LETTER

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Primitive agriculture in a social amoeba

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Agriculture has been a large part of the ecological success of humans[1]. A handful of animals, notably the fungus-growing ants, termites and ambrosia beetles[2–4], have advanced agriculture that involves dispersal and seeding of food propagules, cultivation of the crop and sustainable harvesting[5]. More primitive examples, which could be called husbandry because they involve fewer adaptations, include marine snails farming intertidal fungi[6] and damselfish farming algae[7]. Recent work has shown that microorganisms are surprisingly like animals in having sophisticated behaviours such as cooperation, communication[8,9] and recognition[10,11], as well as many kinds of symbiosis[12–15]. Here we show that the social amoeba *Dictyostelium discoideum* has a primitive farming symbiosis that includes dispersal and prudent harvesting of the crop. About one-third of wild-collected clones engage in husbandry of bacteria. Instead of consuming all bacteria in their patch, they stop feeding early and incorporate bacteria into their fruiting bodies. They then carry bacteria during spore dispersal and can seed a new food crop, which is a major advantage if edible bacteria are lacking at the new site. However, if they arrive at sites already containing appropriate bacteria, the costs of early feeding cessation are not compensated for, which may account for the dichotomous nature of this farming symbiosis. The striking convergent evolution between bacterial husbandry in social amoebas and fungus farming in social insects makes sense because multigenerational benefits of farming go to already established kin groups.

The social amoeba *Dictyostelium discoideum* is well known for its social interactions. When prey bacteria become scarce, amoebae aggregate by the tens of thousands and produce a multicellular migratory slug that becomes a fruiting body in which about 20% of cells die to form a sterile stalk. The stalk aids the dispersal of the remaining cells, which differentiate into spores in a spherical structure called the sorus[16,17] (Fig. 1a). We show that about one-third of wild-collected clones husband bacteria through the sporulation and dispersal process. We call these clones farmers because they carry, seed and prudently harvest their food, but the farming is primitive because no active cultivation is known.

At first glance, beneficial interactions occurring between *D. discoideum* and the bacteria would be unexpected. In the unicellular state, *D. discoideum* are solitary predators of bacteria[18], and bacteria use a wide range of strategies to deter predation[19]. Early life-history work on another species, *Dictyostelium mucoroides*, suggested the possibility of symbiosis with bacteria[20,21] but did not document this interaction experimentally. The possibility fell out of favour, particularly after work[18] finding no support for a symbiotic relationship and much support for a predatory one. However, that study considered only a single *D. discoideum* clone, and the extent of natural variation remains largely unexplored. In our study, we used a population of 35 wild *D. discoideum* clones isolated from soil collected at Mountain Lake Biological Station, Virginia, and Lake Itasca Biological Station, Minnesota (Supplementary Table 1). We observed that the sorus contents of some of the clones contained bacteria in addition to *D. discoideum* spores (Fig. 1b). To confirm this observation, we initially chose four clones that seemed to have bacteria in their sori and four clones that did not. We picked up the sorus contents of six random fruiting bodies from each and then spotted these individually on nutrient agar plates to test for bacterial growth. This assay confirmed that sori from some clones consistently contained bacteria that could initiate new populations (Fig. 1c, top panel) and that sori from other clones did not (Fig. 1c, bottom panel). Bacteria also grew when directional light induced the multicellular slugs to migrate away from the original locations to bacteria-free zones of a plate before fruiting, indicating that bacteria are carried in the slugs. Four sets of wild isolates tested in this way yielded 36% farmers: 13 of 35 tested overall; 4 of 9 from Minnesota; 5 of 14, 3 of 9 and 1 of 3 from three sample dates from Virginia. These data suggest that farmer clones are common, are found in the same locations as non-farmer clones and are therefore likely to have access to similar bacteria.

To confirm that farmers and non-farmers belong to the same species, we constructed a Bayesian phylogenetic tree created from combined mitochondrial, ribosomal and variable nuclear-DNA sequence data

