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Cite this article: Biernaskie JM, West SA. 2015 Cooperation, clumping and the evolution of multicellularity. *Proc. R. Soc. B* **282**: 20151075.
<http://dx.doi.org/10.1098/rspb.2015.1075>

Received: 6 May 2015

Accepted: 6 July 2015

Subject Areas:

evolution, ecology, theoretical biology

Keywords:

eusociality, major evolutionary transitions, public goods, quorum sensing, social evolution, synergy

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2015.1075> or via <http://rspb.royalsocietypublishing.org>.

Cooperation, clumping and the evolution of multicellularity

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The evolution of multicellular organisms represents one of the major evolutionary transitions in the history of life. A potential advantage of forming multicellular clumps is that it provides an efficiency benefit to pre-existing cooperation, such as the production of extracellular ‘public goods’. However, this is complicated by the fact that cooperation could jointly evolve with clumping, and clumping could have multiple consequences for the evolution of cooperation. We model the evolution of clumping and a cooperative public good, showing that (i) when considered separately, both clumping and public goods production gradually increase with increasing genetic relatedness; (ii) in contrast, when the traits evolve jointly, a small increase in relatedness can lead to a major shift in evolutionary outcome—from a non-clumping state with low public goods production to a cooperative clumping state with high values of both traits; (iii) high relatedness makes it easier to get to the cooperative clumping state and (iv) clumping can be inhibited when it increases the number of cells that the benefits of cooperation must be shared with, but promoted when it increases relatedness between those cells. Overall, our results suggest that public goods sharing can facilitate the formation of well-integrated cooperative clumps as a first step in the evolution of multicellularity.

1. Introduction

The evolution of life on the Earth has involved approximately eight major transitions in individuality, where a group composed of individuals that could previously replicate independently form a more complex life form that can only replicate as a group [1]. For example, genes form genomes, cells form multicellular organisms and multicellular organisms form eusocial societies. Two key steps in many of these transitions are individuals forming cooperative groups and division of labour (differentiation) within those groups (figure 1; [2,3]).

We consider the first of these steps, the evolution of undifferentiated cooperative groups, in the transition from single-celled to multicellular organisms [4,5]. One factor that can select for cells to clump or cluster together is the pre-existence of some cooperation between cells, where clumping (clustering) increases the efficiency of this cooperation [6–10]. For example, cells may release extracellular factors that provide a benefit to the local population of cells (‘public goods’), and the use of these factors can be more efficient when cells are grouped at higher density [8,11–13]. Empirical evidence supporting this hypothesis comes from budding yeast [8,9] and myxobacteria [14,15]. More generally, cooperative public goods are of widespread importance in microbial populations [16], and so their production may have been a precursor in multiple origins of multicellularity [8].

However, there are several factors that can complicate this argument. First, cooperation could jointly evolve with clumping. In particular, high levels of clumping or cooperation could make the other trait more beneficial, and low levels of clumping or cooperation could make the other trait less beneficial (evolutionary synergy [17–19]). This suggests that the evolution of cooperative clumping could be an all-or-nothing strategy, depending on a threshold level of pre-existing cooperation. Second, the higher cell densities that result from clumping could either reduce or increase the extent to which non-cooperators are able to exploit cooperators [7,20,21]. This potential for exploitation should depend on (i) whether clumping increases the number of interacting cells in a

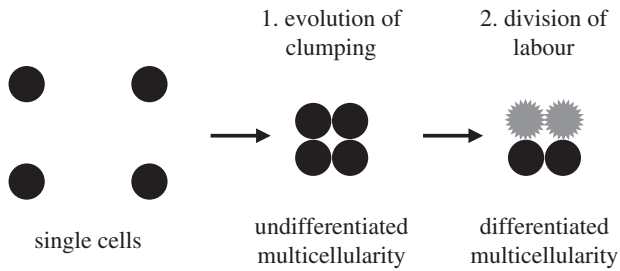


Figure 1. Two key steps in the major transition to multicellularity.

group, in addition to their proximity [20] and (ii) whether groups form in ways that either increase or restrict the extent to which non-cooperators are associated with cooperators—for example, depending upon whether cells remain with their parents (clonal grouping) or aggregate with potentially unrelated cells [22].

Our aim is to examine theoretically how these different factors interact. Using mathematical models, we analyse the adaptive evolution of a pre-existing cooperative public good and a costly factor that leads to cell clumping. Hence, we consider the step from simple microbial cooperation to cells being stuck together in cooperative multicellular clumps. As in real populations [9], our models treat clumping and public goods production as continuously varying traits (as opposed to discrete traits, switched either on or off [23]), and we apply the multi-trait kin selection framework of Brown & Taylor [18] to predict evolutionarily stable trait values. We first consider the evolution of clumping or public goods production alone, holding the other trait fixed, and then compare this with the joint evolution of both traits. We then examine how the evolution of cooperative clumping can change when clumping affects the number of interacting cells in the group, rather than just their proximity, and the genetic relatedness among them.

