OPINION

Division of labour in microorganisms: an evolutionary perspective

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Abstract | The division of labour, whereby individuals within a group specialize in certain tasks, has long been appreciated as central to the evolution of complex biological societies. In recent years, several examples of division of labour in microorganisms have arisen, which suggests that this strategy may also be important in microbial species. In this Opinion article, we explore the set of conditions that define division of labour and propose that cooperation between different phenotypes is a defining feature of division of labour. Furthermore, we discuss how clarifying what constitutes division of labour highlights key evolutionary questions, including what form division of labour takes and why it is favoured by natural selection.

Microbial cells in a population often show extreme phenotypic variation, which can arise through several mechanisms (BOX 1). For example, when *Escherichia coli* cells are growing in a batch culture, a proportion of the cells are in a transient non-growing state, termed persister cells, whereas the rest of the cells are growing normally[1]. A standard explanation for this phenotypic variation is that it represents a bet-hedging strategy, whereby different phenotypes are more successful in different environments, and therefore the fitness of cells is increased by varying the phenotype[2,3]. In the case of *E. coli*, the persister phenotype promotes survival under conditions of environmental stress, such as the presence of antibiotics[4].

However, phenotypic variation often cannot be explained as bet-hedging. These other examples seem to involve the specialization of some cells in a population to carry out cooperative tasks that benefit other cells that do not carry out the cooperative task (FIG. 1A). For example, in populations of *Bacillus subtilis* at stationary phase grown in liquid medium, a proportion of the cells produce and excrete proteases that degrade proteins in the environment into smaller peptides, which can be used as nutrient sources[5]. As the proteases and their degradation products freely diffuse, the production of proteases is beneficial for the local cell population and not just for the protease-secreting cells. In this Opinion article, we propose that cooperation between different phenotypes is a defining feature of division of labour[3,6,7].

Determining whether examples of phenotypic variation represent division of labour raises new questions. Why would natural selection favour division of labour? Why would it be beneficial for just a proportion of cells in a given population to carry out a trait, such as protease production, rather than all cells carrying out that trait at a lower rate? If the division of labour is cooperative, why can lineages that produce a lower proportion of the cooperative phenotype (that is, cheats)[8] not invade the population, which would eventually lead to the loss of division of labour? Why is division of labour favoured by natural selection in certain environments, for certain tasks, but not others? Or why are different mechanisms, such as phenotypic noise or environmental cues[2] (BOX 1), used to promote phenotypic variation in different situations?

A better understanding of division of labour may also provide insights into the evolution of other biological processes, including virulence and multicellularity. The division of labour seems to be central to the success and virulence of pathogenic species, such as *Cryptococcus gattii*, which is the causative agent of fungal meningitis. During infection, host reactive oxygen species, an essential part of the host immune response, induce a tubular mitochondrial phenotype in a proportion of *C. gattii* cells[9]. When phagocytes engulf cells of both types, the cells with tubular mitochondria are able to protect the normal cells and increase their intracellular proliferation[9]. Finally, the division of labour between cells has had a crucial role in the evolution of multicellularity, and therefore to elucidate how complex life on earth has evolved, we need to understand why division of labour is favoured in microorganisms[10–12].

In this Opinion article, we explore what constitutes division of labour within a microbial species. We propose a set of conditions that define division of labour and discuss whether several previously described examples of phenotypic plasticity represent adaptive division of labour. A precise definition is crucial, because imprecise definitions and ambiguity can obscure the fundamental problems and impede conceptual unification[13,14]. We outline key questions in the study of division of labour in microorganisms, focusing on what division of labour is, why it is favoured by natural selection and what forms it can take.

Complementary approaches

Before defining division of labour, it is useful to distinguish between mechanistic and evolutionary approaches to studying traits or behaviours[15]. The mechanistic (proximate) approach is to ask questions about how traits are controlled, such as what are the molecular or genetic mechanisms that control a particular trait (how questions; BOX 1). The evolutionary (ultimate) approach is to ask questions about the fitness consequences of that trait, and why it has been favoured by natural selection (why questions).

The majority of previous work on phenotypic heterogeneity has been mechanistic — our aim in this Opinion article is to ask evolutionary questions[2,3,6,7,16,17]. The crucial point

Box 1 | **Mechanisms of phenotypic variation**

There are at least six possible mechanisms for generating phenotypic variation within a species[2,17]. There can be overlap between these mechanisms.

Genetic differences. Standing genetic variation or mutations can lead to different phenotypes[27].

Epigenetics. Different phenotypes could be maintained by epigenetic inheritance, such as DNA methylation, which leads to a correlation in phenotype across generations[67].

Noise. If random fluctuations in the biochemical reactions of the cell (noise) are coupled with a gene network that amplifies small differences in reaction levels, this can lead to phenotypic variation[2,17]. For example, the phenotypic variation between *Salmonella enterica* subsp. *enterica* serovar Typhimurium cells that do and do not express type III secretion system 1 (*ttss-1*), or exoprotease secretion in *Bacillus subtilis*, both arise from noise[5,22].

Signalling. In cyanobacteria, such as *Anabaena* spp., signalling peptides that are exported from one cell induce the development of a neighbouring cell into a nitrogen-fixing heterocyst[16]. This produces a regular pattern of heterocysts every relatively fixed number of vegetative cells among the filamentous colony. This number can vary from approximately 4–15 cells, depending on the species.

