The evolution of mechanisms to divide labour in microorganisms

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Abstract

Division of labour, where cooperating individuals specialise to perform complementary tasks, plays a fundamental role at all levels of biological complexity, from genes to complex animal societies. Different labour-dividing species employ different mechanisms to determine how tasks are allocated between individuals, including coordinated, random, and genetic determination of phenotype (caste). It is not clear if this diversity is adaptive—arising because different mechanisms are favoured in different environments—or is merely the result of non-adaptive, historical artifacts of evolution. We use theoretical models to analyse the relative advantages of the two dominant mechanisms employed for reproductive division of labour in microorganisms: coordinated and random specialisation. We show that fully coordinated specialisation is more likely to evolve over random specialisation in well-mixed groups when: (i) social groups are small; (ii) cooperation has a greater relative impact on fitness (i.e., is more "essential"); and (iii) there is a relatively low metabolic cost to coordination. We find analogous results when we allow for spatial structure in a more mechanistic model of growing cyanobacteria

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filaments. More generally, our results show that the diversity in mechanisms to divide labour are likely to have arisen through the action of adaptation by natural selection, and not as an historical artifact of non-adaptive evolution.

Key words: random specialisation, phenotypic noise, adaptive coin-flipping, coordination, signalling, cellular differentiation, adaptation, evolutionary theory, cyanobacteria, social microbes

Introduction

Different species use different mechanisms to divide labour between reproductives and their helpers (Figure 1) [1–3]. In some cases, there is coordination across individuals to determine which individual will perform which role (*coordinated specialisation*). This coordination could use signals, cues or a developmental programme to provide information about the phenotypes adopted by other individuals in the group. This happens, for example, when honey bee workers feed royal jelly to larvae to produce reproductive queens (Figure 1A), or when the local density of a signalling molecule determines whether cyanobacteria cells develop into sterile nitrogen-fixing heterocysts (Figure 1B)[4–6]. In other cases, each individual adopts a helper phenotype with a certain probability, independently and without knowledge of the phenotypes adopted by other individuals (*random specialisation*). For example, in *Salmonella enterica* co-infections, random biochemical fluctuations within each cell's cytoplasm are used to determine whether the cell sacrifices itself to trigger an inflammatory response that eliminates competitor species (Figure 1D)[7,8]. In yet further cases, phenotype is influenced by the

individual's genotype (genetic control). For instance, in some ant societies whether individuals develop into queens, major, or minor workers can be determined, in part, by their genes (Figure 1C)[3,9–11]. Across the tree of life some species employ one mechanism to divide labour whereas in other species mixed forms exist with a combination of coordinated specialisation, random specialisation, or genetic control[3,10,12–17].

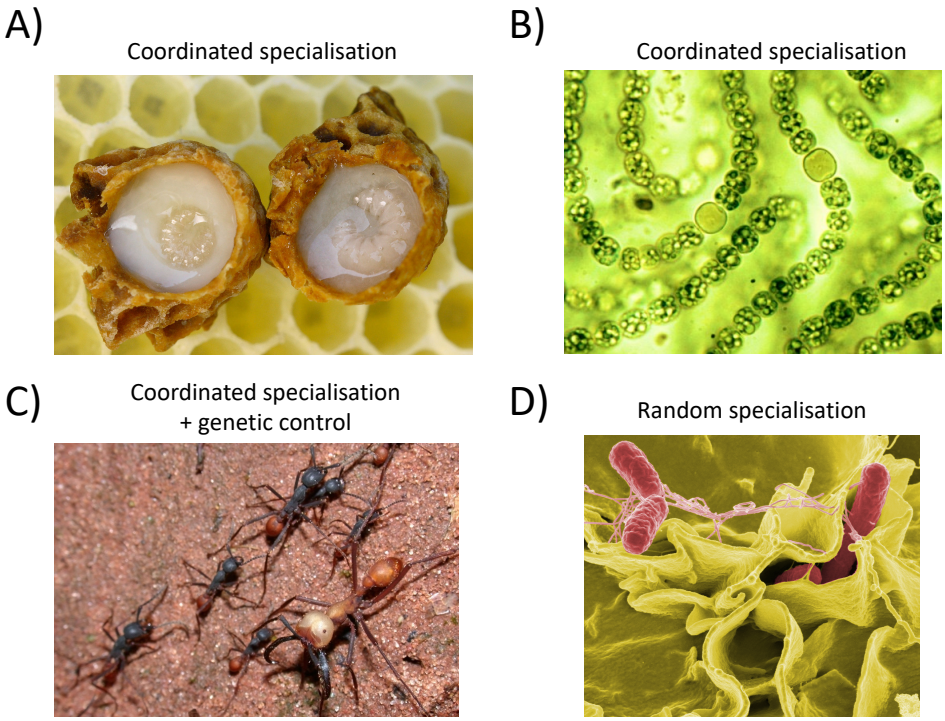

Figure 1. Different mechanisms for division of labour in nature. A) In honey bee hives (Apis mellifera), larvae develop as sterile workers unless they are fed large amounts of royal jelly by adult workers (coordinated specialisation) [4]. (Photo by Wausberg via the Wikimedia Commons.) B) In *A. cylindrica* filaments (cyanobacteria), some individuals develop into sterile nitrogen fixers (larger, round cells) if the amount of nitrogen fixed by their neighbours is insufficient (coordinated specialisation). This leads to a precise allocation of labour, with nitrogen-fixing cells distributed at fixed intervals along the filament. [5] (Picture taken by Robert Calentine.) C) In the army ant (*Eciton Burchelli*), whether individual ants become a major or minor worker has a genetic component (genetic control) [11]. (Photo by Alex Wild via the Wikimedia Commons, cropped.) D) In *S. enterica* infections (serovar Typhymurium), each cell amplifies intra-cellular noise to determine whether it will self-sacrifice and trigger an inflammatory response that

eliminates competing strains (random specialisation). [8] (Photo by Rocky Mountain Laboratories, NIAID, NIH via Wikimedia Commons.)

