

Review

Multispecies Swarms of Social Microorganisms as Moving Ecosystems

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Microorganisms use collective migration to cross barriers and reach new habitats, and the ability to form motile swarms offers a competitive advantage. Traditionally, dispersal by microbial swarm propagation has been studied in monoculture. Microorganisms can facilitate other species' dispersal by forming multispecies swarms, with mutual benefits. One party (the transporter) moves a sessile partner (the cargo). This results in asymmetric associations ranging from temporary marriages of convenience to long-term fellow travellers. In the context of the 'microbial market', the parties offer very different services in exchange. We discuss bacteria transporting bacteria, eukaryotic microorganisms moving bacteria, and bacteria facilitating the spread of eukaryotes – and ask what the benefits are, the methods of study, and the consequences of multispecies, swarming logistics networks.

Spread of Microbes in the Environment

Instances of both competition and cooperation between microorganisms are found in most environments from the rhizosphere to the human body. Microbiologists are mapping the interactions between microbes within the same habitats; for example, in a single soil granule using genomics and imaging [1] or between related species in marine habitats using metagenomics [2]. Evolutionary biologists are developing new ways of calculating cost–benefit relationships (microbial markets) that allow quantification of the relationships between microorganisms – who does what and who benefits [3]. These recent advances give new approaches to long-standing questions, such as what constitutes a microbial community and to what extent cooperation occurs within and between species.

Natural environments are interconnected to varying degrees, and most species occupy multiple but geographically distinct niches, existing as a metacommunity [4]. There are routes between different environments, and microorganisms use these to spread. The barriers are often natural (e.g., air gaps in the soil), and different microorganisms have diverse capabilities to cross these obstacles [4,5]. In order to spread, microbes seek to disperse at an optimal rate – maximal dispersal is not necessarily the best as it can dilute the impact or survival potential of the invader, whilst minimal dispersal has an obvious limitation of range [6].

In a broad ecological and evolutionary sense, we can also ask to what extent does the spread of microorganisms matter. Given potential exponential growth and highly favourable conditions, in theory a single microbe could achieve dominance in a particular location in a very short time. So, if 'everything is everywhere but the environment selects' then dispersal may be less important than factors that affect competition during growth [7]. However, studies on microbial speciation

Trends

Increasingly, microorganisms are studied in relation to other microorganisms.

Many microorganisms can collectively migrate over surfaces to new ecological niches as a swarm.

In recent years, some swarms have been shown to transport other microorganisms as cargo. This leads to interesting, asymmetric relationships whilst 'on the move' – which can be considered a mobile ecosystem.

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⁵*In memoriam*. This article is dedicated to one of the authors, Professor Eshel Ben-Jacob (1952 to 2015), who sadly died during the preparation of the manuscript.

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suggest that physical and geographical barriers can be important, in both extreme and temperate habitats [8–10]. It follows that there are multiple strategies that microbes use to cross these obstacles, including adaptation to transport by water currents or the wind. Another such method is to be carried by a larger organism. For example, freshwater zooplankton can transport bacteria across density gradients in lakes which are otherwise impenetrable to bacteria [11].

The role of microbial motility is surprisingly poorly understood in the context of dispersal. Some microorganisms move rapidly in liquid or over surfaces; the latter is commonly a collective phenomenon and uses a variety of molecular motors with the common factor that they require significant genetic investment, typically over 50 genes [12,13]. The motors can drive the rotation of flagella (swarming) or a series of diverse, non-flagellate force-generating systems resulting in movement [13]. Both terms, gliding and swarming, can be confusing. Gliding can occur by multiple, unrelated mechanisms (Box 1). However, some of the mechanisms of bacterial motility may turn out to have more in common than has previously been suspected. Flavobacterial gliding, despite being dependent on a very different set of proteins to those that turn bacterial flagella [14], has recently also been shown to also involve a rotary motor [15]. The term ‘swarming’ can also be used in different ways; here we refer to swarming as being specific to collective bacterial movement over a surface only when driven by flagella [12]. We will use the concept of a swarm, more loosely, to describe decision making and mass migration of agents, which does not have a clear leader, and intend this term to also cover robots and other nonliving swarms (Box 1). The microorganisms providing the force for motion are defined as the transporter, and the more sessile, hitchhiking organism is the cargo [16].

Despite diversity of mechanisms, and whether swarms are composed of microorganisms, multicellular organisms, or even cybernetic agents, swarms have certain commonalities, including trigger events to start/stop (organisms do not swarm all the time), and collective action (possibly cooperative, with no leader, and exhibiting capabilities beyond the individual). Mathematical modelling can examine swarming across multiple, diverse systems and look at different scales of organization. Imaging provides experimental validation for modelling and independently generates new insights. We describe the powerful combination of imaging and modelling (Box 2) as related to transporter–cargo interactions later in this article.

Box 1. General Aspects of Microbial Motility

Many microorganisms are self-propelling, either swimming in liquids or moving over surfaces [12,13].

Motility on surfaces is often via specialized mechanisms (e.g., gliding or surface-adapted swarming cells). Surface motility is frequently collective; for example, in bacterial swarming, whilst each individual bacterium has functional flagella, groups of bacteria are required for movement to occur. Individual bacteria move within swarms, for example in ordered streams [16] or as a complex random motion pattern [65]. Gliding occurs by multiple mechanisms and, unlike flagella motility, occurs only on surfaces [12].