2. Models and results

(a) Model description

We consider a population of cells that may produce (i) a cooperative public good that provides a benefit to the local group of cells, such as a secreted enzyme and (ii) a trait that causes cells to clump together, such as stickiness and/or behavioural aggregation. We denote the public good and clumping traits by x and y , respectively, and we assume that there is no genetic correlation between the traits, such that they can jointly evolve in any direction.

The population is structured into groups of n interacting cells. We assume that public goods sharing among the n cells occurs over a space s , and so the density of cells in the group (number of cells per unit of space) is $d = n/s$. Clumping may increase the density of interacting cells, and hence the efficiency of public goods sharing, in two non-mutually exclusive ways (i) by bringing cells closer together, thus reducing the space over which public goods sharing occurs (reducing s); (ii) by increasing the number of interacting cells in the group (increasing n). In this section, we describe the basic model, in which clumping increases cell density by simply bringing cells closer together (reducing s , holding n fixed; analysis in appendix A). Later, we also allow clumping to increase the number of interacting cells in the group (appendix B).

We model natural selection as the successive invasion of mutant alleles coding for a variant level of public goods production and/or clumping, denoted by x_m and y_m respectively. We assume that the mutant allele is rare in the global population; however, owing to relatedness, the allele may be common in the local group. Following Brown & Taylor [18], we suppose that a focal mutant cell's fitness (W) is the sum of the fitness costs ($-C_i$) and benefit (B) associated with local cooperative interactions: $W(x_m, y_m, x_g, y_g) = B(x_g, y_g) - C_1(x_m) - C_2(y_m)$, where x_g and y_g are the average public goods and clumping traits, respectively, in the focal group (including the focal cell). Hence, we assume that the fitness costs to the focal cell increase with its own trait values only (assuming linear costs throughout)—reflecting, for example, the expense of manufacturing the public good and a substance to stick cells together. In contrast, the fitness benefit to the focal cell depends on the average trait values in its group. We assume that the total amount of public goods in the group (G) is the product of the average public goods trait and cell density, which is itself a function of the average clumping trait in the group ($G = x_g d(y_g)$). The fitness benefit to a focal cell is then

$$B(x_g, y_g) = \frac{b(G)}{p + (1-p)n}, \quad (2.1)$$

where b is the group benefit from the public good, and p ($0 \leq p \leq 1$) determines how the group benefit is divided among the n cells in the group. At one extreme, if $p = 1$, then all cells in the group enjoy the group benefit (the good is 'non-rivalrous' [24]), such as when the public good is used to defend the group from predators [25]. Alternatively, if $p = 0$, then the benefit of the public goods is divided equally among cells in the group (the good is 'rivalrous'), such as when the public good is used to acquire extracellular nutrients [26].

In order to predict the possible outcomes of evolution, we first derive the direction of selection for public goods production and clumping. Assuming that mutant alleles differ only slightly from a resident allele in the population, we calculate the fitness effects on a focal cell resulting from expression of the mutant allele in the focal cell itself and in the group as a whole, where the local frequency of the mutant allele is R (a 'neighbour-modulated' fitness approach [27–29]). This is equivalent to measuring the 'inclusive fitness effect' of the mutant allele from the perspective of the focal cell [30], where R is interpreted as the relatedness of the focal cell to a random cell in the group, including itself ('whole-group' relatedness [31]). Hence, for public goods production and clumping, the inclusive effects (H and K , respectively) are

$$H(x, y) = \frac{\partial W}{\partial x_m} + R \frac{\partial W}{\partial x_g} \quad (2.2a)$$

and

$$K(x, y) = \frac{\partial W}{\partial y_m} + R \frac{\partial W}{\partial y_g}, \quad (2.2b)$$

where all derivatives are evaluated at $x_m = x_g = x$ and $y_m = y_g = y$. In the basic model, we treat relatedness R as a fixed parameter. For example, the interaction group could involve a single clonal clump ($R = 1$), or it could involve many different clonal clumps or clumps made from the aggregation of cells with varying degrees of relatedness ($R < 1$). Later, we

