SYNOPSIS

By combining laboratory experiments with field work, I have looked at the following aspects of cellular slime mould (CSM) biology: (a) the genetic structure of social groups (fruiting bodies) in the wild and its relation to the role of large mammals as dispersal agents; (b) social behaviour in clonal, intra-species polyclonal and interspecies social groups and (c) fitness-related trade-offs with respect to life history traits as a possible mechanism for coexistence and cooperative behaviour in CSMs. The major findings of this study are as follows: (a) individuals belonging to different strains of a species, different species and genera occur in close proximity, even on a speck of soil (250µm–1mm) or the same dung pat; (b) social groups formed in the wild by *Dictyostelium giganteum* and *D. purpureum* are generally multiclonal; (c) genetically diverse strains can co-aggregate and form chimaeric social groups; (d) in chimaeric social groups, strains differ in their relative sporulation efficiencies; (e) the fact that strains co-exist in spite of this may be attributable in part to trade-offs between various fitness-related traits as can be demonstrated in the case of wild isolates of *D. giganteum* in pairwise mixes.

The Dictyostelids or CSMs are haploid, eukaryotic, soil dwelling social amoebae with an unusual life cycle (Bonner, 1967; Raper, 1984). They exist as single cells in the presence of food (bacteria, yeast, fungal spores). Once the food is exhausted, they enter the social phase of their life cycle. Approximately $10^2$ to $10^6$ amoebae aggregate at a common collection point and form a starvation resistant structure called the fruiting body. In many species a fruiting body is made up of an aerial stalk of dead cells and a ball of viable spores on top. In other CSM species (not part of this study), all amoebae in a fruiting body differentiate into spores and the stalk is an extracellular secretion.

The CSM life cycle raises fundamental questions related to the evolution of an extreme form of ‘altruism’ in the form of reproductive division of labour in social groups. The spore–stalk distinction in the CSMs is analogous to the germ–soma distinction in metazoans, although, the CSMs achieve multicellularity not by repeated divisions of a zygote but via the aggregation of many cells which may or may not be clonally related (Bonner, 1982; Kaushik and Nanjundiah, 2003). Social behaviour in the CSMs offers interesting parallels to what is seen in the social insects.
(Gadagkar and Bonner, 1994). The origin and maintenance of ‘altruism’ has been a long-standing issue in sociobiology. Because of their simple life cycle and experimental tractability, the CSMs are ideal for studying the evolutionary origin and maintenance of social behaviour, in particular of ‘altruistic’ behaviour. By elevating spores above soil level, stalk cells, protect them from noxious compounds and predators present in soil and also facilitate their passive dispersal. In the course of doing so they die. The death of stalk cells appears to be an extreme form of altruism.

Knowledge of the genetic structure of social groups and populations including patterns of kinship is essential for modelling the evolution of ‘altruism’. Thus, it is important to understand the genetic structure of CSM social groups in the wild. For this, social groups (fruiting bodies) of CSMs were isolated from undisturbed forest soil of the Mudumalai forest reserve in South India. Soil and animal dung samples were brought to the laboratory and quasi-natural social groups were generated by inoculating the samples on non-nutrient agar. The fruiting bodies from various CSM species were formed by these isolates. Since soil and dung samples were not perturbed in any way, the fruiting bodies were formed as they would have in nature.

When compared to soil, dung samples contained a higher CSM diversity and more CSM propagules. The presence of CSMs in fresh animal dung makes it likely that they were transported and dispersed over long distances through the gut of these animals. Such dispersal is likely to be preceded by a thorough mixing of spores in the gut. That increases the probability of co-occurrence of different genotypes in a social group. This possibility was confirmed by genetically characterizing spores in social groups of Dictyostelium giganteum and D. purpureum collected from the wild. Random amplification of polymorphic DNA (RAPD), a simple and reliable molecular technique, was used for genotyping spores within a fruiting body. 17 fruiting bodies (8 from animal dung and 9 from soil) were studied. 15 out of 17 (9 out of 11 of D. giganteum and 6 out of 6 D. purpureum) were polyclonal; the minimum number of distinct clones in a single fruiting body was 3 to 7 (animal dung) and 1 to 9 (soil). Therefore in D.giganteum and D. purpureum, chimaeric social groups seem to be the
norm. This suggests that other species of CSMs form intra-species chimaeric social groups in wild, though clonal fruiting bodies occur too.