Whilst the velocities of swarms can exceed 1 cm/h in many cases, relatively little information is available on how motility impacts long-range dispersal in the environment. Most studies concentrate on local motile behaviour (cm to μm scale range) [12,13,66,67].

Life on this scale is different to our own experience. Liquids are highly viscous relative to bacterial mass (low Reynolds number), and individual bacteria are prone to Brownian motion. Surfaces also exert strong effects on microorganisms with factors such as surface tension and the availability of water as critical physical parameters [68].

Microorganisms actively alter their local environment during motility through the secretion of compounds (such as surfactants or lubricants) [12,68,69]. Microorganisms also affect their immediate environment [70] through motility itself, for example, a high density of actively rotating flagella can decrease the viscosity of their environment, even converting the properties of water to a superfluid [71]. The force generated by single [72,73] or multiple [16] microorganisms is sufficient to move objects, often considerably larger than an individual bacterium. For many forms of surface motility the roles of decision making and active motility are unclear.

Box 2. Imaging and Modelling of Microbial Swarming and Cargo Transport

Until recently, the information contained within scientific movies was not highly accessible to quantification beyond manual extraction [74,75]. However, modern video analysis methods, such as particle image velocimetry and optical flow analysis, offer efficient and robust algorithms for inferring the dynamics visualized in movies at high temporal and spatial resolution. Advanced algorithms can identify changes in contrast, colour, field of view, or viewing direction. Similarly, improved particle tracking methods can track individuals in dense populations and predict the location of particles even if they are temporarily obstructed. Such developing technologies facilitate the study and analysis of bacterial dynamics.

Bacterial swarming is a challenging subject for image processing, given the high density and large number of rapidly moving particles. Moreover, the flow of biological matter is particularly difficult to analyse due to discontinuous flows, and varying contrast and shapes. Specific experimental techniques, such as the fluorescent labelling of bacterial flagella [76] and cells [65], are used to improve image capture. Once digitized, the datasets can be analysed directly and compared to models that simulate the behaviour of the swarm.

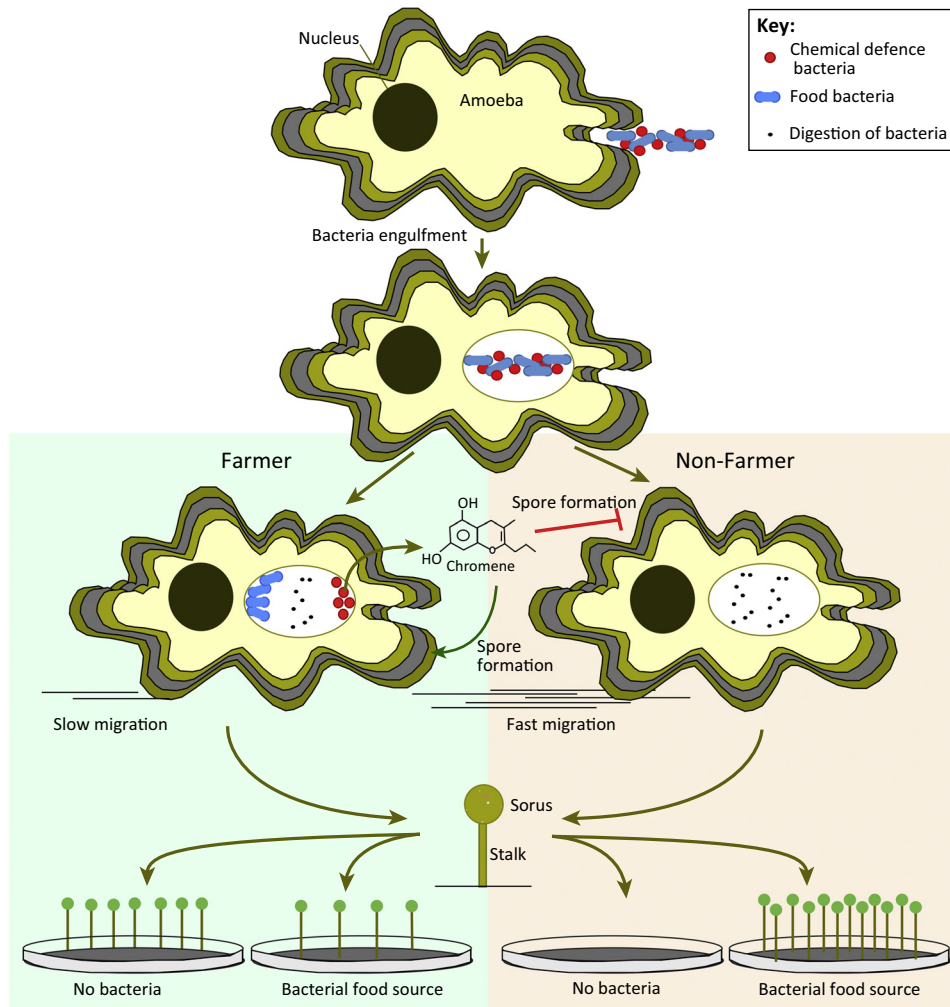
Mathematical modelling of bacterial swarms poses difficult and open mathematical questions regarding the development of a consistent formalism for their description as well as analytical and computational methods. A wide range of modelling approaches has suggested explanations for the emergence of collective motion in bacteria. These can be categorized as either continuous models described by integro-differential equations or discrete agent-based models. Continuous models describe the coarse-grained density of bacteria and other system constituents as continuous fields, for example, as coupled reaction-diffusion equations [77]. Additional methods follow a kinetic approach to describe a population of bacteria as a hydrodynamic or a Boltzmann equation [78,79]. On the other hand, agent-based simulations are useful for describing the internal mechanism of each agent (e.g., a group of bacteria) and their mutual interactions [62]. However, both approaches are limited by the multiscale nature of the dynamics within the colony as it is currently not possible to capture and describe an entire colony across all temporal and spatial scales using a single model.

