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Report

Group Formation, Relatedness, and the Evolution of Multicellularity

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Summary

The evolution of multicellular organisms represents one of approximately eight major evolutionary transitions that have occurred on earth [1–4]. The major challenge raised by this transition is to explain why single cells should join together and become mutually dependent, in a way that leads to a more complex multicellular life form that can only replicate as a whole. It has been argued that a high genetic relatedness (r) between cells played a pivotal role in the evolutionary transition from single-celled to multicellular organisms, because it leads to reduced conflict and an alignment of interests between cells [1–17]. We tested this hypothesis with a comparative study, comparing the form of multicellularity in species where groups are clonal ($r = 1$) to species where groups are potentially nonclonal ($r \leq 1$). We found that species with clonal group formation were more likely to have undergone the major evolutionary transition to obligate multicellularity and had more cell types, a higher likelihood of sterile cells, and a trend toward higher numbers of cells in a group. More generally, our results unify the role of group formation and genetic relatedness across multiple evolutionary transitions and provide an unmistakable footprint of how natural selection has shaped the evolution of life [1].

Results and Discussion

The evolution of life on earth, from the simplest replicating molecules to complex animal societies, has involved approximately eight major evolutionary transitions in individuality [2–4, 18]. In each of these transitions, a group of individuals that could previously replicate independently joins together to form a new, more complex life form that can only replicate as a whole. For example, genes come together to form genomes, cells join together to form multicellular organisms, and multicellular organisms join together to form eusocial societies. The major challenge raised by each of these transitions is to explain why individuals should join together and become mutually dependent in a way that leads to a more complex individual [2–4, 18].

Here, we use a phylogenetically based [19] comparative study to test how the relatedness between interacting cells influenced both the likelihood of the major evolutionary transition to obligate multicellularity and the level of sociality in multicellular groups. We obtained data on 168 species, with representatives from all multicellular lineages except diatoms and charophyte algae (Figure 1; Figure S1 available online). We used life-history data on how groups form to infer relatedness. Specifically, when groups form by cells remaining with their parents, then groups are clonal ($r = 1$; 149 species). This usually involves the group going through a single-celled (unicellular) stage [10, 11]. In contrast, if groups form by cells aggregating together, then relatedness could be anything from zero to one ($r \leq 1$) but is likely to be nonclonal ($r < 1$; 19 species) [20].

Transitions to Obligate Multicellularity

We first examined whether relatedness influenced the likelihood that groups underwent the transition to obligate multicellularity. Our distinction here is between obligately multicellular species, which can only complete their life cycle as a multicellular organism, and facultatively multicellular species, which are able to complete their life cycle as unicells and only become multicellular under certain environmental conditions. For example, *Dictyostelium* species can remain in their unicellular state for many generations, without the need to form a multicellular fruiting body, which they do only under certain harsh conditions, and so we classify them as facultatively multicellular. In contrast, in mammals, the unicellular stage is finite and must always ultimately lead to the multicellular stage, and so we classify them as obligately multicellular. Consequently, we are focusing on whether one of the key requirements for a major evolutionary transition in individuality has occurred, termed "contingent irreversibility" [2, 4].

We found that species with clonal groups were significantly more likely to have made the transition to obligate multicellularity (Figures 2 and 3A; pMCMC = 0.0002). Overall, 75% (9/12) of the lineages with clonal group formation had made the transition to obligate multicellularity, whereas the five lineages with potentially nonclonal group formation had only led to facultative multicellularity. Obligate multicellularity is feasible in species with nonclonal group formation—all it requires is that cells always aggregate to complete a necessary part of their life cycle (i.e., not just under certain conditions). Given that we found no evidence for nonclonal organisms having evolved obligate multicellularity, this suggests that the genetic conflict that arises from lower relatedness ($r \leq 1$) inhibits this major transition in individuality.

If there is a lack of conflict within obligate multicellular groups, such that the group is thought of as an individual, then they will have made a major evolutionary transition in individuality [2–4]. Clonality leads to no within-group conflict [13]. Consequently, because obligate multicellularity has only evolved in species with clonal groups, all of these species have made a major transition in individuality.

