Evolution and Diversity of Dictyostelid Social Amoebae

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Abstract

Dictyostelid Social Amoeba are a large and ancient group of soil microbes with an unusual multicellular stage in their life cycle. Taxonomically, they belong to the eukaryotic supergroup Amoebozoa, the sister group to Opisthokonta (animals + fungi). Roughly half of the ~150 known dictyostelid species were discovered in the last 5 years and probably many more remain to be found. The traditional classification system of Dictyostelia was completely over-turned by cladistic analyses and molecular phylogenies of the past 6 years. As a result, it now appears that, instead of 3 major divisions there are 8, none of which correspond to traditional higher-level taxa. In addition to the widely studied "Dictyostelium discoideum", there are now efforts to develop model organisms and complete genome sequences for each major group. Thus Dictyostelia is becoming an excellent model for both practical, medically related research and for studying basic principles in cell-cell communication and developmental evolution. In this review we summarize the latest information about their life cycle, taxonomy, evolutionary history, genome projects and practical importance.

Keywords: Dictyostelium, Evolution, Genomics, Taxonomy

Contents

1. An introduction to social amoebae

Dictyostelia or social amoebae are ubiquitous soil microbes and bacterial predators. They play an integral role in recycling of soil nutrients (Landolt et al. 1992; Stout 1973), which makes them potentially useful indicators of soil microbial activity. However, the dictyostelids are best known for their remarkable life cycle, which consists of an alternation of single and multicellular phases (Fig. 1). The trophic stage is strictly unicellular, consisting of independent myxoamebas (Depraitere and Darmon 1978; Kessin 2001; Raper and Smith 1939) that multiply by binary fission. However, unlike most other soil microbes, which usually encyst when food becomes scarce, dictyostelids have three different possible responses to limited resources. These are the formation of either microcysts, macrocysts or the well-studied multicellular fruiting body (Escalante and Vicente 2000; Strmecki et al. 2005).

The dictyostelids were at first of interest to only a handful of biologists, but they are now used experimentally in hundreds of laboratories worldwide. Their phylogenetic position was a long-standing controversy due to the fact that aspects of their life cycle share characteristics with fungi, plants or other protists. Most of their early study was carried out by mycologists, and, as a result, current dictyostelid nomenclature is based on the Code of Nomenclatural Botany and their systematics follows botanical rules. Nonetheless, molecular phylogeny now clearly places them within the eukaryotic supergroup Amoebozoa, which is the closest sister group to Opisthokonta (Fungi and Metazoa) with the possible exception of the Apusozoa (Cavalier-Smith and Chao 2010; Kim et al. 2006). Within Amoebozoa, which consists mostly of solitary naked amoebae (Cavalier-Smith et al. 2004, Pawlowski and Burki, 2009), the dictyostelids form a clade of "macromycetozoa" together with the Myxogastria or plasmodial slime molds (Fiore-Donno et al. 2009). However, it should be noted that aggregative development has evolved multiple times in eukaryotes, and social amoeba are found in at least four major eukaryotic groups scattered across the tree. These are the Discoba (*Acrasis*, Roger et al. 1996, Baldauf et al. 2000), Holomycota (*Fonticula alba*, Brown et al. 2009), and Ciliophora (*Sorogenia*, Dunthorn et al. 2008), as well as elsewhere in the Amoebozoa (Brown et al. 2011).

1.1 *A Brief history of their study*

The dictyostelids were first described by the mycologist Brefeld (1869) and the botanist van Tieghem (1880), who were searching natural substrates for new microorganisms. At first, they were considered to be a group of Fungi (Cappuccinelli and Ashworth eds. 1977) based on the superficial similarity of their sorocarps to fungal fruiting bodies. However, it was soon realized that the dictyostelids lack hyphae, at which point they were classified with acrasids, another group of amoeba with aggregative development (Olive 1975). We now know that dictyostelids differ from acrasids in many fundamental ways. These include the cytological nature of the amoeboid cells, the way dictyostelid amoeba align into streams during aggregation, and the dictyostelids' production of well differentiated stalk and spore cells that produce highly developed sorocarps with cellulosic stalk tubes. A large evolutionary distance between dictyostelids and acrasids has also been confirmed repeatedly by molecular phylogeny using a variety of genes (Baldauf et al. 2000; Roger et al. 1996), and the acrasids are now placed in an entirely different region of the eukaryote tree (Adl et al. 2005; Baldauf 2008; Simpson et al. 2006).

Brefeld was the first to describe a cellular slime mold of any type, *Dictyostelium mucoroides*, and he also discovered the first *Polysphondylium*, *P. violaceum*. It was also Brefeld who suggested the generic name *Dictyostelium*, which combines *Dictio-* (from gr. δίκτυον, net), the prefix used in botany to refer to something that forms net-like structures (Font Quer 2000) and *stelium* (tower) referring to the positioning of cells in a stalk. Brefeld thought that the myxoamoebae aggregated to form a true plasmodium, an enormous single cell with thousands of nuclei such as those found in the Myxogastria. It was Philippe van Tieghem (1880) who realized that dictyostelid myxoamoebae remain independent even after aggregation and denoted the aggregate as a pseudoplasmodium. Van Tieghem's published account (1880) of acrasid and dictyostelid slime molds, which together he called Acrasiées, provided the criteria for their eventual classification as distinct from the Myxogastria due to the lack of a true plasmodium. It was also Van Tieghem's experiments with dictyostelids that led him to anticipate the role they would play in the field of developmental biology.

In the beginning of the 20th century, L.S.Olive (1902) was the first author to write about Dictyostelia as a group, and his book on Mycetozoa is still highly informative and authoritative on this taxon as well as the related groups of Myxogastria and the protototstelids. J.T.Bonner wrote the first monograph of the group, “The Cellular Slime Molds" in 1959. Only 9 species were known at that time. He conducted numerous elegant experiments throughout his career into their biology, behavior and biochemistry, Arguably the most influential of these was the series of experiments that led to the identification of cyclic AMP as the signaling molecule (acrasin) of Dictyostelium discoideum. K.B. Raper has been one of the most influential people in studying the group as a whole. He began his work in the earliest 30´s and after more than 40 years of study wrote what is the most authoritative and detailed book on the taxon, “The Dictyostelids” which still serves as a major reference on ecological, behavioral and taxonomic aspects of 50 described species. Raper's work was continued in the 70´s by that of H. Hagiwara who in 1989 published the book: “The Taxonomic Study of Japanese Dictyostelid Cellular Slime Molds”. The greatest living authority still active in the field is J.C. Cavender. He started as a student of Raper, has described numerous species and is an expert on their culture, isolation, behavior, and ecology. Although retired, he is still actively studying and collecting the dictyostelids worldwide together with J. Landolt and E. Vadell.