Environment. Variation can be generated by environmental cues. For example, in cyanobacteria, in addition to the role of signalling, nitrogen stress can lead to a higher proportion of cells that develop into heterocysts[16].

Condition dependence. Variation can be generated by differences in cell condition. In *Volvox carteri*, a series of asymmetric cell divisions, during early embryonic development, lead to the generation of small and large cells, which develop into soma and germ, respectively[68,69]. This process involves a gene that ancestors used to reduce reproduction during stressful conditions, and is being co-opted to produce a non-reproductive phenotype[68,70,71]. Variation in condition can also be dependent on the environment; for example, during nutrient-depleted conditions, cells in poorer condition might be more likely to become a non-reproductive altruist.

here is that these two approaches are complementary and not competing. Mechanistic answers cannot be given for evolutionary questions and vice versa, but an understanding from one perspective can aid the other perspective[18]. For example, an evolutionary approach can suggest when we might find different mechanisms in different species, whereas a mechanistic understating of what factors stimulate phenotypic heterogeneity can help us understand why that heterogeneity is favoured.

What is division of labour?

We define the division of labour as when cooperating individuals specialize to carry out specific tasks. This requires three conditions: individuals carry out different tasks (phenotypic variation); some individuals carry out cooperative tasks that benefit other individuals (cooperation); the division of tasks provides an inclusive fitness benefit to all of the individuals involved (adaptation).

We and others[7] emphasize the importance of division of labour as being cooperative. A behaviour or trait is cooperative when it benefits another individual and has been selected for, at least partially, because of this benefit[14].We emphasize cooperation because we are interested in cases in which individuals are working together, and have been selected to divide tasks, to the benefit of all individuals (see Supplementary information S1,S2 (boxes)). Therefore, division of labour represents a social adaptation across several individuals.

The second and third conditions (cooperation and adaptation) distinguish division of labour from cases in which phenotypic variation has arisen from self-interested traits, such as diversification to exploit different niches, or when one phenotype evolves to exploit another. We focus on inclusive fitness because it is our most general description of Darwinian fitness — natural selection favours traits that lead to an increase in inclusive fitness[19–21] (BOX 2). Consequently, if we are interested in whether a social trait, such as division of labour, can be favoured, we examine the inclusive fitness consequences. Our definition makes no claim as to whether a population must be clonal, although we discuss how clonality can influence whether and what form of division is favoured.

Examples of division of labour. During infection of a vertebrate host, a subpopulation of *Salmonella enterica* subsp. *enterica* serovar Typhimurium cells remains in the gut lumen to reproduce, whereas other cells invade the gut tissue and express the type III secretion system 1 (*ttss-1*), which triggers an inflammatory response that eliminates competing bacteria from different species[22,23] (FIG. 1A). As the cells that enter the gut tissue are killed by the host immune system, this represents an altruistic cooperative behaviour, which is costly to the invading cells but benefits the cells that remained in the gut lumen (FIG. 1B; see Supplementary information S3 (box)). Another example of altruistic division of labour is provided by the fruiting bodies of slime moulds, such as *Dictyostelium discoideum*[24], in which non-viable stalk cells hold up and help disperse the viable spore cells. Similarly, spore cells also exist in the fruiting bodies of *Myxococcus xanthus*[25]. These examples are all analogous to the division of labour between germ cells and soma cells in multicellular species, in that some cells give up any opportunity to reproduce, to help reproductive cells[10].

Not division of labour. Our definition excludes several examples of phenotypic variation that are not cooperative and hence not division of labour. Under liquid culture conditions, *Pseudomonas fluorescens* exhibits different phenotypes, including 'smooth', 'wrinkly spreader' and 'fuzzy spreader' (REF. 26). This diversification represents the specialization of different lineages to exploit different niches: smooth phenotypes inhabit the liquid phase; wrinkly spreader phenotypes form a mat at the air–broth interface; and fuzzy spreader phenotypes inhabit the less aerobic environment at the bottom of the broth. These different phenotypes do not benefit each other cooperatively, and so this diversification does not represent division of labour. Similarly, various phenotypes are also observed in *P. fluorescens* colonies growing on agar, which can be explained by the self-interest of the different phenotypes[27].

Our definition also excludes cases in which cells can be divided between different lineages, whereby some lineages exhibit cooperative behaviour and others do not carry out, or carry out less of, the cooperative tasks. For example, *Pseudomonas aeruginosa* cells produce and secrete siderophores that scavenge iron from the environment. The benefits of iron scavenging are shared between the local cells, and so this is a cooperative behaviour[28]. However, lineages evolve, both in laboratory broth cultures and in the lungs of humans with cystic fibrosis, that produce less or no siderophores[29,30]. These lineages seem to act as cheats, which exploit the siderophores that are produced by other cells[8]. In this example, the cheats gain a selfish fitness benefit, but decrease the fitness of the lineages that produce

