One cell, two cell, red cell, blue cell: the persistence of a unicellular stage in multicellular life histories

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As developmental biologists come closer to understanding at the molecular and genetic levels how a zygote becomes an adult, it is easy to forget that the very phenomenon that gives them an occupation remains a vexing problem to evolutionary biologists: why do unicellular stages persist in life histories of multicellular organisms? There are two explanatory hypotheses. One is that a unicellular stage purges multicellular organisms of deleterious mutations by exposing offspring that are each uniformly of one genotype to selection. Another is that a one-cell stage reduces conflicts of interest among genetically different replicators within an organism.

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Although many multicellular organisms multiply asexually via many-celled 'vegetative' propagules[1,2], a strikingly consistent feature of multicellular life cycles is the intercalation of a unicellular phase, often (but not always) a sexually produced zygote[2–5]. A one-cell stage introduces a size-related ecological vulnerability to predators, competitors, and abiotic factors, which may be countered by prezygotic parental investment and postzygotic parental care. In developmental terms, a superficially undifferentiated unicell must give rise, through the complexities and risks of embryogenesis, to a differentiated, integrated clone of cells – a multicellular organism[3]. Once built by ontogeny, why do so many multicellular organisms, generation after generation, decompose to a single cell?

Hypotheses for the persistence of a unicellular stage concern the distribution of genetic variation among cell lineages and fall into two categories: elimination of deleterious mutations and reduction of conflicts between genetically different replicators within organisms. These hypotheses overlap in that one category of deleterious mutation produces parasitic cell lineages[6], and both hypotheses yield similar predictions.

The first hypothesis proposes that generation of each offspring from one cell (be it a gamete or ameiotically generated propagule) reduces genetic variation among cells within offspring and distributes the variation among progeny. Some of these offspring will carry a large burden of deleterious mutants, whereas others will carry relatively few. This opposes the ratchet-like accumulation of deleterious genes by maximally exposing harmful mutations to selection[2,4–8]. It has been explicitly stated several times without cross citation[4,5,7,8] and is also implicit in several discussions of the evolutionary role of somatic mutations[9–11] and selection among siblings[12,13].

The second hypothesis concerns the control of parasitic replicators by their multicellular hosts. The parasitic replicators could include cancerous mutant cells, pathogens, and cells acquired in contacts between conspecific multicellular individuals. A unicellular propagule is in the interest of the parasitized host when it purges some offspring of the parasitic lineage. On the other hand, a multicellular propagule that carries non-parasitic host cells aids the transmission of parasitic cells. Thus a gene in the parasite that favored the production of multicellular propagules by the host would benefit the parasite lineage, whereas a gene of the host that increased unicellular propagation would benefit the host lineage. This hypothesis pertains to the evolutionary transition from individual cells to multicellular individuals as units of evolution[5–7,14] and to the continuing control of conflict arising from genetic heterogeneity among cells in a multicellular organism.

Purging deleterious mutations

Mutations within a multicellular organism can accumulate and proliferate, producing a chimaera with related cell lineages that differ genotypically and perhaps phenotypically[7,9,11,15]. The chance that a sexual or asexual propagule will carry the descendants of one or more mutant lineages depends upon the rate of proliferation and motility of these mutant lineages; whether they are located in somatic, germinal, or multipotent tissues; mutation rate; an organism's lifespan; and the number of cells that constitute the propagule.

The effects of a unicellular bottleneck are approximated when cells in a propagule are recently descended from the same ancestral cell. Kondrashov[2] modelled the effects of the number and relatedness of cells in a vegetative propagule on mutation load at equilibrium between mutation and selection against deleterious mutants. At one extreme, the mutation load does not increase with number of cells initiating an offspring if all cells are recent descendants of one cell. At the other extreme, with distantly related initiating cells forming propagules, the mutation load approaches one as the number of initiating cells increases.

