgeoA ΔmibAB*[‡]	0 *vs* 0	**3.15** *vs* 0	1.2 *vs* 1.35	46	27 (59%)

*Chalcogran was used for adjusting the amounts of headspace when testing the *ΔgeoA ΔmibAB* mutant.

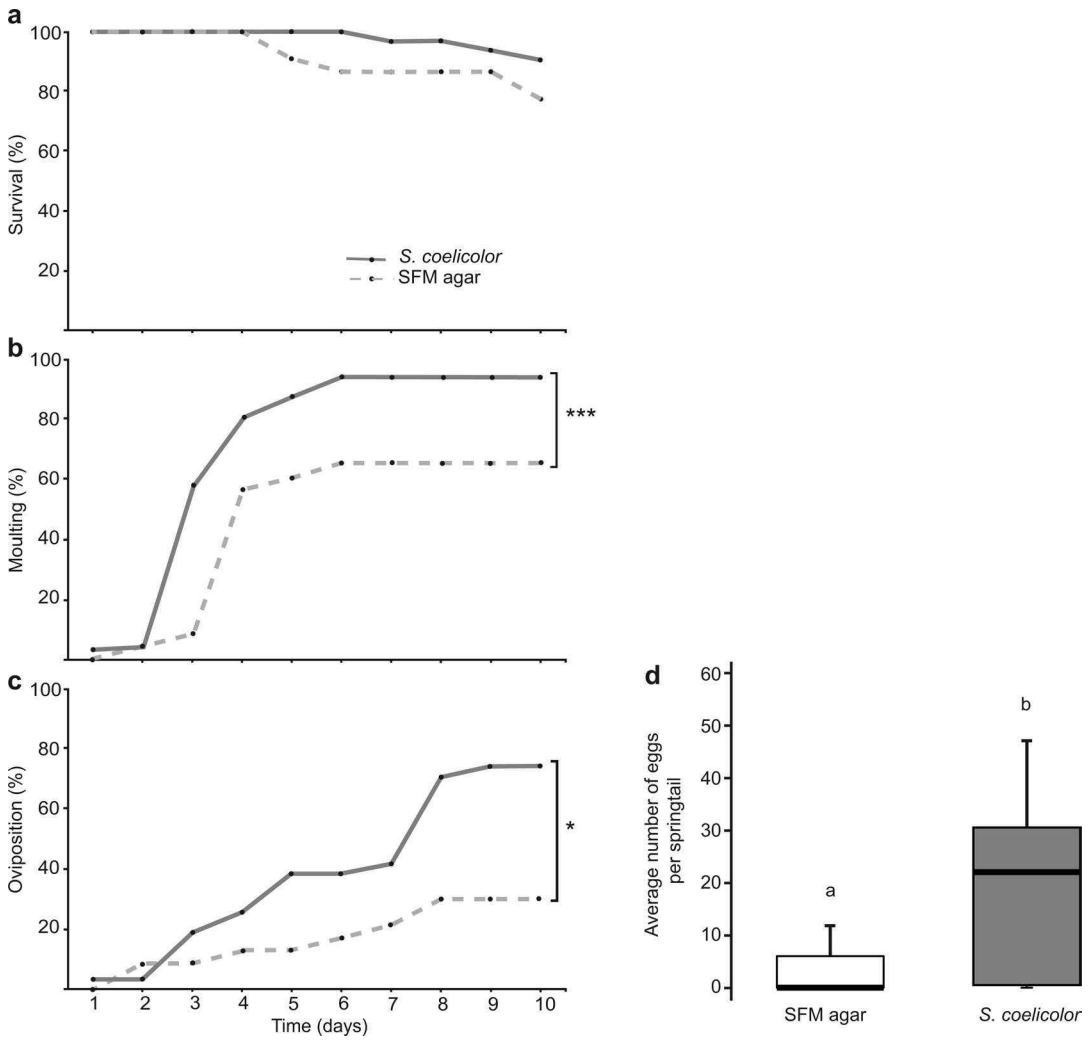
[†]Only animals responding (i.e. walking upwind and choosing one of the arms of the Y-tube) were considered for analysis.