The interplay between video analysis of experiments and modelling is a powerful method for discovering the underlying principles in the emergence of macroscopic order in dynamic and otherwise hard-to-study systems related to swarms, including swarming bacteria [80–83]. Further, once established, modelling may be used to investigate issues of scale and identify common mechanisms between swarms, even between kingdoms of life or non-living systems.

If swarming is important, what is the effect on nonswarming organisms and why do not all microbes swarm? Aside from passive spread by water and air movement, the recent discovery that nonmotile microorganisms use motile microorganisms to spread provides new insight. The phenomenon of microbial ‘hitchhiking’ has interesting dynamics; one microbe (the transporter) generates the force whilst one or more cargo microorganisms come along for the ride. Microbial transport raises questions as to the ecological impact and evolutionary benefits. In this review, we discuss diverse examples of microbial transport and how this phenomenon may be studied, and highlight open questions and future applications.

Eukaryotic Transport of Bacteria: Rudimentary Farming

The myxomycetes, or slime moulds, are amoeboid eukaryotes notable for their complex life cycle, with multiple motile stages including flagellate swarm cells and extended networks of the amoeboid form. Individual amoebae of the myxomycete *Dictyostelium discoideum* are motile and graze on bacteria [17] (Figure 1). When nutrients are limited, amoebae use a cyclic AMP signal to organize, and cells stream into an aggregate of thousands [18,19] to produce a multicellular, migratory slug [17]. Roughly 20% of the cells die in the process of forming the stalk, an apparent example of altruism in nature, and the remaining cells differentiate into a sorus that contains spores [17–19]. Up to a third of all wild strains of *D. discoideum* have been described as ‘farmers’ that transport the bacteria that are the source of nutrients but, unlike other strains, do not graze the food supply to the point of extinction [17,20]. The farming strategy has both costs and benefits for the transporting amoebae. The strains of *Dictyostelium* loaded with bacteria travel less far, and investments in cotransmission do not pay off when spores germinate in places where food bacteria are already present. On the other hand, farmers are able to capitalize on locations with no nutrients by carrying their own food bacteria. This is an interesting capacity, effectively extending the habitat of the strain by codispersing useful cargo. Farmers also transport inedible bacteria that produce small molecules which enhance and depress the farmers’ and non-farmers’ spore production respectively. Therefore, cargo bacteria can have



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Figure 1. Strategies for Subsistence by Motile Amoebae of *Dictyostelium discoideum*. *D. discoideum* feeds on specific strains of bacteria. *D. discoideum* populations can be divided into two types: 'farmers' and 'non-farmers'. The latter group completely consume the bacteria they engulf and then rapidly migrate onwards in the quest for more food. The farmers consume only a fraction of some strains of cargo bacteria; the survivors secrete chromenes that enhance spore production by the farmers while depressing sporulation in the non-farmers. Second, the surviving cargo bacteria are seeded, cultivated, and used as food in case of otherwise barren locations. However, the farmers transporting bacteria move slowly in comparison to non-farmers. Thus, in the environment lacking external prey bacteria, farmers have an advantage over the non-farmers but the reverse is true in an environment with abundant bacteria to graze on.

multiple benefits. The key of profitable farming by *Dictyostelium* is not to specialize on a single bacterial species but to recruit a range of useful cargo bacteria as needed [17,19,21,22].

As noted above, *Dictyostelium* engulfs and kills most bacteria. To protect themselves from predation by amoebae, bacteria have evolved defensive strategies. One such includes survival and replication within the host cell [23,24]. Some of the bacteria consumed by *Dictyostelium* manage to survive in the host cells, and eventually a symbiotic relationship may develop. The endosymbiont/cargo bacteria (*Pseudomonas fluorescens*) gain protection and dispersal while the host enjoys chemical defence via secondary metabolites secreted by the bacteria. The cargo bacteria produce pyrrolnitrin, an antifungal agent that inhibits respiratory electron transport, and a chromene (3-ethyl-2-propyl-2H-chromene-5,7-diol) that enhances spore production of the

host while depressing spore production in amoebae that do not contain cargo bacteria [22]. In the course of evolution, a single point mutation has occurred in the *gacA* gene (part of the *gacA–gacS* regulatory system controlling global antibiotic production) of cargo strains of *P. fluorescens*. This mutation has altered the chemical repertoire of the bacterium, changing its role in the association to being farmed [22]. In some other cases, the shift to a symbiotic relationship of *Dictyostelium* with bacteria has been accompanied by bacterium-to-*Dictyostelium* gene transfer [25]. Lateral gene transfer may help to explain the tendency of the host to gain beneficial bacteria, as well as to evolve resistance to the toxic secondary metabolites secreted by the bacteria [21]. However, farmers' resistance to their 'own' bacteria does not necessarily extend to newly encountered strains [26]. In the latter and more competitive situation, the number of bacteria relative to amoebae determines the likelihood of one outcompeting the other, thus suggesting a positive Allee effect (where increasing group size correlates with increased individual fitness) [6,26]. *D. discoideum* presents various behaviours, such as apparent altruism, by forming the sterile stalk [18], cheating when individuals avoid forming the stalk themselves, yet benefiting from its production by others [27], and producing chimeric slugs and fruiting bodies [28]. Each of those strategies can have an impact on cargo bacteria. For example, creating chimeric fruiting bodies that consist of amoebae with distinct genetic backgrounds, and contain different bacterial species in the farmer's spores, can result in a greater variety of bacteria in the new location [28]. Amoebae provide other examples of long-term associations – for example, *Acanthamoeba* engulfs and transports the pathogenic bacterium *Listeria monocytogenes* [29].

