A wax ester promotes collective host finding in the nematode *Pristionchus pacificus*

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Survival of nematode species depends on how successfully they disperse in the habitat and find a new host. As a new strategy for collective host finding in the nematode *Pristionchus pacificus*, dauer larvae synthesize an extremely long-chain polyunsaturated wax ester (nematoil) that covers the surface of the animal. The oily coat promotes congregation of up to one thousand individuals into stable 'dauer towers' that can reach a beetle host more easily.

ematodes go through four larval stages before they mature for reproduction. These stages are designated as larva 1 (L1) to L4 or juvenile 1 (J1) to J4. However, under harsh environmental conditions, for example, high temperature, low food supply and high population density, many free-living nematodes stop developing after the second larval (J2) stage and enter into a stage called the dauer larva^{1,2}. Dauers can dwell for months without feeding. A dauerspecific cuticle covers the orifices of the animals and restricts the chemical exchange with the environment³. Dauer larvae or infective larvae (in parasitic species) are the main form of nematode dispersal, i.e., finding a new host or changing the habitat⁴. For dispersal, dauer larvae of multiple nematodes have evolved a specific behavior called nictation or waving: worms stand on their tail and wave their body, allowing for attachment to larger animal vectors⁴. Nictation of individuals and attachment to insects under laboratory conditions is also described in *P. pacificus*, a satellite genetic model organism⁵. This behavior is crucial for P. pacificus as, under natural conditions, these nematodes rely on a tight necromenic association with scarab beetles5: dauer larvae invade the host, wait for its natural death and then resume development by feeding on microorganisms that grow on the host carcass⁶.

Herein we report that *P. pacificus* nematodes synthesize a dauer stage-specific, extremely long-chain polyunsaturated wax ester that we name nematoil (from 'nematode-derived oil'). Dauer larvae secrete nematoil to the body surface as an adhesive lipid that promotes the congregation of multiple individuals into large tower-like structures that we call dauer towers. The formation of the dauer towers is part of a new host finding strategy, collective nictation, a process in which the whole dauer tower waves, presumably to maximize the chance of attaching to a new host organism.

RESULTS

P. pacificus form dauer towers for collective nictation

We have discovered that, in addition to nictating intensively as individuals, dauer larvae of *P. pacificus* also nictate collectively in crowded populations, which might facilitate host finding. Specifically, we noticed that on agar culture medium, dauer larvae often congregated in large tower-like waving masses of up to 1,000 individuals, which we refer to as dauer towers (Fig. 1a-c and Supplementary Video 1). Some towers could be seen even without a microscope and photographed with a macro lens (Supplementary Results, Supplementary Fig. 1). Reaching a height of up to 1 cm, the towers were 20-30 times higher than an individual worm. Similar structures have been reported before for Caenorhabditis elegans found in its natural habitat in the vicinity of rotting plant material7. Initially, we observed dauer towers only on plates contaminated with mold, and mold fruiting bodies served as foundations of many small towers (Fig. 1c). This indicated that in nature, vertically protruding objects such as mold fruiting bodies might be required for the formation of the towers. Indeed, when we propagated P. pacificus worms on the larvae of the beetle Tenebrio molitor or on the grubs of the moth Galleria mellonella, the dauer towers always formed on the appendages of the beetle larvae or on the bristles of the grubs, respectively (Fig. 1d,e and Supplementary Fig. 2).

Hydrophobic interaction supports the dauer towers

We found that dauer towers of *P. pacificus* were mechanically stable: they remained intact on the agar surface for at least 72 h, and we could not readily disrupt them by manipulation with a platinum wire (data not shown), suggesting a strong adhesion between the dauer larvae within the towers. We hypothesized that this adhesion is promoted by a strong hydrophobic interaction mediated by a lipid material secreted on the surface of P. pacificus dauer larvae. To test this, we made use of chemical agents that disrupt hydrophobic interactions, such as detergents and organic solvents. When we relocated a dauer tower from the agar plate to water or detergent-free buffer, the dauer larvae started actively swimming. The mechanical force of this movement was, nevertheless, not sufficient to disrupt the association between the larvae, and they remained attached to each other (Fig. 2a). When we added a detergent such as Triton X-100 to the water or buffer, the towers quickly dissociated until they were completely separated to individually swimming larvae (Fig. 2b). The dissociation took about 1-5 min. Moreover, when placed in hydrophobic organic solvent such as hexane, individual dauer towers dissociated within 1-2 s (Supplementary Fig. 3). To visualize the association between individual larvae, we stained dauer towers