We lack general evolutionary explanations for why different species use different mechanisms to divide labour between reproductives and their helpers[2,3,18,19]. Previous work has focused on non-reproductive division of labour in the social insects, and the proximate mechanisms that lead to different worker castes[11,20–25]. However, the focus in that literature is on a different question – how different proximate mechanisms can produce coordinated specialisation – rather than the broader question of whether coordinated specialisation should be favoured over random specialisation or genetic control in the first place. It is with reproductive division of labour that these three very different mechanisms have been observed in different species and for which there is an absence of evolutionary explanations[2,3,18,19,26].

Bacteria and other social microbes offer excellent opportunities for studying why different labour-dividing mechanisms are favoured in different species[1,2]. Across microbes, the two primary mechanisms used to produce reproductive division of labour are coordinated and random specialisation (Figure 2). Furthermore, while the form of cooperation and life histories of social microbes share many similarities, they also vary in factors that could influence the evolution of division of labour, such as social group size [27,28]. We develop theoretical models to examine whether the relative advantage of random versus coordinated specialisation can depend upon social or environmental conditions. Our aim here is to use microbes as a 'test system' to address the broader question of whether

evolutionary models can explain the diversity in the mechanisms that produce division of labour.

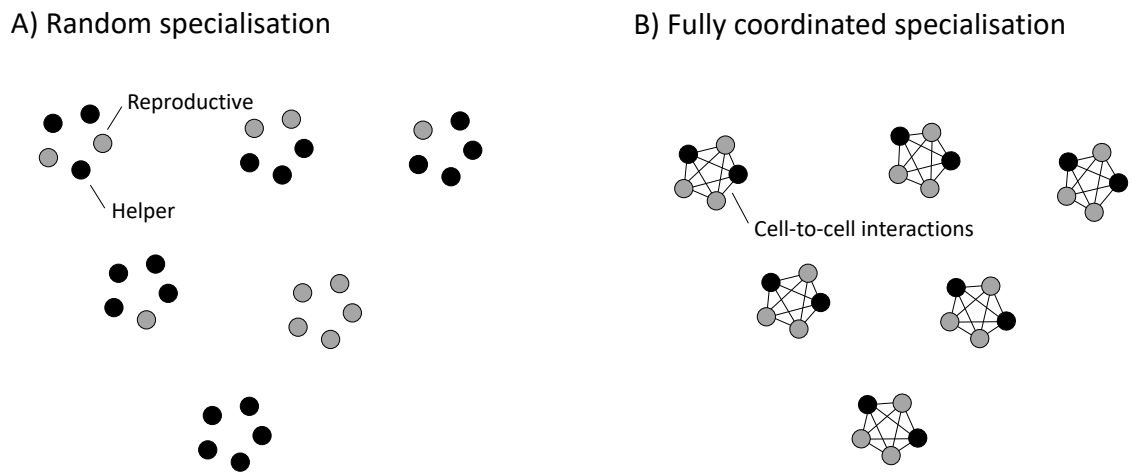

Figure 2. Mechanisms to divide labour in social microorganisms. We examine the relative advantages and disadvantages of the two key mechanisms for reproductive division of labour in social microorganisms. (A) Random specialisation occurs when cells randomly specialise into helpers or reproductives independently of one another. This can occur when a genetic feedback circuit is used to amplify small molecular fluctuations in the cytoplasm of each cell (phenotypic noise) [7,29–33]. (B) Coordinated specialisation occurs when cells interact with one another, and share (or gain) phenotypic information while they are differentiating. This could occur through the secretion and detection of extracellular molecules (signals or cues), or with a shared developmental programme (epigenetics)[1,2].

Results and Discussion

We compare the relative fitness advantages of dividing labour with either coordinated or random specialisation. Our first aim is to capture the problem in a deliberately simple model, which is easy to interpret, and can be applied across diverse microbe species[34,35]. We begin by assuming that coordinated specialisation always produces the optimal proportion of helpers and reproductives (fully coordinated specialisation) and that there

is no within-group spatial structure (well-mixed groups). We then test the robustness of our results by examining a number of alternate models for different biological scenarios and by developing a more mechanistic model of growing cyanobacteria filaments that includes the effects of within-group spatial structure and partial coordination. Throughout, we assume a form of cooperation that is common in microbes, where some individuals produce a 'public good' that benefits all cells.

<u>Random specialisation vs fully coordinated specialisation</u>

We assume that a single cell arrives on an empty patch and, through a fixed series of replications, produces a clonal group of n individuals that consists of k sterile helpers and $n-k$ pure reproductives ($k \in \{0,1,2,\dots,n\}$). We denote group fecundity, $g_{k,n}$, as the reproductive success of a particular group in the absence of mechanism costs. This is measured as the per capita number of offspring that disperse at the end of the group life cycle, given by

$$g_{k,n} = \frac{1}{n}(n-k)f_{k,n}, \tag{1}$$

where $n-k$ is the number of reproductives in the group, and $f_{k,n}$ is the fecundity of each reproductive in the absence of mechanism costs. We assume that $f_{k,n}$ increases with the number of helpers in the whole group (k).