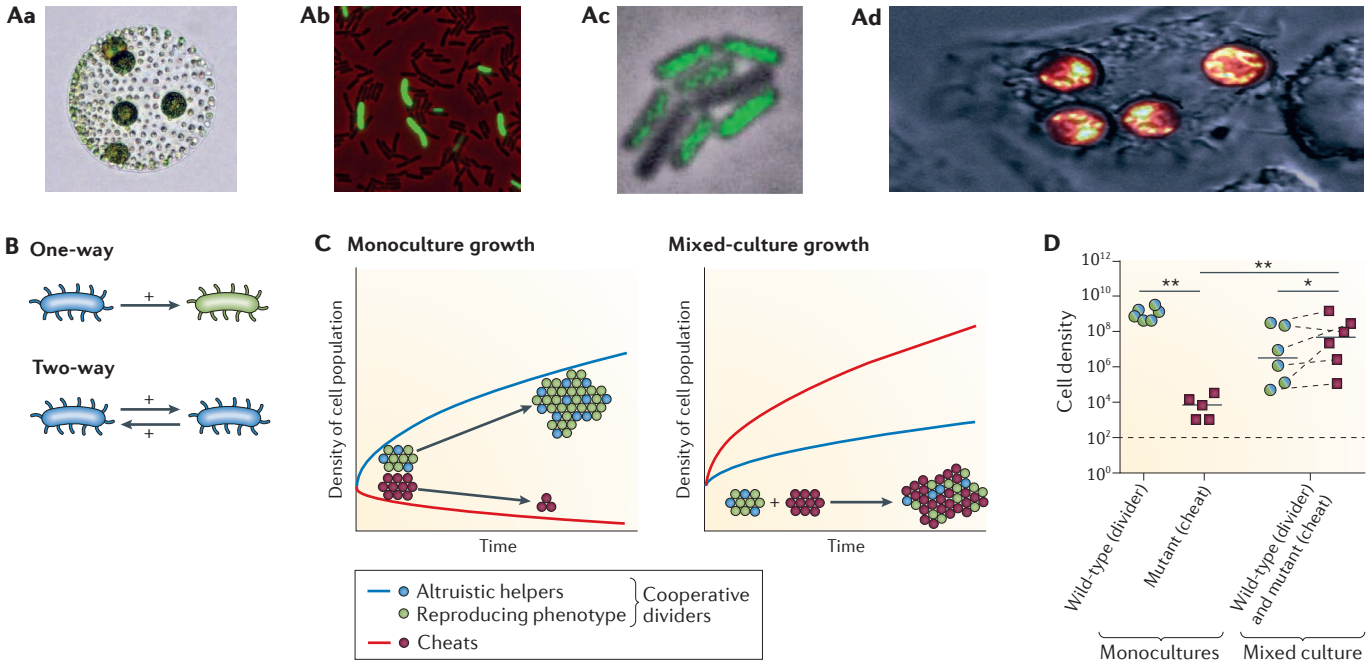

Figure 1 | **Division of labour. A** | Potential examples of division of labour. **Aa** | Phenotypic variation in *Volvox carteri*, which forms large germ cells and small soma cells. **Ab** | Some *Bacillus subtilis* cells (green) produce and excrete proteases that degrade proteins in the environment into smaller peptides. These peptides can be used as nutrient sources by *B. subtilis* cells and cells that do not produce and secrete the protease (grey). **Ac** | During infection, a subpopulation of *Salmonella enterica* subsp. *enterica* serovar Typhimurium cells (green) remains in the gut lumen to reproduce, whereas other cells (grey) invade the gut tissue and express the type III secretion system 1 (*ttss-1*), which triggers an inflammatory response that eliminates competing bacteria from different species. **Ad** | During infection, host reactive oxygen species induce a tubular mitochondrial phenotype in a proportion of *Cryptococcus gattii* cells (mitochondria are shown in yellow). **B** | Division of labour can involve either cooperation in one direction, whereby individuals of one phenotype help another phenotype (top panel; usually altruistically), or cooperation in both directions, whereby individuals of each phenotype cooperate and help the other (bottom panel; possibly to their mutual benefit; see Supplementary information S3, S5 (boxes)). **C** | Division of labour can be demonstrated by growing strains that do and do not carry out the putatively cooperative trait in both monocultures and mixed cultures. We consider: a cooperative strain, with division of labour, in which cells develop into both the altruistic helping phenotype (blue cells) and the reproducing phenotype (green cells); and a cheat strain, in which all cells develop into the reproducing phenotype (red cells). In monocultures (left panel), the cooperative strain, with division of labour, grows at a faster rate than a cheat strain. By contrast, in mixed cultures, the cheats are predicted to grow at a faster rate, because non-cooperative cheats can exploit the benefits that are provided by the altruistic helping phenotype. **D** | An example of this predicted pattern is provided by data from two strains of *S.* Typhimurium — the wild-type with division of labour (that is, cells that do and do not express *ttss-1*, which triggers an inflammatory response), and an *ahilD* mutant, which does not trigger inflammation. The wild-type exhibits an increased fitness when grown in monoculture, as measured by cells per gram of faeces, but decreased fitness when grown in a mixed culture, as the mutant benefits from the wild-type. Part **Aa** is adapted with permission from REF. 76, Springer. Part **Ab** is adapted from REF. 5. Part **Ac** is adapted from REF. 77. Image in part **Ad** courtesy of R. May, University of Birmingham, UK. Part **D** is adapted from REF. 23, Nature Publishing Group.

more siderophores. This contrasts with the examples of division of labour that are discussed above, in which all individuals benefit. In particular, in those examples, the helping and reproductive phenotypes are expressed conditionally, and therefore the helping phenotype is able to gain an inclusive fitness benefit from aiding its reproducing relatives. The contrast here is between an adaptation to exploit others (cheating), rather than an adaptation to help others (cooperative division of labour). An analogous example of cheating is lineages that do not produce or respond to quorum sensing molecules[31,32].