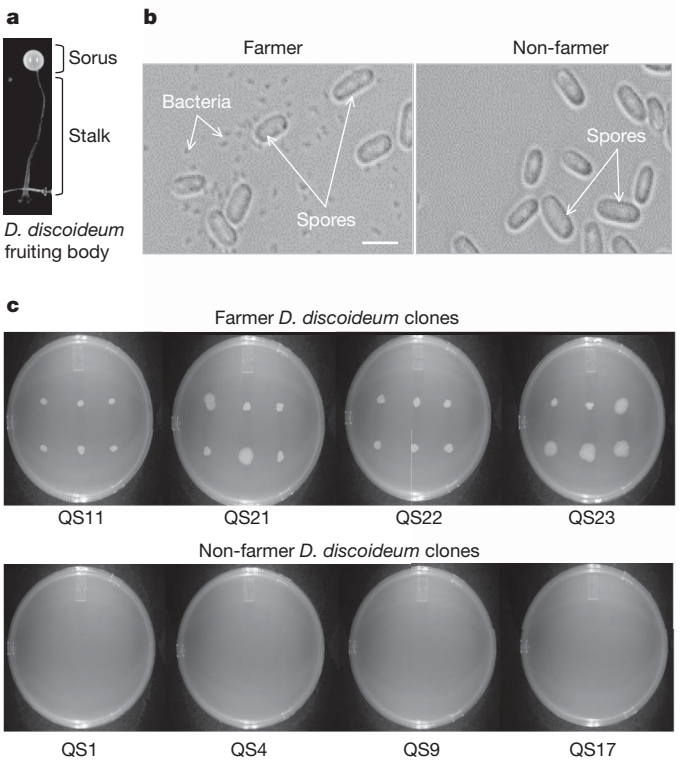

Figure 1 | Fruiting body structure and sorus contents from farmer and non-farmer *D.discoideum* clones. **a**, The fruiting body is composed of two main parts: the sorus, which contains the fertile spores, and the stalk, which holds the sorus aloft to facilitate spore dispersal. **b**, Bacteria and *D. discoideum* spores are present in the contents of a farmer sorus (left) but only spores are present in the contents of a non-farmer sorus (right). Scale bar, 5 μm. **c**, Sorus contents for six random, individual fruiting bodies from each of four farmers and four non-farmers were spotted on individual nutrient agar plates. All farmer sorus contents showed bacterial growth, whereas no bacterial growth was observed in sori from non-farmers. Plates were photographed after two days of growth.

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from 14 clones (five farmers and nine non-farmers; Supplementary Fig. 1). Farmers were interspersed in the phylogeny with non-farmers, as expected if the trait is shuffled through the species by sex[22]. We also calculated pairwise genetic distances between farmer–farmer pairs, non-farmer–non-farmer pairs and farmer–non-farmer pairs and found no differences in the distributions (Supplementary Fig. 2).

We sequenced a portion of the bacterial 16S ribosomal gene to identify the species of carried bacteria. Farmers carry a variety of species of bacteria, with diversity both within and between farmer clones, and diversity is likely to be underestimated because not all bacteria are culturable by our methods (Supplementary Table 2). About half of these bacterial strains serve as good food sources for *D. discoideum*, generally for farmers and non-farmers alike (Supplementary Table 2). The function of the other half, if any, is unknown, but all farmer strains transport and use the food bacteria supplied in the lab (either *Klebsiella aerogenes* or *Escherichia coli*), and we focus the remainder of the paper on food carrying.

Carrying bacteria is a consistent clone-specific trait, and these clones show a number of differences from non-farmer clones that affect life history and fitness. To establish the consistency of the trait, we eliminated all living, carried bacteria from four farmers and four non-farmer controls by treating them with antibiotics. We then grew them on dead *K. aerogenes* as a food source, and confirmed using spotting tests as in Fig. 1 that they became bacteria free. When these bacteria-free clones were then grown on live *K. aerogenes*, all farmers regained an association and had these bacteria in their sori whereas non-farmers did not (Fig. 2).

To examine costs and benefits in the farmer–bacteria interaction, we compared farmers with non-farmers under several conditions. Soil is a very structured, heterogeneous environment in the wild, where bacteria could occur in patchy, monospecific colonies of variable cell numbers[23,24], and the patchiness is accentuated because some dictyostelids have distinct food preferences[25]. We therefore mimicked spore dispersal to both bacteria-poor and bacteria-rich sites. For the former, we transferred *D. discoideum* spores to plates with nutrients for bacteria, but without added bacteria (Fig. 3a). We found that bacteria transported by the farmer spores proliferate and are consumed by the farmers that also proliferate and then sporulate after the social stage, whereas non-farmers with no bacterial partners produce hardly any spores ($F_{1,6} = 58.97$ (derived from F-test; subscripts, degrees of freedom), $P < 0.0001$). Farmers are thus able to capitalize on available nutrients by carrying their own food bacteria in their sori. This difference disappears, and farmers do as poorly as non-farmers, if they are previously made bacteria free using antibiotics ($F_{1,6} = 0.49$, $P = 0.8082$; data not