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Figure 1 | Dauer towers formed by *P. pacificus* for collective host finding.
(a-c) A large upright (-200 larvae) (a), a large bended (-500 larvae) (b) and a small tower (several dozens of individuals) (c) in scale to each other. A fruiting body of a fungus (arrow) serves as a base of the small tower. On the tip of the small tower, a single waving individual can be seen (arrowhead). In a-c, scale bar represents 250 μm.
(d,e) A lower-magnification (d) and higher-magnification (e) micrograph of a dauer tower (arrowheads) formed on the carcass of a *T. molitor* beetle larva. Note that the dauer larvae use the appendages of the insect to form the tower. In d and e, scale bars represent 1 mm and 500 μm, respectively.

with unconjugated boron-dipyrromethene (BODIPY) dye that intercalates into lipophilic milieu and observed intensive staining of the surface between the cuticles of neighboring larvae (**Fig. 2c,d**). Together, these observations strongly support lipid-mediated adhesion between *Pristionchus* dauers within the towers.

Surface lipids promote the formation of dauer towers

P. pacificus dauer larvae do indeed secrete surface lipids. When we propagated them in liquid cultures, the dauer larvae showed a tendency to aggregate and float on the water (**Fig. 3a**). This resembles an observation made by Gilbert Fuchs (who also coined the term 'dauerlarva') made in 1914: dauer larvae of a diplogastrid nematode species closely related to *Pristionchus* and associated with bark beetles were adhesive owing to a surface coat made of a fatty substance ('Schützhülle')⁸. In addition, the surface of dauers was strongly stained by BODIPY (**Fig. 3b**). *P. pacificus* dauer larvae often retain the old J2 cuticle after the dauer molt, and, for an extended period, they have both J2 and dauer cuticles. When analyzed by Nomarski microscopy or electron microscopy, such dauers displayed oil droplets between the old unshed J2 cuticle and the new dauer cuticle (**Fig. 3c, d**).

To elucidate the source of the secreted lipids and the precise timing of secretion, we followed the development of J2 larvae undergoing dauer arrest. We found that during the J2 phase, a large number of BODIPY-stained lipid droplets (LDs) formed in the epidermis of



Figure 2 | Hydrophobic interaction mediates the adhesion of dauer larvae. (a) A tower placed in water does not dissociate. (b) A tower placed in water containing Triton X-100 dissociates within minutes. Photos were taken 10 min after relocation of the towers. In **a** and **b**, scale bars represent 500 μm. (**c,d**) Bright field (**c**) and fluorescent (**d**) micrographs of BODIPY-stained dauer larvae from a tower. At the interphase between the cuticles of the dauers there are BODIPY-stained layers of surface lipids (arrowheads). In **c** and **d**, scale bars represent 10 μm.

the larva in close proximity to the body surface (Fig. 3e.f). These LDs seemed to be uniformly distributed among the different cells of the epidermis (hypodermal syncytium, seam cells and so on) throughout the length of the animals (Fig. 3e,f). We found that the lipid secretion occurred shortly after the J2 to dauer molt, and, concomitantly, the sizes of the BODIPY-stained LDs decreased with secretion, suggesting that the LDs were the source of the secreted lipids (Fig. 3g,h). Figure 3i, j shows higher-magnification Nomarski images of the epidermal LDs before and after the secretion. Furthermore, we observed dauer larvae undergoing lipid secretion in situ using time-lapse Nomarski microscopy (Supplementary Video 2). After the J2-to-dauer molt, the body of dauer larvae shrinks radially9. The radial shrinkage starts at the anterior end and then progresses to the posterior end of the larvae, as shown in Supplementary Video 2. We found that the lipids were secreted from the region of the body that was undergoing radial shrinkage, and the LDs in the vicinity of the shrinking site became less reflectile. Taken together, these observations suggest that lipids contained in epidermal LDs are secreted during the radial shrinkage, which acts as a mechanical force to press the secreted lipids to the surface. So far, multiple Nomarski and electron microscopy experiments have failed to identify specific pores in the cuticle through which the lipids are secreted. Thus, the secretion probably occurs through the dauer cuticle, which is immature and more permeable shortly after the J2 to dauer molt^{1,10}.