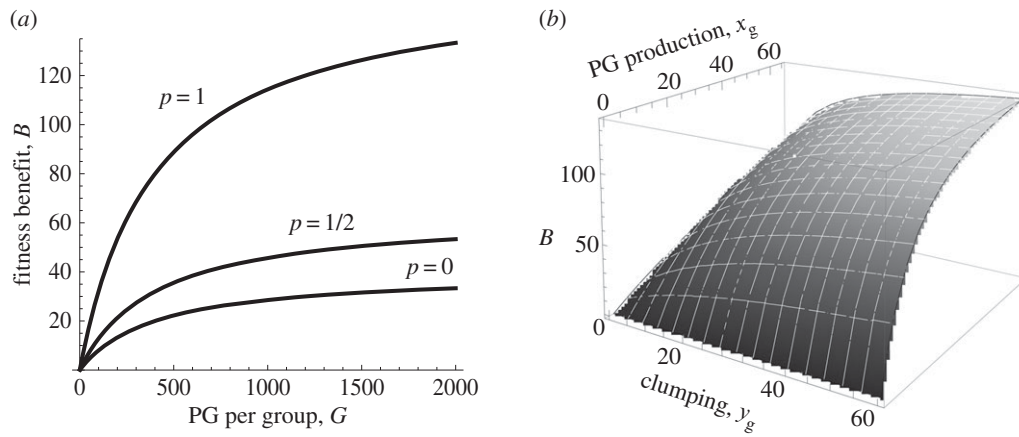


Figure 2. Fitness benefit functions. (a) We assume that the per-cell fitness benefit from local public goods (PG) is a diminishing function, given by $B(G) = b(G)/(p + (1 - p)n)$, where $b(G) = \beta(\gamma G/(\varepsilon G + \lambda))$, using the following parameters throughout: $\beta = 10$, $\gamma = 8$, $\varepsilon = 1/2$, $\lambda = 200$. (b) The per-cell fitness benefit as a function of the average PG production and clumping traits in the group (x_g and y_g , respectively), when $p = 1$ (i.e. all cells enjoy the group benefit of the public good). We assume that the amount of PG in the group is given by $G = x_g d(y_g)$, where $d(y_g) = n/((\alpha y_g + \delta)/(\theta y_g + \delta))$, using the following parameters throughout: $\alpha = 0.1$, $\theta = 2$, $\delta = 10$. Other parameters used: $n = 4$.

allow clumping to increase the genetic relatedness among cells in the group (appendix C).

Using the inclusive fitness effects above, we solve for the equilibrium trait values where directional selection ceases ($H = 0$ and/or $K = 0$). We consider two cases (i) public goods production or clumping evolve separately (holding the other trait fixed), resulting in a single equilibrium trait value (\hat{x} or \hat{y} , respectively); (ii) public goods production and clumping jointly evolve, resulting in multiple joint equilibria (\hat{x}, \hat{y}) . In both cases, we determine whether or not selection drives the population towards an equilibrium (convergence stability [32]; details in appendix A). This is particularly relevant when multiple equilibria exist, where stable states may be separated by a convergence–unstable equilibrium, or evolutionary ‘repeller’, which acts like a threshold trait value for sharp change in evolutionary outcome [18]. We do not consider whether convergence–stable equilibria can be invaded by any other mutant allele (evolutionary stability [33]), as this remains difficult to compute in models of social evolution, especially when multiple social traits evolve [34]. Accordingly, we refer to a convergence–stable equilibrium as a ‘candidate’ evolutionarily stable strategy (ESS), denoted by x^* or y^* , for public goods production or clumping, respectively.

To illustrate the main results of the model, we present numerical results based on the following functions. First, as described above, the density of cells in a group is given by $d(y_g) = n/s(y_g)$, where s is the space in which cells interact. We assume that s takes the form of a rational function, $s(y_g) = (\alpha y_g + \delta)/(\theta y_g + \delta)$ with $\alpha = 0.1$, $\theta = 2$, $\delta = 10$. This function initially declines with higher clumping and then reaches an asymptote, representing the smallest possible space for n cells to coexist. Second, we assume linear fitness costs for public goods production and clumping, with $C_1(x_m) = c_1 x_m$ and $C_2(y_m) = c_2 y_m$. Third, we assume that $b(G)$, the group benefit from public goods, is an increasing function with diminishing returns, $b(G) = \beta(\gamma G/(\varepsilon G + \lambda))$ with $\beta = 10$, $\gamma = 8$, $\varepsilon = 1/2$ and $\lambda = 200$, as shown in figure 2a. Diminishing returns could be biologically realistic for microorganisms because the benefit of extracellular factors will not increase indefinitely [35,36], especially if there is reduced access to resources (e.g. light or nutrients) in multicellular groups. Figure 2b shows how the per-cell

fitness benefit (B) increases with higher public goods production and/or clumping in the group.

(b) Basic model with fixed public goods production or clumping

We first examine the evolution of clumping or public goods production separately, while holding the other trait fixed. This acts as a baseline for comparison with the joint evolution of public goods and clumping, considered below. We find the following.