Further examples of cooperative relationships between fungi and bacteria are known, where the fungus helps to disperse bacteria whilst the latter directly or indirectly provide other benefits [30–32]. For example, the soil fungus *Morchella crassipes* aids the spread of *Pseudomonas putida* along fungal hyphae – the bacteria using their flagella for adherence to growing hyphae, thus offering an explanation for the maintenance of flagella even in soils unsuited for bacterial swimming or swarming [31]. Bacteria can also swarm along fungal hyphae, using them as so-called 'fungal highways' [32,33]. The relationship has been described by the authors as an example of microbial farming – the fungus gains nutrients and stress-resistance, and influences the distribution and abundance of the bacterial crop [31]. Interspecies signalling may be involved between the fungal and bacterial partners, including the interception of bacterial quorum sensing signals by the fungus [33]. In summary, a nonmotile, mycelial eukaryote can aid the dispersal of motile prokaryotes in exchange for other benefits.

Myxomycetes' Logistics Networks Distribute Algae

The development of extended pseudomycelial networks by the myxomycete *Physarum polycephalum* has attracted considerable attention as an organism which can nevertheless solve surprisingly complex tasks in logistics. Such logistical problems include the optimization of nutrient sources and an ability to navigate mazes [34,35]. Solving mazes, that is, finding the optimum path in a situation with complex alternatives to enhance food supply, requires movement plus a record of the past (a 'memory' slime trail that the organism is repelled from) to allow the organism to optimize decision making by taking into account previous, unproductive trails. Whilst myxomycetes are generally predatory, engulfing bacteria and fungi for food, there are indications that some species of algae can survive and are transported within the pseudomycelial networks. For example, a close association forms between the slime mould *Fuligo cinerea* and *Chlorella* spp. [36]. Metabolic labelling studies, using $\text{NaH}^{32}\text{PO}_4$, suggest interchange of otherwise undefined metabolites between slime mould and algae in both directions [37]. Further, when such networks are illuminated in part, the algae thrive and express chlorophyll in the light but not in the darkened areas (Figure 2). The slime mould becomes more stress resistant, grows better, and can form spores – all processes aided by the algal cargo [36,37]. The algae gain in two ways: protection from environmental stresses and movement through (within) the pseudomycelial network [36,37]. Most of the work in the area of slime mould logistics

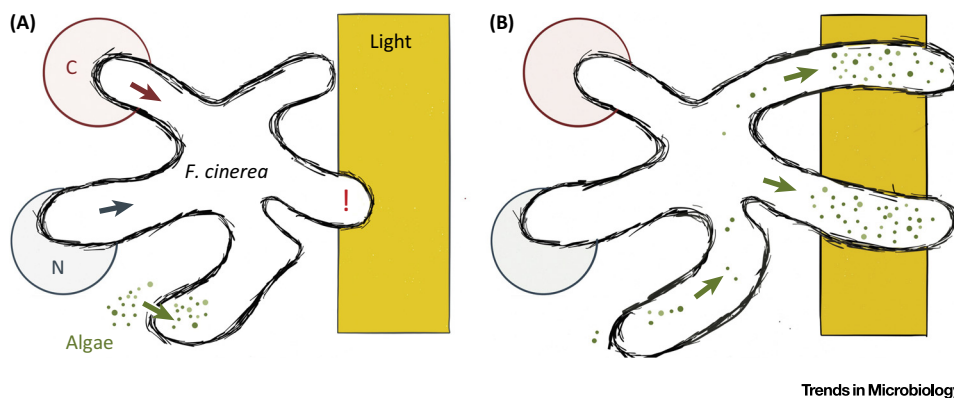


Figure 2. Illustration of Network Formation in Slime Moulds, and Incorporation and Distribution of Transported Algae. (A) Formation of a pseudomycelial network by *Fuligo cinerea* with optimization of logistics for the uptake of beneficial goods; that is, carbon sources (C) and nitrogen sources (N) and uptake of beneficial algae. *F. cinerea* without cargo algae or stressful situations including strong light (I). (B) Intracellular transport allows the algae to use the pseudomycelial network to make the slime mould light-resistant and therefore reach new niches. Benefits for the slime mould may include stress resistance and access to nutrients fixed by the algae.

is not recent [36,37], and given significant advances in slime mould genetics and genomics since the 1960s [38] the area deserves revisiting with modern techniques in order to define genes involved in intracellular transport of algae, the nature of metabolic exchanges between the two parties, and quantifying who gains what in the relationship.

The examples we have described so far are amoeboid eukaryotes. Whilst somewhat out of the scope of this article, we also note that some microbial pathogens, including *Aspergillus fumigatus*, resist phagocytosis by white blood cells and can be dragged to new locations. Whilst this is often linked to a killing mechanism, the potential also exists that survivors will be transported to infect new areas of the human body [39].