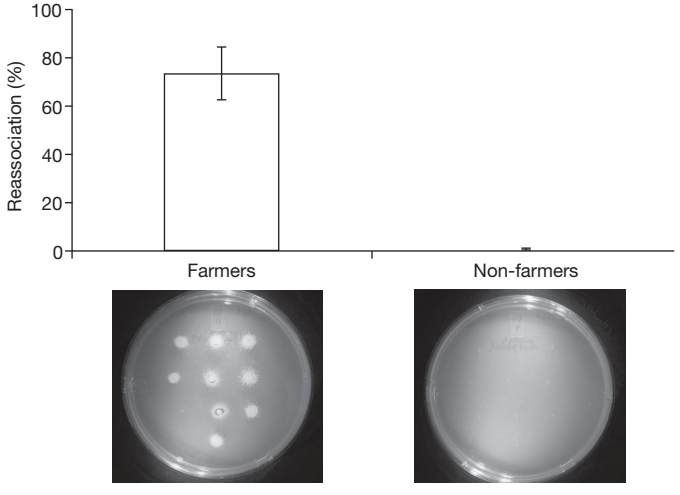

Figure 2 | Farmers readily reassociate with bacteria, suggesting a persistent interaction. After antibiotic treatment to eliminate carried bacteria, reassociation with bacteria was tested by growing four farmer and four non-farmer clones on *K. aerogenes*. We spotted ten random sori from each clone individually on nutrient agar plates, and counted positive/negative growth of bacteria in each sorus after two days. Farmers significantly differ from non-farmers in their ability to associate with bacteria ($F_{1,6} = 48.864$, $P < 0.001$; error bars, s.e.m.). Pictured below the graph are representative examples of sorus contents for farmer and non-farmer clones after one round of growth with live bacteria. Farmer clone sori contain bacteria; no bacterial growth was detected for non-farmers, even after ten days.