These examples show that division of labour requires more than the production of a novel 'joint' phenotype or the ability to carry out a task, which would not be possible without phenotypic diversity. For example, the different morphological variants of *P. fluorescens* can thrive in the liquid media of a beaker because of the production of various phenotypes[26]. These other types of phenotypic diversity are very interesting and result from different selection pressures. For example, cheats arise from conflict and not cooperation, and they do not require the efficiency benefits that are discussed below.

How to demonstrate division of labour?

First, it must be shown that there is phenotypic variation, with different individuals specializing to carry out different tasks. Second, it must be established that this division is cooperative and provides a fitness benefit to all of the cells that are involved.

Considering the example of *S.* Typhimurium discussed above, the differential expression of *ttss-1* in invading and non-invading cells represents phenotypic variation[22], but is this variation cooperative? To test this, strains that carry out the trait can be grown in both monocultures and mixed cultures with genetically manipulated strains that carry out less of, or do not carry out, the trait[18]. This design makes use of the fact that strains with less division of labour, and which carry out less of the potentially cooperative trait, would act as cheats[8]. If the trait is cooperative and provides a benefit to others, then cells that carry out the trait grow best as monocultures (cooperators outperform cheats), whereas cells that do not carry out the trait would grow best in mixed cultures[7,18] (cheats can exploit cooperators; FIG. 1C).

Box 2 | **Natural selection and adaptation**

Natural selection favours genes that are better at being transferred to the next generation[72]. However, researchers often talk about natural selection in the context of individual behaviour, leading to individuals that maximize their fitness. The formal justification for considering natural selection at the individual level is that genes that increase fitness will accumulate, and hence natural selection, through gene dynamics, will generate organisms that behave as if they are trying to maximize their fitness[72,73]. Thus, the gene and individual approaches are not competing, they are flip sides of the same coin — gene dynamics lead to the maximization of individual fitness.

Genes can influence their transmission to the next generation, not only by influencing the reproductive success of the individual they are in, but also by influencing the reproductive success of other individuals that carry the same gene. Hamilton[19] showed that natural selection will lead to individuals that behave as if they are maximizing not their personal reproductive success, but what he called 'inclusive fitness'. Inclusive fitness is the sum of fitness that is obtained directly, through reproduction, and indirectly through influencing the reproduction of relatives. Indirect fitness must be weighted according to relatedness, which is a statistical measure of the genetic similarity between individuals.

Hamilton's theory is often discussed in terms of kin selection and Hamilton's rule[19]. Hamilton's rule shows that an altruistic trait, such as becoming a sterile stalk cell in a fruiting body, will be favoured when $rB–C>0$ (where C is the fitness cost of carrying out the trait, B is the fitness benefit to other individuals, and r is the genetic relatedness to the individuals that receive the benefit). The most common ways for interacting cells to be related are by either limited dispersal keeping relatives together, or mechanisms of kin discrimination, which allow individuals to preferentially interact with relatives[19]. This approach emphasizes how we can explain division of labour, and especially altruistic division of labour, by examining the inclusive fitness, or kin selected, consequences.

Division of labour is often discussed as benefiting the population or community, with cells behaving analogous to a multicellular organism. It is useful to ask whether this is justified. More formally, we can ask when would gene dynamics lead to individuals that are trying to maximize their group or population fitness? This requires extremely restrictive conditions, in which there is effectively no conflict within groups, such as in clonal populations of cells[63,74,75]. The cells that make up complex multicellular organisms, such as humans, fit this criterion, but populations or communities of microorganisms might not. Consequently, thinking about adaptations such as division of labour at the group or population level is not formally justified and can lead to errors with microorganisms.

As mentioned above, the expression of *S.* Typhimurium *ttss-1* in a subpopulation seems to be an altruistically cooperative trait, and hence represents division of labour[23,30]. In agreement with this, studies have shown that when grown in a monoculture, a mutant *S.* Typhimurium lineage that does not express *ttss-1* is avirulent and unable to spread within hosts (FIG. 1D). By contrast, when grown in a mixed culture with a ttss-1-expressing wild-type strain, the mutant lineage is at an advantage and expands (FIG. 1D). Similar data, showing that more cooperative strains can be exploited by less cooperative strains, have been reported with the fruiting bodies of *D. discoideum* and *M. xanthus*, and the tubular mitochondrial morphology in *C. gattii*[9,24,25].

It is necessary to test whether a trait is cooperative in the environmental conditions under which that trait evolved, or as near to it as possible[8]. The costs and benefits of traits vary under different environmental conditions, and wrong conclusions can be made if experiments are carried out in inappropriate environments[33]. For example, if an experiment is carried out under conditions in which a trait was not required, then the cooperative benefit to other cells would not be observed[31]. Determining whether division is cooperative can be harder when traits are more complex and/or when labour is divided into more types. For example, in *B. subtilis*, two cell types are required to facilitate migration (one task): cells that produce surfactin, a surfactant that decreases water surface tension, and cells that produce an extracellular polysaccharide matrix that glues cells together[34].