The pattern of divisions and movements of cells in lineages that give rise to a multicellular propagule together determine the structure of the cellular population, hence the relatedness of cells that constitute that propagule. Mobility of cells within the organism distinguishes animals from most other major groups of multicellular organisms[16]. If mobile cells from an animal's body are recruited to stolons, buds, or fragments, mutations that have accumulated over repeated mitotic cycles of propagation are likely to be included in the cells of the propagule. Even in an immobile syncytium, analogous processes could occur among nuclei. In contrast, propagules formed from immobile uninucleate cells recently descended from one unicellular meristem are more likely to be genetically uniform.

The mutation load carried by a multicellular propagule also depends on the way selection operates. Mutation loads should be low in organisms in which cell lineages carrying deleterious or beneficial mutants are exposed to selection before being incorporated into a propagule[4,17,18]. Alternatively, when two or more cell lineages interact synergistically to enhance fitness, or when the phenotype of one cell lineage masks the expression of deleterious mutants carried by other cell lineages, mutation load may be preserved. From this perspective, a one-celled stage will disrupt the transmission of rare synergistic combinations of cell lineages.

A unicellular stage will also promote the establishment of beneficial mutations

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in all cells of an offspring. However, the relative rarity of beneficial mutations suggests that selection to purge deleterious mutants is more important[4,8].

Conflict and units of evolution

Mutation, fusion between different genotypes, and infection by symbionts are the key processes that can introduce genetic heterogeneity into the population of cells that constitutes a multicellular organism. The presence of genetically distinct replicating units in a multicellular organism can lead to several different kinds of conflict among cell lineages. The conflicts may arise from competition among dividing cell lineages for opportunities to propagate or for access to the germ line[15,19]. Conflicts can also arise among the products of meiosis[20], among symbionts[21], and between hosts and endosymbionts[8,21,22]. Here, we focus on conflicts at the level of cell lineages, on how these conflicts can disrupt the stability of higher levels of biological organization, and on how a unicellular bottleneck can, at least partially, minimize these conflicts by periodically reducing genetic heterogeneity (and increasing kinship) among the cells that constitute multicellular organisms.

The unrestrained multiplication of a mutant or infected cell lineage that occurs at the expense of other lineages, as well as the whole organism, exemplifies one of the simplest and most disruptive conflicts among cell lineages[6]. A malignant cell lineage that mingled with other lineages could easily be transmitted to progeny in a multicellular propagule. Only the creation of unicellular propagules would fully expose transmitted malignant cell lineages to selection, and in each generation minimize a source of conflict that could impair function of a differentiated multicellular soma[23].

Cell lineages may also compete for access to the germ line, potentially at the expense of the soma[15,24]. In the majority of multicellular organisms, the descendants of most cells die with the organism (somatic cells). Depending upon when, or if, the germ line is sequestered, relatively few ancestral cells give rise to the germ cells that contribute to future generations[10]. To the extent that the cells composing the organism are genetically uniform, there will be no conflict of interest over access to the germ line. However, a mutant lineage could gain an immediate increase in fitness by shirking its somatic responsibilities in order to contribute cells to the germ line in disproportionately large numbers[24]. Routine production of multicellular propagules could favor the accumulation of mutant cell lineages that competed successfully for access to the germ line yet impaired function at the level of the whole organism. On the other hand, if these parasitic cell lineages by themselves produce inferior phenotypes, then reduction of the life cycle to a unicell should promote their elimination by presenting their phenotypes to selection at the organismal level.

Yet such somatic cell parasites appear to persist in at least some species[24–26]. For example, in the slime mold *Dictyostelium mucoroides*, unicellular amoebae in the soil periodically associate to form a fruiting body that disperses propagules. As in myxobacteria[27], aggregating amoebae can contribute to the non-reproductive stalk or become dispersed spores. Buss[24] isolated naturally occurring strains of *D. mucoroides* that were incapable of forming stalks (soma). On their own, such mutant strains may have lower fitness than strains that produce normal fruiting bodies, because the absence of a stalk reduces the probability of germination and dispersal of the spores[28]. If, however, amoebae of these stalkless mutants combine with amoebae of a stalked strain, a normal fruiting body forms, but the spore population contains a disproportionately large fraction of the mutant cell lineage[24].