Swarm Logistics of Social Bacteria

Mass migration of bacteria over the surface of agar plates is a familiar phenomenon to microbiologists [12–14]. Typically, these swimmers are studied in monoculture. Indeed, some swarming bacteria, such as *Proteus mirabilis*, actively exclude other strains of the same species [40]. However, cases of coswarming are known – situations where two swarming bacteria, often not closely related species, travel together with mutual advantage. For example, coswarming *Pseudomonas aeruginosa* and *Burkholderia cepacia* can overcome combinations of antibiotics that would defeat both parties individually [41]. This association appears to be highly specific, and a combination of laboratory experiments and modelling suggests that this phenomenon is mediated by quorum sensing interactions [41,42]. Further, coswarming *Bacillus megaterium* and *Ketogulonicigenium vulgare* are capable of metabolic cross-feeding. The relationship between these two bacteria appears to be primarily a form of nutritional cooperation [43]. These studies on swarming bacteria are not necessarily simply in a dispersal phase, but can also be considered as a dynamic, travelling ecosystem where microbes grow and trans-species interactions occur. Additionally, even monoclonal swarms may be far from homogeneous and contain multiple phenotypic variants [12,44–46]. An interesting example is the Gram-positive bacterium *Paenibacillus vortex*, which creates elegant patterns on agar plates, with a preponderance of rapidly swarming, slowly reproducing explorer cells towards the edge and rapidly dividing but poorly motile builder cells in the interior [47] (Figure 3). All swarming bacteria show a degree of traffic organization, streaming, and often some vortex formation [12], but *P. vortex* forms particularly structured and stable streams and other forms of dynamic patterns. The dynamics of *P. vortex* colony development and motile organization has been studied extensively

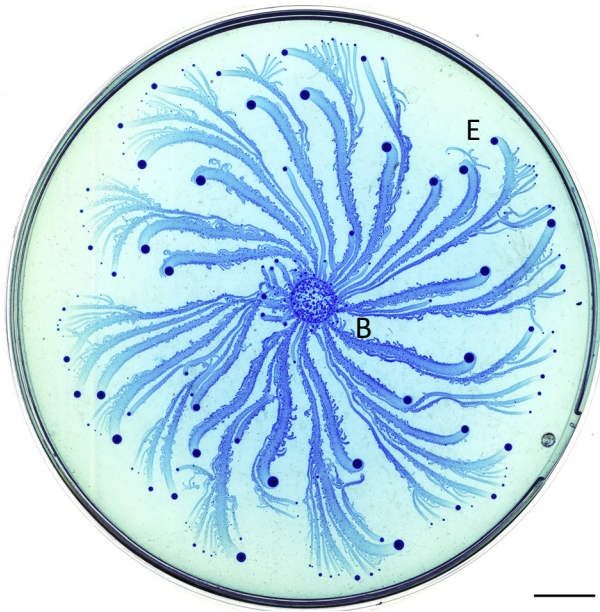


Figure 3. Example of a Differentiated, Extended Colony of *Paenibacillus vortex* on Hard Agar in a 9 cm Diameter Petri Dish. *P. vortex* was cultivated on agar for a week then stained with Coomassie blue. Towards the centre of the colony the builder cells (B) are primarily reproductive but have limited motility whilst the edges are composed of highly motile explorers (E), organized into rotating self-lubricating colonies spinning out into new territories. Scale bar, 10 mm.

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at the level of modelling (Box 3). Such formations are notable for their stability despite the cells making up these networks being in constant motion. Further, the paenibacilli are notable for moving objects [16,48]. A movie available online (see Video S1 in the supplemental information online) illustrates both streaming and cargo capacity: in some cases over 15% of the volume of the swarm consists of foreign objects – in these examples the foreign objects are polymer beads which can be individually >100 times the mass of a single bacterium. The beads appear entrapped by the flagella of adjacent bacteria rather than being moved in the fluid streams generated during bacterial swarming [16]. However, the most interesting aspect of the transport capacity of *P. vortex* is the relationship with other microorganisms within a moving swarm. This will be discussed in the next two sections and in Box 3.

Bacteria Transport Bacteria

When *P. vortex* swarms it can carry other bacteria within the motile colonies. The latter can be considered cargo if they are moved but do not contribute to the motility of the consortium/swarm [48]. If the cargo bacteria assist the survival of the swarm then they can form a persistent part of the moving colony. For example, *P. vortex* swarms continuously in monoculture but, despite a limited refractory resistance to antibiotics during swarming, it cannot enter environments containing high concentrations of β -lactam antibiotics [48]. A less motile cargo bacterium expressing a β -lactamase capable of detoxifying this group of antibiotics could survive in such an environment but could not spread. The combination of *P. vortex* and antibiotic-resistant cargo creates alternating waves of detoxification and cargo transport, allowing both parties to invade new territory and expand their populations (Figure 4). The value of the cargo matters, as more highly resistant cargo facilitates faster spread in the presence of an antibiotic due to faster degradation of the antibiotic [48]. From the perspective of the cargo bacterium, this suggests that there may be a selection pressure for high levels of resistance facilitating local dispersal.