Sociality in Multicellular Groups

We then examined whether relatedness influenced the level of sociality in multicellular groups. We collected data for four life-history variables: the number of different cell types that can occur in each group, whether or not species had sterile cells, the percentage of the total number of cells that was sterile in species that had sterile cells, and the total number of cells in the group (organism). The number of different cell types that can occur in a group is analogous to the number of castes in eusocial insect colonies, and hence represents the extent to which different group members specialize in different roles

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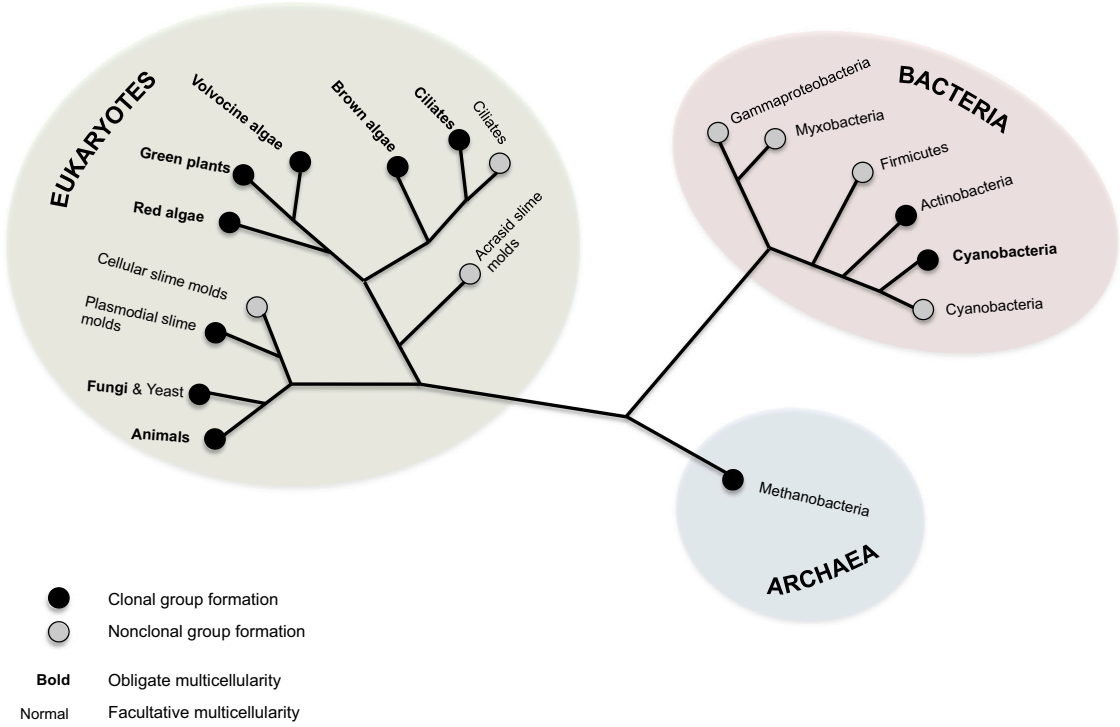

Figure 1. Multicellularity across the Tree of Life

The figure shows an overview phylogram of the taxa in our data set. We have labeled each taxon according to whether multicellular groups are either clonal (black circles) or nonclonal (gray circles) and whether multicellularity is an obligate (bold font) or a facultative (normal font) part of the life cycle. The relatively low number of times that multicellularity has evolved, especially in taxa with nonclonal groups, emphasizes the importance of collecting data on specifically targeted groups to increase the statistical power of phylogenetically based analyses.

[4]. Sterile cells represent a case of extreme altruism, analogous to sterile workers in eusocial insects, forgoing any opportunity to reproduce directly in order to help others [5]. The total number of cells in the group is seen as one of the traits that contribute to and correlate with group complexity [4].

We found that species with clonal groups had significantly higher numbers of cell types and a significantly higher probability of having sterile cells. Species with clonal groups had, on average, approximately six times as many cell types as species with nonclonal groups (Figures 2 and 3B; pMCMC = 0.0008) and were approximately twice as likely to have sterile cells (Figures 2 and 3C; pMCMC = 0.02). These significant influences of relatedness are particularly impressive given the following: (1) ecological costs and benefits also matter, not just relatedness [17]; (2) relatedness may still be extremely high (for example, $r = 0.98$ [20] in the nonclonal groups); and (3) our statistical power is limited by the small number of evolutionary transitions to multicellularity. This emphasizes not only that relatedness matters, but that it matters a lot.

