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Evolution

Somatic cell parasitism and the evolution of somatic tissue compatibility

(chimera/*Dictyostelium mucoroides*/frequency-dependent selection/fusion-rejection/self/not-self)

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ABSTRACT **Selection pressures proposed to account for the convergent evolution of self/not-self recognition systems in lower organisms include defense against microbial parasites and somatic cell variants. Direct support for the existence of somatic cell parasites in natural populations has been lacking. I here report the occurrence of a somatic cell parasite in the cellular slime mold *Dictyostelium mucoroides* and discuss the implications of this phenomenon to the evolution of mechanisms of somatic tissue compatibility.**

Somatic mutations may arise in any proliferating cell lineage. In many organisms, such variants may become incorporated into gametes or asexual propagules. An organism that cannot control the proliferation of somatic variants could actually be parasitized by a member of its own species. I here report the occurrence of one such somatic cell parasite in natural populations of the cellular slime mold *Dictyostelium mucoroides*, review the potential fitness costs and benefits of somatic variation, and argue that somatic cell parasitism may be an important selective force underlying the convergent evolution of self/not-self compatibility mechanisms in lower organisms.

METHODS

There are three possible fates of any somatic variant (1). The mutant may be incapable of increasing in frequency in the presence of the cell lineage from which it arose and hence will be eliminated. This is presumably the fate of most mutations. Alternatively, the mutant lineage may be capable of increasing when rare in the presence of the original lineage and eventually establishing an independent existence by incorporation into gametes or asexual propagules. However, if such a form is incapable of maintaining itself without the presence of the original form, the variant could still persist in natural populations by acting as a somatic cell parasite on competent forms (1). Demonstration that such a condition occurs requires (*a*) collection of chimeric individuals from natural populations and (*b*) demonstration that the mutant individual can increase when rare in the presence of the original cell line and persist within the soma of susceptible individuals for extended intervals. The following methods were developed to determine whether these conditions are realized in natural populations of cellular slime molds.

Field Sampling. The detection of individual fruiting bodies in natural populations of the cellular slime mold *D. mucoroides* has never been reported, to my knowledge. The small size of fruiting bodies, the free-living nature of the amoebae, and the cryptic habit of occupying the interstices of the soil render direct methods of field sampling ineffective (2). Hence, an indirect method was developed which exploits the fact that amoebae will travel a limited, species-specific distance to produce an aggregation (i.e., the aggregation territory) (3). If a sample obtained on this scale yields a large number of slime mold isolates, the conclusion is inescapable that the sampling includes either a large number of amoebae, an aggregation center, or a fruiting structure, or a combination of these.

Soil cores were taken by using 100-μl micropipettes (diameter, 1 mm) tied together in bundles of either 25 or 30 micropipettes. The sampling bundle was depressed by hand no more than 1 cm into the soil at each of 14 haphazardly selected locations, for a total of 380 micropipette cores. Cores were extruded, placed in individual test tubes, and suspended in 1 ml of sterile water within 2 hr of collection. After brief agitation on a vortex mixer, 0.2 ml of this soil suspension was added to 100 × 15 mm Petri dishes containing 0.1% lactose-peptone agar. Each Petri dish was inoculated with 0.4 ml of *Escherichia coli* (strain b/r) as a food source and the *E. coli*/soil suspension was spread evenly over the entire plate. Two replicate plates were made for each soil sample. The plates were maintained for 4 days and then observed under a dissecting microscope at ×25 for slime mold growth. Observations were continued at ≈8-hr intervals for an additional 2 days, and all fruiting bodies of *D. mucoroides* appearing were isolated and individually subcultured for further observations.