[‡]Headspace collected *S. coelicolor* strains M145 (wild-type), J3003 (*ΔgeoA*), and J2192 (*ΔgeoA ΔmibAB*).

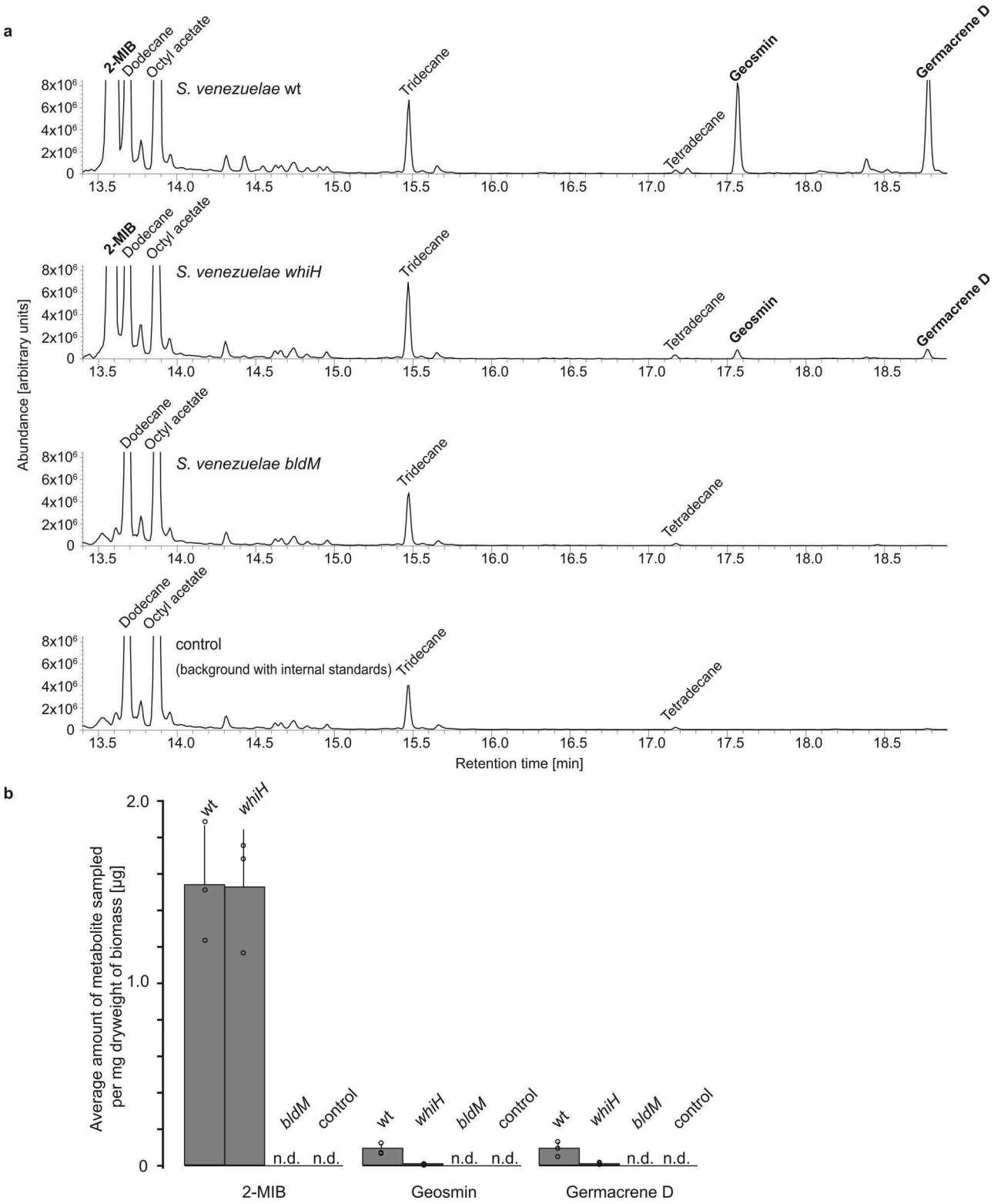
Extended Data Fig. 2 | Absolute amounts (ng) of geosmin and 2-methylisoborneol (2-MIB) tested as individual compounds or as components of *Streptomyces* headspace in the Y-tube choice assay.