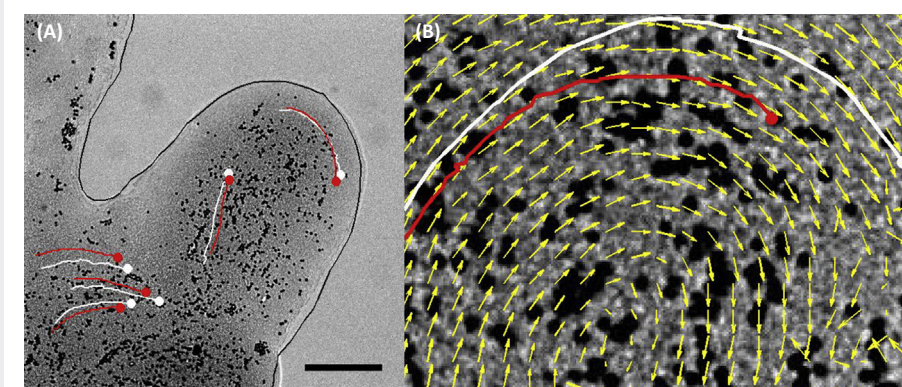
Whilst swarming is commonly studied in the laboratory, examples of swarming (and other forms of surface motility) in the environment are still rare [12]. *Paenibacillus* spp. robustly colonize many habitats, including organic surfaces. On such surfaces a complex mix of microorganisms will exist and potentially interact with swarm-proficient *Paenibacillus* spp. For example, the plant

Box 3. Lessons from Modelling *Paenibacillus vortex* Traffic Streams and Transport

Typically, models of swarms assume equal capabilities for all agents within the swarm. However, not all agents are equal within swarms of bacteria such as *P. vortex*; individuals vary in their motility and transport capability, and cargo objects also require different treatments, requiring the extension of existing modelling approaches. Agent-based modelling techniques have been used to analyse the traffic structure, navigation potential, and transport of cargo objects within *P. vortex* swarms (Figure 1). This has included:

- (i) Vortex formation, structure, and maintenance [80].
- (ii) Traffic structure. Culture on low-solidity agarose using tracking beads to aid visualization revealed long-term stable traffic of motile bacteria (see Video S1 in the supplemental information online); this is in contrast to the relatively disorganized swarming of bacteria such as *Escherichia coli* [76].
- (iii) Transport. Movies of *P. vortex* transporting fungal spores were compared to agent-based simulations making different assumptions about the mechanism of transport. A high degree of convergence between movie and model was obtained if the spores are assumed to be surrounded by a relatively firmly attached group of bacteria [16].
- (iv) Contacts between bacteria and cargo were modelled based on the connection between *P. vortex* and cargo, with the dynamics of the connection being consistent with flagella [81].

Using such multi-agent bacterial swarming models, several different putative generic mechanisms that may underlie the observed swarming logistics and cargo-carrying abilities in *P. vortex* were tested and analysed by comparing simulations with experiments. In particular, cell–cell and cell–cargo interactions, response to chemical gradients, and interactions between the bacteria and the boundary of the layer of lubricant collectively generated by the swarming bacteria were studied. Using realistic parameters, models captured the observed phenomena with semi-quantitative agreement in terms of the velocity as well as the dynamics of the swarm and its envelope. This agreement implies that the bacteria's interactions with the cargo and swarm boundary play a crucial role in mediating the interplay between the collective movement of the swarm and the internal traffic dynamics [77–83].

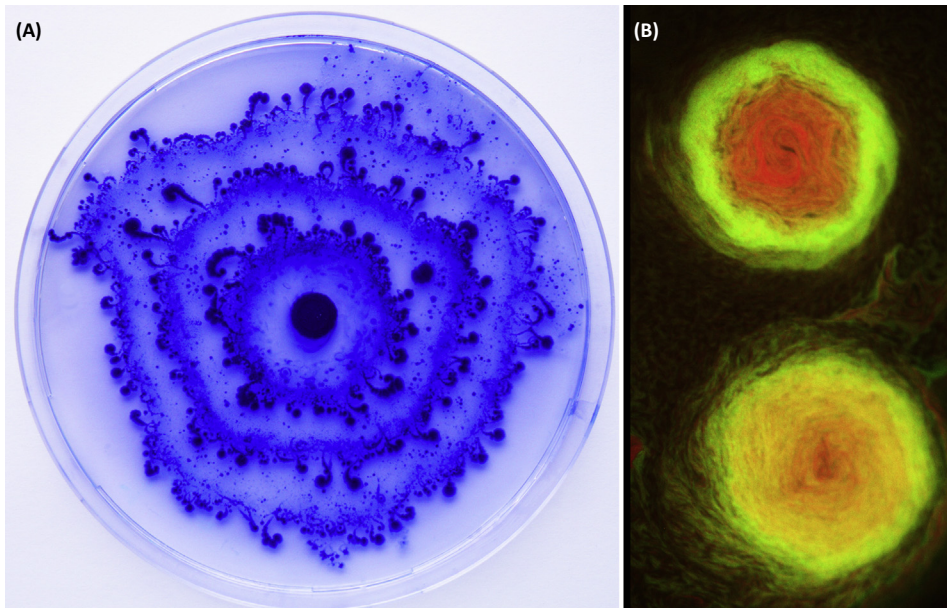


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Figure 1. Comparison of Modelling and Image in Cargo Transport. (A) Frame from a movie of *Paenibacillus vortex* transporting spores, with the trajectory of specific spores (red) compared to a model that assumes that virtual spores (white) were closely connected to local bacteria. (B) Zoomed-in section of the previous panel showing individual spores (dark spheres, 5 μm diameter) with the addition of yellow arrows showing the velocity field calculated from successive frames showing a local rotational movement. Image adapted from [16].

pathogen *Xanthomonas perforans* is capable of stimulating the motility of *P. vortex* and using this to spread over the surface of a tomato leaf [49]. The stimulation of motility and attraction of the transporter by the cargo (using unknown volatile compounds) suggests an element of species specificity. Therefore, it is likely that transporter–cargo networks exist productively in the environment and that some relatively sessile species actively promote their own dispersal by stimulating the motility of nearby bacteria that may be able to render assistance.