Cell Lineage Competition. To determine whether variants obtained from chimeric fruiting bodies are capable of increasing in frequency and persisting in the presence of normal genotypes, a series of competition experiments was initiated. Spores from pure cultures of two strains found to be chimeric in nature were suspended in 1.0 ml of sterile water and counted in a Levy chamber. These single-strain suspensions were used to produce mixed-strain suspensions in which the initial relative frequencies of the two forms were systematically varied over 4 orders of magnitude (10^{-3} to 10^{1}). These suspensions were plated at high density (10^4 spores per plate) on 100 × 15 mm Petri dishes, along with 0.5 ml of *E. coli*, and allowed to fruit. This plating density was sufficiently high that amoebae belonging to each strain coaggregated to produce chimeric fruiting bodies. The resulting sorocarps were collected with a wire loop, suspended in sterile water, and divided into two equal-sized fractions. The first fraction was diluted to the appropriate frequency and plated on L/P agar to produce a minimum of 200 single-spore plates. The relative frequency of each strain was determined from these single-spore isolates. By using the spores from the second fraction, a second generation was initiated. This process was repeated for 10 generations, with enumerations for each of the first 5 generations and for the 10th generation. An increase in the frequency of one strain relative to another in these experiments could arise either because one strain placed a lower proportion of its cells in the reproductive cell lineage relative to the supportive cell lineage or because one strain had a greater

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rate of increase during the vegetative phase (i.e., germination and growth), or both.

To distinguish between these possibilities, a second series of experiments was performed. Slime molds grown in liquid culture delay aggregation and do not irreversibly differentiate into prespore and prestalk fractions (4). If removed from liquid culture and plated in the absence of a food source, their vegetative growth ceases and aggregation begins soon thereafter. Thus, it is possible to mix isolated strains and study their interactions in the differentiation phase alone. To exploit these facts, pure isolates of each strain were grown in liquid culture (5) for 3 days and the cells were counted. Cells from each liquid culture were concentrated by centrifugation (5), mixed at a wild-type/variant ratio of 100:1, and plated at a density of 2×10^6 cells per plate on L/P agar in the absence of a food source. The resulting sorocarps were collected, suspended in sterile water, diluted appropriately, and plated. The resulting single-spore isolates were then used (*a*) to enumerate the relative frequency of each strain, (*b*) to initiate new single-strain liquid cultures, and (*c*) to initiate a control experiment identical in protocol to the experiments described above. After these cultures had grown for 3 days, the amoebae in each were counted, mixed to produce a suspension with the same relative frequency as that obtained after the first generation, and plated on L/P agar in the absence of a food source. The process was continued for five generations.

Somatic Compatibility Assays. A variant form may spread from one slime mold to another via coaggregation of amoebae derived from different sources (1). To determine whether the chimeric strains isolated from nature would coaggregate with other strains isolated from the same location, a series of compatibility tests was initiated. High densities (10^5 spores) of each of 10 test strains were plated with high densities (10^5 spores) of standard strain (obtained from a naturally occurring chimeric sample) on L/P agar plates. For each test there were five replicates. The resulting fruiting bodies were observed for evidence of coaggregation. A minimum of three fruiting bodies were collected from each replicate, suspended separately in sterile water, diluted appropriately, and plated to produce a minimum of 100 single-spore isolates. Two strains were considered compatible if any of the three fruiting bodies sampled proved to be chimeric.

RESULTS AND DISCUSSION

Naturally Occurring Chimeras. Of the 14 bundles of soil cores, only 7 contained cellular slime molds. Of 380 micropipette samples, only 11 (2.9%) produced fruiting bodies. The horizontal distances separating each of the 11 isolates are summarized in Fig. 1. In only one case was a large number of individual fruiting bodies isolated from a single micropipette sample; sorocarps blanketed the entire plate in both replicates. This sample must have included either a sorocarp or a large mass of vegetative amoebae.

Single-spore isolates from this dense sample revealed that the original isolate was chimeric. Two strikingly different forms were present. Each replicate yielded a form having normal morphology as well as an apparently aberrant form. The aberrant form (hereafter called "stalkless") produced a fruiting structure composed of a ball of spores lying on the substratum without a stalk.