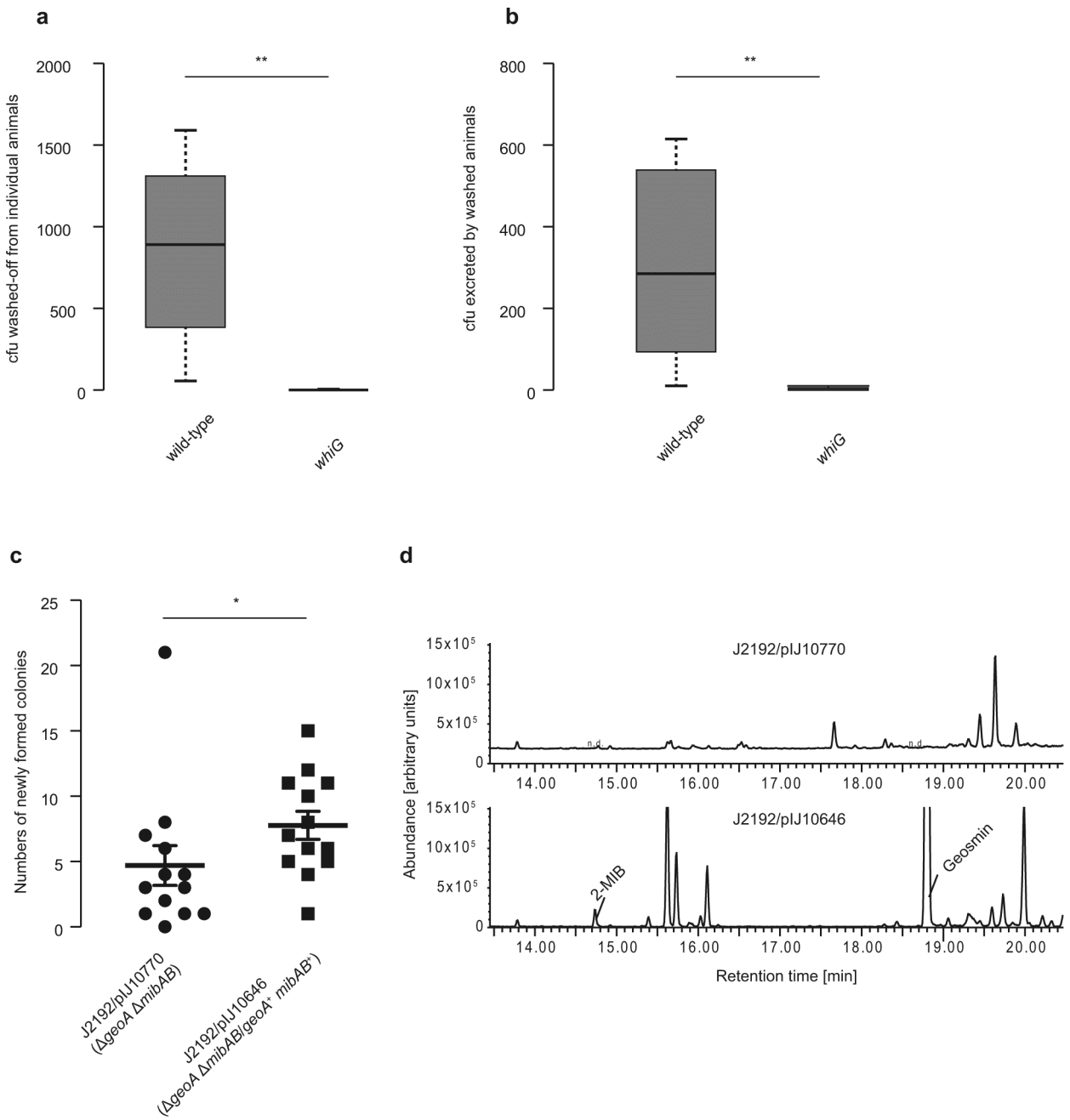
Extended Data Fig. 3 | *Folsomia candida* springtails feed on *Streptomyces coelicolor* mycelium. (**a**) *Folsomia candida* springtails feeding on sporulating *Streptomyces coelicolor*. The bacteria were grown as lawns on agar covered with cellophane membranes, and then scraped off and transferred to *F. candida* kept on a Petri dish lined with gypsum plaster. The photo shows the dark bacterial biomass visible inside the guts of the animals as well as faecal pellets covering the plaster. (**b**, **c**) Agar plugs with developed colonies of *S. coelicolor* strain M145 were placed in containers with autoclaved potting soil, either without springtails (**b**), or with 10 *F. candida* individuals (**c**). After 6 days of incubation, the colonies in soil without springtails remained intact, while the colonies in soil with springtails were consumed by grazing of the animals. Control experiments showed that the animals did not graze on uninoculated agarose plugs (not shown). Scale bars, 10 mm. Similar observations were made in three independent experiments. In addition to the grazing, the ability of springtails to disperse *S. coelicolor* was clearly reflected in the large number of microcolonies (seen as white spots in the photographs) developing throughout the microcosms containing *F. candida* individuals.