We found that species with clonal groups had a higher proportion of sterile cells (in the species with sterile cells) and a greater number of cells, but that these differences were nonsignificant. In species with sterile cells, those that formed clonal groups had approximately three times the proportion of sterile cells in comparison to species with nonclonal groups (Figure 3D; pMCMC = 0.41). Species with clonal groups had, on average, approximately 100 times as many cells in their group as species with nonclonal groups (Figures 2 and 3E; pMCMC = 0.27). This lack of statistical significance despite the magnitude of differences reflects both high variability and a limited statistical power, due to the small number of independent evolutionary origins of multicellularity, especially those with potentially nonclonal group formation (Figures 1 and 3A). For example, the comparison of the proportion of sterile cells was limited primarily to a comparison of clonal volvocine algae and cyanobacteria with the nonclonal cellular slime molds (Figure 3D). The high variability may reflect that although a higher relatedness can favor more sterile cells and larger groups, the ecological costs and benefits of these traits could vary across taxa.

Causality and Assumptions

We have examined how relatedness influences both whether a transition to obligate multicellularity has occurred and the different traits that determine the level of sociality, such as whether there are sterile cells in the group. Previous work has examined multiple traits simultaneously by comparing simple and complex multicellular species, where complexity is defined by the presence of sterile cells, an early germ-soma split, a high number of cell types, and a large number of cells [4, 10, 11]. However, although the simple/complex distinction is likely to be correlated with whether a major transition has occurred, it is not a defining feature, and the correlation can break down [7]. For example, *Dictyostelium purpureum* and *Volvox carteri* are both simple, having a sterile soma and the same number of cell types, but only *V. carteri* has undergone the major transition to obligate multicellularity. Furthermore, although complex multicellularity has evolved only in species with a single-cell stage (unitary development) that leads to clonality ($r = 1$) [10, 11], there is no significant correlation between relatedness and whether multicellularity is simple or complex (pMCMC = 0.21). A problem here is that