(i) Result 1

When each trait is considered separately, both clumping and public goods production are promoted by higher relatedness (higher R) and a higher per-cell fitness benefit from the public good (higher p ; figure 3a,b; appendix A). This is because a higher per-cell benefit increases the fitness of the focal cell and its group mates, higher relatedness increases the indirect fitness benefit of helping group mates and clumping increases the efficiency of this cooperation. Figure 3a,b shows that when the benefit from public goods is sufficiently high, both public goods production and clumping can invade the population at a threshold level of relatedness and then gradually increase with increasing relatedness.

(c) Basic model with joint evolution of public goods production and clumping

We now examine the more realistic scenario in which public goods production and clumping can evolve together. For example, suppose that a population initially expresses the optimal level of public goods production in the absence of clumping (as in figure 3a). Such a population may produce enough public goods to favour the invasion of clumping, at which point the public goods and clumping traits can evolve to a candidate joint ESS. Our aim is to determine how the predicted joint evolution of clumping and public goods production differs from the single-trait analyses above (result 1). We find the following.

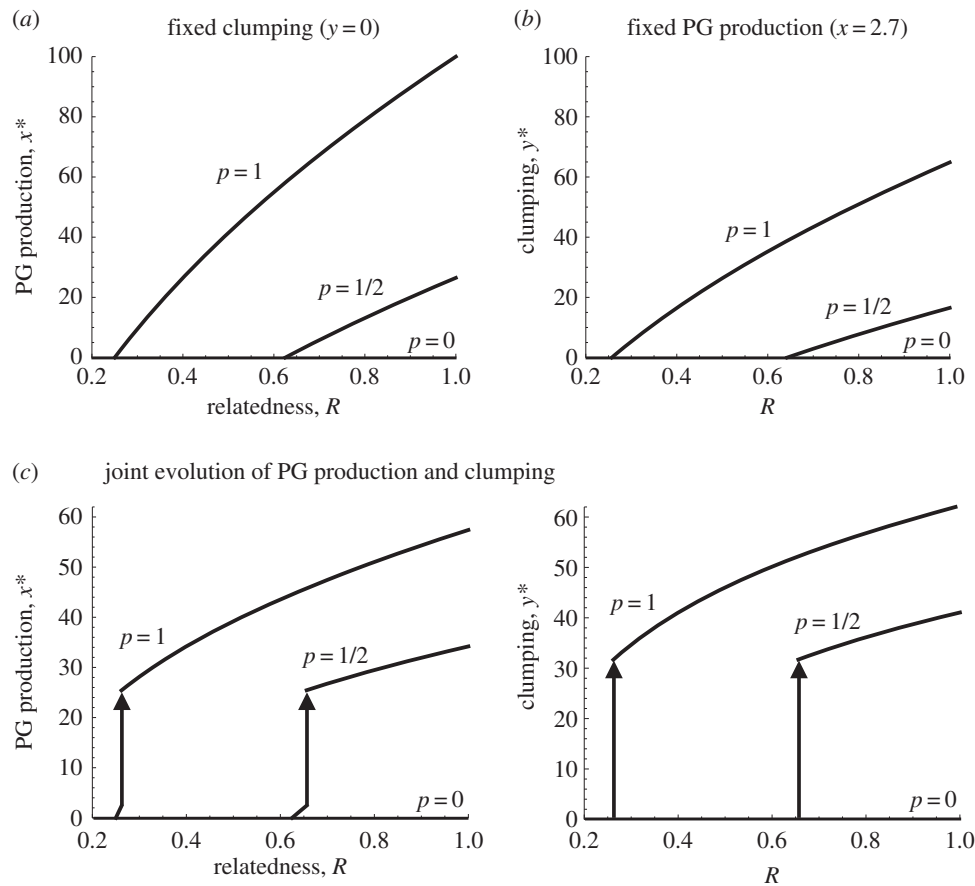


Figure 3. Optimal public goods (PG) production and clumping (candidate ESS values). The optima depend on how the benefit of the public good is divided among cells in the group (when $p = 1$, all cells enjoy the group benefit; when $p = 0$, the group benefit is divided equally among cells). In (a,b), we solve for the optimal value for one trait while holding the other trait fixed. In this case, PG production and clumping may invade at a threshold relatedness and then gradually increase with increasing relatedness. In (c), the traits evolve jointly, assuming that the population initially expresses the optimal PG production in the absence of clumping (as in (a)). In this case, there may be a major shift from a non-clumping state to a cooperative clumping state, occurring at a threshold level of relatedness (arrows). Other parameters used: $c_1 = 0.4$, $c_2 = 0.2$ and otherwise as in figure 2.