1.2 *Life cycle:*

The trophic stage of dictyostelids consists of uninucleate amoebae (myxamoebae) with bulbous pseudopodia. Despite the apparent antiquity of the group, dictyostelid myxamoebae appear to be indistinguishable at the light or electron microscopic level. The cells lack polarity and can form pseudopodia from any edge and in response to a variety of stimuli. Lack of food seems to be the universal cue, at least in the lab, for microcyst formation (encystment of individual amoeba), macrocyst formation (sexual stage) and aggregation leading to multicellular development (fruiting bodies). The first is a common response in soil amoebae, and probably evolved earlier in the Amoebozoa (Schaap et al. 2006). Macrocysts have two functions, as a resistant stage and a sexual stage, and although macrocysts were probably present in the last common ancestor of Dictyostelia, there are many species for which this stage has yet to be observed. However, the greatest attention has focused on aggregative development (Fig. 1). This true multicellular stage consists of distinct cell types within a motile slug producing a fruiting body comprised of a cellulosic stalk supporting a bolus of spores. Thus, the dictyostelids have evolved, among other things, differentiated cell types and the ability to regulate their proportions and morphogenesis (Jang and Gomer, 2010). This process has been especially studied in considerable detail in the experimental model *Dictyostelium discoideum*, and the following is based largely on these studies.

In *D. discoideum*, the transition from growth to development is regulated by a complex interplay of extracellular factors that serve as autocrine sensors of food availability and cell density (reviewed in Gomer et al. 2011). A specific gene expression program is then triggered by starvation, which ensures the synthesis of the proteins necessary for the production and detection of an extracellular chemoattractant and relay of the signal (Clarke and Gomer 1995; Mahadeo and Parent 2006). Thus, the cells become sensitive to the chemoattractant that they secrete, which is cAMP in the case of *D. discoideum* or glorin, folate or often unknown molecules in other species (Burdine 1995; Burdine and Clarke 1995).

Once aggregation begins, the cells polarize, forming anterior pseudopods at the leading edge by regulating the local actin cytoskeleton. Simultaneously, myosin II, a component of the cytoskeleton, is assembled laterally and at the back of the cell to prevent formation of lateral pseudopods, which is essential to maintain cell polarity. This polarity is important for efficienct chemotactic directed movement. This movement follows a gradient of cAMP created by its being released in pulses, every six minutes under laboratory conditions (Goldbeter 2006). Responding cells move towards the gradient and in turn produce another cAMP pulse, thereby greatly amplifying the response. Each pulse is followed by a refractory period during which background cAMP is cleared by a phosphodiesterase (Meili and Firtel 2003; Ridley et al. 2003). Eventually cells organize themselves in streams (Coates and Harwood 2001; Kessin 2003) and, ultimately, the formation of an aggregation center results.

The signal transduction pathways underlying chemotaxis have shown a remarkable conservation between *Dictyostelium* and mammalian cells (Parent 2004), and have been wonderfully reviewed over the last years (King and Insall 2009; Swaney et al. 2010; Wang 2009;). Some of the mechanisms that allow the cell to sense the gradient of chemoattractant have been revealed such as the localized formation of signaling lipids at the leading edge of chemotactic cells where the pseudopods emerge (Chen et al. 2007; Comer and Parent 2002).

Soon after formation, the aggregated cells surround themselves with a complex extracellular matrix of protein, cellulose and polysaccharides (the slime sheath), that isolates the developing structure (Freeze and Loomis 1977). One slug or pseudoplasmodium (a true multicellular polarized unit) arises from each aggregation center in *D. discoideum*, although this differs among species. The slug migrates in response to temperature, relative humidity, solute concentration and light, moving as a unit by means of a coordinated helical motion of the individual cells inside the slime sheath (Clark and Steck 1979; Dormann and Weijer 2001). Movement is organized from the tip (Rubin and Robertson 1975; Weijer 2004), where cAMP production continues in a pulsatile fashion (Bretschneider et al. 1995). Waves of cell contraction and elongation appear to proceed from tip to rear at regular intervals.

The cells of the *D. discoideum* slug are partially differentiated (Bonner 1952), with the anterior approximately 20% being pre-stalk cells, destined to form the stalk, while the posterior ~80% pre-spore cells are destined to become spores. The exception is the rear guard cells, which form the basal disc upon which the stalk is supported (Raper 1940). Different cell types have been identified in the prestalk population showing that the slug structure is more complex than expected (Williams 2006). The morphogen DIF-1, an alkyl phenone secreted by prespore cells, has been identified as being essential for the regulation of prestalk cell differentiation (Thompson and Kay 2000a, 2000b).

Upon completion of migration, the slug develops a vertical orientation. The decision to culminate (begin sorocarp formation) depends on environmental cues such as light, humidity and ammonia, among others (Kirsten et al. 2005). For *D. discoideum*, this begins with the prestalk cells secreting a stalk tube that is brought to the agar surface by flattening of the slug. This results in the “mexican hat” stage (Raper and Fennell 1952), although in other species, the stalk is formed during migration. As the stalk tube forms, an inverted fountain movement occurs as prestalk cells migrate up and then into the tube (Dormann et al. 1996). Once inside the tube, these cells vacuolate, construct cellulose walls, and then die in a process that is reminiscent of autophagic cell death (Calvo-Garrido et al. 2010; Tresse et al. 2007; Uchikawa et al. 2011). At roughly the same time, the rear guard cells of the slug that form the basal disc also vacuolate and die. Meanwhile, the prespore cells move up the growing stalk where they eventually differentiate into spores encompassed by slime. In *D. discoideum,* construction of the sorocarp takes about eight hours, and the proportions of stalk to spore cells are precisely controlled (Bonner and Slifkin 1949). However, in other species, such as *D. minutum*, development is simpler and quite different from *D. discoideum* (Schaap et al. 1981). The fact that stalk cells are fated to die opens fascinating questions about the evolution of social behavior and altruism (Li and Purugganan 2011).

Once formed, Dictyostelium spores are covered by a protective barrier, the spore coat, which is assembled from secreted proteins and cellulose (West 2003). This structure enables survival of the spore for extended periods of time, allowing the amoeba to emerge when the environmental conditions are again appropriate. This is probably most importantly in terms of food availability and humidity (Cotter et al. 2000). Spore differentiation in Dictyostelium depends on the activation of an intracellular cAMP signaling pathway involving the protein kinase A (Thomason et al. 1999) and a late gene expression program dependent on the transcription factor SrfA (Escalante et al. 2004a,b). It should be noted that this developmental program is not a sexual cycle and thus, the amoebas of *Dictyostelium discoideum* remain haploid throughout their differentiation.