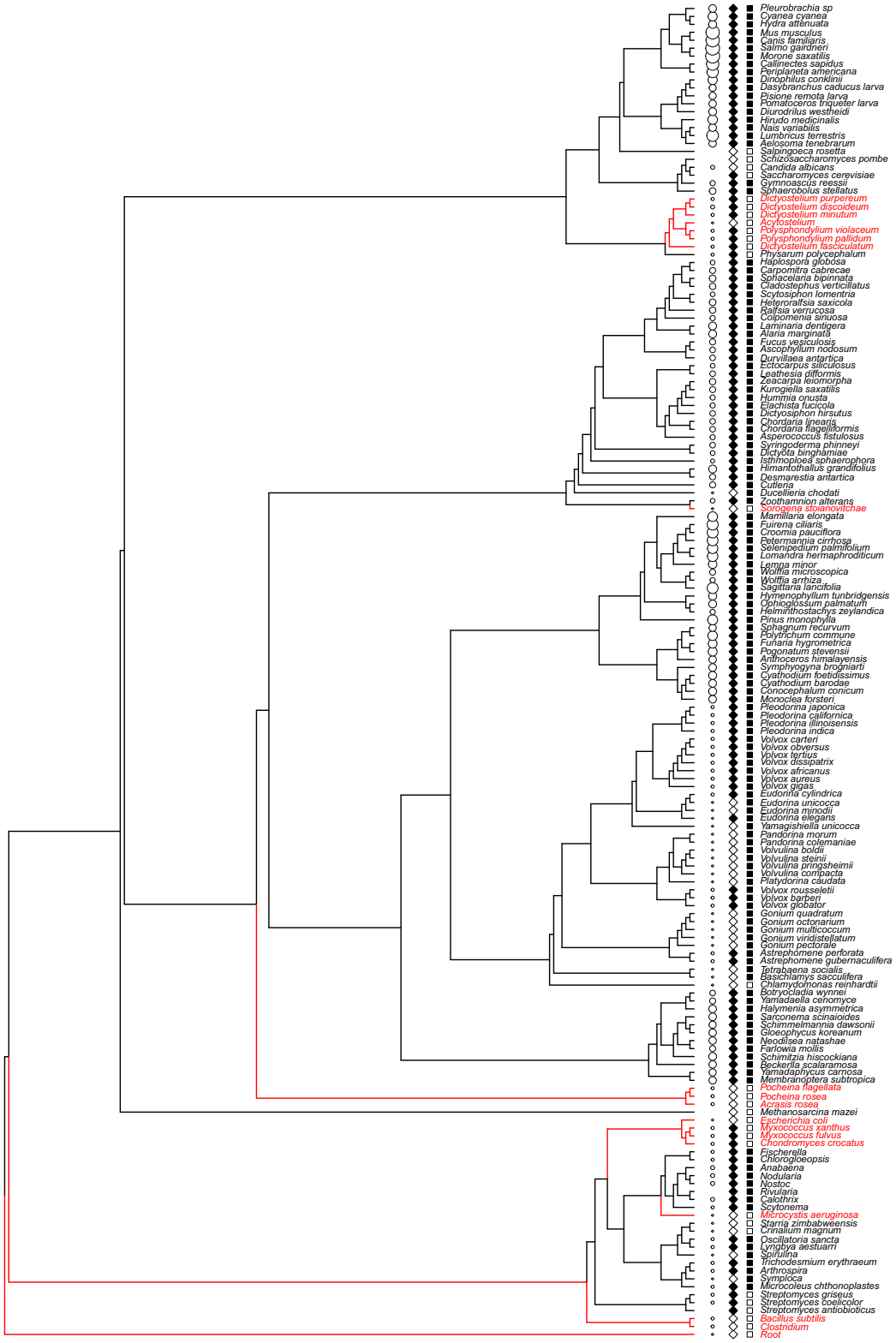

Figure 2. Evolutionary Relationships between Clonality and Multicellular Traits

The figure shows the evolutionary transitions between clonal (black tips and edges) and nonclonal (red tips and edges) group formation and its correlation with obligate (black squares) versus facultative (white squares) multicelluarity, the presence of sterile soma (black diamonds represent sterile soma; white diamonds represent no sterile soma), and the number of different cell types (the size of the white circles represents the natural logarithm of the number of cell types). Ancestral reconstruction of clonal and nonclonal states was conducted using Bayesian phylogenetic mixed models in MCMCglmm (see also Figures S1 and S2).