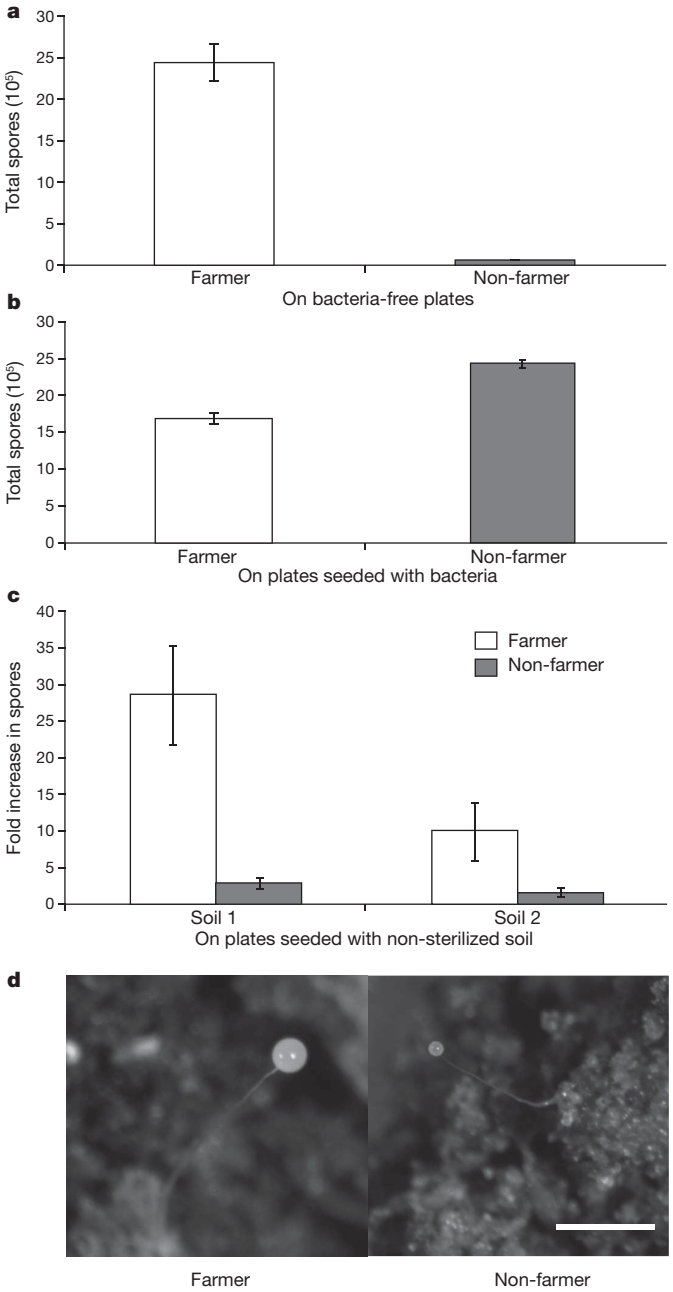

Figure 3 | The advantage of carrying food is context dependent. **a**, Farmers produce more spores than non-farmers when colonizing plates with bacterial food substrates but without bacteria ($F_{1,6} = 58.97$, $P < 0.0001$). We used three replicates of each of four farmer and four non-farmer clones. **b**, Farmers produce fewer spores than non-farmers when provided a fixed amount of live bacteria ($F_{1,12} = 64.36$, $P < 0.0001$). We used three replicates from each of five farmer and nine non-farmer clones (the results are similar when only the eight clones in **a** are used). **c**, Farmers produce more spores than non-farmers in unsterilized soil (soil 1: $F_{1,10} = 14.82$, $P = 0.0032$; soil 2: $F_{1,10} = 26.21$, $P = 0.0005$). We used individual sori collected from six farmers and six non-farmers to test spore production after one round of the social stage. All error bars, s.e.m. **d**, Representative examples of farmer and non-farmer fruiting bodies formed after the social stage in unsterilized soil. Scale bar, 1 mm.

shown). Next we tested whether farming was costly when spores are transferred instead to a site where edible bacteria are abundant, reducing or eliminating the advantage to farmers of bringing their own bacteria. Farmer clones then produce fewer spores than non-farmers from a given number of live bacteria ($F_{1,12} = 64.36$, $P < 0.0001$; Fig. 3b). Therefore, carrying seed stocks can be advantageous or disadvantageous, depending on bacterial availability at new sites.

Sites entirely lacking bacteria in nature are rare, but farmers could still gain by bringing preferred bacteria (whatever allowed them to flourish and sporulate in their previous site), just as humans seed preferred plants in an already green world. We therefore tested spore production for farmers and non-farmers in unsterilized soil collected from two separate locations (soil 1 and soil 2) at the Houston Arboretum and Nature Center, Texas. We determined the numbers of colony-forming units of culturable bacteria in soil 1 and soil 2 to be $(1.3–2.3) \times 10^8$ and $(0.6–0.64) \times 10^8$ per gram of soil, respectively. However, the bacteria already present in the soil do not make bacteria carrying superfluous. Under these conditions, farmers produced more spores than non-farmers for both soil locations (soil 1: $F_{1,10} = 14.82$, $P = 0.0032$; soil 2: $F_{1,10} = 26.21$, $P = 0.0005$; Fig. 3c, d), as well as many more fruiting bodies (soil 1: $F_{1,10} = 35.78$, $P = 0.0001$; soil 2: $F_{1,10} = 9.31$, $P = 0.0122$; data not shown). Farmer sori continued to carry their original bacteria (43 of 44 isolates checked by sequencing) whereas no bacteria were isolated from non-farmer sori. This suggests the bacteria available in the two test soils were not very suitable for both non-farmers and farmers, so the bacteria carried by the farmers allowed them to flourish in comparison with the non-farmers.

We proposed that the lower success of farmers when bacteria are provided stems from prudent harvesting. *D. discoideum* amoebae normally leave the solitary stage and enter the social stage when food is exhausted[16,17], but farmers may do so sooner to save some bacteria for transport. We therefore measured the number of uneaten bacteria present along a developmental time course (Fig. 4a). During the solitary amoeba stage (day 1), there was no difference in bacterial density among treatments (also, day-1 farmers and non-farmers did not differ significantly in number of amoebas: $F_{1,17} = 0.6733$, $P = 0.4233$; data not shown). During the social stage, however, bacterial usage differs significantly between farmers and non-farmers at all time points. Non-farmers eat all the bacteria whereas farmers leave many bacteria unconsumed, roughly half the number present as compared with bacteria grown alone. Figure 4b shows representative farmer and non-farmer clones photographed at day 5, revealing that only the farmers entered the social stage and formed fruiting bodies in the presence of uneaten bacteria. Thus, it seems that farmer clones forgo considerable food to save some for co-dispersal.

Farmer clones also migrate significant less far than non-farmers during the mobile slug stage that immediately precedes fruiting ($F_{1,14} = 87.59$, $P < 0.001$; Fig. 4c). This might be a cost caused by bacterial interference, or it might be an evolved response of not needing to move as far when farmers carry their own bacteria. Either way, it adds to the list of significant differences between farmers and non-farmers.