In addition to spores, dictyostelids can also form microcysts and macrocysts. Microcysts are formed from single dictyostelid amoebas which enter a resting stage for survival when conditions are suboptimal, as was first noted by Cienkowski in 1873 and then confirmed by many others (Hagiwara 1989; Olive 1902; Raper 1984). This process of encystment is common to solitary as well as social amoebae (Ekelund and Ronn 1994; Kessin 2001). Laboratory conditions for microcyst formation are related to starvation and osmotic pressure and perhaps also ammonia concentration (Kessin 2001).

The macrocyst resting stage is the culmination of the sexual cycle, which is much less well understood than multicellular fruiting body formation. This stage is widespread among dictyostelids, but has not been observed for many species (Kessin 2001). It begins with acquisition of fusion competence (gamete formation) (Blaskovics and Raper 1957). Macrocysts also may require environmental factors for induction, particularly darkness (Hirschy and Raper 1964), excess water (Weinkauff and Filosa 1965), and ethylene (Amagai 1984). Once formed, the zygotes attract surrounding myxamebas to form small aggregations that secrete a protecting sheath around the collective. The zygote progressively increases in size by engulfing and digesting the other cells, hence the term "giant cell". A wall is then secreted, inside of which the macrocyst matures. Division of the giant cell before germination reconstitutes uninucleate cells.

There is evidence for macrocyst meiosis from studies of segregation patterns (MacInnes and Francis 1974; Okada et al. 1986) as well as the observation of a synaptonemal complex within the macrocyst (Erdos et al. 1972). Recently, thanks to the completion of the *Dictyostelium discoideum* genome sequence and available molecular genetics, new genes involved in the regulation of the sexual cycle are now beginning to be discovered. One of them is a novel regulator of cAMP signaling, which is specific to this process (Urushihara and Muramoto 2006). Also recently the mating-type locus for the model species *D. discoideum* has been identified (Bloomfield et al. 2010)

2. Ecology

The primary habitat of dictyostelids appears to be the surface layers of forest soils (Cavender and Raper 1965a; Raper 1984). These have yielded most of the more than 150 described species. However, dictyostelids have also been found in other habitats such as soils from agricultural lands (Agnihothrudu 1956), prairies (Smith and Keeling 1968), deserts (Benson and Mahoney 1977), under decaying plants and mushrooms (Hagiwara 1992), on epiphytes in tropical forests (Stephenson and Landolt 1998) and on animal dung (Waddell et al. 1982).

Geographically, dictyostelids are distributed around the world, from the coldest regions, such as Alaska (Romeralo et al. 2010a; Stephenson et al. 1997), to the tropics (Cavender 1973; Swanson et al. 1999). In general, diversity appears to decrease with increasing altitude and latitude (Cavender 1973; Hagiwara 1984; Swanson et al., 1999). As in many groups of plants and animals, species diversity seems to be highest in the tropics (Cavender, 1978; Kawabe, 1980), although some species are probably endemic to temperate (Cavender, 1978; Hagiwara, 1982) or subalpine zones (Traub et al. 1981).

Escherichia coli or *Klebsiella aerogenes* are the most used bacteria food source to cultivate the species in the laboratory (Raper 1984). Optimal temperatures (20–25°C) for growth and development of most species overlap. The larger species of dictyostelid respond to several factors during development indicating considerable environmental sensitivity. These factors are light and temperature (Bonner et al, 1950; Kessin 2001; Raper 1940), humidity (Bonner and Shaw 1957), gases (Bonner and Dodd 1962; Bonner and Lamont 2005), and solutes (Slifkin and Bonner 1952). The smaller species may be even more sensitive to some of these factors since they are more difficult to culture (for example, *A. ellipticum, D. menorah*, *D. oculare*, *D. stellatum*).

Most dictyostelid species are phototrophic (Bonner 2006; Raper 1984). It seems to be an adaptative mechanism to aid the slug in locating positions for sorocarp formation that maximize spore dispersal. Long distance dispersal of dictyostelid spores is not by wind but rather by water and animal vectors such as insects, rodents, amphibians, bats, and birds (Stephenson and Landolt 1992; Suthers 1985). This allows dictyostelids to be dispersed in large numbers to a single location, creating clonal patches giving rise to new clonal fruiting bodies (Strassmann et al. 2011).

Some general patterns in the ecology of social amoeba have been suggested over the years. There appears to be a relationship between vascular plants and dictyostelid species, so that certain species of plants are associated with different species of amoebas (Cavender and Kawabe 1989; Cavender and Raper 1965a, 1968). Forest soils, preferably ones that are slightly acidic, appear to be the best habitat in terms of numbers of amoebas and species diversity (Cavender and Raper 1965a; Landolt et al. 2006). However, some species are also tolerant of alkaline or neutral conditions and a few, such as *D. mucoroides*, are tolerant of a wide pH range. In general it is thought that species diversity and composition change with forest type (Cavender and Raper

1965b), soil moisture gradient (Sutherland and Raper 1978), vegetation diversity (Hagiwara 1976), altitude (Cavender 1983; Hagiwara 1976; Romeralo and Lado 2006), and latitude (Cavender 1973).

By applying statistical modeling to a set of data obtained from an extensive survey in southwestern Europe, Romeralo et al (2011a) were able to estimate the main environmental factors (both biotic and abiotic) influencing the distribution and diversity of dictyostelids in temperate climates. Their results show that a combination of climatic (temperature, water availability), physical (pH) and vegetational (plant species richness) factors favor dictyostelid species richness. In the Iberian Peninsula, dictyostelid diversity is highest in colder and wetter environments, indicating that this group has likely diversified in relatively cold places with high levels of water availability. It also appears that both water availability and plant species richness could facilitate dictyostelid diversity indirectly, via their prey—bacteria. Thus, dictyostelids may merely be responding directly to a higher diversity of bacteria. More surveys including bacteria, dictyostelids, and other factors (Romeralo et al. 2011a) are needed in order to disentangle whether the environmental effects of plants are direct or indirect, or if both, which are more important.