Why divide labour?

Why would natural selection favour a division of labour, with different individuals carrying out different tasks, rather than just have each cell carry out all of the tasks? Considering a simple case with two tasks, A and B, in which investment into these two activities must be traded off against each other, because time and energy spent on task A cannot be spent on task B. For example, task A might be reproduction and task B might be the secretion of a factor that causes an inflammatory response in the host, as is the case for *S.* Typhimurium[22,23]. A division of labour can be favoured when two conditions are met: there is an efficiency benefit from having different individuals carry out different tasks (specialization); the reproductive interests of the different individuals are aligned, such that cooperation is favoured.

Efficiency benefits. Division of labour requires an efficiency benefit from different individuals specializing in different tasks[7,10,35–39]. This means that when a cell puts a large effort into a task, it obtains a larger return per unit invested. Assuming linear costs per unit invested into a task, this requires that the slope of the relationship between the proportion of resources that a cell allocates to a task and the fitness return is accelerating[7,10,35,40] (FIG. 2a). An accelerating slope could arise if a task becomes more efficient as more effort is put into it, or if carrying out one of the tasks affects the ability to carry out the other task (they are best carried out in different locations). By contrast, if the fitness returns from a task are decelerating, with cells becoming less efficient at carrying out their task, natural selection would favour that all cells carry out both tasks (FIG. 2a; see Supplementary information S4 (box)).

A major problem in the study of division of labour is the lack of experimental data showing that the fitness return is accelerating[41]. To date, arguments for efficiency benefits have relied on indirect extrapolations, rather than direct experimental tests. For example, in some species of cyanobacteria, cells are divided into cells that photosynthesize and cells that convert nitrogen gas into ammonia (heterocysts)[16]. It has been argued that this division is favoured because nitrogenase, the nitrogen-converting enzyme, is rapidly degraded in the presence of oxygen, which is produced during photosynthesis[42]. A direct experimental test of this hypothesis would require the manipulation of the extent of the division of nitrogen fixation and photosynthesis between cells. Such a manipulation would enable a direct measurement of how the amount of nitrogen fixed varied with the amount of effort that was put into nitrogen fixation versus photosynthesis.

There are several other factors that could influence selection for division. For example, the relative returns from different tasks could vary depending on the physiological condition or size of the cell[43]. In some volvocine green algae, such as *Volvox carteri*, multicellular groups are composed of large germ cells that reproduce and smaller somatic cells that beat their flagella to keep the colony afloat[44,45]. This division seems to be favoured because large

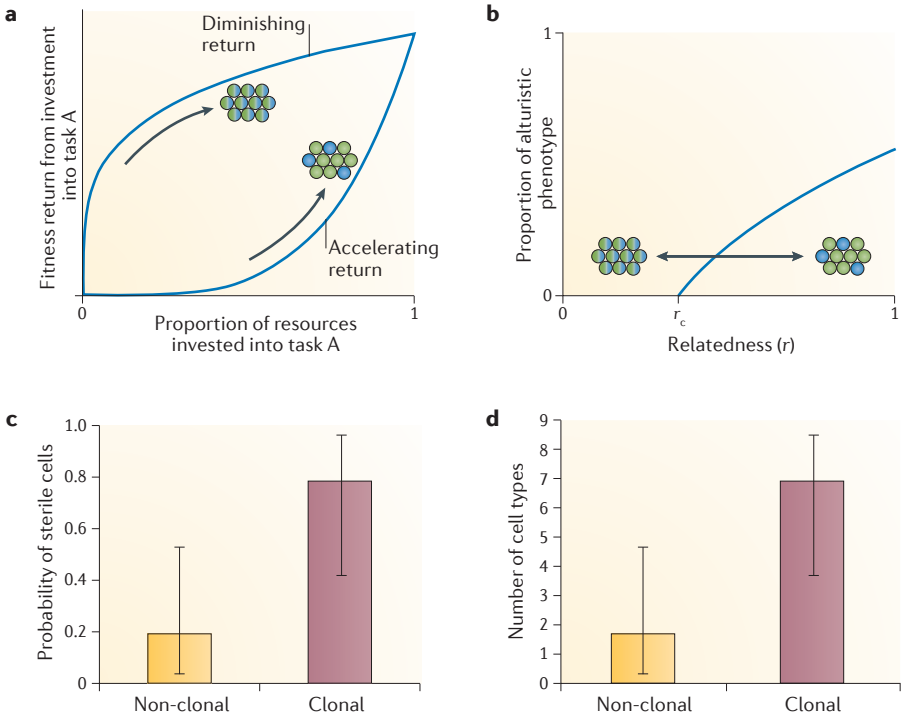

Figure 2 | **Why divide labour? a** | The relationship between the proportion of resources that a cell invests into task A and the fitness return from that task. We assume that a cell invests a proportion of resources (*X*) into task A, and the remaining proportion (1-*X*) into task B. Division of labour can be favoured when the returns from investment are accelerating. Cells can either exhibit division of labour, developing into a mixture of the altruistic helping phenotype (blue cells) and the reproducing phenotype (green cells), or exhibit no division of labour, developing into cells that carry out both the helping phenotype and reproduction (blue and green mixed-colour cells). **b** | The hypothetical relationship between the proportion (or likelihood) of individuals specializing in task A, and the relatedness (*r*) between interacting individuals in a social group. Division of labour is only favoured above a threshold value of relatedness (r_c). **c**,**d** | Among the kingdoms of life, a higher relatedness in multicellular groups correlates with a higher likelihood of sterile cells (part **c**), and more cell types (part **d**). The relatedness comparison is between groups that form clonally ($r=1$) and groups that form non-clonally ($r<1$). Part **c** and part **d** are adapted from REF. 53.