An alternative explanation for the apparent costs—leaving some bacteria unharvested and reduced slug migration—is that the bacteria are pathogenic and harm *D. discoideum*. However, the pathogen hypothesis does not account for why the farmer strains would be infectible by many bacteria and why infection is highly consistent, even after curing. Neither does it explain why all farmers carry food. Moreover, it does not explain why infection causes no cost to growth in the solitary stage ($F_{1,11} = 0.72$, $P = 0.4132$; Supplementary Fig. 3). Instead, the costs appear precisely where the farming hypothesis predicts (saving some food for transport) or where it provides a plausible explanation (less need for slug migration). However, a mixed explanation seems possible. Carrying food bacteria could have the side effect of sometimes taking up useless or harmful bacteria. The existence of the farmer polymorphism will allow additional within-species comparisons that should help in exploring the mechanisms, which are as yet unknown,

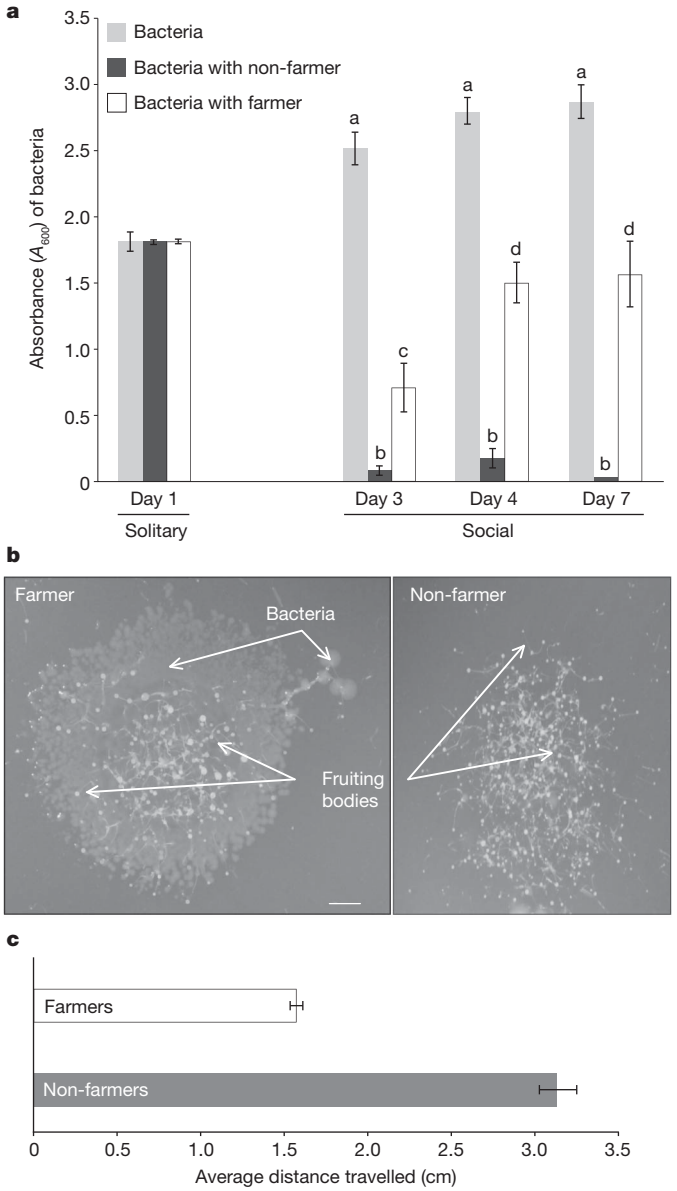

Figure 4 | Life-history traits differ between farmers and non-farmers. **a**, Bacterial usage. For 11 non-farmer clones and nine farmer clones, we individually spotted a fixed number of spores mixed with the food bacterium *K. aerogenes* on nutrient agar plates. We also spotted *K. aerogenes* alone as a control. Bacterial density for each spot was determined at various time points during development. During the social stage (days 3, 5, 7), farmers leave more bacteria unconsumed than non-farmers at all time points (type: $F_{2,19} = 106.18$, $P < 0.0001$; day: $F_{2,38} = 10.23$, $P = 0.0003$; type × day: $F_{4,38} = 10.60$, $P < 0.0001$; significant differences found within each day are indicated by different letters, which reflect results of a *post hoc* Tukey HSD test). At the solitary-amoeba stage (day 1), no difference in bacterial density was found among the three treatments ($F_{2,19} = 1.2943$, $P = 0.2972$). **b**, Bacterial usage of representative clones. Farmer clones develop fruiting bodies before all bacteria are exhausted whereas non-farmers consume all accessible food sources before fruiting. Examples of fruiting bodies are marked with arrows and appear as white dots. Clones were photographed on day 5. Scale bar, 3 mm. **c**, Migration. The average distance farmer slugs migrate towards light is less than for non-farmer slugs ($F_{1,14} = 87.59$, $P < 0.001$). Eight clones of each type were used, with two replicates. All error bars, s.e.m.

but could be very simple alterations in timing of aggregation, sensitivity to bacteria or ability to produce a specific enzyme or toxin. The abundant scientific resources available for this model organism have recently proven very useful in understanding the genetics of their social interactions[26–29]. They should prove similarly useful here, providing a

unique model system for probing the genetics of eukaryotic–bacterial symbioses.