The occurrence of this stalkless strain under natural conditions is surprising. The prey of dictyostelid slime molds lie adherent to soil particles, as do the amoebae that feed upon them. The cellular stalk serves to raise the spore mass from the surface of soil particles into the interstices that separate them. Here,

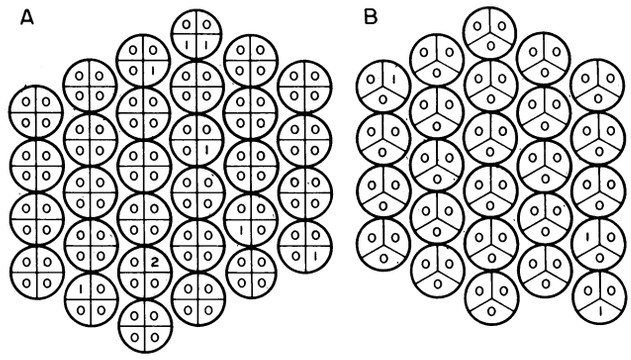

FIG. 1. Microdistribution of morphologically distinct isolates of *D. mucoroides* from soil cores. Each circle represents a single core 1 mm in diameter. Each quadrat within a circle represents the results of a replicate of a bundle sample. The cluster of cores in *A* represents four bundle samples of 30 cores per bundle; the cluster in *B* represents three samples of 25 cores per bundle.

the sticky spores adhere to the legs and carapaces of passing soil invertebrates (2). Any genotype that fails to produce an erect fruiting body is unlikely to disperse in this fashion, raising the question of how these forms are maintained in natural populations. The discovery of such a stalkless form may represent a mutant fortuitously sampled that would soon be selected against in nature. However, the rate of mutation to similar forms in the laboratory is on the order of 10^{-4} to 10^{-8} per generation (6) and the occurrence of 1 such form in 11 isolates is thus difficult to account for on the basis of mutation rate alone. The alternative suggestion, demonstrated by Filosa (1) for laboratory strains, is that these developmentally aberrant genotypes are maintained at some frequency within the sorocarps of normal stalked forms.

Cell Lineage Competition. If such an aberrant strain is acting as a somatic cell parasite it must be capable of (*a*) increasing when rare in the presence of a normal form and (*b*) stabilizing within the population of that form at some frequency. The results of the cell lineage competition experiments are frequency-dependent (Fig. 2). When the relative frequency of the stalkless genotype was above a stable equilibrium point of about 0.05, its frequency rapidly increased and the stalked form was eliminated. When the frequency of the stalkless form was below the stable equilibrium point, its relative frequency approached equilibrium within five generations. These results clearly illustrate that when the stalkless strain is at low frequency it is capable of increasing in frequency to some equilibrium value. The results of the liquid-culture experiments are presented in Fig. 3. The initial and final frequencies obtained in the vegetative phase plus differentiation treatment and the differentiation alone treatment were similar. Hence, the increase in frequency of the stalkless form in the original experiment was due primarily to the greater per capita contribution of the stalked form to production of supportive tissue.

The ability of the stalkless strain to increase when rare and become fixed in a population of the stalked strain is clear. In the presence of the stalkless strain, the reproductive output of the stalked strain was reduced by approximately 20% (Fig. 3). The stalkless strain benefits by its association with the stalked strain in that it acquires a fruiting structure to which it has contributed no supportive cells. By definition, the stalkless genotype is a parasite.

Somatic Cell Compatibility. The results of the somatic compatibility tests were dramatic. The single-spore isolates from high-density mixed cultures demonstrated that all nine strains collected in the original field sampling failed to form chimeras