Expression (1) highlights the trade-off between the number of reproductives in the group $(n-k)$, which is higher when there are fewer helpers (lower k), and the amount of help that those reproductives obtain ($f_{k,n}$), which is higher when there are more helpers (higher

130 k). The balance of this trade-off often results in an optimal number of helpers, k^*, that is
131 intermediate (i.e., $0 < k^* < n$).
132
133 In species that divide labour by coordination, the outcome of individual specialisation
134 depends on the phenotypes of social group neighbours. Our first model is deliberately
135 agnostic to the details of how phenotype information is shared between group members
136 in order to facilitate predictions across different systems. For instance, individuals may
137 share phenotype information via signalling between cells or with a common
138 developmental program (Figure 2B)[1,2,36]. We make the simplifying assumption that
139 individuals coordinate fully, so that coordinated groups always form with precisely the
140 optimal number of helpers, k^*. The disadvantage of coordinated specialisation is that the
141 mechanism could incur metabolic costs, such as the production of extracellular signaling
142 molecules. The fitness of a group of coordinated specialisers is given by:

$$w_C = (1 - c_C) g_{k^*,n} \ , \qquad (2)$$

143 where $g_{k^*,n}$ is the group fecundity with the optimal number of helpers (i.e., $g_{k^*,n} = \max_k g_{k,n}$)
144 and $0 \leq c_c \leq 1$ is the metabolic cost of coordination, whose form we leave unspecified
145 but could in principle depend on further factors such as group size. A number of different
146 models have examined how different proximate mechanisms can produce coordinated
147 division of labour in specific systems [20,23–25].
148
149 In species that divide labour by random specialisation, each individual in the group
150 independently becomes a helper with a given probability and a reproductive otherwise
151 (Figure 2A). Hence, the final number of helpers in the group is a binomial random variable.

We assume that the probability of adopting a helper role is equal to the optimal proportion of helpers ($\mathrm{p}^* = \mathrm{k}^*/\mathrm{n}$). Thus, the expected fitness of a group of random specialisers is given by:

$$\mathrm{w_R} = (1 - c_\mathrm{R}) \sum_{\mathrm{k}=0}^{\mathrm{n}} \binom{\mathrm{n}}{\mathrm{k}} \mathrm{p}^{*\mathrm{k}} (1 - \mathrm{p}^*)^{\mathrm{n}-\mathrm{k}} \mathrm{g}_{\mathrm{k,n}}, \qquad (3)$$

where $0 \leq c_R \leq 1$ is the metabolic cost of random specialisation, which we assume is independent of the number of helpers in the group, k. The potential advantage of random specialisation is that there may be fewer upfront metabolic costs from, for example, between cell signaling (i.e., if $c_R < c_C$ holds). The downside of random specialisation is that groups sometimes form with fewer or more helpers than is optimal (demographic stochasticity). In principle, the probability of becoming a helper could be regulated by environmental cues so as to produce on average more or fewer helpers when this is more favourable. However, throughout our analysis we assume a stable environment and ignore such regulation.

We need to specify how reproductive fecundity depends on the number of helpers in the group. We focus here on one of the most common forms of cooperation in microbes, where individuals secrete factors that provide a benefit to the local population of cells ("public goods") [37]. We assume that the amount of public good in the social group depends linearly on the number of helpers in the group and is "consumed" by all group members equally [38,39]. An example of such a public good is found in *Bacillus subtilis* populations, where only a subset of cells (helpers) produce and secrete proteases that degrade proteins into smaller peptides, but where these are then re-absorbed as a nutrient source by all cells [40].

We allow the relative importance of producing public goods to vary between species. Each reproductive has a baseline fecundity, $\mathrm{b} \geq 0$, that is independent of the amount of public good in the group. The fecundity benefit of helpers scales according to $\mathrm{h} \geq 0$ as the amount of public good in the group increases. When reproductives have no baseline fecundity ($\mathrm{b} = 0$), we say that cooperation is essential. When baseline fecundity is non-zero ($\mathrm{b} > 0$), cooperation is non-essential and the ratio h/b provides a useful metric for the relative importance of cooperation.

Our assumptions give the following expression for the fecundity of a reproductive:

$$\mathrm{f_{k,n}} = \mathrm{b} + \mathrm{h}\frac{\mathrm{k}}{\mathrm{n}}. \tag{4}$$

By substituting Equation 4 into Equations 1-3, we can determine when the fitness of coordinated specialisation is greater than the fitness of random specialisation (i.e., $\mathrm{w_C} > \mathrm{w_R}$), which gives the simplified condition:

$$\gamma < \frac{h-b}{n(h+b)}, \tag{5}$$

where $\gamma = (c_C - c_R)/(1 - c_R)$ captures the relative change in metabolic costs paid when switching to coordinated specialisation from random specialisation. If $h < b$, then helpers are not required and the group is composed of all reproductives ($k^* = 0$). Thus, division of labour is favoured to evolve only when $\mathrm{h} > \mathrm{b}$, which we will assume henceforth (supplementary section C). Condition (5) specifies that coordinated specialisation is favoured when the relative change in metabolic costs of switching from random specialisation to coordination (γ), is less than the fecundity benefits gained from doing so

(right-hand side). The condition can be used to predict how key environmental and ecological factors will influence which labour-dividing mechanism is more likely to evolve (Figure 3).