If there is an intermittent or continuous selection pressure for maintenance of the cargo bacteria, the association appears highly robust. However, if there is no selection pressure then cargo bacteria are lost [48]. *P. vortex* deploys small, rapidly moving colonies swarming ahead of the bulk population that do not carry cargo bacteria [48]. This suggests a bet-hedging strategy: if



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Figure 4. *Paenibacillus vortex* Transporting Bacteria. (A) Periodically expanding colony composed of *P. vortex* and an ampicillin-resistant strain of *Escherichia coli* (cargo) on a 14 cm diameter nutrient agar plate containing ampicillin. The plate is colonized in alternating phases of swarming and consolidation; when the antibiotic concentration becomes too high, consolidation and detoxification of the antibiotic by the cargo strain takes place. Neither species can thrive in this environment alone. (B) Detail from the outer edge of colony shown in panel (A). Cargo-transporter microcolony from the edge of the colony in panel (A), >0.4 mm across, stained with fluorogenic dyes (red, *P. vortex*; green, protective cargo bacteria), and imaged by fluorescence microscopy. Figure reprinted with permission from [48].

these monospecies, migratory colonies reach new territory they can thrive without competition from hitchhikers. However, if this effort fails then slower colonies may be able to use phenotypes conferred by cargo bacteria to solve problems such as antibiotic presence and cocolonize an environment as a consortium. Furthermore, *P. vortex* is a sporulating bacterium and can move its own spores. That may be another bet-hedging strategy, where a swarm expends extra energy but highly resistant spores are always present rather than taking an hour or longer to form from vegetative cells [16]. In summary, *P. vortex* physically transports and disperses cargo bacteria that 'pay their fare' by contributing stress or antibiotic resistance and the invasion of new territory.

Lilliputian Transport of Gulliver for Travelling in Harsh Territories

Relative to their mass, bacteria can generate powerful propulsive forces (Box 1). *P. vortex* can move objects larger than the bacteria themselves. Asexual fungal spores (conidia) hundreds of times the mass of an individual bacterium can be moved tens of centimetres before germinating productively. In rare cases, conidial aggregates thousands of times the mass of a bacterium can be moved [16]. A comparison of agent-based modelling with movies of conidial transport suggests that the bacteria surrounding any given spore remain the same during transport. This implies that the conidia are in some way entrapped or connected with the *P. vortex* cells, with only limited turnover. This view is supported by electron microscopy in which conidia appear stably entrapped within flagellar nets (Figure 5). Therefore, there may be two roles for flagella in swarm-mediated transport – force generation to move the swarm and entrapment of the cargo. Conidia gain movement to more productive territories, including rescue from hostile regions, such as those containing antifungals. Conversely, fungi can also disperse bacteria by providing paths through the soil, including over air gaps and other barriers that bacteria alone find hard to

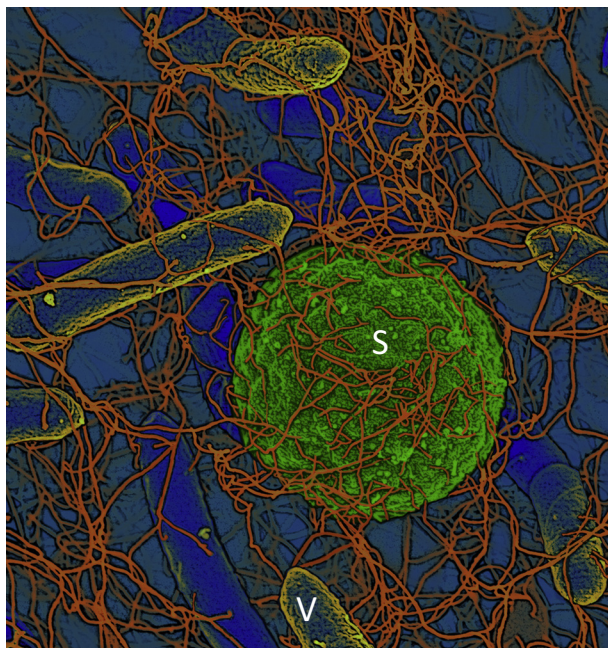


Figure 5. Transport of Fungal Spores. The fungal spores (green sphere, 5 μm diameter, marked S) are apparently entangled by bacterial flagella (orange filaments). The bacteria (e.g., V) are outlined in yellow (colourized scanning electron micrograph). Figure reprinted with permission from (and subsequently modified by adding colour) [16].

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cross [50,51]. Mycelial spread may be via attachment of bacteria to growing mycelia, facilitation of motility along fungal highways, and even within mycelia, for example intrahyphal cyanobacteria in the cyanolichens or nitrogen-fixing bacteria in mycorrhizal fungi [30]. When *P. vortex* disperses spores of *A. fumigatus* the spores germinate during transport. If this happens adjacent to an air gap that cannot be crossed by swarming bacteria then the germinated spores form mycelia that bridge the gap. This, in turn, can allow the bacteria to cross, although it is not clear if bacterial motility is involved. Thus, two very different microorganisms can mutually facilitate each other's dispersal [16].