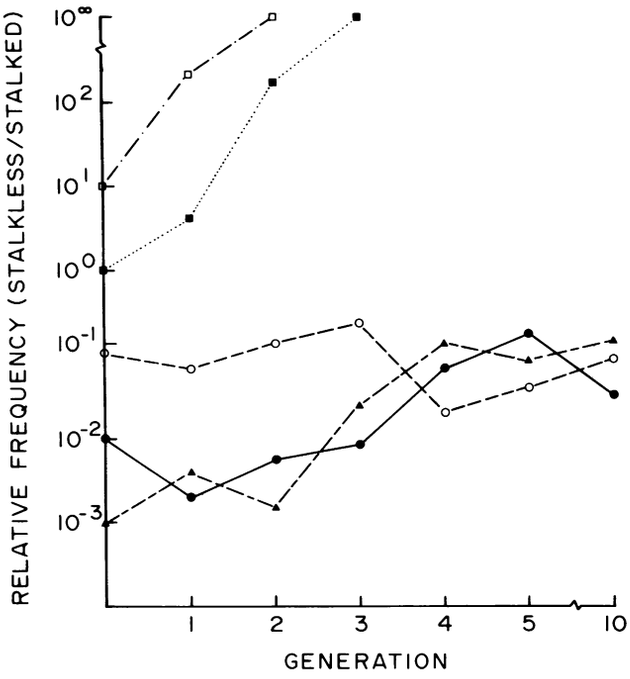

FIG. 2. Cell lineage competition. Each curve represents the relative frequency of the two strains over 10 generations of mixed culture for the fixed initial frequencies. Frequencies of 10^{∞} represent extinction of the stalked form.

with the parasitic form. The somatic cell compatibility systems were effective in defending these strains against invasion by the parasite.

GENERAL DISCUSSION

Although somatic variation has attracted considerable interest among agriculturalists, health scientists, developmental biologists, and geneticists, there is a general lack of discussion of the evolutionary significance of somatic variation. Somatic variation may arise either through mutation within established cell lineages or via fusion of genetically distinct individuals. Fusion is known to exist in protists, plants, and animals, distributed over at least nine phyla (Table 1). Somatic mutation undoubtedly occurs at some frequency in all multicellular organisms. Both

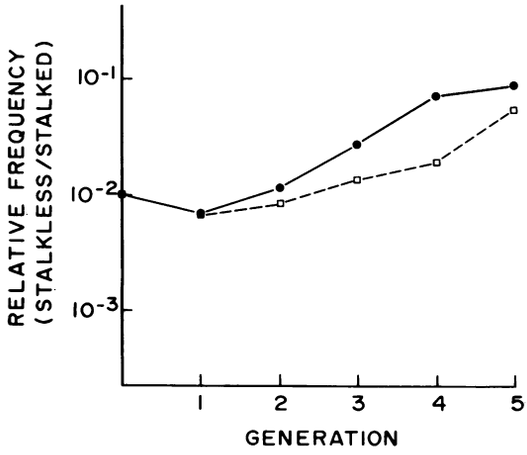

FIG. 3. Cell lineage competition. ○, Treatments (differentiation alone); ●, controls (vegetative phase and differentiation). The contribution of vegetative phase to the relative rate of increase of the stalkless strain can be estimated by the difference between these values at each generation.

Table 1. Phyletic survey of chimera formation and somatic tissue compatibility*

Group	Mechanism of chimera formation	Evidence of somatic tissue compatibility system†
Protists:		
Dictyostelids	Coaggregation, mutation	Failure to coaggregate, separation during migration or culmination
Myxomycetes	Plasmodium fusion	Failure of plasmoidia to fuse
Fungi:		
Phycomycetes	Mutation	Failure of hyphal fusion
Ascomycetes	Hyphal fusion	
Basidiomycetes	Hyphal fusion	
Plants:		
Rhodophyta	Sporeling coalescence	Root fusion
Gymnosperms		
Angiosperms		
Animals:		
Porifera	Larval fusion	Failure of fusion, strain-specific reaggregation
Coelenterates	Planulae fusion	Failure to fuse
Annelids		Graft rejection
Molluscs		
Echinoderms		
Arthropods		Graft rejection
Chordates		
Ascidians	Colony fusion	Failure to fuse
Vertebrates	Bovine twins, malignancy	Lymphocyte-mediated immune response

* Compiled from 188 citations; list available from author upon request.
† Somatic compatibility systems are defined to occur when evidence exists of a mechanism by which individuals selectively accept or reject extended cell–cell contact with genetically distinct individuals of the same species.

phenomena are probably as primitive as the origin of multicellularity itself. It would be surprising indeed if a host of adaptations have not evolved both to enhance the potential benefits of somatic variation and to reduce its costs.