cells are more efficient at reproducing, which involves growth to a large cell size and then division, and small cells are better at both keeping the colony afloat and promoting the diffusion of nutrients across the colony wall[36,46,47]. Another possibility is that cells in poor condition, such as smaller sized or starved cells, could preferentially become altruistic helper cells rather than reproduce, if they have less to lose by not reproducing, compared with other cells in better condition.

A major research aim is to explain why some species divide labour whereas other similar species do not? For example, it seems reasonable that the interaction between nitrogen fixation and photosynthesis could lead to the accelerating fitness return in FIG. 2a, and hence lead to division of labour in species of cyanobacteria such as *Anabaena cylindrica*. However, this raises the question of how we explain other cyanobacteria such as *Trichodesmium erythraeum*[16,42], in which this division of labour has not been favoured.

Alignment of interests. Division of labour requires that the fitness interests of different individuals are aligned. If not, the interaction between these individuals could be destabilized by selfish cheats, which can exploit the cooperative nature of division of labour. Examples of such cheats would be a strain of *S.* Typhimurium that produced more cells that remain in the gut lumen and less cells that migrate to the gut tissue to express *ttss-1*, or a lineage of *D. discoideum* that invested more into spore cells and less into stalk cells[23,24,48].

One way for the interests of different individuals to be aligned is if they are genetically related[19]. This idea, often termed kin selection, proposes that by helping a relative to reproduce, an individual is still indirectly passing on copies of its own genes to the next generation (BOX 2). Consequently, related cells might be favoured to work together by dividing tasks to increase their genetic contribution to the next generation. Hamilton's rule[19] (BOX 2) predicts that altruistic traits (such as invading the gut tissue and expressing *ttss-1* in *S.* Typhimurium) will be favoured when both the relatedness between cells (*r*) and the efficiency benefits of cooperation (*B*/*C*) are sufficiently high (BOX 2). This illustrates that although division of labour is more likely with a higher relatedness, and that many examples of division are clonal ($r=1$), division of labour is also possible in non-clonal ($r<1$) groups.

Four lines of evidence support the hypothesis that relatedness is important in favouring division of labour within species. First, anecdotally, many examples of division of labour occur in groups that are clonal ($r=1$) or that are close to clonal. For example, cyanobacteria differentiate in clonal filaments[16], and the average relatedness in fruiting bodies of the slime mould *D. discoideum* is 0.98 (REF. 49). Second, some species that divide labour exhibit kin discrimination during group formation, which increases the relatedness within social groups. For example, individuals of *Dictyostelium purpureum* preferentially form fruiting bodies with clone mates[50]. Third, the maintenance of cultures with an artificially low relatedness led to the loss of the ability to form fruiting bodies in both *D. discoideum* and *M. xanthus*[51], and a lower investment into somatic functions in the fungus *Neurospora crassa*[52]. Fourth, comparing across species, species with clonal group formation have greater division of labour, with both a higher likelihood of sterile cells and more cell types than species in which group formation is non-clonal[53] (FIG. 2c).

What kind of division?

Having established whether division of labour is favoured, more subtle questions arise. For example, what proportion of individuals should carry out the different tasks? Between how many different cell types will labour be divided? And which mechanisms are expected to give rise to division of labour?

What proportion of individuals should carry out a task? Within the context of the theoretical example, in which individuals carry out either task A or task B, we can ask what the evolutionarily stable strategy[54] (ESS)

fraction of individuals is that should carry out task A and task B[22,35,42]. The concept of ESS is often used in evolutionary biology to denote the strategy or behaviour that would be selected over evolutionary time[55]. Put formally, it is the strategy which, if adopted by everyone in the population, cannot be replaced by any alternate strategy[54].

The ESS depends on the shape of the fitness-return curves (FIG. 2a) and the relatedness (*r*) between interacting cells. One prediction is that, in clonal populations, the fitness of individuals will peak at the ESS fraction of individuals that carry out task A and task B, and then decreases when individuals that carry out either task A or task B become more common[22]. For example, *S.* Typhimurium cells express enough *ttss-1* to trigger an inflammatory response, but any more than that is a waste[22]. Consistent with this, strains with either a lower or a higher proportion of mutant cells that express *ttss-1* showed a reduced fitness[23].

Theory could be developed for specific cases to help explain both what the ESS is for certain situations and in what way the ESS should vary across populations or species. For example, in the volvocine green algae, the ratio of soma cells to germ cells increases with colony size[44]. It has been argued that this represents the correlation between the shape of the fitness curve (FIG. 2a) and the changing group size. Specifically, as colony size increases, it becomes harder to keep the colony afloat and to transport nutrients, and therefore the ESS fraction of soma cells increases[36,46].