The size and stability of the *P. pacificus* dauer towers seem to be genus specific. Although we could sporadically observe small towers also on agar plates with *C. elegans* cultures (**Supplementary Fig. 4a**), these towers were not stable and disintegrated easily when

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Figure 3 | Secreted surface lipids facilitate dauer tower formation. (a) Clusters of floating dauer larvae (arrows) in aqueous solution. Scale bar, 500 μ m. (**b**) The surface of a dauer larva is stained by fluorescent BODIPY (arrow). (c) Lipid droplets (arrowheads) formed as a result of the excessive secretion. In **b** and **c**, scale bars represent 10 µm. (d) Transmission electron micrograph of a longitudinal section of a dauer larva. The surface lipids are visible as lipid droplets (arrowheads) between the old unshed J2 cuticle and the new dauer cuticle. Scale bar, 2 µm. (e,f) Differential interference contrast (DIC) (e) and fluorescent (f) images of a BODIPY-stained J2 larva on the day before the J2 to dauer molt, before the secretion of lipids has occurred. (g,h) DIC (g) and (h) fluorescent image of a BODIPY-stained dauer larva on the day after the J2 to dauer molt, when the lipids have been secreted. Scale bars in **e-h** represent 10 μm. (**i**,**j**) High-magnification DIC images of the epidermal LDs before (i) and after (j) the secretion of lipids. Note the much bigger size of the epidermal LDs in J2 larvae that have not yet secreted the lipids (black arrowheads) compared to the LDs observable in mature dauer larvae (white arrowheads). Scale bars in **i** and **j** represent 5 μ m. (**k**) Fluorescent micrograph of a BODIPY-stained wsd-1 dauer larva. The surface of the larva is not stained (arrow), although some subcutaneous blisters are seen (triangle). Scale bar, 10 μ m. (I) Dauer towers are



not observed on plates with wsd-1 cultures. In wild-type cultures, dauer towers appear with an incidence of ~14% of the plates ($n_{\text{(plates/strain)}} = 90$; error bars show 95% binomial confidence intervals; P < 0.0001, Fisher's exact test).

perturbed with a platinum wire or following transfer to water (**Supplementary Fig. 4b**). In addition, *C. elegans* dauer larvae neither formed aggregates in water nor could be stained by BODIPY (**Supplementary Fig. 4c,d**). Thus, *Pristionchus* nematodes might have a dispersal strategy that relies on a secreted lipid.

To test the possible function of the secreted lipids in the formation of dauer towers and collective host finding, we performed a genetic screen for mutations that block the synthesis and/or secretion of the surface lipid. We isolated several mutants in which dauers did not display or displayed altered lipid secretion judged by BODIPY staining. We designated these mutants wsd (for wax synthesis/secretion defective; detailed information on the mutants will be published elsewhere). One of the mutant strains, wsd-1(tu462), was suitable for the study because of the lack of abnormalities when grown in the reproductive mode and the normal superficial morphology and behavior of the dauer larvae (data not shown). When stained with BODIPY, wsd-1 dauers did not display fluorescence on the surface owing to the lack of secreted lipids (Fig. 3k). Instead, we observed epidermal subcutaneous blisters containing BODIPY-stained lipids (Fig. 3k). The mutation abolished the adhesiveness of the dauers: they neither aggregated nor floated in water. The ability to secrete lipids correlated with the formation of dauer towers: we never observed dauer towers on plates cultured with wsd-1 (Fig. 31). In contrast, ~14% of the plates with wild-type worms contained, on average, 20-30 towers (Fig. 3l).