It is appropriate to consider briefly the fitness costs and benefits to chimeric individuals relative to genetically homogeneous individuals. Four classes of benefits can be attributed to chimeric individuals.

Genetic variability. A chimeric individual has available to it a greater store of genetic variability with which to respond to the viscissitudes of environmental change (1, 7–13). Considerable data exist on the occurrence of "chimeric vigor" in fungi, demonstrating that intraspecific chimeras are capable of utilizing a wider array of environmental conditions than either of the components of the chimera is capable of utilizing in isolation (11–16). A greater store of genetic variability may be of particular importance in two cases: (*i*) in clonal organisms, allowing a prolongation of the asexual phase and protecting favorable genetic combinations from being lost in the sexual process, and (*ii*) in haploid organisms, allowing the masking of disadvantageous recessives. It is probably not coincidental that all of the lower organisms in which fusion events have been reported are clonal in at least part of their life cycle (Table 1) and that all reported instances of chimeric vigor occur in haploid organisms (11–16).

Developmental synergism. The task of producing supportive tissue in a chimeric individual might be shared cooperatively. A number of cases of developmental synergism have been reported in fungal (17, 18), cellular slime mold (19–23), and myxobacterial chimeras (24). In each case, two aberrant forms are

capable of producing normal structures when they occur in the chimeric state. Although laboratory evidence of developmental synergism is common, I know of no documented intraspecific synergisms in nature. This may reflect the rather severe constraints governing the evolution of cooperative acts among individuals (25–27).

Mate location. In sedentary forms, which are largely incapable of controlling the timing and location of contacts with potential mates, the occurrence of chimeras may serve as a mechanism to ensure that mate location will not be difficult should the occurrence of some environmental stress demand it. This suggestion, of course, is limited to organisms in which sexually compatible individuals are also compatible in somatic tissue fusion. Although this often is not the case, the likelihood of this benefit in some groups is clearly demonstrated by the fact that sexuality in many basidiomycete fungi occurs only after the formation of the chimera in the asexual stage (28, 29).

Size-specific ecological processes. Fusion results in a size increase. Size increase has two obvious advantages in terms of fitness. A number of mortality sources (e.g., competition and predation) behave in a size-specific manner, with larger individuals enjoying a higher survivorship than smaller individuals (e.g., refs. 30 and 31). Fecundity also increases with size in many organisms and may do so indeterminately in some clonal groups (32, 33). A particularly important fecundity effect is the potential influence of fusion on the minimum age that must be attained before reproduction can occur. Chimeras formed of two prereproductive individuals may produce an organism of sufficient size for reproduction. Early age of first reproduction, and perhaps the formation of chimeras, is strongly favored in fluctuating environments or expanding populations (34, 35). It is notable that several sessile organisms are known to fuse in the dispersive (and early metamorphic) stages (36–43).

The occurrence of heterocytosis is not always an advantage. A somatic cell lineage is provided with various energy resources that are allocated to continued proliferation of that lineage and to the performance of particular supportive functions. In a chimeric individual, any cell lineage that allocates a greater proportion of the available resources to proliferation relative to supportive functions will increase in relative frequency. Cell lineages within chimeric individuals may compete for limited resources. Such competition may be adaptive, as in the case of chimeric vigor in fungi in which the relative frequencies of various nuclei reflect the environment in which each strain is favored (11–16, 44–47). However, such competition can also be maladaptive. A number of cases are known in fungi (45, 48–51), ascidians (52–54), myxomycetes (55–57), and cellular slime molds (1) in which one cell lineage largely excludes another, leading either to an individual with a greatly reduced efficiency of, or even the entire absence of, a critical supportive function. Such is the case with many mammalian cancers (58).