Other questions include why within species does the proportion of stalk cells in *D. discoideum* vary so markedly between samples taken from the same location[48,49]? And why does the proportion of stalk cells vary among closely related *Dictyostelium* species[56]. Moreover, among more distantly related species, why does the proportion of the altruistic reproductive phenotype vary from approximately 20% of *D. discoideum* cells that become stalk cells, to more than 99% of *Volvox* spp. cells that become soma cells[24,57]? ESS theory provides a tool for finding answers to these questions.

The ESS ratio of different phenotypes is also likely to vary with the relatedness between interacting individuals. However, there is a lack of both theory and empirical work that examines how variation in relatedness would influence the ESS ratio of phenotypes[22,53]. Previous theoretical analyses have focused on the extreme case of clonal populations (r=1). As relatedness decreases, it will lead to a decrease in the kin selected benefit from helping others[19]. Thus, we suspect that a general prediction is that a lower relatedness will lead to a lower proportion of individuals that express altruistic phenotypes (FIG. 2b). Comparing between species, the percentage of sterile cells is twice as high in species with clonal groups, but this pattern is not statistically significant[53]. However, there were only data available for a small number of phylogenetically independent comparisons, and thus this test had low statistical power, emphasizing the need to obtain data from a wider range of taxa.

How many types? Labour is sometimes divided into more than two phenotypes. For example, in cyanobacteria the number of cell types varies among species, up to at least four types[16]. These cell types include photosynthetic cells, nitrogen-fixing heterocysts, resting cells that are able to withstand environmental stress (akinetes) and motile dispersing filaments of cells (hormogonia).

We lack a formal theoretical framework to explain variation in the number of cell types. Various factors are likely to be important, including ecological conditions, molecular mechanisms, relatedness within groups and group size. Consistent with these possibilities, species that form clonal groups (r=1) have more cell types than species in which groups are not clonal ($r<1$) (FIG. 2d), and the number of cell types is positively correlated with group size[53,58]. A caveat here is that not all cell types represent division of labour, as phenotypic variation can arise for other reasons, such as bet hedging[2,3] (see above).

Which way to divide? Given that there are many ways to produce variable phenotypes within a species (BOX 1), should we expect one mechanism to dominate or different mechanisms to mediate phenotypic variation in different species? And if mechanisms vary across species, does this represent adaptive variation, with different mechanisms being better suited to different situations, or is phenotypic variation just the result of noise created by historical artefacts? There has been no theoretical or empirical work that has addressed such questions, and we therefore make several tentative suggestions.

We suspect that it would require restrictive conditions to maintain division of labour within species that are mediated by genetic differences. For example, most interactions in division of labour occur in clonal lineages, in which there will be insufficient genetic differences. Furthermore, when several genetic lineages interact, this can reduce selection for cooperation[59], and so although phenotypic variation can be maintained, it could be hard for such interactions to constitute a cooperative division of labour. For example, the coexistence of producing and non-producing lineages, that have traits such as invertase production in *Saccharomyces cerevisiae* or siderophore production in *P. aeruginosa*, seems to represent the coexistence of cooperators and cheats, and not cooperative division of labour[60,61]. Cooperation between different genotypes (or species) presents the problem of how to keep them together over evolutionary time (Supplementary information S3 (box)). Similar arguments would apply when considering epigenetic mechanisms[62].

By contrast, phenotypic noise seems to be a robust way to produce division of labour. Noise can produce one or more phenotypes from a single genotype. The ratio of these phenotypes could be selected for based on the underlying gene network to produce ratios of different phenotypes that vary according to the ESS in the local environment[2]. However, a limitation is that phenotypic noise could work less well in small social groups, as stochasticity would lead to a chance that there is none, or almost none, of a certain phenotype in a social group, which could result in a large fitness cost associated with maintaining phenotypic variation (FIG. 3). Consequently, we predict that phenotypic noise will more likely be the mechanism that is used when the social group is very large, which is exemplified by the expression of the *ttss-1* system in *Salmonella* spp. cells[22,23].

Coordination between cells by, for example, cell signalling, provides a possible solution to the problem of stochasticity in small social groups. If cells interact and coordinate phenotypes at a local level, then this can ensure a precise and appropriate ratio of different phenotypes, even in small social groups, as occurs in cyanobacterial filaments[16] (FIG. 3). Given this advantage of signalling, why is it not used more frequently to control division of labour? It is possible that this strategy could be costly, or that it would be ineffective in certain environments — for example, when diffusion rates are high. In such cases, and when the problem of stochasticity is less important, phenotypic noise could provide a more efficient mechanism to divide labour.