3. Taxonomy and Evolutionary History

Dictyostelid species have been traditionally recognized by the morphology of their fruiting bodies using a wide array of characters. These include the initial aggregation stage (mound, radiate) and type of chemoattractant signalling molecule (acrasin: cAMP, glorin, folate, etc), type of growth (clustered, gregarious, coremiform or solitary) and branching pattern of sporophore, spore characteristics such as shape (round or elliptical) and the presence or absence of polar granules inside the spores, etc. (Hagiwara 1989; Raper 1984). Based on these characters three genera were recognized: *Acytostelium*, *Dictyostelium* and *Polysphondylium*. Acytostelids produce an acellular stalk tube, therefore differentiating only spore cells at maturity. *Dictyostelium* species have unbranched or irregularly branched sorocarps, and polysphondylids have sorocarps with regularly spaced whorls of branches.

This classification system has been completely overturned by recent phylogenetic analyses of both morphological (Swanson et al. 2002) and molecular data (Romeralo et al. 2007b; Schaap et al. 2006; Spiegel et al. 1995; Swanson et al. 2002). The most detailed of these are molecular analyses based on 18S ribosomal DNA (rDNA) (Romeralo et al. 2011b; Schaap et al. 2006) and its internally transcribed spacer (ITS), (Romeralo et al. 2007b; Romeralo et al. 2010b), and on α-tubulin (Schaap et al. 2006). The most recent analysis, including many newly discovered species, show Dictyostelia to consist of at least eight major divisions, none of them corresponding to traditional genera (Fig. 2; Romeralo et al. 2011b). Instead, *Dictyostelium* and probably also *Acytostelium* are paraphyletic and *Polysphondylium* is polyphyletic, having two independent origins. There are also many cryptic species (i.e. molecularly distinct species with a similar morphology) throughout the phylogeny (Romeralo et al. 2011b). Thus the traditional genera are now more appropriately considered morphotypes. While the dictyostelids await a much-needed taxonomic revision, the major groups are simply referred to by number or provisional names (Fig. 2). These groups are outlined below along with tentative morphological justifications (Romeralo et al. 2011b).

Dictyostelid molecular Group 1 consists of a morphologically diverse set of dictyostelid types (Table 1, Fig. 2). In the original molecular phylogeny, these taxa were noted to have markedly smaller spores than most other dictyostelids, and therefore the name Parvisporids was proposed for the group (Schaap et al. 2006). However, some recently isolated Group 1 species have quite large spores, while species with very small spores are now found in other groups (Romeralo et al. 2011b). The one unifying feature for Group 1 now appears to be that all examined species have consolidated polar granules inside their spores. Group 1 may be the deepest major branch of Dictyostelia (Schaap et al. 20006) or the sister taxon to molecular Group 2A+2B (Schaap et al. 2006; Sucgang et al. 2011), depending upon the position of the root of the tree, which is still an open question.

The original Group 2 now appears to be deeply divided into two very different groups. Group 2A is very homogeneous, consisting exclusively of all sequenced acytostelids except *A. ellipticum* (Table 1, Fig. 2). These species have the distinct combined morphological characters of unbranched acellular stalks and spherical spores. In contrast, Group 2B includes all three morphotypes. *A. ellipticum* appears to form the deepest branch, followed by several branches of *Dictyostelium* morphotypes, within which the majority of the polysphondylids are nest. Furthermore, at least two additional *Dictyostelium* clades are found dispersed among these polysphondylids. Thus there appear to have been multiple switches between morphotypes within this group. Nonetheless Group 2B as a whole shows a trend toward having unconsolidated spore granules and a filose sorophore tip.

Group 3 is a diverse set of dictyostelid-types. They all share the presence of consolidated polar granules inside the spores (Table 1, Fig. 2). The group includes a highly molecularly and morphologically distinct subgroup of species with sorocarps supported by a digitated "crampon" base. The deepest branch in the group belongs to the only cannibalistic species, *D. caveatum*, which preys on other dictyostelids and prevents them from fruiting. This very unusual species was isolated from bat guano in a cave in Arkansas and has never been found again. It has very distinct SSU rDNA and α-tubulin sequences with no close relatives in either molecular phylogenies (Schaap et al. 2006).

Group 4 is the largest group with most of the large robust and commonly encountered species such as the type species *D. mucoroides* and the model organism *D. discoideum*. This extremely molecularly shallow group (based on 18S rDNA) was originally thought to share three common characters: large sorocarps, solitary and unbranched fruiting bodies, and spores that lack polar granules (Schaap et al. 2006). However, recently described new species show exceptions to all three traits (Romeralo et al. 2011b).

Three additional small but molecular distinct clades are found scattered among the 4 original major groups. The "polycarpum" complex lies between Group 1 and the remaining dictyostelids. It consists so far of just two morphologically nearly indistinguishable isolates with clustered sorocarps and polar granules inside the spores. These two isolates are nonetheless extremely molecularly distinct, showing as much sequence difference in 18S rDNA as nearly any two species in Group 4. The "polycephalum" complex forms a distinct branch arising near Groups 3 and 4 and the violaceum complex. Again, the four examined isolates are morphologically nearly indistinguishable but molecularly highly distinct. All are characterized by having small coremiform fruiting bodies, very long thin slugs and spore granules that are sometimes polar. Finally, the "violaceum complex" appears to be a close sister group to Group 4 in rDNA trees, although its position is less clear in α-tubulin phylogeny (Schaap et al. 2006). The group includes both dictyostelid and polysphondylid types, which nonetheless share two strong common traits - violet or purple pigmented sorocarps and consolidated and polar spore granules.

The new phylogenies of Dictyostelia indicate that sorocarp morphology is probably quite plastic and therefore not a reliable indicator of deep evolutionary relationships in the group. In fact, the taxon includes many cryptic species, so morphology is not always reliable even on short time scales (Mehdiabadi et al. 2009; Romeralo et al. 2010, Romeralo et al. 2011b). Nonetheless, over 50 new species have been isolated in the last 5 years, all on the basis of morphology alone, and all of these were later confirmed by molecular phylogeny (Romeralo et al. 2011b).

One of the interesting questions in dictyostelid evolution is the origin of chemoattractant signaling with acrasins, which is at the heart of dictyostelid aggregation. Although the best known chemoattractant is cAMP, which is used exclusively and probably universally among Group 4 species, at least eight different acrasins have so far been identified. These are used by various different species and probably many more remain to be determined (Bonner 1983; Schaap et al. 2006). Recently Winckler and colleagues showed that glorin (N-propionyl-gamma-L-glutamyl-L-ornithine-delta-lactam ethylester) is used by species in at least four major groups of Dictyostelia (Groups 1, 2, 3 and the violaceum complex). This suggests that it was probably the chemoattractant of the last common ancestor of Dictyostelia, and it has been repeatedly replaced by other systems during dictyostelid evolution (Asghar et al. 2011). The use of cAMP as chemoattractant is clearly a derived state in Group 4, as it has evolved by duplication of cAMP cell surface receptor genes that are found in all dictyostelids and possibly also in their ancestors among the solitary amoebozoans (Alvarez-Curto et al. 2005).