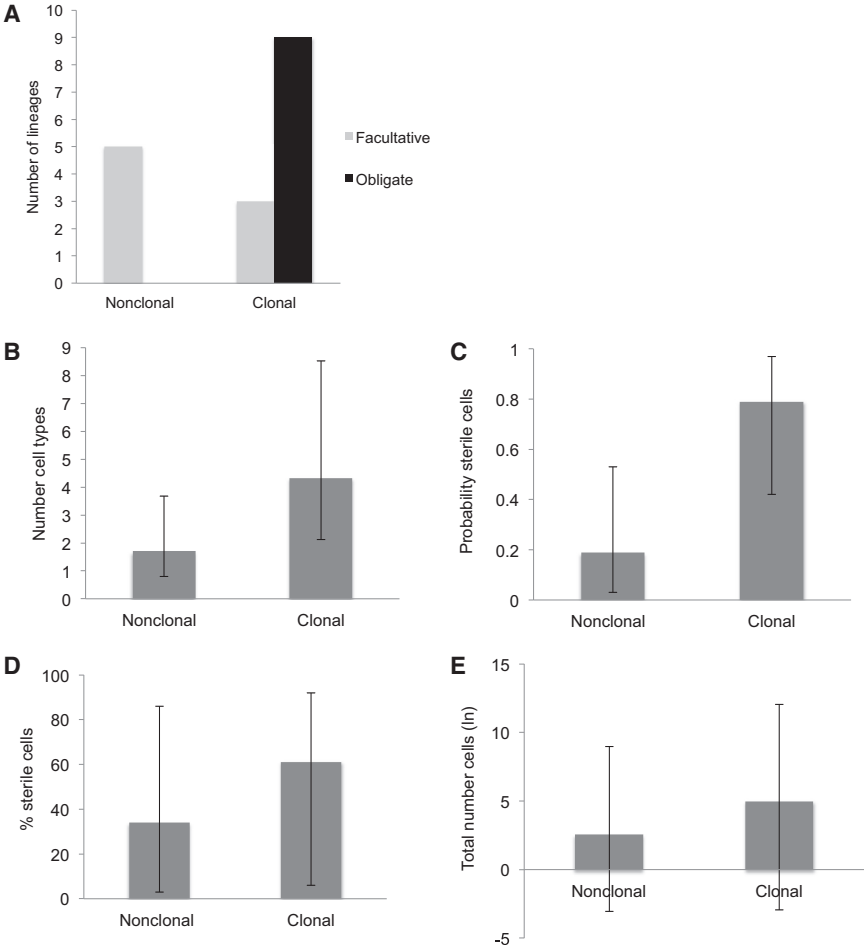

Figure 3. Group Formation, Evolutionary Transitions, and Sociality

The graphs show, for multicellular groups that are either clonal or potentially nonclonal, the (A) number of lineages in which facultative and obligate multicellularity have evolved; (B) number of cell types; (C) probability of having steriles cells; (D) percentage of sterile cells in species with a sterile soma; and (E) total number of cells. Estimates in (B)–(E) are posterior modes with 95% credible intervals from Bayesian phylogenetic mixed models. The number of cell types is back-transformed from a Poisson distribution with log link function, the probability of sterile cells from a binary distribution with logit link function, the proportion of sterile cells from a binomial distribution with a logit link function, and total number of cells is on a logarithmic scale (see also Figure S3).

complex multicellularity has evolved only five times, in sister groups: animals, fungi, plants, red algae, and brown algae [4, 11]. Consequently, complex multicellularity hasn't evolved in enough separate places on the tree of life to provide statistical power for a formal comparative analysis [19, 21].

Our results are robust for the assumptions that we make about how relatedness and the various social traits coevolve. We assumed that relatedness does not evolve and causally affects each of the social traits. Although the causal effect of relatedness on the evolution of social traits is strongly supported [22], the variation in relatedness among cells may change over evolutionary time. We therefore reanalyzed our data, allowing both relatedness and the social traits to evolve across the tree, while relaxing our assumption of causality [23]. These analyses gave qualitatively identical results, with a significant statistical correlation between relatedness and obligate versus facultative multicellularity ($p < 0.0001$), number of cell types ($p = 0.003$), and probability of sterile soma ($p = 0.009$), but not number of cells ($p = 0.08$) or the proportion of sterile cells in the species that had sterile cells ($p = 0.34$; Table S3).

In almost all of the species with clonal group formation, a unicellular stage is involved, wherein the group (or organism) arises from a single cell, usually a zygote or spore [10, 11]. The exception to this is in species with multicellular propagules, wherein the propagules are formed by cells remaining with their parents, as in some cyanobacteria [24]. Another consequence of a unicellular stage is that it leads to a shared developmental history that could facilitate the scope for coordinated development among cells, for example into different cell types [11]. This should not be seen, however, as a competing explanation to relatedness, as it is a mechanistic (proximate) and not a selective (ultimate) issue [25, 26]. For example, although the potential for coordination could place a limit on the number of cell types (the strategy set open to natural selection), we still need an explanation for why multiple cell types are favored (which strategy is favored by natural selection). Nonetheless, interactions can be important, as natural selection can only act on the options that are developmentally and mechanistically possible.

Relatedness and the Major Transitions

More generally, when combined with data from previous studies, our results show how relatedness provides a single life-history variable that plays a key role in explaining evolutionary transitions that involve members of the same species joining together to cooperate [3, 4, 7–9, 27]. We have shown how a higher relatedness between cells leads to a higher likelihood of obligate multicellular groups, a higher number of cell types, and a higher likelihood of sterile cells (Figures 2, 3, and S3). A previous experimental study has shown that *Dictyostelium discoideum* loses cooperative fruiting-body formation if kept under conditions of low relatedness [12]. Previous comparative studies have shown how a higher within-group relatedness is correlated with cooperative breeding in both birds and mammals [28, 29] and with the evolution of eusociality in animals [8, 9, 30].

