Social Adaptations in Myxobacteria

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Myxobacteria are unique amongst the procaryotes in displaying a wide range of social adaptations (Kaiser et al. 1979). They live in compact masses, grow better on polymeric substrates when in dense populations (Rosenberg et al. 1977), and collaborate in producing fruiting bodies. Moreover, they generally sporulate only when in dense populations (Chapter 4); while sporulating, most of the cells lyse and presumably nourish those that sporulate (wireman and Dworkin 1977).

Sporulation in myxobacteria as well as their movements in packs are most probably synchronized by a communication system. Although accumulation of various compounds has been correlated with development (Chapter 4), it is difficult to determine, based on our present knowledge, which of those compounds serve as the signals in the communication system.

The above characteristics make the myxobacteria a suitable model system for the study of social behavior. Due to the immense difficulties in conducting experimental evolutionary studies, much of the theory of evolution in maioresuborganisms is based on comparative studies. On the other hand, bacterial evolution is relatively easy to follow experimentally owing to their short life cycle and large population densities that increase the chance of finding appropriate mutations and the possibilities of controlling their environment. Using microbial model systems have proven to be fruitful in many genetic and physiologic fields and, we hope, will contribute to the understanding of social behavior as well. Moreover, looking at the social interactions of myxobacteria from an evolutionary point of view might suggest new approaches to experimentation with myxobacteria. We hope that the approach presented in this chapter will con-

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tribute to the understanding of both the evolution of social adaptations and the complex mechanisms in the life cycle of the myxobacteria.

**Group Selection and Individual Selection**

It is generally accepted that an individual bacterium that grows and survives better than another bacterium would ultimately prevail in a culture if the two types are mixed and grown together. The success of one type of bacterium over the other types is a consequence of the advantage of the individual over the other cells. The selection mechanism involved is referred to as *individual selection*, because the adaptation spreads in the population by the selection of individuals.

Individual selection, however, is not the only type of selection that is theoretically possible. Wynne-Edwards (1962) and Wilson (1975) have suggested that adaptations may be selected for by the selective elimination of groups or populations, that is, *group selection*. Hamilton (1964) has suggested that social adaptations may be selected for through investments in individuals that are genetically related to one another, that is, *kin selection*. Among organisms such as myxobacteria, which reproduce asexually and do not move over large distances, there is not much difference between models based on group selection as suggested by Wynne-Edwards (1962) or those based on kin selection as suggested by Hamilton (1964).

There is no question that a group composed of individuals, all of which are superior to others, would outcompete other groups composed of inferior individuals. But is it possible that an adaptation that enables inferior individuals to cooperate better would enable them to outcompete, as a group, individuals that are otherwise superior to them? Group selection theory suggests that under certain conditions that could be the case.

Maynard-Smith (1976) discussed the conditions necessary for the operation of group selection. The main weakness in any group selection theory is the possibility that a group composed of cooperating individuals that are otherwise inferior would be infiltrated by superior types that are not cooperative. The latter individuals would prevail and outcompete the cooperating types through their superior adaptations as individuals. Group selection would succeed only if new cooperating groups are generated quicker than those destroyed by superior noncooperative individuals. Bacteria and other microorganisms may fulfill the conditions necessary for the operation of group selection more than macroorganisms. Although Maynard-Smith (1976) did not believe that group selection plays a primary role in evolution, he nevertheless suggested it as the mechanism by which less virulent myxomatosis has been selected from the virulent type.

Group selection is especially attractive in interpreting the evolution of social adaptations in myxobacteria. Group selection theory furnishes an apparently easy answer. To explain why individual myxobacteria (1) invest in secr
enzymes whose products are used by other bacteria and (2) participate in a sporulating mass in which they are most likely to perish. Populations of myxobacteria that possess these adaptations thrive and outcompete groups that do not possess them. Zahavi (1981) suggested that what appears to be group selection in animals can be interpreted as a more complex mode of individual selection. Could this interpretation be applied to myxobacteria as well? We will suggest some mechanism to explain the social adaptations of myxobacteria as a consequence of individual selection and present some preliminary experimental data that are in accordance with this notion.