Another intriguing question is the origin of dictyostelid multicellularity. *Acytostelium* species were long considered the most "primitive" dictyostelids, due to their acellular stalks, which result from the absence of cellular differentiation into stalk- and spore-cells. However, their position as members of Group 2A suggests instead that their simplicity is secondarily derived, as previously suggested by Bonner (1982). The position of *A. ellipticum* as the deepest branch of Group 2B is especially intriguing. If correct, it means that the Group 2B *Dictyosteliums* and *Polysphondyliums* are nested within acytostelids. This means that these species arose from an ancestor that lacked cellular differentiation and that this ability was reinvented during the evolution of the group.

The evolution of polyspondylids is especially intriguing as their striking morphology, which consists of a series of regularly spaced whorls of side branches, has clearly evolved twice independently (Romeralo et al. 2011b; Schaap et al. 2006). Thus the majority of polysphondylids, particularly the species with small pale sorocarps, are found in Group 2B closely allied with the small sorocarp-forming acytostelids. Meanwhile the type species, the robust violet colored *P. violaceum*, is found in a completely different clade (the "violaceum complex"), together with the robust violet dictyostelid, *D. laterosorum.* Thus the two types of polysphondylid have not only arisen independently but have done so from very different ancestors.

4. Practical Importance

Dictyostelium discoideum was first isolated by Raper in 1933, from partially decomposed leaves from a hardwood forest at Little Butts Gap in the Craggy Mountains of western North Carolina (Raper 1935). His slug-grafting experiments with this species (1940), which demonstrated that the stalk and spores develop from the front and rear of the migrating pseudoplasmodium, respectively, are classical, and the first application of modern, creative experimental approaches to this organism. Investigations of cell aggregation by J. T. Bonner (1944) culminated in experimental proof for the existence of a chemotactic agent responsible for cell aggregation, which he named "acrasin" (1947). The identification of the acrasin as cyclic adenosine monophosphate (cAMP) in *D. discoideum* in Bonner's laboratory (Konijn et al. 1967) stimulated further growth in studies of the molecular basis of dictyostelid development and differentiation. Cellular slime mold experimental biology was first reviewed by Sussman (1956) and Bonner (1959). Since then numerous reviews and a number of books have been written on *D. discoideum* as a model system, for example by Loomis (1975, 1982), Spudich (1987), Maeda et al. (1997) and Kessin (2001).

The study of dictyostelid genetics developed slowly until the isolation by subculturing of an axenically growing strain, AX1. The full and complicated story of the development of the various laboratory strains is beautifully described in (Kessin 2006). AX1 was the first isolate that could be grown in the laboratory purely on broth medium (Sussman and Sussman 1967). The existence of axenic strains facilitated enormously the experimental manipulation of *D. discoideum* allowing the development of molecular genetic tools such as transformation of exogenous genes (Pang et al. 1999) which, together with their highly efficient homologous recombination allows the rapid generation of loss-of-function mutants. Other techniques include restriction enzyme-mediated integration (Kuspa 2006), RNA interference, antisense-mediated gene silencing (Kuhlmann et al. 2006), and more.
The existence of a diploid phase in the dictyostelid life cycle was only discovered when the relationship between the macrocyst (multicellular resting structure) and sexuality was firmly established (Clark et al. 1973; Erdos et al. 1973). This occurred relatively late in the history of dictyostelid studies, when it was discovered that macrocyst formation in certain species depends upon mixing amoebas of opposite mating type. However, work with macrocysts is difficult and was hampered by problems with induction, germination and recovery of the segregating amoebas, which slowed the progress of sexual genetic techniques (Katz 1978; Newell 1978). However, a working system of parasexual genetics has been developed. Occasional cell fusion events can occur in the population forming a diploid cell allowing non-sexual recombination of the two sets of chromosomes. This has practical advantages such as the generation of multiple knockouts or the study of lethal genes (King and Insall 2003; King and Insall 2006).

Thus, *D. discoideum* is now a well-developed genetic system (Escalante and Vicente 2000). This has been further aided by completion of the *D. discoideum* genome sequence (Eichinger et al. 2005) and ensuing large scale post-genomic studies (Torija et al. 2006). The latter include large scale analyses (microarray) of gene expression patterns, a description of the complete protein repertoire, and the potential to develop a complete set of gene knock out mutations, to name just a few. Comparative genomics of *D. discoideum* and related species, such as *D. purpureum* (Sucgang et al. 2011), will lead to the definition of amoebozoa-specific genes, which may open new avenues of research aimed at controlling amoebic diseases. This will be further enhanced by the development of genome sequences and model systems from all major dictyostelid groups.

With all these techniques available, *D. discoideum* has become a relevant model to study processes at the cellular level such as cell motility, chemotaxis, cytokinesis, phagocytosis, pynocytosis and more. At the multicellular level it has been used to study processes such as cellular differentiation and development (Escalante and Vicente 2000; Hudson et al. 2002; Maeda et al. 1997; Ratner and Kessin 2000; Strassmann et al. 2000; Strmecki et al. 2005).

Interestingly *D. discoideum* shares more genes with Metazoa, including ones associated with development (e. g. Williams et al. 2006), than either taxon shares with plants and retain some similarities with Metazoa that have been lost in the more closely related but highly derived model organism, *Saccharomyces cerevisiae.* Consequently, a number of important genes have been conserved between *D. discoideum* and human that are absent in the yeast model *S. cereviasie*. This is the case for example of several genes coding for relevant proteins involved in basic cellular functions such as autophagy, a regulated degradation of cell´s own material (Calvo-Garrido et al. 2008; 2010). Therefore, *D.discoideum* can now complement the studies from other experimental systems to shed light in the function of these highly conserved proteins and their possible role in higher organisms including human.

In this line, *D. discoideum* has also begun being exploited as a useful model for basic aspects of human diseases (Barth et al. 2007; Williams et al. 2006). Certain features of dictyostelid biology offer a convenient framework to address disease-related topics such as the study of pathogen infection. The selective pressure that soil amoeba have exerted during evolution on environmental bacteria is likely to have been tremendous. As a consequence, bacteria have developed virulence factors to escape and survive the attack of predatory amoebas. It is believed that these defense mechanisms have been adapted by certain bacteria to allow them to infect and survive in other organisms, including humans. In this regard, many of the bacterial virulence mechanisms involved in human pathogenicity are functioning in a similar way during the bacterium's interaction with dictyostelids. Thus the host-pathogen interplay can be conveniently studied using the interaction between *D. discoideum* and diverse pathogens such as *Legionella*, *Mycobacterium* and *Pseudomonas* among others (Lima et al. 2011; Steinert 2011).