Competition for limited energy resources is only one form of cell lineage competition. In any multicellular organism with some degree of cellular differentiation, there is some limit on the number of cells that may differentiate into germ cells. The occurrence of two genotypes within the same body raises the possibility of competition between lineages for positions in the germ line. Any genotype that is more effective in placing its cells into those lineages destined to become reproductive rather than supportive tissue will be at a distinct advantage. Several investigators, working on fungi (45, 48, 51), ascidians (54), myxomycetes (56, 57), and cellular slime molds (3), have noted that, after fusion of organisms of equivalent size and reproductive condition, one strain has successfully placed a disproportionate number of cells in reproductive cell lines. Particularly compelling evidence for the importance of competition in chimeras for positions in the germ line comes from morphological studies in the fungi. These organisms are often coenocytic—i.e., nuclear division is not associated with cell division—and hence nuclei share a common cytoplasm. In the basidiomycetes, the formation of a chimera is immediately followed by differentiation of elaborate clamp connections which serve to control the free access of nuclei to the site of proliferation of reproductive tissue (29). In the ascomycetes, septation between nuclei sharing the common cytoplasm occurs in the crozier, again at the site of proliferation of reproductive tissues (28). The lack of these structures in homokaryons is persuasive evidence that, at least in part, clamp connections and septa function to mediate potential competition for reproductive positions. The extreme form of competition for germ-line positions is reported here—the occurrence of a strictly parasitic form which forms no supportive tissues at all.

Cell-lineage competition is clearly a potentially severe cost to the chimeric state. The extent of such costs, however, is dependent on the degree of genetic relatedness of the components of the chimera. The possibility that kin selection has operated in chimeric systems is suggested by the observation that fusion-rejection loci are linked to fertility loci in many species of fungi (28, 59), myxomycetes (60), and ascidians (61). This linkage limits chimera formation to closely related individuals, hence reducing the impact of cell-lineage competition on the inclusive fitness of the individuals involved.

It is important, nevertheless, to realize that the costs associated with somatic variation are potentially substantial and not limited to the organism in which the variant arises. Somatic variants that compete effectively for germ-line positions may evolve the parasitic habit. Even variants that do not enter the germ line are potentially capable of increase. Somatic variants that are effective competitors for energy resources could act in much the same fashion as an infectious disease. If some mechanism does not control their spread, an individual with an open wound for example, could pass on such a somatic variant to any other individual with an open wound (62). Without some defense against this process, somatic variants could become an important form of infectious disease.

The control of the spread of somatic cell parasites or effective cell-lineage competitors requires a mechanism to prevent fusion and to survey the tissues of the individual for variants. Somatic tissue compatibility systems perform these functions in most organisms (62). From an evolutionary perspective, it is evident that cell lineages within a chimeric individual must compete for limited energy resources and for positions in the germ line. Any activity, such as somatic tissue compatibility, that prevents the invasion or proliferation of such variants serves as a mechanism mediating such competition.

Five lines of evidence suggest that somatic compatibility systems have evolved to combat competition between cell lineages: (*i*) somatic variants, arising via fusion or mutation, are probably as primitive as multicellularity itself; (*ii*) the resources in limited supply—energy sources or germ-line positions—must occur in all multicellular organisms; (*iii*) all protists and animals known to fuse also possess somatic compatibility systems (Table 1); (*iv*) chimeras formed between compatible individuals are often stable (1, 9, 11, 12, 15, 63, 64) whereas forced chimeras between incompatible individuals invariably lead to the exclusion of one form or the other (57, 65–72); and (*v*) the extreme form of cell-lineage competition, that of a somatic cell parasite capable of reproducing itself and spreading infectiously, has now been recorded from a natural population of a primitive multicellular organism and the somatic compatibility system of these organisms is effective in protecting potential hosts from infection.

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