The next objective of this thesis was to test whether genetic heterogeneity had functional consequences. That is, when different strains come together in an aggregate, do they contribute equally to the reproductive (spore) and non-reproductive (stalk) pathways? Amoebae of different clones (strains) of *D. giganteum* or *D. purpureum* were mixed and developed together and the number of spores formed by each strain was counted. These experiments confirmed that strains of *D. giganteum* or *D. purpureum* can aggregate together and form chimaeric fruiting bodies. The ability to mix (measured as the frequency of chimaerism) depended on the strains used and varied from one mix to another. One strain was often found to 'exploit' the other during sporulation, that is, it formed more spores than its expected share. Despite this, strains are found in very close proximity in the soil, which raises an important question: when one strain is more efficient at sporulating than other, how can the two co-exist stably?

To investigate what might lie behind the stable co-existence of strains, I studied various fitness-related traits in the life cycle of *D. giganteum*. They included the rate of cell division, the time taken to go through multicellular development, the efficiency of slug migration through various depths of soil and the probability of differentiation into a spore. Measurements were carried out on strains taken separately and on their pairwise mixes. Five different *D. giganteum* wild strains (46a3, 46d2, 48.1a1, F5 and F16) were used. All were isolated from the Mudumalai forest (India). 46a3 and 46d2 came from soil within 10 cm of each other, 48.1a1 from soil about 200 m away from 46a3; and F5 and F16 from the same fruiting body (Kaushik et al., 2006; Sathe et al., 2010). Members of a pair differed significantly in the measured fitness-related traits. For example, in the case of 48.1a1 and 46d2, 48.1a1 grew faster than 46d2 both individually and in a mix. After starvation, 48.1a1 formed fruiting bodies faster than 46d2; a mix of the two developed at the rate of the faster member, implying that the slower one (46d2) gained from the association with 48.1a1. During slug migration, slugs formed by 48.1a1 came up through a higher depth of soil than 46d2 slugs and did so earlier. Chimaeric slugs were like the
more efficient member, 48.1a1, in terms of the maximum depth of soil that was covered, but like the less efficient member, 46d2, in terms of the time taken for slugs to be seen on the soil surface. 48.1a1 seems to have an advantage over 46d2 in all these respects. However, during sporulation in chimaeras, 48.1a1 formed relatively fewer spores than 46d2. Similar trade-offs were seen in all mixes. F5 and F16 displayed an unexpected feature during sporulation; the spore-forming efficiency of either strain depended on its proportion in the initial mix in a frequency-dependent manner that was consistent with a stable equilibrium. Thus, trade-offs between different fitness-related traits contribute to the co-existence of strains.

Next, I studied interactions between members of different CSM species. Several species of CSMs were isolated from the same environment (Sathe et al., 2010); a question of interest was to see if amoebae of different species came together to form a chimaeric multicellular body. Five strains (two D. purpureum and three D. giganteum) were used in this study. Amoebae of D. giganteum and D. purpureum co-aggregated. However, there were factors that caused amoebae of the two species to sort out thereafter. The extent of segregation differed between strains, a characteristic that inter-species mixes share with intra-species mixes.

In conclusion, the ability of cellular slime moulds to form multiclonal social groups in the wild suggests that one should look to factors in addition to close relatedness to understand the evolution of CSM social behaviour. The existence of fitness-related trade-offs between different traits indicates that individual-level selection can also contribute to the maintenance of chimaeric social groups.

References