In the same line, the motility and chemotactic properties of dictyostelids have proved useful for modeling cell-motility pathologies in the immune and neurological systems such as lissencephaly (Carnell and Insall 2011; Meyer et al. 2011). Other examples include human diseases associated with endocytic traffic such as Chediak Higashi Syndrome, Ceroid Lipofuscinosis and Niemann Pick Disease that cause severe symptoms and whose molecular bases can be studied in *D. discoideum* (Maniak 2011). Moreover, certain human mitochondrial diseases are being addressed using *D. discoideum*, such as a signalling pathway regulated by the AMP-activated protein kinase (AMPK) that has been involved in the underlying cytopathological symptoms (Carilla-Latorre et al. 2010; Francione et al. 2011). *D. discoideum* has also proved useful in pharmacogenomics as a model for studying the mechanisms of action of drugs such as the chemotherapeutic drug cysplatin and the mood-stabilizing drugs valproic acid and lithium (Alexander and Alexander 2011; Ludtmann et al. 2011).

5. Highlights from three genome sequences

The first completed dictyostelid genome sequence was that of *D. discoideum*, published in 2005 (Eichinger et al. 2005). The genome is 34 megabases (Mb) in size with six chromosomes encoding an estimated 12500 proteins. This makes it quite small and compact, similar to the genome of the model organism, brewer's yeast (*Saccharomyces cerevisiae*). The *D. discoideum* genome is extremely AT-rich (70-80%), with large tracts of triplet repeats in many of the protein coding genes (known as simple sequence repeats or SSRs). These repeats are translated and retained in the mature proteins (Eichinger et al. 2005). *D. discoideum* SSRs appear to be under negative selective pressure (Eichinger et al. 2005), but comparisons with the second sequenced genome, that of *D. purpureum*, shows that dictyostelid SSRs also evolve relatively quickly (Sucgang et al. 2011). Most genes also contain introns, which are small in size (150 base pairs on average), similar to the situation in most examined eukaryotic microbes.

The *Dictyostelium* genomes offer interesting insight into the evolution of multicellularity. Although Metazoa and Dictyostelia evolved multicellularity independently, some pathways have been recruited for developmental roles in both systems, for example STAT signaling (Williams 2000). In fact, a broad survey of proteins known to be required for multicellular development shows a number of them to be present in *D. discoideum* but missing in *Saccharomyces*. Since Fungi are more closely related to Metazoa than are the Amoebozoa, these proteins were presumably lost at some point during fungal evolution (Eichenger et al. 2005). Thus it now appears that some of the proteins involved in processes like cell adhesion and signaling modules, which were originally assumed to be associated exclusively with Metazoa, are in fact much older. The *D. discoideum* genome also encodes more than 40 proteins involved in cellulose metabolism, which are probably involved in fruiting body formation. Some of these are homologous to proteins found in plants, and are therefore likely to play similar roles in both systems (Eichinger et al. 2005).

Since publication of the *D. discoideum* genome (Eichinger et al. 2005), and the first molecular phylogeny of Dictyostelia (Schaap et al. 2006), efforts have been underway to develop model systems across the taxon. In addition to the recently completed genome sequence of *D. purpureum* (Sucgang et al. 2011), three other genome sequences are nearing completion, those of *Polysphondylium pallidum*, *D. lacteum*, and *D. fasciculatum*. *D. discoideum* and *D. purpureum* are both Group 4 species, and their genomes are similar in size, coding for at least 7,619 orthologous proteins. The two genomes also display a substantial amount of synteny, with most orthologs present in conserved clusters. Nonetheless, there is a high overall level of sequence divergence, roughly equivalent to that across the vertebrates, suggesting that these two Group 4 species shared a common ancestor roughly 400 million years ago. Thus, while some large gene families are highly conserved, others are not. For example

ABC transporters and histidine kinases have undergone little change whereas the polyketide synthases have suffered a high diversification. None of the *D. purpureum* SSRs are not found in homologous positions in *D. discoideum* proteins, suggesting that their presence is more a random tendency rather than the consequence of ancestral homopolymer tracks with functional meaning. A comparison between genes with specific expression patterns have shown that the genes involved in multicellular development have evolved more rapidly, which could indicate either relaxed selection or accelerated evolution due to the complexity of social behavior (Sucgang et al. 2011).

6. Conclusions

Up until about 15 years ago it was thought that soil was not a particularly good environment for prolific speciation and since most of the major soil zones and vegetational regions of the world had already been sampled, there were probably not many distinctive species remaining to be described. Nonetheless the number of described dictyostelid species has been doubled since then and in only the last 5 years almost 100 new species have been discovered. This was in part due to the work of the "Global Biodiversity of Eumycetozoa" survey group based at the University of Arkansas (S. Stephenson, F. Spiegel co-PIs), which had a mandate to conduct exhaustive sampling of all major divisions of Eumycetozoa (myxomycetes, protostelids and dictyostelids) from all major terrestrial biomes. The new dictyostelids identified by this survey are spread across the entire phylogeny, indicating among other things that previously isolated long branches probably correspond to major groups (Romeralo et al. 2011b).

A large diversity of undescribed dictyostelids is also suggested by recent studies using culture independent molecular sampling techniques (metagenetics or ciPCR). Similar studies have revealed a hidden diversity of every major group of protist so far examined (e.g., Howe et al. 2011; Jones et al. 2011; Marande et al. 2009; Massana 2011). Our initial ciPCR studies of total DNA extractions using dictyostelid specific rDNA primers have yielded novel phylotypes across the tree. These include new deep branches in all major divisions, some of which could correspond to novel morphologies (Romeralo and Baldauf, ms in prep). Isolating and characterizing the species that correspond to these novel deep branches should lead to a greater understanding of the evolution of development in Dictyostelia.

In conclusion, although much is now known about the molecular, behavioral and developmental biology of *D. discoideum*, much less is known about nearly the entire rest of this deep and ancient lineage. A global analysis of sequence divergence suggests that the genetic diversity of the Dictyostelids is similar or even higher to that of the vertebrates, from the bony fishes to the mammals (Sucgang et al. 2011). With a detailed and well-resolved phylogeny and genome sequences from across the group, the dictyostelids have tremendous potential as an evolutionary model system.