(i) Result 2

When public goods production and clumping jointly evolve, a small increase in relatedness can lead to a major shift in evolutionary outcome—from a non-clumping state with low public goods production (low x^* , $y^* = 0$) to a cooperative clumping state with relatively high values of both traits (high x^* , y^* ; figure 3c). This is due to synergy between the traits (appendix A): at a threshold level of relatedness (arrows in figure 3c), enough public good is produced to favour clumping, which in turn favours more public goods production, and so on. Hence, in contrast to the single-trait analyses, figure 3c shows that the candidate joint ESS can increase in a stepwise manner with increasing relatedness.

(ii) Result 3

Synergy between clumping and public goods traits can lead to bistability, where either a non-clumping or a cooperative clumping state are possible evolutionary outcomes (figure 4). For example, figure 3c shows that when the per-cell benefit of the public goods is relatively small ($p = 0$), selection does not favour enough public goods production for clumping to invade the population. Yet if the population could get over an initial threshold level of public goods production or clumping (e.g. if the cost of public goods were temporarily reduced), then the population could ‘escape’ the non-clumping state and evolve to a stable level of

cooperative clumping (figure 4b). Figure 4a shows that this threshold declines as relatedness increases.

(d) Clumping affects cell number and relatedness

We next examine the case in which clumping may have a positive effect on the number of interacting cells in the group, rather than just on their proximity. In comparison with when cell number is fixed, we find the following.

(i) Result 4.1

Clumping can be either promoted or inhibited when clumping increases cell number (appendix B). This is because, on the one hand, higher cell number has two inhibiting effects: (i) it may lower the focal cell’s relatedness to the whole group, because the focal cell itself becomes a smaller fraction of the group and (ii) when the group benefit of the public goods is divided equally among individual cells ($p = 0$), higher cell number reduces the benefit going to each cell. On the other hand, higher cell number can increase cell density and therefore the amount of public goods in the group (G). This promoting effect of higher cell number can be greatest when all cells enjoy the group benefit of the public good ($p = 1$) and when the interaction group involves a single clonal clump ($R = 1$) because, in this case, relatedness is not reduced by higher clumping.

Finally, we consider the case in which clumping may have a positive effect on the relatedness of interacting cells in the group. This could occur if clumping involves staying

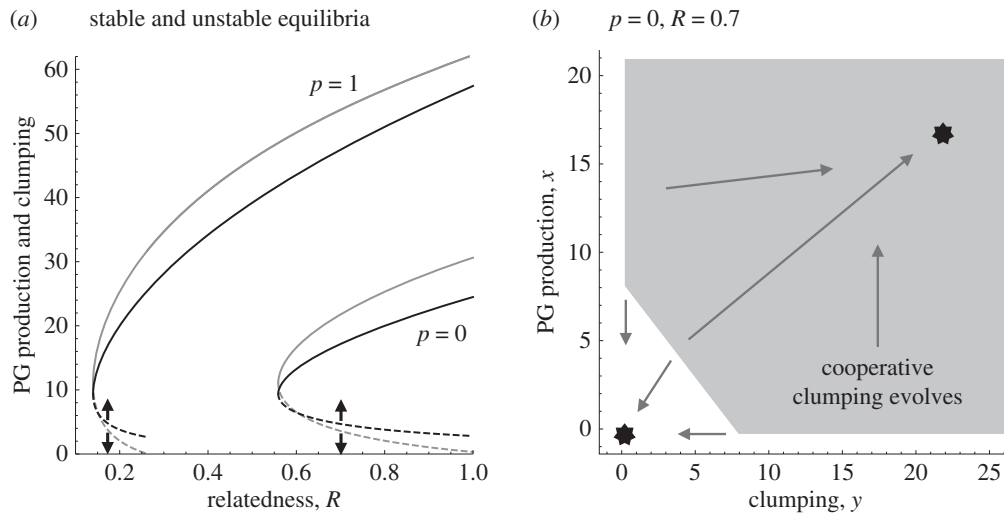


Figure 4. Bistability in the joint evolution of public goods (PG) production and clumping. In (a), solid lines show convergence–stable equilibria and dashed lines show convergence–unstable (repelling) equilibria, where black lines are for the PG trait and grey lines are for the clumping trait. When $p = 1$, all cells enjoy the group benefit of the public good, and when $p = 0$, the group benefit is divided equally among cells. The example in (b), with $p = 0$ and $R = 0.7$, illustrates two possible stable outcomes (candidate joint ESSs): a non-cooperative, non-clumping state ($x^* = 0, y^* = 0$) and a cooperative clumping state ($x^*, y^* > 0$). The cooperative clumping state can be reached only if the population exceeds a threshold level of PG production and/or clumping (grey area). Panel (a) shows that this threshold declines as relatedness increases.

with parents (i.e. incomplete cell separation [8,9]) or an element of kin discrimination [22,37,38]. To illustrate the main result, we define the ‘realized’ relatedness as $\hat{R} = R + (\mu(1 - R))/(1 + 1/y_g)$, where R is the baseline whole-group relatedness and μ mediates the increase in relatedness with increased clumping. In comparison with when relatedness is fixed, we find the following.