This is an example of cooperation between very different organisms. The size disparity between cargo and transporter and the distance covered challenges the way we think about scales in microbiology. Mycelial bridges may be important 'bacterial highways' in crossing barriers in the soil [23,24], and fungi are known to farm and disperse bacteria [25], but that bacteria can contribute to building their own roads is surprising. Along with bet-hedging strategies discussed above, it suggests that there are novel strategies, in terms of microbial networks, not easily seen by metagenomics or other molecular techniques.

Synthetic and Virtual Swarms

Understanding heterogeneous swarms of microorganisms may lead to new theories and eventually technologies. Algorithms are already derived from motile microorganisms and used outside microbiology; for example, swarm search and optimization methods motivated by microbial foraging optimization, including elements of chemotaxis, have been found to be advantageous for searching big data structures [52,53]. Man-made swarms of a large number of simple cooperating robots can perform complicated tasks even with only minimal communication and sensing capabilities [54].

From the point of view of medical applications, efforts towards modelling and optimization of biological swarms carrying therapeutic cargo are currently being directed towards the targeting

Box 4. Tools and Approaches for Further Advances

- An understanding of the genetics and genomics of microbial logistics and transport is needed, starting with key eukaryotic and bacterial model microorganisms.
- Enhanced imaging and analytical systems are needed that are capable of tracking individual cells within dense microbial swarms.
- Development of biologically realistic mathematical models is needed to describe the new, highly organized swarms.
- Modelling tools should be developed that are insightful on a small scale (short times, and/or short distances – e.g., agent-based simulations).
- Modelling tools should be developed that connect the macroscopic to microscopic scale.
- Microfluidics and Lab-on-a-Chip systems that allow calculation of forces generated are needed.

of modified bacteria or microrobots with the aim of developing cancer therapies [55,56]. In terms of synthetic cargo, individual microorganisms can move cargo far larger than the mass of a cell. Motile algae, for example, can carry covalently coupled beads, guided and targeted by light. The expectation is that swarms will add cargo capacity, targeting, and decision making capabilities. However, achieving this is challenging and requires the development of new tools (Box 4). One possibility lies within ‘swarm intelligence’, when the group collectively makes better decisions than the individual. The degree to which collective sensing, communication, and decision-making impacts microbial cargo transport, that is, to what degree it can be directed, is unclear. However, it is notable that microorganisms that are cargo transporters can solve problems in ways not accessible to other microorganisms. Programmable microscale swarms may seem far-fetched, but the interface of microbiology, synthetic biology, and cybernetics is advancing rapidly [57]. Further, individuals within cybernetic swarms are not necessarily agents of high complexity and may have interesting directable properties, such as cells containing magnetic monopoles [58]. Given this, cargo transport in nature can be expected to provide further inspiration for miniaturized swarms of increasing sophistication.

Concluding Remarks and Future Directions

This review describes apparently diverse situations in which transporting and sessile microorganisms form associations where the cargo gains by dispersal and the transporting organism benefits in other ways. By transporting, we refer to motility and the generation of force moving a load. However, mycelial or pseudomycelial eukaryotes may contribute to cargo dispersal by growth or intracellular transport [59,60], or by mycelia crossing barriers such as air gaps [16].

These observations put forward a range of open questions. For example, are there specific, currently unknown loading and unloading mechanisms? What are the intracellular and extracellular processes enabling bacteria to decide which cargo to transfer, when to pick it up, and when to discard?

To date the most prominent examples derive from two groups of transporting organisms: amoebae and swarming bacteria. Amoeboid eukaryotes form highly specific associations with bacteria or algae; the cargo microorganisms are often internalized but survive, spread, and prosper. In this situation, the transfer of cargo DNA to the transporter suggests a long association in evolutionary terms. In contrast, at first sight the transporting bacterium *P. vortex* appears relatively opportunistic and indiscriminate. Swarms of *P. vortex* can move a wide variety of cargo organisms and inanimate objects very effectively. However, when the cargo is not productive it is discarded, and this appears a beneficial strategy. Further, some cargo bacteria gain from stimulating *P. vortex* motility, supporting the idea of specific transporter–cargo interactions [49].

When it comes to long-range dispersal there are still many unanswered questions (see Outstanding Questions). We can expect genomics to develop in order to predict which strains within the microbiome of any given habitat can move, and possibly transport. In terms of transport

Outstanding Questions

Do individual cells cooperate within a swarm, do they compete, or are both possible within the same swarm?

To what extent do microbial logistics networks form in nature?

What are the advantages of complex (heterologous) swarms?

Are microbial swarms more capable, in terms of information processing and decision making, than individual cells; to what extent is swarm intelligence relevant to microbiology?

To what extent does this phenomenon impact microbial dispersal and the colonization of new niches?

What are the loading/unloading mechanisms responsible for picking up and discarding cargo? To what extent does interspecies specificity matter, and how does it evolve?

To what extent are insights from microorganisms applicable to swarms of more complex organisms or the organization of cells within multicellular organisms?

How can we couple multiple species in synthetic networks in biotechnology applications?

Can we use biomimetics to design heterologous, nonliving swarming agents?

capacity, experiments looking at the genetics of transporter–cargo interactions appear feasible, including coevolution experiments over many generations. At another level, we need more sophisticated models to simulate how heterogeneous, motile ecosystems behave [61]. Development of simplified models, extending some well-studied homogeneous models [62] to include cargo and cooperation between species, may allow in-depth study and analysis to identify the key interactions and properties that allow cooperation and coordination while on the move and help us to understand how cells build to communities and then to ecologies [63,64].

Supplemental Information

Supplemental information associated with this article can be found, in the online version, at doi:10.1016/j.tim.2015.12.008.

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