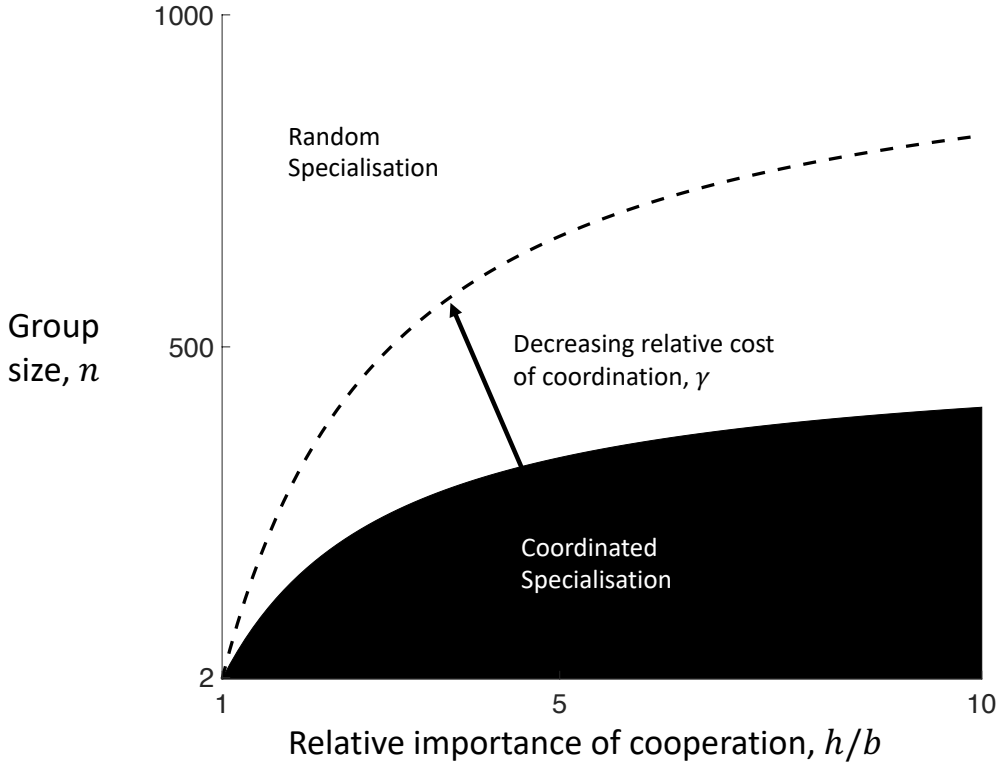

Figure 3. Random versus coordinated specialisation. Small group sizes (lower n), relatively more important cooperation (higher h/b), and lower relative metabolic costs to coordination (lower γ) favour division of labour by coordinated specialisation (black) over division of labour by random specialisation (white). Here we have used $\gamma = 2 \times 10^{-3}$ (solid boundary) and $\gamma = 1 \times 10^{-3}$ (dashed boundary). We note that the limit as the relative importance of cooperation goes to infinity (very large h/b) illustrates the outcome when cooperation is essential ($b = 0$).

Prediction 1: Smaller relative metabolic costs of coordination favour coordinated specialisation.

When the metabolic cost of coordination is smaller (lower c_C) and the metabolic cost of random specialisation is larger (higher c_R), then the relative cost of switching from random specialisation to coordinated specialisation is lessened (smaller γ), which favours the evolution of coordinated specialisation (smaller left-hand side of Condition 5). If the metabolic costs of random specialisation are equal to or larger than the metabolic costs of coordination ($c_R \geq c_C \Rightarrow \gamma \leq 0$), then coordinated specialisation is always the favoured mechanism (Condition 5 always satisfied). Conversely, random specialisation can only ever be the favoured strategy ($w_R > w_C$; Condition 5 not satisfied) if the metabolic costs of random specialisation are less than the metabolic costs of coordination ($c_C > c_R \Rightarrow \gamma > 0$; a necessary but not sufficient condition). This arises directly from our starting assumption that coordinated specialisation always produces groups with the optimal proportion of helpers whereas random specialisation only does so sometimes.

Larger metabolic costs of coordinated specialisation ($c_R < c_C \Rightarrow \gamma > 0$) may be a reasonable assumption for many biological systems. The metabolic costs of random specialisation are determined by the production costs of the regulatory proteins employed in the genetic feedback circuit that amplifies intra-cellular noise[29,41–43]. In contrast, coordinated specialisation requires both an intracellular genetic feedback circuit and some mechanism by which phenotype is communicated between cells, such as the costly production and secretion of extra-cellular signaling molecules[1,2,36,44–46].

As a result, in many scenarios, the optimal mechanism to divide labour depends on how the potentially higher metabolic costs of coordination ($c_C > c \Rightarrow \gamma > 0$) balance against

the benefit of avoiding the stochastic costs of random specialisation (right-hand side of condition 5). The stochastic costs of random specialisation are determined by: (i) the relative likelihood that random groups deviate from the optimal proportion of helpers, and (ii) the degree to which those deviations from the optimal proportion of helpers leads to a reduced fecundity for the group (supplementary section C). Equation (5) shows how the importance of these two factors depends upon the size of the group (n) and on the relative importance of cooperation (h/b).

Prediction 2. Smaller social groups favour coordinated specialisation.

The number of cells in the group has a large impact on the relative likelihood that random groups deviate from the optimal proportion of helpers (Figure 3). In smaller groups, there are fewer possible outcomes for the proportion of helpers ($\mathrm{n}+1$ possible allocations of labour for groups of size n). Consequently, random specialisation can more easily lead to the formation of groups with a realized proportion of helpers that deviates significantly from the optimum ($\mathrm{p} \ll \mathrm{p}^*$ or $\mathrm{p} \gg \mathrm{p}^*$). In contrast, in larger groups there are more possible outcomes and the resulting proportion of helpers will be more closely clustered about the optimal composition with highest fitness (with $\mathrm{p} \approx \mathrm{p}^*$ for very large group sizes).

This effect of group size on the stochastic cost of random specialisation is a consequence of the law of large numbers. For example, outcomes close to 50% heads are much more likely when tossing 100 coins in a row compared to only tossing 4 coins in a row where no heads or all heads may frequently occur. Our prediction is related to how, when mating occurs in small groups, small brood sizes select for more precise and less female biased

sex ratios as there would otherwise be a high probability of producing a group containing no males at all[47–49]. In another analogue, Wahl showed a mechanistically different effect when division of labour is determined genetically and the number of group founders is small: groups may sometimes form that do not contain all of the genotypes required to produce all of the necessary phenotypes in the division of labour [19].

Prediction 3: The higher the relative importance of cooperation, the more coordinated specialisation is favoured.