The connection between farming and sociality may not be coincidental, because social species have suitably structured populations. In this social microbe, the advantage of prudent harvesting and seeding is large because it can benefit many generations of cell descendants before fruiting. Moreover, the high relatedness of natural fruiting bodies[27] minimizes any potential exploitation by non-farmers, which could either consume the bacteria that the farmers would save to carry, or freeload when co-dispersed with farmer spores and their bacteria. This same advantage—long-lived groups of kin—provides similarly fertile ground for agriculture in the ants and termites that are the most sophisticated non-human farmers.

METHODS SUMMARY

Isolation of wild *D. discoideum* strains. Isolation techniques followed published methods[30] with the exception that we collected soil samples of 20 g or more, instead of 0.2 g, at each location.

Culture conditions. We grew all wild isolates from spores on SM/5 agar plates (2 g glucose, 2 g BactoPeptone (Oxoid), 2 g yeast extract (Oxoid), 0.2 g $MgCl_2$, 1.9 g $KHPO_4$, 1 g K_2HPO_4 and 15 g agar per litre) in association with bacteria *K. aerogenes* or *E. coli* at room temperature (21 °C).

PCR amplification and sequence identification of novel bacterial isolates. We followed the procedures outlined in "Identifying unknown bacteria using biochemical and molecular methods" (http://www.nslc.wustl.edu/elgin/genomics/Bio3055/IdUnknBacteria06.pdf) with one exception. Bacteria to be cloned and identified were grown on and collected from SM/5 agar plates. PCR fragments generated (using the above procedure) were sequenced at Lone Star Labs (Houston, Texas). We used the NCBI website http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi as the search tool for sequences to identify bacteria to species.

Data analyses. We analysed our data using standard analysis-of-variance methodology with fixed (farmer and non-farmer) effects and a random effect (clone) for all experimental assays. The data analysis was generated using SAS software (version 9-1 of the SAS System for Microsoft Windows).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Contributions D.A.B. identified the symbiosis, performed the experiments and analysed the data. T.E.D. constructed and analysed the phylogeny. D.A.B., T.E.D., D.C.Q. and J.E.S. designed the experiments, discussed the results and wrote the manuscript.

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Data analyses. We analysed our data using standard analysis-of-variance methodology with fixed (farmer and non-farmer) effects and a random effect (clone) for all experimental assays. The data analysis was generated using SAS software (version 9-1 of the SAS System for Microsoft Windows).

Experimental assays. Farmer test. We picked up the sorus contents of 6–12 random fruiting bodies developed on SM/5 plates in association with *E. coli* or *K. aerogenes* from each wild clone to be tested using a filtered pipette tip. The sorus contents were spotted individually on SM/5 agar plates and assessed for bacterial growth after two days at room temperature.

Reassociation assay. We used a population of four farmers and four non-farmers treated to eliminate all living, carried bacteria. To accomplish this, clones were grown on SM/5 agar plates containing antibiotics (0.1 g ampicillin, 0.3 g streptomycin sulphate per litre) using dead *E. coli* as a limited food source to allow the spores to hatch and develop. Dead *E. coli* was prepared by autoclaving a suspension of *E. coli* and KK2 (2.25 g KH_2PO_4 and 0.67 g K_2HPO_4 per litre H_2O) for 20 min at 121 °C. After autoclaving, the absorbance of dead bacteria was set to A_{600} 6.0. Treated clones were allowed to form fruiting bodies, spores were collected and the process was repeated. We performed the farmer test after the second round and no bacterial growth was seen in the sorus contents of test clones even after ten days. We harvested spores from the bacteria-free, cured clones in KK2 and the spore density for each clone was determined by serial dilution using a haemacytometer under a light microscope. Spores from these treated clones were then reintroduced to live *E. coli*, plated on SM/5 agar plates, and allowed to form fruiting bodies. Ten random sori were collected individually from each clone and tested for bacterial growth following the farmer test above.