In all cases, relatedness is determined by how groups are formed. A higher relatedness arises from (1) offspring staying with their parents (termed "subsociality") rather than individuals of the same generation aggregating together (termed "parasociality" or "semisociality") and (2) either asexuality or lower levels of promiscuity. Taken together, these results support a fundamental role of how groups form, via the influence of group formation on relatedness, for the evolution of cooperative breeding, eusociality, and now multicellularity [7–9, 28–30]

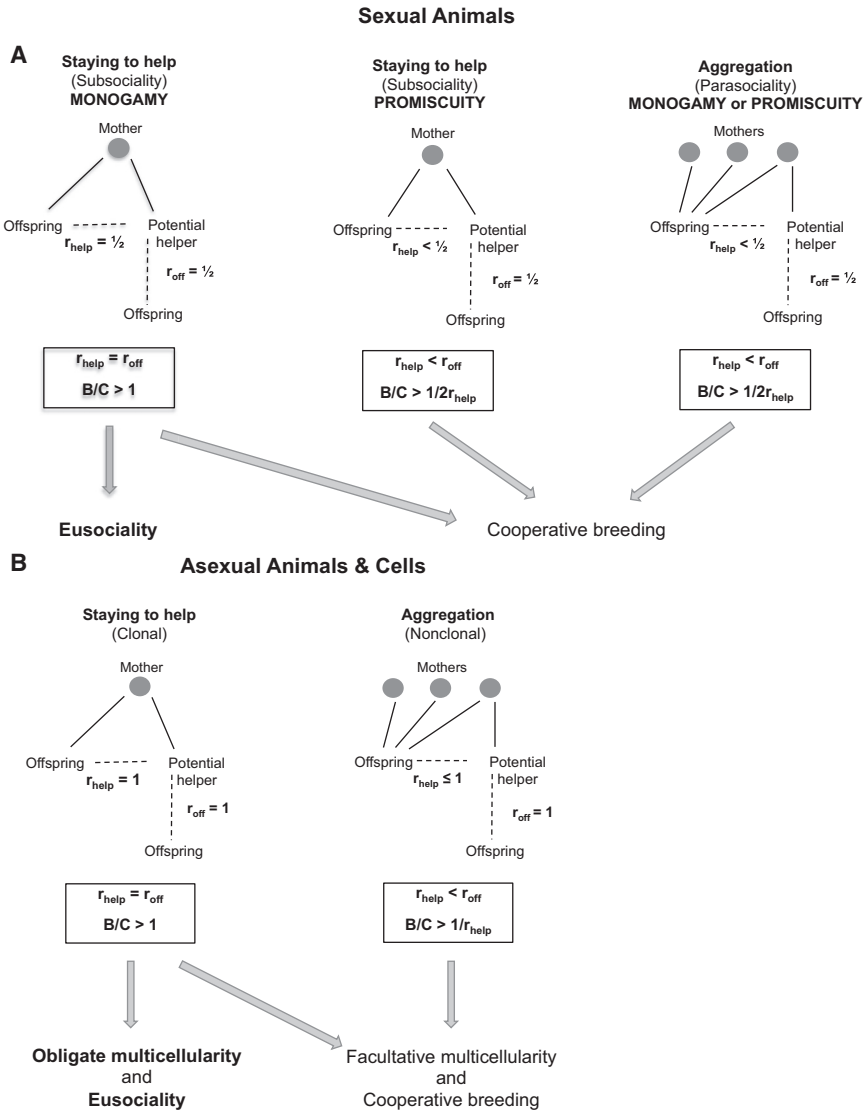

Figure 4. Relatedness, Group Formation, and Evolutionary Transitions

(A) In sexual animals with strict lifetime monogamy, when groups form by offspring staying to help their mother (subsociality), potential helpers are equally related to their own offspring (r_{off} = 1/2) and to their mother's offspring (r_{help} = 1/2), who they could help raise. This means that there is no conflict over which offspring to raise ($r_{off} = r_{help}$), which facilitates the evolution of reproductive dependency between group members and means that any small efficiency benefit of helping will favor altruistic cooperation [8, 13] ($B/C > 1$, where B and C are the benefit and cost terms in Hamilton's rule [17]). In contrast, if groups form by individuals from the same generation aggregating together (parasociality), such as sisters, or if females mate multiply during their lifetime (promiscuity), then potential helpers are more related to their own offspring (r_{off} = 1/2) than to the offspring who they could help raise (r_{help} < 1/2). In this case, it is much harder to evolve complete reproductive dependency between group members, and a larger efficiency benefit of helping is required to favor cooperation.