The apparent depth of Dictyostelia as indicated by the two molecular markers examined so far, together with the large number of new species identified in the last few years (Romeralo et al. 2011b), suggest that there may be a far greater diversity of extant taxa than currently known. This is also suggested by the fact that some of the deepest branches in the Dictyostelia are occupied by small delicate species which are the most difficult to isolate. The possibility of a large hidden diversity of dictyostelids is now confirmed by metagenetic analyses using rDNA sequences obtained from total soil DNAs. These data reveal new major branches throughout the dictyostelid tree, as well as new sequences breaking up previously isolated long branches (Romeralo and Baldauf, unpublished). Isolating and characterizing these new species should greatly expand our understanding of dictyostelid diversity, ecology and evolutionary biology.

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Figure Legends

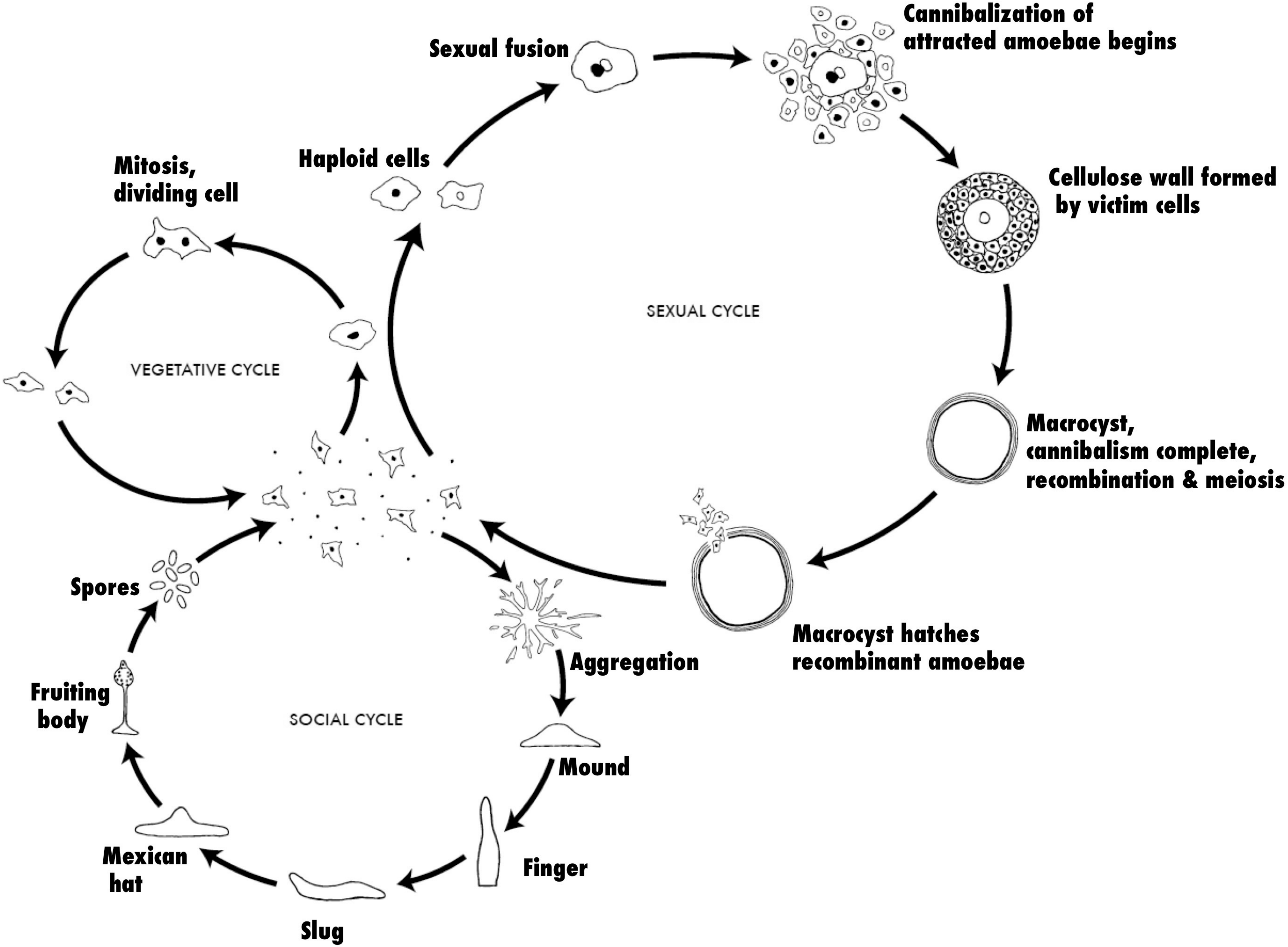
Fig. 1. Life cycle by David Brown and Joan E. Strassmann. CC 3.0 copyright (http://www.dictybase.org/Multimedia/DdLifeCycles/index.html)

Fig. 2. The Phylogeny of Dictyostelia as indicated by analyses of nuclear small subunit (18S) rDNA sequences. Modify from Romeralo et al. (2011b). Pictures by Romeralo, M.