When the relative importance of cooperation is larger (higher $\mathrm{h/b}$), the fitness costs incurred from producing too few helpers increases. In addition, as the relative importance of cooperation increases (higher $\mathrm{h/b}$), the optimal proportion of helpers increases to 50% helpers ($\mathrm{p}^* \approx \frac{1}{2}$). This increases the variance in the proportion of helpers produced by random specialisers, and so sub-optimal groups may arise more frequently (supplementary section C). Thus, a higher relative importance of cooperation increases both (i) the likelihood that groups deviate from the optimal proportion of helpers and (ii) the scale of the fitness cost when they do. Both of these effects increase the stochastic costs of random specialisation (larger right-hand side of Condition 5), and thus favour the evolution of coordinated specialisation (Figure 3).

<u>Alternative forms of cooperation</u>

The above analysis employs a deliberately simple public goods model, focusing on factors that are expected to be relevant across many microbial systems. This facilitates

277 the interpretation of our results and generates broadly applicable predictions that are less
278 reliant on the specifics of any particular biological species.
279
280 In order to test the robustness of our results (predictions 1-3) we also developed a series
281 of alternative simplified models corresponding to different biological scenarios
282 (supplementary sections D and E; Figures S1-S3). We examined the possibility that the
283 public good provided by helpers is: (i) not consumed by its beneficiaries, as may occur
284 when self-sacrificing *S. enterica* cells enter the gut to trigger an immune response that
285 eliminates competitors (non-rivalrous or non-congestible collective good); or (ii) only
286 consumed by the reproductives in the group, as may occur for the fixed nitrogen secreted
287 by heterocyst cells in *A. cylindrica* filaments (excludible or club good) [7,46,50,51]. We allowed
288 for reproductive fecundity to depend non-linearly on the proportion of helpers in the group,
289 for helpers to have some fecundity (non-sterile helpers), and for division of labour to occur
290 in each generation of group growth. In all of these alternative biological scenarios, we
291 found broad qualitative agreement across all three predictions of the linear public goods
292 model.
293
294 We found that less specialised helpers (with some fecundity) favours random
295 specialisation over coordinated specialisation. In contrast to prediction 3, more fecund
296 helpers can lead to a scenario where a larger relative importance of cooperation (higher
297 h/b) disfavours coordinated specialisation. This occurs because a high relative
298 importance of cooperation (higher h/b) can produce groups composed predominantly of

non-sterile helpers ($p^* \approx 1$), where the likelihood that random groups deviate from the optimal proportion of helpers is significantly diminished (supplementary section E.4).

In supplementary section F we develop an individual based simulation, which also supports predictions 1-3. In addition, this simulation shows that costly coordination can evolve incrementally from random specialisation, and that intermediate levels of coordination can be favoured (Figure S4).

Division of labour in a cyanobacteria filament

We then developed a more mechanistically detailed model of a growing cyanobacteria filament to investigate the impact of within-group spatial structure (supplementary section G). When there is insufficient fixed nitrogen (N_2) in the environment, some cyanobacteria species will facultatively divide labour between reproductive cells (autotrophs) that photosynthesise light and sterile helper cells (heterocysts) that fix and secrete environmental N_2 (Figure 1B)[5,52,53]. The fixed N_2 diffuses along the filament where it is used by reproductives to grow and produce new cells. Division of labour in cyanobacteria is a canonical example of coordinated specialisation as helpers produce a variety of signalling molecules that diffuse along the filament to ensure that a regular pattern of phenotypes develops (Figure 1B)[5,52,53]. Previous models of cyanobacteria focused on determining the signalling and regulatory network required to recreate the exact pattern of heterocysts along the filament[52,54–58].

Cyanobacteria spores (hormogonium) tend to contain multiple cells [5,52]. We assumed that each filament begins as a clonal sequence of two reproductives (R) and two helpers (H) in the arrangement (H-R-R-H). Over time, the number of cells in the filament increases as reproductives grow and divide to produce within-filament offspring cells, which become either helpers or reproductives (Figure 4A). The group life cycle ends when the filament has reached a maximum size of L cells. At this time, the reproductives in the filament produce dispersing hormogonia that found filaments in the next generation of the group life cycle and all remaining cells die (non-overlapping generations) [5].

Reproductives absorb fixed N_2 in order to replicate. Each reproductive receives fixed N_2 from the abiotic environment at a rate of $\phi \geq 0$ units of fixed N_2 per unit time (uniform background density of fixed N_2)[58]. In addition, each helper in the filament produces fixed N_2, at a maximum rate of $\bar{\phi} > 0$ units of fixed N_2 per unit time. We assume that the fixed N_2 produced by a helper diffuses across the filament, decaying by a factor of $0 < \eta \leq 1$ with each cell position that is further away (cooperation range).