A wax ester is a major component of the surface lipids

We set out to identify the lipid (or lipids) involved in dauer tower formation. We developed a mild liquid-liquid extraction procedure that allowed us to obtain the surface lipids without affecting the internal body organization of dauer larvae. In short, we briefly agitated dauer larvae suspended in water with hexane. After phase separation, dauer larvae remained in the water phase, whereas the surface lipids dissolved in the organic phase. Notably, the larvae were fully viable after this procedure, indicating that the organic solvent did not penetrate the interior of the worms but only extracted the surface lipids. Separately, we homogenized dauer larvae and extracted the total lipids. We then analyzed the total and surface lipids by TLC using a very hydrophobic running system. Figure 4a shows several bands in the surface lipid fraction. One of these bands (Fig. 4a) was specific for the dauer stage: we did not detect it in a total lipid extract of mixed-stage Pristionchus worms grown in the reproductive mode (Fig. 4a). The analysis of properly staged animals revealed that the biosynthesis of this substance occurred during the last stage of dauer formation (Supplementary Fig. 5). We also monitored the dauer-specific lipid in wsd-1 mutant dauer larvae. As seen, the wsd-1 strain had substantially reduced levels of the lipid compared to the wild type (Fig. 4a). Combined, these observations suggest that the compound is a positive regulator of the formation of dauer towers.

To identify its chemical structure, we isolated the dauerspecific lipid in milligram quantities from total dauer lipid extracts. For this, we produced several liters of liquid culture containing dauer larvae. Silica column chromatography followed by preparative TLC led to a highly pure substance (**Fig. 4a**) that at ambient temperature formed a colorless oil. On the basis of its oily liquid state and its presence in *P. pacificus* dauer extracts, we named this lipid nematoil.

Using MS in the positive ion mode, we detected a charged ammonium adduct of a single chemical compound (Fig. 4b; m/z

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Figure 4 | A very long-chain polyunsaturated wax ester specific for the dauer stage is a major compound of the surface lipid coat. (a) TLC of total lipid extracts of dauer larvae shows an additional band (asterisks), which is not detected in reproductive stages. The same band is seen in dauer surface lipids. In *wsd-1* mutant dauers, this lipid was substantially reduced compared to the wild-type dauers. The rightmost lane shows the preparation of lipid that was used for the structural analysis. SE, sterol esters; TAG, triacylglycerols; PL, polar lipids. (b) MS detection of nematoil on a Q Exactive instrument as a singly charged ammonium adduct in the positive ion mode. (c) Synthesis of nematoil (1). a: 1.26 equiv. DIBAL-H, CH₂Cl₂, 0 °C, 2 h, 89%; b: 3 equiv. TsCl, pyridine, 0 °C, 18 h, 84%; c: 1.4 equiv. KCN, DMSO, 70 °C, 1.5 h, 100%; d: 1.3 equiv. DIBAL-H, CH₂Cl₂, -20 °C, 1.5 h, 69%; e: 20 equiv. KOOC(CH₂)₆PPh₃Br, 3 equiv. KOt-Bu, DMSO, THF, -10 °C, followed by addition of 1 equiv. **2** in THF, 2.3 h, 85%; f: 1.1 equiv. Et₃N, 1.1 equiv. i-BuOOCCI, THF, -15 °C, 40 min, then 25 °C, 15 min, followed by -15 °C, 2 equiv. DIBAL-H, 1 h, 86%; g: 1.95 equiv. **3**, CH₂Cl₂, 3.0 equiv. EDC·HCI, 2.2 equiv. DMAP, 25 °C, 3 h, 84%. (d) Comparison of the ¹³C NMR spectra (150 MHz, CDCl₃) in the olefinic region of natural nematoil (1), synthetic nematoil (1), synthetic nematoil (3) and synthetic nematoil (4).

of 866.7677; C₆₀H₁₀₀O₂N). The molecular formula was consistent with that of a wax ester with very-long-chain polyunsaturated fatty acid (VLCFA) and alcohol. Indeed, tandem MS in the positive mode indicated a VLCFA of 30 carbon atoms with 6 putative double bonds (Supplementary Fig. 6a,b). To confirm the high degree of unsaturation of nematoil, we performed TLC on silver salt-impregnated silica plates. Indeed, nematoil was strongly retained (Supplementary Fig. 7a,b). The 600 MHz ¹H NMR and DOSY spectra showed that the isolated nematoil was a single pure compound (Supplementary Figs. 8 and 9). The 150 MHz ¹³C NMR spectrum (Supplementary Fig. 10) and the subsequent full set of two-dimensional NMR spectra (COSY, HSQC, HMBC, HSQC-TOCSY and NOESY; Supplementary Figs. 11-19) enabled the almost complete assignment of the structure for natural nematoil. It appeared that both carbon chains contained a hexaene sequence with five skipped double bonds and a terminal ethyl group. However, we could not assign with absolute certainty the precise location of two additional isolated double bonds, one within each chain.