(B) The same general predictions occur when considering group formation by cells or asexual animals, such as aphids. Specifically: when groups form by offspring remaining with their parents, this leads to potential helpers being equally related to their own offspring and to the offspring that they could help raise ($r_{help} = r_{off}$), as they are all clonal (r = 1); when groups form by individuals aggregating together, this leads to potential helpers being more related to their own offspring than to the offspring who they could help raise ($r_{help} < r_{off}$).

(Figures 2 and 3). Experimental evolution studies suggest that the method of group formation is similarly important for a range of cooperative behaviors in microorganisms [31–33].

Furthermore, our data also support Boomsma's hypothesis [7–9] that although a higher relatedness favors greater levels of cooperation, there is something special about clonality in asexual species or strict lifetime monogamy in sexual species. At these extreme points, the offspring that can potentially stay and help their parents are equally related to the offspring they can help raise (r_{help}) and the offspring they could produce if they breed independently (r_{off}), such that $r_{help} = r_{off}$ (Figure 4). Consequently, as long as there is some ecological benefit to cooperation, this removes any conflict over whether to help or breed [13], and thus facilitates the evolution of complete reproductive dependency between group members. The major evolutionary transitions to obligate multicellularity and obligate eusocial societies have only occurred when $r_{help} = r_{off}$ holds, due to group formation by offspring staying with parents combined with either strict lifetime monogamy or asexual reproduction [7–9, 11, 30] (Figure 3A). The distinction between facultative and obligate multicellularity is of equal importance, and it is directly analogous to that between facultative and obligate eusociality [7, 9, 34].

In contrast, when there is not strict lifetime monogamy in sexual species, or groups form by individuals of the same generation aggregating together, this leads to $r_{help} < r_{off}$, and only transitions to facultative multicellularity or cooperative breeding have occurred [7–9, 11, 28, 29] (Figure 3A). Examples of this include the multiclone fruiting bodies of *Dictyostelid* slime molds, or the slightly promiscuous cooperative-breeding vertebrates. This suggests that $r_{help} = r_{off}$ can be a necessary precursor for "fraternal" major transitions that involve cooperation between members of the same species. If $r_{help} < r_{off}$, then at some point in their life, potential helpers may do better by breeding independently; therefore, they are selected to retain the flexibility to do so [7–9]. Major transitions require, by definition, a complete reproductive dependency between group members [2, 4], and thus the retention of this flexibility can stall a potential major transition (Figure 4).

Experimental Procedures

We searched the literature for information on the evolution and development of multicellularity in as many different taxa as possible and found data for 17 out of the 25 independent evolutionary transitions to multicellularity

(Supplemental Information). Several groups (e.g., plants) include both complex and simple multicellular species. When analyzing the evolutionary transitions (Figure 3) we are interested in the highest level of complexity obtained; therefore, we classified each group according to the more complex species in the group. A single-cell (unitary) stage leads to clonality [10, 11], but clonality can also occur in species with multicellular propagules when the propagules are formed by cells remaining with their parents, as in some cyanobacteria [24]. This matters because, from an evolutionary theory perspective, the key distinction is whether groups are clonal ($r = 1$) or not ($r < 1$), and not just whether there is a single-cell (unitary) stage (although they will be highly correlated [10, 11]).

We examined whether the evolution of multicellularity was influenced by relatedness by testing whether there were differences between clonal and nonclonal taxa for six different social traits: (1) obligate versus facultative multicellularity (binary distribution); (2) total number of cells (Gaussian after log transformation); (3) the number of cell types after controlling for total number of cells (Poisson distribution); (4) presence of sterile cells (binary distribution); (5) percentage of cells that are sterile in taxa with sterile soma (binomial distribution); and (6) complex versus simple multicellularity (binary distribution). We carried out our analyses using Bayesian phylogenetic mixed models (BPMM) with MCMC estimation implemented in MCMCglmm and checked the robustness of our results to assumptions of causality and tree-branch lengths (Supplemental Information).

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, three figures, and seven tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.05.004.

Licensing Information

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