Migration assay. We tested the slug migration ability of eight farmers and eight non-farmers per replicate, for two replicates. Duplicate plates were set up for each clone per replicate. For each plate, we prepared a slurry of 5×10^6 spores of one *D. discoideum* clone, *K. aerogenes* and KK2. We then applied the slurry to the edge of a $150 \times 15\,mm^2$ Petri plate containing 80 ml nutrient-free agar (0.198 g KH_2PO_4, 0.0356 g Na_2HPO_4 and 15 g agar per litre), and allowed the slurry to dry. Each plate was wrapped in aluminium foil and a small pinhole was made directly opposite the spores and bacteria to provide directional light. The wrapped plates were placed in a lit incubator at 21 °C for 84 h to allow slugs to form and migrate. For each clone, we counted the distance travelled by slugs in each of five 1.5-cm bands across the nutrient-free agar plate, and calculated the average distance travelled for all slugs.

Spore production assay. We tested two conditions: first, nutrients for bacterial growth but no outside bacteria were provided as food for the test clones; second, nutrients for bacterial growth as well as outside bacteria were provided as food for the test clones. For the first condition, we used four farmer clones and four non-farmer clones per replicate, with three replicates. We spread 200 µl of 10^5 spores plus dead *E. coli* in KK2 (absorbance, A_{600} 6.0) on $100 \times 15\,mm^2$ SM/5 agar plates. Bacterial growth is possible for farmers in this condition. After development was complete, spores were collected by washing the plates with KK2 plus 0.1% NP-40 (Calbiochem). The total number of spores produced by each clone was determined by counting using a haemacytometer and a light microscope. As a control to assess confounding growth differences between farmers and non-farmers, all eight clones were grown as above but the plates were supplemented with antibiotics (see "Reassociation assay", above) to eliminate any potential food bacteria carried by the clones. No farming is possible in this set-up.

For the second condition, we used five farmer clones and nine non-farmer clones per replicate, with three replicates. For the assay, we prepared nutrient-free agar plates (see "Migration assay", above) laid with a grid of equidistant 13-mm AABP 04700 (Millipore) black filter squares. Filters were spotted individually with 5×10^5 spores in a slurry of live *K. aerogenes* and KK2 (A_{600} 6.0). Clones were spotted in an order determined by a random number generator. Duplicate samples were made for each clone for each experiment. Clones were allowed to hatch, grow and develop under direct light to limit potential movement of slugs before final culmination in fruiting bodies. Development was complete for all clones after four days. Each filter was collected and placed in a 1.5-ml conical Eppendorf tube containing 1.0 ml KK2 plus 0.1% NP-40 alternative. Tubes were vortexed briefly to disperse the spores evenly and counted as above without dilution to determine density.

Soil assay. We collected soil from two separate locations in the Houston Arboretum and Nature Center (29° 46′ N, 95° 27′ W). Thin, non-nutrient agar plates were prepared to provide a humid environment for spores to hatch and to hold the soil in place. Soil was then layered to a ~2–3-mm depth in a half-moon pattern with an empty space at the centre line atop the starving agar on each plate (Supplementary Fig. 4a). We used 12 clones consisting of six farmers and six non-farmers. Farmer–non-farmer clones were randomly paired on each plate (one on each half-moon of soil) as a check for plate environment bias. Each pair was tested on two separate plates for each soil type. Data analysis was reported unpaired as no plate environment bias was detected.

For the assay, fruiting body sori were collected from stock plates of all 12 clones previously prepared on the same day by plating 2×10^5 spores in association with *K. aerogenes* on SM/5 agar plates. The contents of an individual sorus for either a farmer or a non-farmer clone were picked up using a filtered pipette tip and placed on the unsterilized soil in the previously chosen locations (marked by coloured circles in Supplementary Fig. 4b). Three farmer sori and three non-farmer sori were placed on each experimental plate in this manner. After three days at room temperature under direct overhead light, all plates were viewed under a dissecting microscope to locate and collect all fruiting bodies formed. Fruiting bodies found were generally located in the same area as spotted on the soil. Whole fruiting bodies from all three spots per plate for either the farmer or the non-farmer were collected and placed together in an Eppendorf tube containing 1 ml KK2 plus 0.1% NP-40. Tubes were vortexed briefly to disperse the spores, and the spores were counted without dilution using a haemacytometer. The change in spore number for each clone was then calculated. To determine the initial number of spores spotted for each clone without diluting sorus contents, a proxy was used. The sorus contents from ten random fruiting bodies from each clone were collected and counted as above. The average count for these ten fruiting bodies was used to determine the clone's average spore number per sorus spotted on the experimental plates. Additionally, we determined presence or absence of bacteria in the sori as well as bacteria identity for positive growth using a subset of ten clones (five farmers and five non-farmers) in both types of soil. We performed serial dilutions in KK2 of 80 sori contents (44 farmer and 34 non-farmer), and the clonal isolates recovered were used for sequencing following the methods described in "PCR amplification and sequence identification of novel bacterial isolates", above.