Therefore, we decided to assign unambiguously the complete structure of nematoil by total synthesis of the natural product (Fig. 4c and Supplementary Note). For the synthesis of nematoil (1), we converted the commercially available ethyl all-cis-7,10,13,16,19docosapentaenoate via the aldehyde 2 into the C_{30} fatty acid (3), designated as nematoic acid (Fig. 4c). Reduction of 3 led to nematyl alcohol (4), which, on subsequent esterification with 3, provided nematoil (1) (Fig. 4c). We unequivocally confirmed the structure of nematoil, including the positions of the ester group and the double bonds, by comparison of the 600 MHz ¹H NMR and 150 MHz ¹³C NMR spectra of synthetic nematoil with those of the natural compound (Fig. 4d, Supplementary Figs. 20-22, Supplementary Note and Supplementary Table 1). Nematoil is a pseudosymmetric long-chain wax ester formed by condensation of nematoic acid (3) with nematyl alcohol (4) (Fig. 4c). All 12 double bonds have a cis stereochemistry and are symmetrically positioned with respect to the ester bond, located at positions 7, 15, 18, 21, 24 and 27. Our highly efficient synthesis affords nematoil in seven steps with an overall yield of 32% and can be easily elaborated to produce the natural product on a large scale.

We made use of the synthetic nematoil to validate the adhesive properties of this molecule. Indeed, even *C. elegans* dauer larvae started forming clumps on agar plates containing synthetic nematoil on their surface (**Supplementary Fig. 23**). We also considered the possibility that nematoil could act as a chemoattractant that signals to *P. pacificus* dauer larvae to congregate to start building dauer towers. For that, we designed a chemotaxis assay (**Supplementary Fig. 24a**) in which we tested the ability of an aliquot of synthetic nematoil applied to the surface of an agar dish to attract *P. pacificus* dauer larvae. Nematoil displayed very poor chemoattractant properties compared to an aliquot of dietary *E. coli* bacteria assayed in the same manner (**Supplementary Fig. 24b,c**).

DISCUSSION

Taken together, we show that, upon transition from the reproductive stage to the dauer stage, P. pacificus produces a new extremely long-chain polyunsaturated wax ester consisting of two 30-carbon chains with twelve double bonds, representing one of the longest known waxes in animals or plants. It could be compared only to some very minor components of carnauba wax that have aliphatic chains of ~60 carbons¹¹. Nematoil constitutes a substantial fraction of total lipids in the differentiated dauer stage. The major finding of this study is that nematoil, as an adhesive molecule, promotes a new, collective host finding strategy of P. pacificus. We propose that the formation of a dauer tower increases the chance of dauer larvae to attach to a beetle by manyfold compared to a single nictating worm because the tower is much taller and can attach to the host as a whole, leading to the simultaneous transport of multiple individuals. The importance of this strategy is underscored by consideration of the cost for nematoil biosynthesis: we calculate that the production of the two 30-carbon chains with twelve double bonds requires a huge amount of ATP and NAD(P)H (at least 28 ATP and 68 NAD(P)H molecules per nematoil molecule). For comparison, the synthesis of a triglyceride containing three oleic acid moieties consumes 27 molecules ATP and 51 molecules NAD(P)H.

Nematoil has an unusual degree of unsaturation in comparison to the vast majority of naturally occurring waxes. The twelve double bonds contribute to its liquid 'oily' state. In comparison, all very long wax esters with saturated backbones stay solid at ambient temperature¹¹. The fluidity of nematoil might be advantageous in two respects. The secretion of a solid wax would be physically very complicated. In addition, the lipid coat, while remaining adhesive, would not influence the locomotive properties of the larva.