Apparently, the parasitic strain persists, despite a unicellular stage in the life history, because the cells of different genotypes can associate to form chimaeric multicellular organisms. In the long run, selection for or against such cheaters should be frequency dependent: when in chimaeric combinations with non-cheaters, they could be favored; but when combined with other parasites, performance of the whole organism should be reduced.

The fact that fusion chimaeras occur in many other taxa, notably those which grow indeterminately and do not sequester their germ lines early in ontogeny[24], suggests that there is widespread potential for somatic cell parasitism. However, in most cases, the evidence takes the form of the coexistence of two or more genotypes in a chimaeric soma following fusion, rather than direct information concerning the population dynamics of coexisting cell lineages. In any case, virtually all taxa that have mobile cells and are capable of somatic fusion also possess a highly specific allorecognition system, that can distinguish self and close kin from more distantly related conspecifics[15,29]. Thus, a unicellular stage in the life history and rejection of cell lineages whose genotype indicates distant relationship[15,30] are two means of reducing the risks of parasitism by selfish lineages in the many plants and clonal animals that do not sequester a germ line. With such defenses, fusion to form chimaeras could even confer ecological advantages[24,30].

Biparental inheritance of organelles, mutations within a population of endosymbionts inhabiting a host's cells, and multiple infection by pathogens can also lead to heterogeneity and conflict at the level of the cell lineage. Recent papers have focused on the importance of uniparental transmission of cytoplasmic replicators for control of competition among endosymbiotic replicators[21,31], and between cytoplasmic replicators and nuclear genes[22,32]. However, the problem of endosymbiont-mediated conflict among cell lineages that carry them is more complex still, because endosymbionts can be vertically transmitted by even unicellular propagules, as well as horizontally transmitted among the cells of an organism and between different organisms[21].

It is in an endosymbiont's interest to maximize its probabilities of transmission, both vertically and horizontally. For beneficial endosymbionts, the host's interest is also served by increasing the likelihood that the endosymbiont will be initially transmitted to its offspring. For pathogens and parasites, the opposite is true for the host, but not for the endosymbiont. Selection on virulence strongly depends on the mode of transmission and relatedness of pathogens infecting a particular host[21]. Notably, a one-celled bottleneck in the host's life cycle, along with uniparental transmission of endosymbionts, should increase the relatedness of endosymbionts inhabiting the descendants of the founder cell, and thereby select against highly virulent pathogens[22,33].

There are, however, at least two major problems with this scenario. First, in terms of vertical transmission of cytoplasmic replicators, the unicellular bottleneck is far from perfect. Unless every daughter cell receives exactly the same set of cytoplasmic replicators during each mitotic cycle, genetic heterogeneity of the cytoplasmic elements or pathogens within a unicellular propagule can lead to persistent differences among the descendants from that propagule[34]. Second, the reduction of the life cycle to a unicellular stage can do little to control either the horizontal transmission of pathogens and parasites between cells of the organism or the incidence of multiple infections (which increases selection for virulence). Moreover, high relatedness among endosymbionts inhabiting a host promotes horizontal transfer, because selection should favor dispersal to a new host instead of competition within a host. If multiple infections are possible, they will increase the genetic diversity of an endosymbiont population infecting a host and once again favor increased virulence[21].

In sum, a unicellular bottleneck initially makes the interest of all cells in a multicellular organism congruent[5,21], and stabilizes the organism as an individual evolutionary unit, even in the face of mutation and somatic fusion[10]. Thus, a unicellular stage increases the 'effectiveness

of selection at the higher (organism) level relative to the lower (cell)'[5]. Whether a unicellular bottleneck is sufficient to protect the state of multicellular differentiation against invasion by non-cooperative cell lineages will depend on the frequency and types of variation that arise among cell lineages. If the frequency of mutations that increase the replication rate of the mutant cells while decreasing the performance of the multicellular individual and yet remaining transmissible across generations of multicellular individuals is sufficiently high, then modifiers for germ line sequestration or policing could represent additional necessary controls on conflict[6]. There is as yet little direct evidence that such mutations occur at the necessary frequencies to render a unicellular bottleneck ineffective. Moreover, the existence of many multicellular organisms that do not sequester a germ line suggests that a unicellular bottleneck is sufficient.