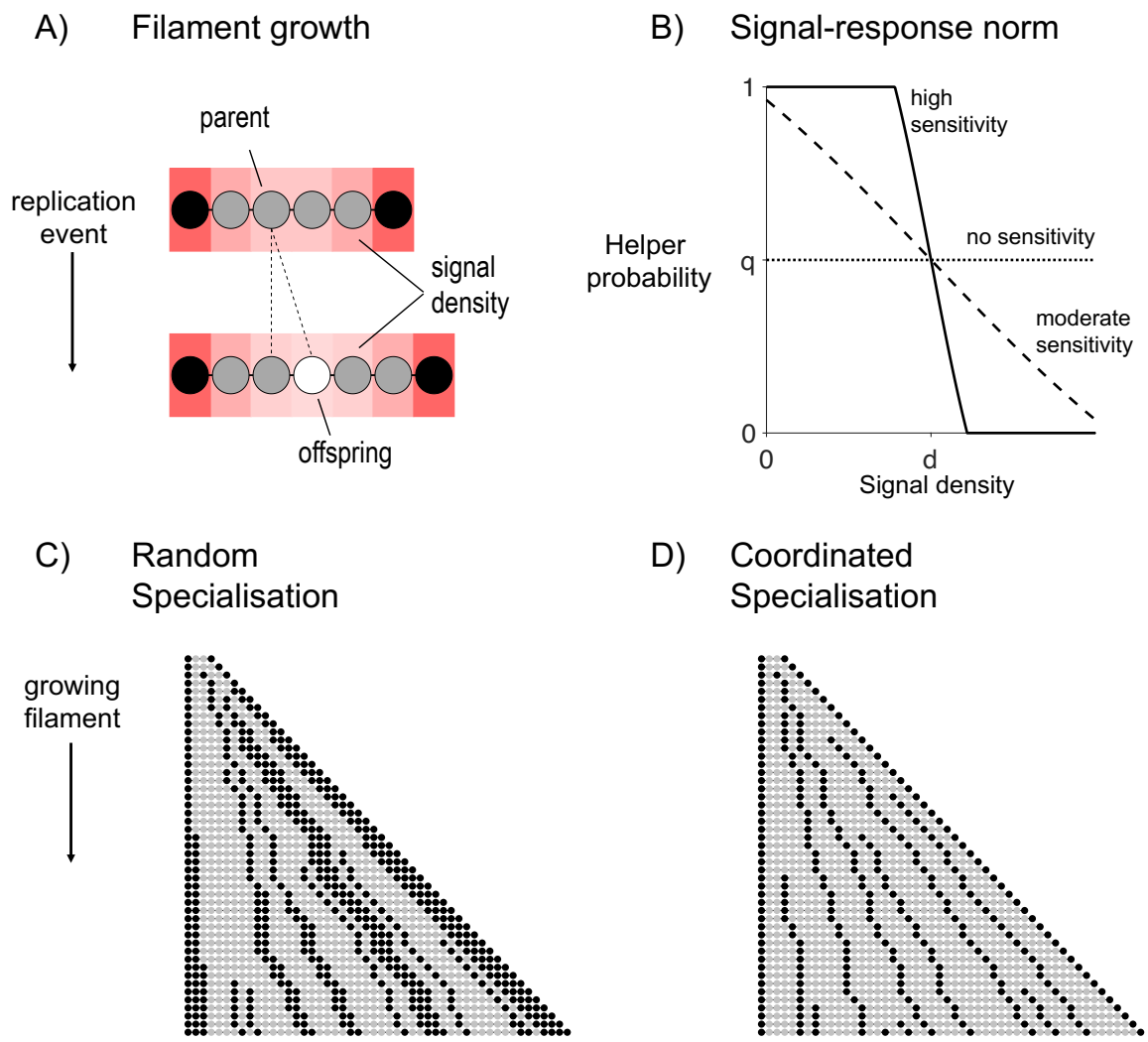

Figure 4: Division of labour in a cyanobacteria filament. (A) Black cells represent helpers, and grey cells reproductives. When a reproductive replicates, the parent cell produces an offspring cell (white cell) to one side of itself along the filament. The red shading shows the density of the signal molecule produced by the helpers as it diffuses along the filament. (B) When an offspring cell is sensitive to the signal ($v > 0$), a greater (lesser) signal density will decrease (increase) the probability that it becomes a helper ($q = 0.5, d = 5, v = 0, 0.2, 1$). (C) A simulated example of a filament growing that employs random specialisation ($q = 0.33, s = 0$, $d = 0$, and $v = 0$). (D) A simulated example of a filament growing that employs coordinated specialisation ($q = 0.33, s = 0.1$, d=1 and $v = 1.5$) (Supplementary information G).

Whether a new cell becomes a helper or a reproductive depends on four evolutionary traits that jointly determine the extent of division of labour and coordination in the filament ($\mathrm{q}, \mathrm{s}, \mathrm{d}$, and v; Figure 4B). The baseline probability ($0 \leq \mathrm{q} \leq 1$) is the underlying probability

352 that a cell becomes a helper in the absence of coordination. The level of signalling ($0 \leq$
353 $s \leq 1$) is the fraction of resources that a helper commits to the production and secretion
354 of signalling molecules. The signalling molecules produced by a helper diffuse along the
355 filament, decreasing in density by a fixed factor for each cell position that is further away,
356 and which we assume is distinct from the N_2 diffusivity (Figure 4A). The local density of
357 signalling molecules allows new cells to estimate how close they are to a helper, or how
358 many helpers there may be nearby.
359
360 Empirically, the reason that new cyanobacteria filaments do not simply use the local
361 density of fixed N_2 as a cue may be that signalling molecules are cheaper and faster to
362 produce and thus coordination can occur even before helpers begin to fix N_2 [57].
363 Furthermore, using a dedicated signal could be more reliable than one based on fixed N_2
364 density alone, which might be biased by transient fluctuations in the background level of
365 fixed N_2 (ϕ).
366
367 Whether and how the new cell responds to the signal depends on the response sensitivity
368 ($v \geq 0$) and the response threshold ($d \geq 0$; Figure 4B). If $v = 0$, then a new cell is
369 insensitive to the signal and adopts the helper phenotype with the baseline probability q
370 (random specialisation). If the daughter cell is sensitive to the signal ($v > 0$) then a local
371 signal density that is greater than the response threshold, d, will lead to that cell being
372 less likely to adopt the helper phenotype (Figure 4B). A higher signal density than the
373 threshold leads to the opposite effect. As sensitivity increases (higher *v*), the response to
374 the signal becomes more deterministic (Figure 4B).

Increasing levels of coordination (higher v and s), allows for a more precise patterning of helpers and reproductives in the filament (compare Figures 4C and 4D). However, we assume that increased coordination is costly. First, as helpers produce more signalling molecule (higher s), they can produce proportionally less fixed N_2. Second, when cells are more sensitive to the signal (higher v), we assume an increasing delay in the time it takes for a replication event to occur.