Bacteria usage assay. We collected spores from a population of 11 non-farmer and nine farmer clones. For each clone, we individually spotted four 30-µl spots of 3×10^4 spores mixed with live *K. aerogenes* (A_{600} 3.0) on a SM/5 plate as well as spotting *K. aerogenes* alone as a control. Bacterial density was determined by using a sterilized inoculating loop to collect all growth from one spot for each clone in a 1.5-ml Eppendorf tube containing 1 ml KK2, vortexing to obtain a uniform suspension, removing hatched amoeba or spores by briefly centrifuging at 2,000*g* to pellet, and then determining the absorbance (A_{600}) of the remaining bacteria. Data points were collected on days 1, 3, 5 and 7. To determine whether confounding differences in spore germination occurred among clones, the number of hatched amoeba was determined for each clone on day 1 by counting using a haemacytometer.

Proliferation assay. To determine vegetative doubling rates during exponential growth, we grew each clone separately by plating 1×10^4 log-phase cells per plate in association with *K. aerogenes* as a food source on replicated SM/5 agar plates. After 12 h of growth, we collected all cells from a plate, diluted the cells in a measured amount of KK2 and counted the number of cells present using a haemacytometer. We repeated this process for plates grown for 18, 24, 30, 36 and 42 h. We conducted the experiment in two temporally independent blocks. To analyse the data, we log-transformed the counts, determined the slope for each clone and performed a full-factor analysis of covariance.

Phylogeny construction. DNA sequencing. We extracted DNA from spores using a Chelex/proteinase K extraction protocol. We amplified a non-coding region of the mitochondrial genome (mtDNA), regions of the nuclear ribosomal DNA (rDNA) and six variable fragments of nuclear DNA by PCR with the primers listed in Supplementary Table 3, using the following protocol (step 1: 2 min at 94 °C; step 2: 30 s at 94 °C; step 3: 30 s starting at 65 °C and decreasing by 1 °C per cycle; step 4: 1 min at 72 °C; step 5: 15 cycles to step 2; step 6: 30 s at 94 °C; step 7: 30 s at 50 °C; step 8: 1 min at 72 °C; step 9: 25 times to step 6; step 10: 6 min at 72 °C). We cleaned the PCR product with USB ExoSAP-IT and then sequenced using Perkin Elmer Applied Biosystems Big Dye 3.1 chemistry and a 3100 genetic

analyser. We analysed approximately 4,300 base pairs of the nucleotide sequence of nuclear 17S, 5.8S, 26S and 5S rDNA regions, approximately 800 base pairs of the nucleotide sequence of mtDNA (LSU intron) and approximately 3,700 base pairs of variable nuclear DNA from chromosomes 1, 2, 3 and 4. We aligned the sequences using the programs LASERGENE SEQMAN (version 7.0.0) and BIOEDIT (version 7.0.5.2).

Data analysis. We used comparative DNA sequence data from 14 individual clones (QS numbers 1, 4, 6, 8-9, 11-12, 14-15, 17-18 and 21-23) to estimate gene trees/phylogenies and to estimate pairwise genetic distances between clones. These 14 clones represent five farmers and nine non-farmers. We used Bayesian methods for phylogenetic reconstruction. Using MRBAYES[31] (version 3.1), we estimated a phylogeny for each data set based on the GTR+I+Γ model of molecular evolution. In addition, two high-frequency, polymorphic indels (one in the mtDNA and one in the variable nuclear DNA) were scored as standard presence/absence characters and were included in the analysis with weighting equal to the nucleotide polymorphisms. For each analysis, four Metropolis-coupled Markov chains were run for 250,000 burn-in generations followed by 1.75×10^6 generations of data collection. We used the software program MEGA[32] (version 4) to estimate pairwise genetic distances between clones using the *p*-distance algorithm. We analysed sequence data from all 14 individual clones. Gaps and missing data were eliminated in pairwise sequence comparisons.

31. Huelsenbeck, J. P. & Ronquist, F. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17,** 754–755 (2001).
32. Tamura, K., Dudley, J., Nei, M. & Kumar, S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* **24,** 1596–1599 (2007).