A unicellular bottleneck will generally be less efficient at controlling the virulence of endosymbionts and conflicts among them because of the potential for horizontal transmission (which usually reduces kinship among symbionts within a host and – all else being equal – favors increased virulence). Also, though the propagule itself is unicellular, the population of replicating symbionts is not reduced to a single replicating unit.

Meiosis and syngamy

Segregation, independent assortment, and recombination (along with mutation) virtually guarantee that no two haploid gametes will be genetically identical, raising a variety of prospects for conflict, that depend upon how syngamy occurs. Gametes could re-associate to form unicellular or multicellular combinations in several ways. These scenarios minimally include (1) aggregation of gametes without fusion to form one large propagule consisting of multiple haploid cells; (2) fusion of gametes without nuclear fusion yielding one large cell, containing multiple haploid nuclei (and their chromosomes) and perhaps cytoplasmic replicators; and (3) fusion of gametes followed by nuclear fusion.

In the first case, a chimaera composed of multiple cell lineages would result, generating the potential for the propagation of deleterious mutations and cell-lineage conflict.

The second scenario could also increase mutational load, following the model described above for vegetative propagation[2]. Furthermore – depending on (1) mode of inheritance of cytoplasmic replicators, (2) number of different mating types, (3) rules for fusion of different mating types, and (4) synchrony of multiplying nuclei and other replicators – the second scenario could lead to extensive cytoplasmic and nuclear genetic conflict[22,35,36]. For example, in the slime mold *Physarum polycephalum*, the plasmodium is a syncytium of millions of nuclei[25]. Syngeneic strains of plasmodia normally fuse; allogeneic strains normally do not fuse. However, when allogeneic strains do fuse to form a heterokaryon, it is usually unstable, with the nuclei of one strain eventually being excluded. Cell lineage conflict could also arise when a large multinucleate cell divided, unless daughter cells received equal allocations of all nuclei[36]. To avoid the problems of conflict among cytoplasmic replicators, fusion of numerous cells might require that only one carried cytoplasmic elements[35].

There are several variations on the third scenario, with fusion of multiple nuclei. However, when karyogamy involves the fusion of only two haploid nuclei to form a unicellular zygote, it amounts to conventional eukaryotic sex. Mitosis will then synchronize reproduction of the maternal and paternal elements of the nuclear genome. In the absence of meiotic drive, meiosis ensures that maternal and paternal homologues have an equal chance of finding their way into a gamete, thereby controlling conflict between the chromosomal lineages that constitute the nuclear genome[4,20,37].

We can imagine other obstacles, some ecological, others developmental, to sexual production of a multicellular propagule consisting of diploid cells. For example, encounters by large numbers of gametes could require elaborate mechanisms to avoid waste of gametes, particularly for anisogamous organisms. If some gametes in a group did not find partners, a partially haploid chimaera would be formed, or cell deaths would be necessary for production of an entirely diploid offspring. There could also be problems of matching the state of differentiation within groups. In sum, despite the potential size-related advantages to producing a multicellular propagule, the ecological, developmental, and genetic obstacles to the evolution of sexually produced multicellular propagules appear to be substantial.

Ancestry and sex

Sexual reproduction involving the production of eggs capable of embryonic development appears to be plesiomorphic for all of the major clades of multicellular eukaryotes[38]. This pattern of embryonic development remains the sole method of both sexual and even asexual reproduction in many clades. To the extent that the evolutionary persistence of eukaryotic sex depends on the products of meiosis and syngamy being unicellular, the production of unicellular ameiotic eggs (and similar stages) may reflect ancestry, rather than current selective value. Therefore, even if vegetative reproduction via multicellular propagules were selectively favored, sexual ancestry could be too recent for the evolution of an alternative to embryonic development in clades that reproduce only via ova.