(ii) Result 4.2

Cooperative clumping is further promoted when clumping increases the relatedness among interacting cells (appendix C; electronic supplementary material, figure S1). Specifically, higher relatedness owing to clumping increases the candidate ESS values of public goods production and clumping, and it also lowers the trait values that are required to get over the initial threshold for cooperative clumping to evolve. Consequently, cooperative clumping can potentially evolve from much lower values of baseline relatedness than would be possible when clumping does not affect relatedness.

3. Discussion

Our models show how the production of a public good can favour the first step in the evolution of multicellularity, where cells become stuck together in cooperative multicellular clumps. We found that (i) when public goods production and clumping are considered separately (holding the other trait fixed), both traits gradually increase with increasing genetic relatedness (figure 3a,b); (ii) in contrast, when the traits evolve jointly, a small increase in relatedness can favour enough public goods production to cause a major shift in evolutionary outcome—from a non-clumping state with low public goods production to a cooperative clumping state with high values of both traits (figure 3c); (iii) high relatedness makes it easier to get over the initial threshold for cooperative clumping to evolve (figure 4) and (iv) clumping can be inhibited when it increases the number of cells in a group that the benefits of cooperation must be shared with,

but promoted when it increases relatedness to the cells that share those benefits. These results illustrate the unique dynamics that can arise when multiple social traits evolve jointly [18]; in particular, our models predict a threshold level of relatedness and public goods production that must be reached for cooperative clumping to evolve.

(a) Synergy and the evolution of multicellularity

We found that the sharp transition from a non-clumping to a cooperative clumping state occurs because of synergy in the joint evolution of public goods production and clumping. Synergy occurs when higher (or lower) expression of one trait makes the other trait more (or less) beneficial [17–19]. Such evolutionary feedback between traits has been recognized as a key driver of the major transitions in evolution, including the evolution of chromosomes [39] and cooperative symbioses [17]. This is because, once two cooperative traits are beyond a threshold level, they may evolve through an irreversible stage where one trait depends on the expression of the other [19,40]. Similarly, we found that synergy can drive the transition from cooperative single cells to highly cooperative cell clumps, where a high level of clumping depends on the expression of high public goods production, and vice versa. In this way, synergy can be crucial for the evolution of multicellularity because it leads to large, well-integrated and highly cooperative clumps that may later evolve cell differentiation and a new level of individuality. These predictions about synergy and the sharp transition to cooperative clumping could be tested with contemporary microbial species such as budding yeast or myxobacteria [8–10,15].

Frank [17] showed that the threshold trait values required for the evolution of a cooperative symbiosis decline with increasing spatial association in trait values between symbiotic partners. Similarly, we found that the threshold trait values for the origin and evolution of cooperative clumping decline with increasing genetic relatedness among cells in the group (figure 4a). This is because high relatedness ensures that cells with high public goods production and/or

clumping will have group mates with similar traits, making it easier to get over the threshold for the evolution of cooperative clumping.

Our threshold result for the evolution of cooperative clumping is similar to a recent study showing that synergy can lead to a threshold group size required for public goods to be favoured [41]. In that study, however, ‘synergy’ was used to describe an accelerating fitness benefit of local public goods, rather than a positive feedback between two jointly evolving traits (see also Queller [42]). In our model of joint evolution, synergy between public goods production and clumping can arise even when the fitness benefit from increased clumping or public goods production is always decelerating (as in figure 2). Our model could also be extended to study accelerating fitness gains, as in scenarios where a public good is beneficial only when some threshold amount is produced [41,43,44].

(b) Effects of clumping on cell number and relatedness

We found that the evolution of cooperative clumping will depend on whether clumping also increases the number of interacting cells in a group, rather than just their proximity, and the relatedness among them. Cooperative clumping is generally promoted when clumping also increases relatedness. This is consistent with the idea that high relatedness and high cell density increase the efficiency of using a public good, owing to the exclusion of cheats that exploit the public good but produce less of it themselves [7–9]. On the other hand, clumping can be inhibited when clumping also increases group size, particularly when the group benefit of public goods are equally divided among cells in the group (the good is rivalrous; $p = 0$). This is consistent with previous theory and empirical work on rivalrous goods, which suggests that larger group size increases the ability of cheats to exploit cooperators, thereby inhibiting the production of public goods [20]. Our results suggest that if clumping typically increases cell number, in addition to their proximity, then the evolution of cooperative clumping may be more probable when all cells in the group enjoy the group benefit of a public good (the good is non-rivalrous; $p = 1$), such as when the public good is used to defend the group from predators [25].