The process of building the towers must be complex: it implies a coordinated action of multiple individuals to congregate at the tower formation site, the multiorganismal orchestration of tower assembly and the coordination of a collective waving motion. What are the signals and behavioral determinants that bring thousands of larvae together? This cannot be solely due to the adhesion induced by nematoil. The clustering of worms in dauer towers supposedly requires a chemical signal or pheromone that initiates the aggregation of worms. This signal can be nematode derived but could also be from an exogenous source, for example, a mold fruiting body or a host organism. Additional behavioral traits should also be considered. For instance, it has been shown that dauer larvae of Caenorhabditis japonica have negative gravitaxis, and this positively correlates with their ability to nictate¹². In addition, the neuronal circuitry of nictation, studied in C. elegans, showed that the mechanosensory function of a subset of neurons is essential for this process¹³.

Finally, it should not be overlooked that in some nematodes, secreted lipids have been reported to protect the organism from extensive water loss and desiccation by acting as hydrophobic barrier¹⁴. Whether nematoil has such a function in *P. pacificus* will be the topic of future investigations. The studies of dauer formation

have already been proven useful in understanding fundamental processes such as aging, energy homeostasis and the stress response. We believe that the continued development of the 'chemistry' of dauer larvae will provide new insights into the strategies of survival under natural and hostile conditions.

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METHODS

Methods and any associated references are available in the online version of the paper.

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Author contributions

S.P. and A.O. performed phenotypic and microscopy studies; S.P. performed lipid analysis; A.O. performed the genetic screen; U.S. performed organic synthesis; D.T. performed the experiments with insect host organisms; V.Z. and S.B. performed MS analysis; M.G. and U.S. performed NMR analysis; D.V. performed the electron microscopy studies; S.P., A.O., R.J.S., H.-J.K. and T.V.K. conceived the project and wrote the paper; S.P., A.O., J.-M.V., U.S., M.G., H.-J.K., R.J.S. and T.V.K. designed the experiments; all of the authors discussed the results.

Competing financial interests

The authors declare no competing financial interests.

Additional information

Supplementary information and chemical compound information is available in the online version of the paper. Reprints and permissions information is available online at http://www.nature.com/reprints/index.html. Correspondence and requests for materials should be addressed to H.-J.K. or T.V.K.

ONLINE METHODS

Materials. All *P. pacificus* strains are from the Sommer laboratory collection. *C. elegans* strains were obtained from CGC. Unless stated, all chemicals were obtained from Sigma-Aldrich (Taufkirchen, Germany).

Maintenance of worms and microscopy. The wild-type strains of *P. pacificus* (RS2333) and *C. elegans* (N2 var. Bristol) were cultured as described previously^{15,16}. Worms were incubated with 2.5 μ g/ml BODIPY 505/515 (Invitrogen) with shaking for 1.5–3 h and observed under fluorescent or confocal laser scanning microscope.

For analysis of dauer towers, worms were starved on NGM agar plates in crowded populations until collectively nictating dauers were detected. The process of tower formation is facilitated by emerging fruiting bodies of contaminant mold species, which are usually used by dauers as a primary site for nictation. For BODIPY 505-515 staining, whole towers were treated as described above.

For quantification of incidence of dauer towers, only plates contaminated with mold were analyzed. The number of plates containing dauer towers was counted, and the percentage of plates containing towers was calculated. To test the statistical significance of the difference observed between the wild-type and the *wsd-1* strains, a two-tailed Fisher's exact test was performed. The confidence level was fixed to 95%.

For the dissociation of dauer towers, whole towers were relocated to water or M9 buffer with added 0.25% Triton X-100 (v/v). Alternatively, the towers were placed in 100% hexane.

The pictures of lipid secretion by dauer larvae were recorded at 1 frame per 10 min and were assembled into a movie with the rate of 3 frames per second (30 min in real time per 1-s movie).

For the examination of the lipid content and secretion during the J2 to dauer transition, we let the bleached eggs hatch and prepared arrested J2 larvae in S medium without cholesterol (50,000 worms per ml). Larval development was resumed by adding 1.4% food bacteria to the medium (on day 0). The worms were sampled every day and were subjected to staining with BODIPY for microscopy or to lipid extraction.