Not all asexual lineages are, however, recently derived from sexual ones and not all employ unicellular propagules derived from eggs[39,40]. Moreover, asexual production of embryos or larvae, presumably through a unicellular bottleneck, may occur in colonial animals that have alternative methods of vegetative propagation through budding, fission, or fragmentation[1,41]. This coexistence of unicellular and multicellular modes of asexual propagation implies that the persistence of a unicellular stage cannot be explained solely by the constraints imposed by sexual reproduction and the recency of sexual ancestry.

Mitigating limitations of a one-cell stage

Although there appear to be substantial benefits to passage through a unicellular bottleneck, its intercalation into a multicellular life cycle entails potentially significant costs. Even though small size may be associated with better performance of functions such as dispersal or diapause, or be favored by tradeoffs between size and number of offspring, offspring released as a single cell lack capabilities that several kinds of specialized cells could provide. Small size and dispersal can be combined with the functional advantages of multicellularity; for example, some species release multicellular dispersing larvae that are smaller than the zygotes of other species. Widespread features of multicellular life cycles, such as parental provisioning and protection of propagules, may compensate for the vulnerability of a unicellular bottleneck[42]. However, some forms of parental care bring genotypically distinct cell lineages into close proximity. In so doing, parental care can open up new arenas for conflict.

Internal brooding of sexually produced offspring has evolved numerous times in both animals and plants. It often involves transfer of resources to offspring from parental cells (e.g. pregnancy in placental mammals[43]) or via products of meiosis (e.g. gametophyte tissues in angiosperms[44]). This form of parental care combines two or more cell lineages, one from the brooding parent and others from brooded offspring. Internal brooding therefore increases the potential for direct conflict between parents and offspring[43], between siblings[45], and among the haploid (the gametophyte), diploid (parental sporophyte and embryos), and triploid (endosperm) tissues of

an angiosperm's developing seed[44]. When the parental tissues are themselves chimeric, the potential for parent–offspring conflict is greater still for sexually produced offspring, and extends even to amictic progeny.

Parents can also protect vulnerable unicellular offspring by enclosing groups in structures such as egg capsules, maternal body cavities, seed pods, fruits, prey, and similar structures. The many instances of sibling cannibalism in such structures[46–48] indicate the potential for sibling conflict in this mode of protection. A parent can reduce such conflicts by blocking development of some products of meiosis or syngamy, and in the process convert some of the potential competitors into resources[42]. Alternatively, polyembryony (asexual multiplication of a sexually produced zygote), which occurs primarily in taxa that brood offspring (cyclostome bryozoans) or enclose them within the confines of a host (e.g. some parasitoid wasps and endoparasitic hydrozoans), can also reduce sibling conflict[49]. However, polyembryony occurs in organisms such as red algae, where sibling zygotes are not in close proximity, suggesting that reduction of sibling conflict is not its only cause. Curiously, in hymenopterans polyembryony may retain association of products of meiosis via a nutritive trophamnion derived from the meiotically produced polar bodies[50].

Even when propagules are individually released as gametes or zygotes, they may be provisioned with maternal cells and cell products (including yolk and genetic information for further syntheses, such as messenger RNA). The simplest interpretation of this parental provisioning is that it relieves the developing offspring of some synthetic burdens. However, in the process, offspring may relinquish some control of their phenotype. For example, in some species of ascidians, diploid maternal follicle cells enclosing a developing embryo provide buoyancy; in others they provide adhesion[51]. In these roles, maternal follicle cells can affect offspring dispersal and thereby could influence the expected conflict over dispersal distance between parents and their sexually produced offspring[52]. Offspring may also be provisioned with protective parental cells, such as cnidocytes from a medusa[53]. Transdifferentiation of cells is known from medusae[54,55]. If these vertically transmitted cnidocytes are capable of de-differentiating into multipotent cells, then parental cell lineages could compete for access to their offspring's germ line.