Extended Data Fig. 4 | *Folsomia candida* springtails are positively affected by being fed *Streptomyces coelicolor* mycelium. Survival, moulting and oviposition of female *F. candida* kept for 10 days on sporulating cultures of *S. coelicolor* (n=31, grey solid lines) or SFM agar controls (n=23, light grey dashed lines). Survival on *S. coelicolor* was as high as on SFM control (**a**, $X^2_{1,54}$ =1.7, P=0.195). Exposure to *S. coelicolor* had a positive effect on the initiation of moulting (**b**, $X^2_{1,54}$ =11, P<0.001***) and oviposition (**c**, $X^2_{1,54}$ =6.4, P=0.011*) with more eggs being laid (**d**, $X^2_{1,54}$ =6.064, P=0.0138*; the boxplot shows the medians, lower- and upper quartiles as well as the span of data points that are within 1.5 times the interquartile range).



Extended Data Fig. 5 | Effects of developmental regulatory genes *bldM* and *whiH* on production of geosmin and 2-methylisoborneol (2-MIB) in *Streptomyces venezuelae*. (**a**) Gas chromatograms analysed for the presence of 2-MIB, geosmin and germacrene D in *Streptomyces venezuelae* headspace sampled from wild-type, *whiH*, *bldM* mutants or a system control. The chromatograms and the quantification of *Streptomyces* metabolites (**b**; n=3; error bars give the standard deviation of the mean) illustrate that in comparison to wild-type, geosmin and germacrene D were reduced in the *whiH* mutant. None of the three *Streptomyces* metabolites was detected (n.d.) in the *bldM* mutant or the control. C12 –C14 hydrocarbons were used for calculation of retention indices. Octyl acetate was introduced with the internal standard heptyl acetate (not shown), which was used for quantification.



Extended Data Fig. 6 | Assays of spore adherence and dispersal. To compare dispersal of sporulating and non-sporulating strains, individuals of *F. candida* (n=12) were fed for 3 days with developed mycelium of *S. coelicolor* strain M145 or its congenic non-sporulating *whiG* mutant J2400. CFUs that could be washed off (0.05% Tween-20) from individual animals were quantified by plating on SFM agar. Significantly more spores than hyphae had adhered to the springtail body (**a**, two-tailed Mann Whitney test, P<0.001 **). To monitor the release of viable cells by defaecation, each washed springtail was allowed to roam for 20 h on MM agar before colonies were allowed to develop. Significantly more CFU were excreted from springtails that had been feeding on the sporulating *Streptomyces* strain (**b**, two-tailed Mann Whitney test, P<0.001 **). The boxplots show the medians, lower- and upper quartiles as well as the span of data points (smallest to largest). In a dispersal assay (**c**), *S. coelicolor* strains J2192/pIJ10646 (*geoA mibAB* mutant with *geoA* and *mibAB* genes provided *in trans* on integrated plasmid) and J2192/pIJ10770 (*geoA mibAB* mutant with empty vector) were allowed to develop and sporulate (5 days) in small spots in center of MM agar plates. Two springtails (*F. candida*) (n=13) were released for 2 h on each plate. Animals were then removed, plates incubated, and *Streptomyces* colonies counted. The presence of *geoA* and *mibAB* genes (strain J2192/pIJ10646) resulted in significantly more CFU being dispersed by springtails (**c**, two-tailed Mann Whitney test, P=0.022 *). Error bars show the standard error of the mean. Volatiles from cultures of these strains were collected for 24 h on an air filter, eluted with hexane, and analysed by GC-MS. Chromatograms (**d**) show that geosmin and 2-MIB were present in strain J2192/pIJ10646 but not detectable (n.d.) in the non-complemented J2192/pIJ10770 strain. The analyses were independently repeated three times with similar results.