To obtain dauer towers of *P. pacificus* on insect larvae, the following procedure was used: Larvae of *T. molitor* and *G. mellonella* were obtained commercially from HW Terra (Muenchaurach, Bavaria, Germany). A fresh larva was put on an NGM plate and sacrificed with a sterile surgical blade. Twenty dauers were placed individually near the carcass and let to propagate on the plate by feeding on the carcass. After approximately 15 d of expansion of the worm population on the carcass, the dauer towers became visible on the appendages of the *T. molitor* or on the bristles of *G. mellonella* larvae, respectively.

Behavioral and adhesiveness assays. For the chemotaxis tests, 5 µL of synthetic nematoil dissolved in hexane at a concentration of 3.5 mg/ml was applied to the surface of NGM agar plate with a diameter of 5.5 cm, such that the diameter of the nematoil droplet on the agar surface was ~1 cm (Supplementary Fig. 24a). The distance between the center of the plate and the center of the droplet was 2 cm. Diametrically opposite to the nematoil droplet, also 2 cm away from the center of the plate, an aliquot of hexane (5 µL) was applied as a solvent control. After short drying of the aliquots, a sample of P. pacificus mixed stage larvae obtained from a crowded plate with multiple dauers was applied to the center of the plate. Before that, the larvae were washed three times with M9 buffer. The larvae were let to distribute over the test plate for 3 h, after which two parameters were scored: (i) the percentage of dauer larvae located to the half of the plate to which nematoil was applied relative to the total amount of dauer larvae on the plate and (ii) the percentage of dauer larvae found over the perimeter of the nematoil droplet relative to the number of dauers located to the half of the plate to which nematoil was applied. In a control experiment, instead of nematoil, a 15-µL aliquot of dietary E. coli bacteria of the NA22 strain was applied. The bacteria was obtained from saturated LB liquid culture, washed three times and concentrated ten times in M9 buffer. As a solvent control, pure M9 was used. The test was performed in the same way as for nematoil, and the diameter of the E. coli droplet on the agar surface was also ~1 cm. To test the statistical significance of the difference in the chemoattraction displayed by nematoil and *E. coli*, Pearson's uncorrected χ -squared test was performed. The confidence level was fixed to 95%.

For the adhesiveness of nematoil tests, *C. elegans* dauer larvae were routinely obtained by growing the dauer-constitutive mutant strain *daf-2(e1370)* at 25 °C.

Under these conditions, 100% of the animals form dauer larvae¹⁷. The dauer larvae were collected and washed three times in M9 buffer, after which they were applied to an NGM agar plate containing a large spot of nematoil on the surface. This spot was prepared by applying 30 μ L of synthetic nematoil dissolved in hexane in concentration of 3.5 mg/ml to the surface of the agar and letting it dry for several seconds. The dauer larvae were applied directly over the nematoil spot, let to distribute for ~15 min and microscoped. As a solvent control, the same experiment was performed using pure hexane to produce the spot on the agar surface.

Isolation of mutants defective in lipid secretion. Single F2 progeny from EMS mutagenized RS2333 (wild-type) animals were picked into S medium supplemented with 1% OP50 in 96-well plates. After several rounds of additional feeding of 1% OP50 and exhaustion of culture, dauer larvae were incubated with BODIPY 505/515 and were examined for the surface staining. We have isolated ten wax synthesis- and/or secretion-defective mutant strains out of ~2,500 F2 animals. In this study, we use the strain RS2592 carrying *wsd-1(tu462)*, which was outcrossed two times.

Extraction of lipids followed by TLC analysis. For extraction of total lipids, between 50,000 and 100,000 worms were homogenized by freezing and thawing in 1 ml of water and extracted as described before, by sequential addition of 3.75 ml of chloroform/methanol (1/2), 1.25 ml of chloroform and 1.25 ml of water¹⁸. For extraction of body surface lipids, a liquid-liquid partitioning was designed in the following way: approximately 100,000 dauer larvae were collected and washed three times with water, and the volume of the sample was adjusted to 2 ml. To this sample, 2 ml of hexane were added, followed by brief and vigorous mixing. After phase separation, the upper organic phase was collected. The hexane wash was repeated three times in total. Organic phases were pooled, dried and stored at -20 °C for further analysis.