<u>Simulations</u>

We simulated an evolving population to estimate the strategy that is favoured by natural selection in different scenarios ($q^*,\ s^*, d^*, v^*$) (supplementary section G). We started with a uniform population that specialises randomly ($s = d = v = 0$), and allowed the helper probability (q) to mutate and evolve for 500 generations, until an approximate equilibrium was reached. We then held the baseline helper probability (q) fixed and allowed the coordination traits (s, d and v) to mutate and evolve for 3500 generations. Each generation, the mutant strategy successfully replaces the resident strategy if it has a higher estimated average fitness. We calculate the fitness of individual filaments as the summed fecundity of reproductives in the last generation of the group life cycle, divided by the amount of time that it took the filament to grow to L cells. An equilibrium was generally reached within a few hundred generations.

We found that the degree to which specialising cells evolve to coordinate can depend on social and environmental factors. In particular, both a smaller background density of fixed

N_2 (low ϕ) and a smaller cooperation range (low η) lead to the evolution of higher signaling levels (higher s^*; Figure 5A) and higher response sensitivities (higher v^*; Figure 5B). This produced filaments with more precise allocation of labour across the filament (Figure 5C), where we calculate the variance in the number of helpers in a contiguous sub-block of 10 cells relative to the variance that would be expected for a binomial random variable of the same mean (supplementary section G.4.). These results also illustrate that intermediate coordination can be favoured in certain scenarios.

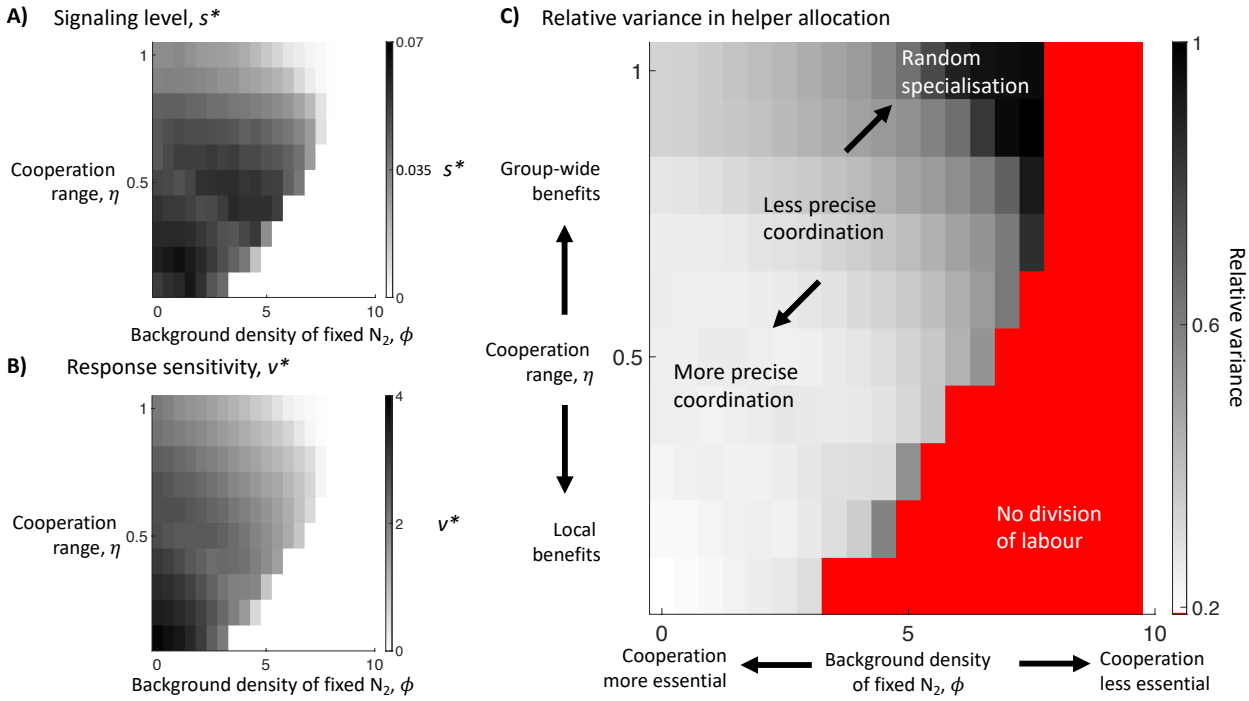

Figure 5: The optimal level of coordination. We present simulation results for two key factors that affect the optimal level of coordination (supplementary section G.4.) A lower background density of fixed N_2 (lower ϕ) and a smaller cooperation range (lower η) favours both: (A) the evolution of a higher level of signalling (larger s^*); and (B) a higher response sensitivity to the signal (larger v^*). (C) The combined effect of higher levels of signalling (larger s^*) and higher response sensitivity (larger v^*) is that groups form with an estimated lower variance in the allocation of help across the filament, where the estimated variance is calculated relative to a binomial distribution with the same mean (supplementary section G).

The predictions of our cyanobacteria model agree broadly with those of our simpler analytical model. When the benefits of cooperation are shared more locally (low η) this leads to a smaller social group (analogous to lower *n*). With random specialisation, a smaller social group can lead to proportions of helpers that show greater deviations from the optimum, increasing the benefit that can be obtained by coordination (Figure 3). When the background density of fixed N_2 is small (low ϕ), this increases the relative benefit of cooperation (analogous to higher *h/b*). With an increased benefit from cooperation there is a greater advantage from coordinating to produce the optimum proportion of helpers (Figure 3).