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Software and code

Policy information about availability of computer code

Data collection	Statistical analyses: Analyses were carried out in R (v. 3.3.3; R Foundation for Statistical Computing, Vienna, AT). GC-MS analysis: Before verification with reference compounds, bacterial volatiles were tentatively identified by matching their mass spectra with those in the MS Libraries (NIST 11, Wiley) using the software ChemStation (MSD Chemstation D.01.02.16 Agilent Technologies). Electroantennography (EAG and EAD): Antennal signals were recorded using a GC-EAD software (GC-EAD 2000, version 1.2.3, Syntech). Transcription Profiling: Affymetrix Arrays were scanned as described by the manufacturer and further processed by software on the scanner. This resulted in .cel files. ChIP-Seq: ChIP and total genomic DNA was sequenced by a sequencing contractor and reads received as fastq files.
Data analysis	All analysis was carried out on the Linux (Fedora 27) platform. Bash and Perl scripts were used for general purpose file manipulations. Statistical data analysis was carried out on the most recent version of R and BioConductor available at the time.

Transcription Profiling:
Data in .cel files was normalized using RMA as implemented in the Bioconductor package "affy" of R. Replicates were combined using the lmFit and eBayes functions of the Bioconductor package "limma". The resulting expression value (log2) were written to a text file.

ChIP-Seq:
R BioConductor packages Rsubread and edgeR were used for ChIP-Seq data analysis.

GC-MS analyses:
Before verification with reference compounds, bacterial volatiles were tentatively identified by matching their mass spectra with those in the MS Libraries (NIST 11, Wiley) using the software ChemStation (MSD Chemstation D.01.02.16 Agilent Technologies).

Statistics:
Statistical procedures were conducted using R version 3.3.3 and packages specified in the manuscript.

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The transcriptional profiling data from Affymetrix arrays and the ChIP-Seq data in Fig. 3 have been deposited at the ArrayExpress Archive of Functional Genomics Data (http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-5853 for the whiH mutant array data, E-MTAB-2716 for the bldM mutant array data, E-MTAB-6702 for the WhiH ChIP-Seq data, and E-MTAB-2698 for the BldM ChIP-Seq data). Source data for Figs. 1a,b,e,f,g and 4b,c and Extended Data Figs. 1a,b,c, 4, 5 and 6a,b,c are included in this article and its Supplementary Information files. Other data that support the findings of this study are available from the corresponding authors upon reasonable request.

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Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Pre-study estimations were done for all tests based on simulation of hypothetical data sets, based on expected data variability and based on experience/previous studies. For analysis of data, test assumptions like data independence, distribution and variances were considered.
Data exclusions	None. Few samples in field and glass house studies could not be analysed (no data) as they got damaged by irrigation or lost for other reasons. One sample also got lost in the laboratory dispersal assay (springtails escaped). Lost samples are mentioned in source data files.
Replication	Biological triplicates for Affymetrix. Biological duplicates for ChIP-Seq. Replication is specifically stated for all other experiments.
Randomization	For field and glasshouse tests, a random number generator was used to decide upon the location of the treatments in the plots; plot and date treated as random factors in the analyses. For all laboratory experiments with springtails, animals were selected randomly from the culture. For EAG testing, test compound stimuli were randomized.
Blinding	CFU counts for different strains applied in springtail dispersal assays were done blind with respect to strain phenotype (with/without voaltile release). For the Y-tube assays data collection could not be blinded because of the nature of the assay. For the large-scale microarray adn ChIP-seq analyses, blinding is irrelevant for data analysis.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☒	☐ Unique materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☐	☒ Research animals
☒	☐ Human research participants

Antibodies

Antibodies used

For anti-FLAG-WhiH ChIP-seq, protein-DNA complexes were immunoprecipitated using ANTI-FLAG® M2 Affinity Gel (Sigma cat # A220). For BldM ChIP-seq, protein-DNA complexes were immunoprecipitated with a custom made anti-BldM polyclonal antibody, raised for us by Cambridge Biochemicals Ltd using purified BldM protein provided by us.