Analytic TLC was performed on 10-cm HPTLC plates (Merck, Darmstadt, Germany). Total and surface lipids were analyzed using petroleum ether/diethyl ether/glacial acetic acid (90:10:1) as a running system. For visualization of lipids by charring, TLC plates were sprayed with copper acetate/phosphoric acid reagent and incubated for ~10 min at 150 °C. For impregnation with silver salt, the TLC plates were sprayed with 12.5% aqueous solution of AgNO₃ and dried before TLC. TLC was performed as described above.

Purification of nematoil. Purified nematoil was obtained from ~2,000,000 dauer larvae. Worms were homogenized by freezing and thawing and extracted according to Bligh and Dyer to obtain total lipids¹⁸. Lipid extracts were applied to a silica gel column (Kieselgel 60, 0.04–0.063 mm, Roth) equilibrated with hexane, and subsequent two-step elution with (i) hexane and (ii) hexane/diethyl ether (99:1) was performed. The hexane/diethyl ether fraction was enriched with very hydrophobic neutral lipids, including nematoil. To isolate nematoil from the residual impurities in this fraction, it was concentrated and loaded to 20-cm TLC plates (Merck, Darmstadt, Germany) for preparative TLC using petroleum ether/diethyl ether/glacial acetic acid (90:10:1) as a running system. The silica gel from the regions corresponding to the R_f of nematoil was removed from the TLC plates and extracted with hexane. Nematoil was pooled, dried and used for TLC, MS and NMR experiments.

Structural analysis by MS. Samples were dissolved in isopropanol/methanol/ chloroform 4:2:1 (v/v/v) mixture containing 7.5 mM ammonium acetate. MS and MS/MS measurements were performed on Q Exactive tandem mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a robotic nanoflow ion source TriVersa NanoMate (Advion BioSciences, Ithaca, NY) operated at gas backpressure of 1.25 psi and ionization voltage of 0.95 kV. The MS inlet capillary was heated to 200 °C. Spectra were acquired in MS mode with target mass resolution of $R_{m/z} = 200 = 140,000$ under automatic gain control (AGC), targeted value of 1×10^6 and maximum injection time of 1s. For MS/MS experiments, automatic gain control (AGC) was targeted to a value of 2×10^5 and a maximum injection time of 500 ms. The lock mass m/z = 680.4802 was used for internal online calibration. Fragmentation was performed in the HCD cell at the normalized collision energy (NCE) of 25%.

Structural analysis by NMR spectroscopy. NMR spectra were measured in CDCl₃ on a Bruker Avance III 600. The DOSY spectrum was recorded to confirm the purity of the isolated natural product. An almost complete assignment of the ¹H and ¹³C NMR signals for nematoil has been achieved by a complete

set of the following two-dimensional NMR spectra: COSY, HSQC, HMBC, HSQC-TOCSY and NOESY.

followed by aqueous lead citrate. Acquisitions were taken with a transmission electron microscope (TECNAI12, FEI) operated at 100 kV.

- **High-pressure freezing and electron microscopy.** Dauer larvae of wild-type *P. pacificus* were fixed by high-pressure freezing (EMPACT2, Leica) followed by automated freeze substitution (EM AFS2, Leica) for 22 h at progressively rising temperatures, from –90 °C up to room temperature, in acetone containing 1% osmium tetroxide, 0.1% uranyl acetate and 5% water. Samples were infiltrated with Epon LX112, thin-layer embedded and cut with an ultramicrotome (UCT, Leica) into 70-nm sections. After collecting sections on Formvar-coated copper grids, the contrast was enhanced by 1% uranyl acetate in 70% methanol
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CORRECTIONS

ERRATUM

A wax ester promotes collective host finding in the nematode Pristionchus pacificus

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In the version of this article initially published online, the legend to Figure 4c describing step e incorrectly stated that 1 equiv. **3** was added when it should have been 1 equiv. **2**. The error has been corrected for the HTML version of this article.