However, care is required when examining factors in mechanistic models that can have additional effects unaccounted for by their analogues in simpler models. For instance, an increase in the background density of fixed N_2 (higher ϕ) means that cooperation is relatively less important (lower h/b), which we have found favours less coordination (Figure 5). However, relatively less important cooperation (lower h/b) in the mechanistic model also means that helpers may be willing to dedicate more effort to signal production (higher s) as there is then a relatively lower fitness cost to producing less of the public good. Another example is how helpers that produce more fixed N_2 (larger $\bar{\phi}$) leads to cooperation that is relatively more important (higher h/b) but can also lead to larger effective social groups sizes (larger n) as the increased good that helpers produce can then diffuse further along the filament and benefit reproductives that are farther away.

Our simulations show that coordination ($s^* > 0, v^* > 0$) is often favoured over random specialisation ($s^* = 0, v^* = 0$). In groups with fixed spatial structure and local cooperation (lower γ), an effective division of labour requires a regular distribution of helpers across the group. We hypothesize that random specialisation is particularly disadvantageous when groups grow over time because it is more likely to lead to contiguous groups of helpers that expand as the whole group grows (compare Figures 4C and 4D). The helpers within these clumps can neither reproduce to break up the clump, nor are they close enough to reproductives to provide fixed N_2. Consequently, even a little bit of coordination ($s^* > 0, v^* > 0$) can lead to a substantial fitness advantage by decreasing the chance that such "dead zones" can form and grow.

<u>Distribution of labour dividing mechanisms in the natural world</u>

Our analyses provide a theoretical framework to help explain why different species of microorganisms use different mechanisms to divide labour [2]. While testing our predictions with a formal comparative analysis would require data from more species, our predictions can help to understand the mechanisms that have evolved in well studied examples.

There are many reasons why coordinated specialisation was favoured to evolve in cyanobacteria filaments. First, cyanobacteria only divide labour when fixed N_2 is growth-limiting and so the relative importance of cooperation is high (low ϕ and high $\mathrm{h/b}$)[5,53,58]. Second, the fixed nitrogen produced by helpers diffuses across the filament, preferentially aiding nearby reproductives and so the effective social group size is small

(low η and small n)[45,59,60]. Third, the initial costs of coordination may have been quite small as new cells could use the local level of fixed N_2 as a cue (low γ) [61]. Finally, cyanobacteria filaments have a rigid linear structure that can grow to very large sizes (higher L) and thus random specialisation could have lead to the accumulation of large sterile clumps.

Colonies of *Volvox carteri* and *Dictyostelium discoideum* use coordination to divide labour, despite the fact that these groups are composed of large numbers of cells (high n; on the order of 1000s of cells or more) [15,62–64]. However, the initial specialisation of helpers in each case appears to make use of a shared developmental programme and so the relative costs of coordination could be quite small for both species (low γ) [62,63,65]. Further, in both cases an absence of helpers would lead to cell death and thus cooperation is important (higher h/b) [1,2,63,66]. These conflicting effects highlights that no single factor can fully explain empirical patterns. Furthermore, some details of the mechanism of division of labour are still not well understood. For instance, it is possible that there is also an initially random component to pre-stalk specialisation in *D. discoideum* [63].

There are multiple reasons why random specialisation would have been favoured to evolve in other well-studied species. In *Salmonella enterica*, the self-sacrificing helper cells provide a competitive advantage that eliminates other microbes but is not "essential" to the replication of *Salmonella* cells (lower $\mathrm{h/b}$)[7,8]. Further, the benefits of cooperation are provided to all cells in the co-infection ($\eta = 1$) and so the effective

485 social group size is reasonably large (higher n). Finally, division of labour occurs in one
486 generation of the group life cycle so there is no scope for the accumulation of growing
487 helper clumps as for cyanobacteria filaments. In *B. subtilis*, a subset of cells become
488 helpers that produce and secrete protein degrading proteases[40]. However, these helper
489 cells are not sterile and so the consequence of deviating from the optimal caste ratios is
490 much smaller (supplementary section E.4).
491

492 Conclusion

493 Most previous work on reproductive division of labour has tended to be either
494 mechanistic, focusing on how different phenotypes are produced (caste determination),
495 or evolutionary, focusing on why division of labour is favoured in the first place [2–4,18–
496 24,26,45,63,67–72]. We have used evolutionary models to explain the broader question of why
497 different mechanisms are used in different species [2,3,7,18–20]. We have shown that
498 coordinated specialisation is more likely to be favoured over random specialisation in
499 small groups, when relative coordination costs are low, and when there are larger fitness
500 costs to deviating from optimal caste ratios. We have also shown how these patterns can
501 hold in groups with spatial structure and where different degrees of coordination can be
502 favoured. These results identify social and environmental factors that could explain the
503 distribution of mechanisms for division of labour that have been observed in bacteria and
504 other microbes. Beyond microorganisms, our results also suggest a hypothesis for why
505 random caste determination has not been widely observed in animal societies: during the
506 initial evolution of complex animal societies, group sizes were likely to be small and

relative costs of coordination might have been minor compared to each individual's day-to-day organismal metabolic expenditure.

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Data and Code Availability

All simulated data was generated using C and Matlab. The codes and generated data used for this study are available at:

https://github.com/mingpapilio/Codes_DOL_Mechanisms.

Author Contributions

G.A.C., S.A.W., and J.P. conceived the study. G.A.C. and J.P. and designed and analysed the analytical models, G.A.C. and M.L. designed and analysed the simulation models. G.A.C. and S.A.W. wrote the first draft. All authors contributed toward writing the final manuscript.

Declaration of Interests

The authors declare no competing interests.

Supplementary information:

A. Overview

B. Labour dividers and their fitness

C. Linear public goods

D. Alternative modelling assumptions

E. Alternative forms of cooperation

F. The optimal level of coordination

G. Dividing labour in a cyanobacteria filament

Figure S1. Alternative modelling assumptions

Figure S2. Non-linear public goods

Figure S3. Alternative biological scenarios

Figure S4. The optimal level of coordination

Figure S5. Simulation results for other traits

Table S1: Cyanobacteria model

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