Validation

The use of ANTI-FLAG® M2 Affinity Gel (Sigma cat # A220) in ChIP-Seq experiments with S. venezuelae is well established and has been validated in a series of previous publications (Bush MJ, et al., 2013. mBio 4:e00684-00613; Bush MJ, et al., 2016. mBio 7:e00523-00516; Bush MJ, et al., 2017. Mol. Microbiol. 104:700-711.
Similarly, the use of specific batch of anti-BldM polyclonal antibody has been established and validated in Al-Bassam MM, et al., 2014. PLoS Genet. 10:e1004554.

Research animals

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Animals/animal-derived materials

Only springtails and insects were tested or sampled in this study. Insects are legally not considered as test animals. For laboratory experiments we used females (parthenogenetic species) of the springtail Folsomia candida. Trap catches of insects, springtails and spiders from the field and greenhouse experiments were not determined to species level.

Method-specific reporting

n/a	Involved in the study
☐	☒ ChIP-seq
☒	☐ Flow cytometry
☒	☐ Magnetic resonance imaging

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
May remain private before publication.

WhiH:
Affymetrix data: http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-5853
ChIP-Seq data: http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-6702

BldM:
Affymetrix data: http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-2716/
ChIP-Seq data: http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-2698

Files in database submission

E-MTAB-5853.processed.1.zip
E-MTAB-5853.raw.1.zip
E-MTAB-5853.idf.txt
E-MTAB-5853.sdrf.txt
E-MTAB-5853.idf.txt_original

E-MTAB-6702.processed.1.zip
E-MTAB-6702.processed.2.zip
E-MTAB-6702.idf.txt
E-MTAB-6702.sdrf.txt
E-MTAB-6702.idf.txt_original

E-MTAB-2716.processed.1.zip
E-MTAB-2716.raw.1.zip
E-MTAB-2716.idf.txt

E-MTAB-2716.sdrf.txt

E-MTAB-2698.processed.1.zip
E-MTAB-2698.idf.txt
E-MTAB-2698.sdrf.txt
For the reads of ChIP-Seq data links are provided to the ENA (European Nucleotide Archive) on the ArrayExpress web pages for these submissions.

Genome browser session
(e.g. UCSC)

Genomic sequence fasta file, genome features bed file and ChIP peak locations bedgraph file have been provided as supplementary material. These can be used to browse the locations of the ChIP peaks on a genome browser such as IGB or IGV.

Methodology

Replicates

Two biological replicates of immunoprecipitated and non-immunoprecipitated samples.

Sequencing depth

Approximately 25 million single-ended 100 nucleotides long reads for each sample. Overall alignment rate using bowtie2 was above 95 percent in all cases. High quality sequencing depth > 250 in all cases.

Antibodies

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters

bowtie2 version 2.2.9 at default settings was used for read mapping to a bowtie2 index made from the genome nucleotide sequence of S. venezuelae. The genome sequence was divided into 25 nucleotide segments and the number of reads mapping to each of these segments were counted using the featureCounts() function of the BioConductor package Rsubread. Differential analysis of the read counts was carried out using functions calcNormFactors(), estimateDisp() and exactTest() (in this order) provided in the BioConductor package edgeR.

Data quality

Reads with mapping quality of less than 40 were excluded from analysis. There are 24 peaks above 5-fold enrichment at FDR 5%.

Software

100 nucleotide single ended reads were generated on an Illumina HiSeq 2000 sequencer. Bowtie2 version 2.2.9 was used to align reads to the S. venezuelae genomic nucleotide sequence. BioConductor packages Rsubread version 1.30 and edgeR version 3.2.2 were used for counting and differential analysis of read counts mapping to 25 nucleotide sections of the genome. Gnu Parallel (https://www.gnu.org/software/parallel) was used to run commands in parallel when possible.