These conflicts are unavoidably associated with many kinds of parental care. However, unlike conflicts between distantly related lineages that extend across generations, these conflicts involve close kin and probably do not extend beyond a fraction of one life cycle, limiting their cost. In contrast, when parents enclose numerous apparently beneficial replicators (such as mitochondria, chloroplasts, or cellular endosymbionts) in a propagule, the descendants may remain with the offspring throughout its lifetime. Conflicts between lineages of such endosymbionts should be reduced by vertical transmission to offspring through a unicellular bottleneck. However, unless these replicators themselves have recently passed through a bottleneck, a unicellular bottleneck cannot completely purge deleterious mutant lineages or eliminate the potential for conflict between these replicators[2]. Selection can favor cooperative policing to limit cheating among replicating units[56], but the genetic circumstances promoting such policing may be uncommon[57].

Obligate vegetative propagation

In the clam *Lasaea*[58] and bdelloid rotifers[40] it appears that asexual lineages propagating from unicells persist for millions of years; however, there are relatively few examples of obligate propagation via multicellular propagules. Asexual lineages propagated from groups of cells may persist for millions of years, as inferred for the asexual fungal symbionts of attine ants[39], but it is unclear if these clonal lineages are occasionally purged by passing through unicellular bottlenecks[59]. Some lichens, perhaps to maintain their obligate symbiosis, produce multicellular propagules that include both fungal and algal cells[2]. If these lichens (and other similarly constrained organisms) lack a unicellular bottleneck in both fungal and algal cell lineages, they also must suffer a diminished ability both to purge mutations[2] and to reduce the potential for conflict among genetically distinct cell lineages.

Conclusions

Unicellular stages affect diverse features of life histories, creating the subject of embryology and most of the subjects of life-history ecology. A unicellular bottleneck increases a propagule's vulnerability to biotic and physical hazards, both as a consequence of small size per se and the extended period of development necessary to rebuild a complex multicellular organism. Passage through a unicellular stage also limits, at least during the initial phases of development, advantages inherent in the possession of specialized cell types. Parental care reduces the vulnerability of unicellular stages but introduces new conflicts among genetically distinct replicators.

The unicellular phase in the life cycles of multicellular organisms appears to reflect the overwhelming advantages of such a bottleneck in reducing mutational load and limiting the potential for conflict among lineages of replicators, particularly with sexual reproduction. Deciding which hypotheses best account for the origin and persistence of a unicellular stage depends on identifying the relative importance of different sources of genetic heterogeneity. The sources may have changed historically and may differ among species. Deleterious mutants and pathogens are ubiquitous and undoubtedly represent important selective agents favoring unicellular propagules. Sexual reproduction creates genetic differences among cells. Mixing of cell lineages may occur by fusion or coaggregation of some multicellular organisms and represents an avenue for horizontal transmission of parasitic cell lineages. Fusion chimaeras are clearly a special case, but for some species they may represent a significant source of conflict among cell lineages within a multicellular organism. Of deleterious mutations, those impairing function of both the mutant cells and the entire multicellular individual (-/- mutants) can be contrasted with mutants that increase replication of the mutant cell lineage while impairing function of the multicellular individual (+/- mutants). The potential conflicts introduced by +/- mutants have received much attention[6,15], but these may be rarer than -/- mutants and a less important source of selection for unicellular propagules. Pathogens present another kind of +/- relationship but with the potential for horizontal as well as vertical transmission. At present we lack data to compare the costs and benefits of purging pathogens to those of purging mutations.

Although there are many parallels between our analysis of conflicts between replicating cell lineages and discussions of conflicts among other kinds of independently replicating units[21,35], there is a key distinction between conflicts between cell lineages and conflicts between genomic elements. If cells have a legislature of lineages like the parliament alleged for genes[37], then a multicellular organism is a clonal congress. It is the unicellular bottleneck that maintains a voting block of genetically identical cells that is overwhelmingly large.

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