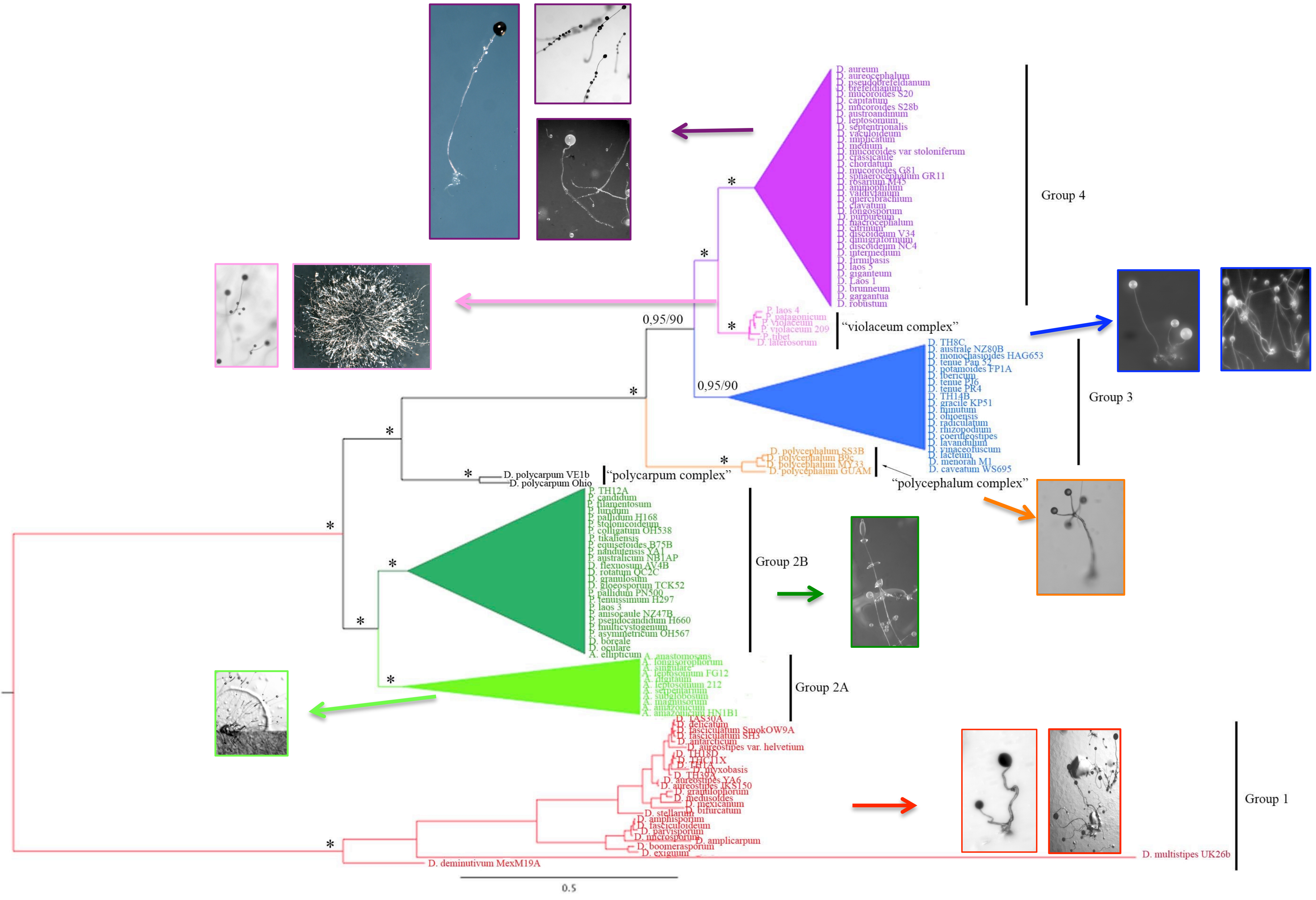
Tables

Major Group	Morphotypes	Diagnostic
Group 1	D	consolidated polar granules
Group 2A	A	unbranched acellular stalks and spherical spores
Group 2B	A, D, P	trend toward unconsolidated spore granules, filose sorophore tip
Polycarpum complex	D	sorocarps adhere near the base, multiple sorogens arising from single aggregation, polar granules
Group 3	D	consolidated polar granules except *D. minutum*
Group 4	D	“gigantic species” clade
Polycephalum complex	D	small coremiform fruiting bodies, very long thin slugs, spore granules sometimes polar
Violaceum complex	D, P	violet or purple sorocarps, consolidated and polar spore granules

Table 1. Eight major groups of dictyostelids according to Romeralo et al. (2011b). Morphotype A: *Acytostelium*; D: *Dictyostelium*; P: *Polysphondylium*



Cannibalization of attracted amoebae begins
Sexual fusion
Haploid cells
Mitosis, dividing cell
Cellulose wall formed by victim cells
SEXUAL CYCLE
VEGETATIVE CYCLE
Macrocyst, cannibalism complete, recombination & meiosis
Macrocyst hatches recombinant amoebae
Spores
Aggregation
Fruiting body
SOCIAL CYCLE
Mound
Mexican hat
Finger
Slug


D. aureum
D. aureocephalum
D. pseudobrefeldianum
D. brefeldianum
D. mucoroides S20
D. capitatum
D. mucoroides S28b
D. austroandinum
D. leptosomum
D. septentrionalis
D. vaculoideum
D. implicatum
D. medium
D. mucoroides var stoloniferum
D. crassicaule
D. chordatum
D. mucoroides G81
D. sphaerocephalum GR11
D. rosarium M45
D. ammophilum
D. valdivianum
D. quercibrachium
D. clavatum
D. longosporum
D. purpureum
D. macrocephalum
D. citrinum
D. discoideum V34
D. dimigraformum
D. discoideum NC4
D. intermedium
D. firmibasis
D. laos 5
D. giganteum
D. Laos 1
D. brunneum
D. gargantua
D. robustum
Group 4
*
*
0,95/90
P. laos 4
P. patagonicum
P. violaceum
P. violaceum 209
P. tibet
D. laterosorum
*
"violaceum complex"
D. TH8C
D. australe NZ80B
D. monochasioides HAG653
D. tenue Pan 52
D. potamoides FP1A
D. ibericum
D. tenue PJ6
D. tenue PR4
D. TH14B
D. gracile KP51
D. minutum
D. ohioensis
D. radiculatum
D. rhizopodium
D. coeruleostipes
D. lavandulum
D. vinaceofuscum
D. lacteum
D. menorah M1
D. caveatum WS695
0,95/90
Group 3
*
*
D. polycephalum SS3B
D. polycephalum B9c
D. polycephalum MY33
D. polycephalum GUAM
*
"polycephalum complex"
*
D. polycarpum VE1b
D. polycarpum Ohio
"polycarpum complex"
*
P. TH12A
P. candidum
P. filamentosum
P. luridum
P. pallidum H168
P. stolonicoideum
P. colligatum OH538
P. tikaliensis
P. equisetoides B75B
P. nandutensis YA1
P. australicum NB1AP
D. flexuosum AV4B
D. rotatum QC2C
D. granulosum
D. gloeosporum TCK52
P. pallidum PN500
P. tenuissimum H297
P. laos 3
P. anisocaule NZ47B
P. pseudocandidum H660
P. multicystogenum
P. asymmetricum OH567
D. boreale
D. oculare
A. ellipticum
*
*
Group 2B
A. anastomosans
A. longisorophorum
A. singulare
A. leptosomum FG12
A. digitaum
A. leptosomum 212
A. serpentarium
A. subglobosum
A. magnusorum
A. amazonicum
A. amazonicum HN1B1
*
Group 2A
D. TAS30A
D. delicatum
D. fasciculatum SmokOW9A
D. fasciculatum SH3
D. antarcticum
D. aureostipes var. helvetium
D. TH18D
D. THC11X
D. TH1A
D. myxobasis
D. TH39A
D. aureostipes YA6
D. aureostipes JKS150
D. granulophorum
D. medusoides
D. mexicanum
D. bifurcatum
D. stellarum
D. amphisporum
D. fasciculoideum
D. parvisporum
D. microsporum
D. amplicarpum
D. boomerasporum
D. exiguum
*
D. deminutivum MexM19A
D. multistipes UK26b
Group 1
0.5