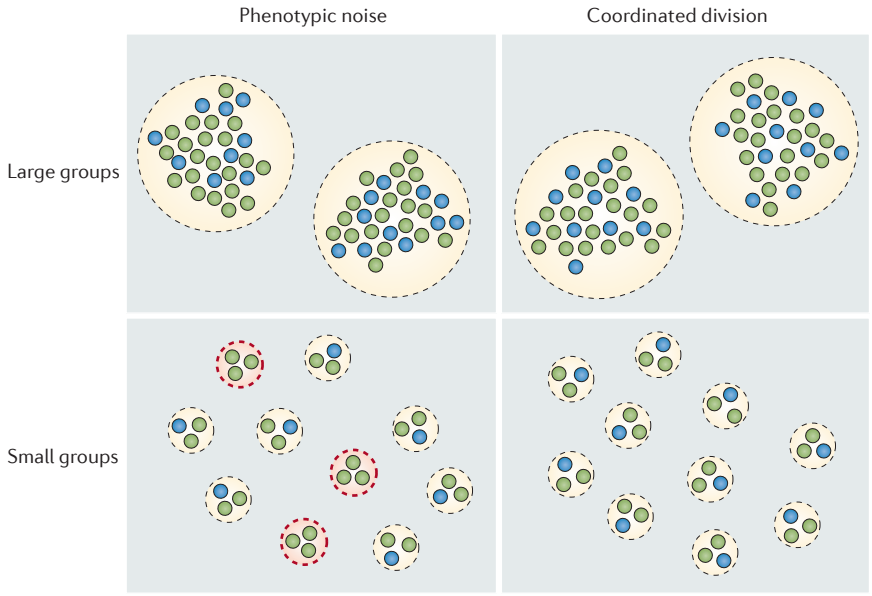

Figure 3 | **How to divide?** Considering a population that comprises altruistic cells (blue) and reproductive cells (green). Determination of phenotype occurs through phenotypic noise or through a mechanism, such as cell signalling, that coordinates division. The number of cells that interact in a social group can be large or small. In large groups, both phenotypic noise and coordinated division lead to groups with the evolutionary stable strategy (ESS) number of altruists. Consequently, both mechanisms could be favoured to establish division of labour. If coordinated division is more costly; for example, because it is metabolically costly to produce a signal, then phenotypic noise will be the favoured mechanism. In small social groups, owing to the stochastic nature of phenotypic noise, there is a chance that the group contains no altruists, and this group will consequently have poor fitness (these groups are circled in red). By contrast, coordinated division provides a mechanism to ensure that all groups contain altruists. Consequently, phenotypic noise is less likely to be favoured as a mechanism to divide labour in species in which the number of interacting cells is small.

Outlook

In this Opinion article, we have provided a definition of division of labour and discussed its implications. Is our definition useful? We have taken an evolutionary approach, focused on how individuals are adapted to their environments, and emphasized that division involves the cooperation of individuals to provide an inclusive fitness benefit to the entire social group. An alternative approach, focused on outcome rather than evolutionary adaptation, would be to define division of labour more loosely, such as when phenotypic diversity enables more complex tasks to be carried out[27]. This alternative approach would include the examples that we have excluded, such as diversification and one phenotype exploiting another.

The advantage of our stricter definition is that it integrates cases in which the same problems arise, and in which there is the potential for unifying understanding. For example, our stricter definition has highlighted the importance of shared interests and nonlinear fitness returns in the evolution of division of labour (FIG. 2). By contrast, a looser definition would integrate traits that have evolved for very different reasons and hence obscure underlying similarities. For example, the evolution of exploitation, or cheats that do not produce iron chelating siderophores, does not require nonlinear fitness returns, and is less likely to be favoured when individuals have shared interests (cheating is favoured by a lower and not a higher *r* value). This illustrates the advantage of drawing a clear distinction between processes that arise for different reasons[63].

Phenotypic heterogeneity between cells could arise in two ways; either through persistent specialization, whereby some cells only carry out task A, and other cells only carry out task B; or through transient specialization, whereby the same cell switches between carrying out task A or task B at different times. In the social insect literature, it has been suggested that persistent specialization is required for division of labour[64]. By contrast, by emphasizing the role of cooperation, our definition clarifies that both persistent and transient specialization can lead to division of labour.

Moreover, it has been suggested that the mutation of cooperators into non-cooperative cheats had a pivotal role in the evolution of the division between reproductive cells (germ cells) and helper cells (soma cells), and hence the evolution of complex multicellularity[65,66]. However, if we consider this hypothesis from an evolutionary perspective, both theory and empirical data contradict this notion. Theory suggests that selection would favour cheats that exploit cooperators and cooperators that are less likely to be exploited, which would lead to selection in the opposite direction than towards multicellularity[8,11]. Empirical data have shown that dividing labour between germ cells and soma cells occurs more often in species that have clonal group formation, in which there is no selection for cheating[53] (FIG. 2c).

Based on the several examples that have been described in recent years, these are exciting times for the study of division of labour in microorganisms. However, much more theoretical and empirical data are required. In many cases, it even remains to be shown whether phenotypic variation really represents division of labour. In cases in which division of labour can be established, further questions arise (see Supplementary information S5, S6, S7, S8, S9 (boxes)). Why is division favoured? How many different phenotypes exist, and what proportion of each phenotype? What mechanisms drive phenotypic diversity and why? Can we explain variation among species, as well as specific cases? Can we apply the same concepts to explain division of labour between species? By answering these questions, we can unify our understanding of division of labour, not only with regards to mechanistically different microbial examples, but also for other taxa, including animal societies.

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Competing interests statement

The authors declare no competing interests.

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