(c) Links to quorum sensing and eusociality

There are clear links between our model and those for cell-to-cell signalling, termed quorum sensing (QS), in bacteria. In our model, clumping is favoured because the fitness benefit of public goods production is greater at higher densities. Similarly, it has been argued that QS is used to turn on the production of public goods at high cell densities, because the production of public goods provides a greater benefit at higher cell densities [45,46]. These suggest different solutions to the same problem—with cells either raising their density (clumping), or only switching on the production of public goods at high cell densities (QS). These solutions are not mutually exclusive, as species could both clump and use QS. While relevant empirical studies are rare, they support the underlying assumption of both classes of models, by showing that the production of public goods provides a relatively greater benefit at higher cell densities [8,11,14,47].

Finally, our models help clarify the conceptual links between the major transitions to multicellularity and

eusociality [1–3,48,49]. The eusocial insects can be divided into two types, depending upon whether the evolution of eusociality was driven by life insurance or fortress defence [50,51]. Life insurance is when there is a period of extended parental care, and a significant chance that the parent will die during that period, such that there is an efficiency benefit in forming cooperative groups to rear young (e.g. wasps [52–54]). Fortress defence is when individuals live in sites where food is obtained and there is an advantage in forming cooperative groups to defend that site (e.g. termites [50,55]). The benefits of forming multicellular clumps can be divided into two analogous categories. First, multicellular clumps may provide an efficiency benefit to cooperative behaviours, such as public goods production (this study) or the formation of fruiting bodies [56]. Second, multicellular clumps may be better at avoiding predation [2,10], and this could be made more efficient by sharing anti-predator public goods [25]. It has also been shown that high relatedness plays a directly analogous role in promoting the transitions to multicellularity and eusociality [22,49]. Hence, kin selection theory—which separates out the roles of ecological benefits and relatedness [29]—illuminates the incredibly similar forces that have driven these two major transitions.

Authors' contributions. J.B. carried out the modelling work; J.B. and S.W. conceived the study and wrote the paper. All authors gave final approval for publication.

Competing interests. We declare we have no competing interests.

Funding. We were funded by the European Research Council.

Acknowledgements. We thank Daniel Cornforth, Roberta Fisher, Kevin Foster, Stephanie Kapsetaki, and Gregory Wyatt for comments.

Appendix A. Basic model

Here, we present a general analysis of the basic model. Following our description from the main text, the fitness of a focal mutant cell can be written as

$$W(x_m, y_m, x_g, y_g) = \frac{b(x_g, y_g)}{p + (1-p)n} - C_1(x_m) - C_2(y_m), \quad (\text{A } 1)$$

where the fitness benefit to the group (b) is a positive function of the average public goods trait in the group (x_g) and of the average clumping trait in the group (y_g), owing to the effect of clumping on the space in which cells interact; the costs C_i are positive functions of the mutant's public good and clumping trait values. The inclusive fitness effects of public goods production and clumping (H and K , respectively, from equation (2.2)) are

$$H(x, y) = R \frac{b'_x}{p + (1-p)n} - C'_{1,x} \quad (\text{A } 2)$$

and

$$K(x, y) = R \frac{b'_y}{p + (1-p)n} - C'_{2,y}, \quad (\text{A } 3)$$

where $b'_x = \partial b / \partial x_g$, $b'_y = \partial b / \partial y_g$, $C'_{1,x} = dC_1 / dx_m$ and $C'_{2,y} = dC_2 / dy_m$, all evaluated at $x_m = x_g = x$ and $y_m = y_g = y$.

We first consider the evolution of public goods production or clumping separately, holding the other trait fixed. In this case, an equilibrium (\hat{x} or \hat{y}) will be convergence stable only if the selection gradient (inclusive fitness effect) is positive at trait values below the equilibrium and negative at trait values above the equilibrium [57]. This implies $H'_x < 0$

and $K'_y < 0$ or

$$R \frac{b''_x}{p + (1-p)n} - C''_{1,x} < 0 \quad (\text{A4})$$

and

$$R \frac{b''_y}{p + (1-p)n} - C''_{2,y} < 0, \quad (\text{A5})$$

for public goods production and clumping, respectively, where $b''_x = \partial^2 b / \partial x_g^2$, $b''_y = \partial^2 b / \partial y_g^2$, $C''_{1,x} = d^2 C_1 / dx_m^2$ and $C''_{2,y} = d^2 C_2 / dy_m^2$, all evaluated at $x_m = x_g = x$ and $y_m = y_g = y$. Convergence stability therefore depends on the shape of the fitness cost and benefit functions. For example, if costs are linear ($C''_{1,x} = C''_{2,y} = 0$), then an equilibrium value of public goods production or clumping will be convergence stable only if the corresponding benefit function is decelerating ($b''_x < 0$ or $b''_y < 0$, respectively), as assumed in the main text.

Next, we consider the joint evolution of public goods production and clumping. In this case, a joint equilibrium (\hat{x}, \hat{y}) will be convergence stable only if the 'restoring' force of selection, driving a population towards the equilibrium, is greater than the 'disturbing' effect of synergistic interactions, where an increase (or decrease) in one trait selects for higher (or lower) values of the other trait [18]. This implies $H'_x K'_y > H'_y K'_x$, or

$$H'_x K'_y > \frac{R^2 b''_{x,y}}{(p + (1-p)n)^2}, \quad (\text{A6})$$

where $b''_{x,y} = \partial^2 b / \partial x_g \partial y_g$, evaluated at $x_g = x$ and $y_g = y$. This shows that when the single-trait convergence stability conditions are met ($H'_x K'_y > 0$), a joint equilibrium can be convergence unstable, or repelling, when the slope of the fitness benefit function with respect to one trait (e.g. public goods production) sufficiently increases with higher values of the other trait (e.g. clumping; $b''_{x,y}$ is positive and sufficiently large; figure 2b).

Appendix B. Clumping increases cell number

Here, we extend the basic model by allowing clumping to also increase the number of interacting cells in the group. For simplicity, we treat cell number as a continuous variable that increases with the average clumping trait in the group ($n(y_g)$), which in turn increases the total amount of public good in the group (G). This leads to the following fitness function

$$W(x_m, y_m, x_g, y_g) = \frac{b(x_g, y_g, n(y_g))}{p + (1-p)n(y_g)} - C_1(x_m) - C_2(y_m). \quad (\text{B1})$$

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In addition to its effect on public goods, higher cell number may also reduce the relatedness between a focal cell and the whole group ($R(n)$). This is because whole-group relatedness includes the focal cell's relatedness to self, and the focal cell becomes a smaller fraction of the whole group as cell number increases. When clumping affects relatedness, the inclusive fitness effects are

$$H(x, y) = \frac{\partial W}{\partial x_m} + R(n) \frac{\partial W}{\partial x_g} \quad (\text{B2})$$

and

$$K(x, y) = \frac{\partial W}{\partial y_m} + R(n)(1 + R'_n) \frac{\partial W}{\partial y_g}, \quad (\text{B3})$$

where $R'_n = dR/dn(y_g)$, evaluated at $y_g = y$, accounts for the effect of the mutant allele on whole-group relatedness. Here, we focus on the inclusive fitness effect of clumping, and for simplicity we evaluate this when all cells enjoy the group benefit of the public good ($p = 1$) or when the group benefit is equally divided among cells in the group ($p = 0$):

$$K(x, y)|_{p=1} = R(n)(1 + R'_n)(n'_y b'_n + b'_y) - C'_{2,y} \quad (\text{B4})$$

and

$$K(x, y)|_{p=0} = R(n)(1 + R'_n) \frac{(n'_y(n(y_g)b'_n - b) + nb'_y)}{n(y_g)^2} - C'_{2,y}, \quad (\text{B5})$$

where $n'_y = dn/dy_g$ and $b'_n = \partial b / \partial n$, all evaluated at $x_g = x$ and $y_g = y$. These effects show that (i) clumping is inhibited by a strong negative effect of the mutant clumping trait on relatedness (large R'_n) but promoted by strong positive effects of cell number on the group benefit from public goods (large $n'_y b'_n$); (ii) when $p = 0$, clumping is further inhibited by larger cell number because the group benefit of the public good gets divided among a larger number of cells.

Appendix C. Clumping increases relatedness

Here, we extend the basic model by allowing clumping to increase the relatedness among interacting cells (e.g. clumping involves clonal grouping or kin recognition). We do not consider changes in cell number here, so whole-group relatedness ($R(y_g)$) will always increase with increased clumping. This leads to the following inclusive fitness effect for clumping

$$K(x, y) = R(n)(1 + R'_y) \frac{b'^2_y}{(p + (1-p)n)^2} - C'_{2,y}, \quad (\text{C1})$$

where $R'_y = dR/dy_g$, evaluated at $y_g = y$. This shows that, as long as groups are not already clonal ($R = 1$ and $R'_y = 0$), clumping will always be promoted when it increases relatedness.

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