Social Behavior During Growth

It has been suggested that the cooperation among myxobacteria facilitates their growth because together they can use their substrates better than if each of them operated alone (Chapter 11). This suggestion was supported by experiments that showed that dense cultures of Myxococcus xanthus grew on casein better than the sparse population did (Rosenberg et al. 1977). This phenomenon of cell density dependent growth did not occur when the bacteria were grown on casein (casein hydrolysate). The bacteria produce much less protease activity under this latter condition (Guesspin-Michel personal communication). Although the myxobacteria have the capability of regulating their protease activity, it has repeatedly been observed that when myxobacteria are growing on solid casein medium they hydrolyze casein over a large area (several centimeters) around the colony. Thus, the myxobacteria produce and secrete the proteolytic enzymes in sufficiently large amounts so that the population as a whole may benefit from the breakdown of the protein. Although this phenomenon can be explained by either group or individual selection theory, each theory makes a different prediction regarding the properties of individual cells within the population. If casein use is a group characteristic selected for on the basis of the group, a mutant deficient in protease activity may exploit the niche within cultures of wild-type cells growing on casein. If, on the other hand, protease excretion is individually advantageous, mutants deficient in protease activity would be less competitive than the wild type would be. Recently, Guesspin-Michel (unpublished) and Zahavi and Ralt (unpublished) have independently isolated protease deficient mutants of M. xanthus. It is interesting to note that neither laboratory was able to isolate a mutant with no activity; the mutants still retain 5% of the wild-type protease activity on casein. A protease-deficient mutant enabled Zahavi and Ralt to conduct competition experiments with the wild type and the mutant on liquid and solid casein media. In preliminary experiments Zahavi and Ralt have shown that the wild type outcompeted the mutant on casein medium and had no advantage on casein medium. These results are compatible with the assumption that the adaptation to produce extracellular protease is selected for on the basis of individual selection.
This conclusion raises an interesting question: What is the advantage to the individual in the production of excessive amounts of extracellular protease? We may argue that there is no other way for myxobacteria to secrete a protease beside that which is involved with the waste, later used by other members of the population. Although such an explanation is possible, it is known that other bacteria can hydrolyze casein efficiently, and their cooperation is not required for the use of the protein. For example, individual *Bacillus subtilis* cells plate on casein medium and show no cell density dependent growth (Fattum and Rosenberg 1977). An alternate explanation to cooperation in the use of casein is that there is an additional benefit to the individual from the secretion of the protease besides its function in breaking down the casein. Zahavi (1977) developed a model to explain allo feeding amongst cooperating bidders. This model suggests that the feeding is used by the bidders as a status signal (i.e., only a strong bird can afford to feed another bird). Following this model, we suggest that the protease or the breakdown products produced by its action function as an attractive signal attracting the myxobacteria into the pack. A mutant that does not excrete the protease, cannot "signal," is thus less attractive, and may be left outside the mass of cells with no collaborators.

Why is it necessary for the myxobacteria to live in packs in order to exploit their niche? One answer could be that feeding on certain substrates, or predation on other bacteria, requires the cooperative effort of more than a single bacterium. Alternatively, it could be that living in compact masses enables the myxobacteria to withstand desiccation better and thus enable them to exploit comparatively dry environments. It is interesting to note that extracellular polysaccharides, similar to those found in the slime (Kaiser et al. 1979), have been suggested to function in protection against desiccation (Dudman 1977).

Hence, assuming that it is advantageous for the myxobacteria to live in a pack, it is reasonable to suggest that individual myxobacteria would rather move toward a collaborator that produces excessive extracellular enzyme (or break down products) than toward a cell that produces less of them. It follows that it is advantageous for myxobacteria to produce and excrete enzyme since they thus ensure collaborators.

**Social Behavior During Development**

**Synchronization of Sporulation**

The selective advantage of collaboration for survival requires that the bacteria should synchronize their sporulation. Otherwise those that sporulate out of phase will not participate in fruiting body formation and will find themselves solitary or in smaller groups. It is thus reasonable that myxobacteria would signal their "intention" to sporulate and respond to the signal by initiating developmental sporulation. Bacteria that respond to the signal would benefit from that signal in proportion to the reliability of the signal. The reliability of the signal would be maintained if bacteria that produce the signal must
sporulate. Such a link between the signal and its message, as suggested theoretically by Zahavi (1977), would reduce mistakes brought about by growing cells that would try to exploit the tendency of other cells to respond to the signal without themselves participating in the collaborative sporulation. For example, a reliable signal may be a chemical that harms growing cells; it would be reliable because it cannot be produced by growing cells. A toxic chemical signal has another advantage because it forces other cells to stop their growth. It is interesting to note that recently it has been shown that myxobacteria produce antibiotics against myxobacteria just before the lysis of the culture (Chapter 5). Such antimyxobiotics may serve as sporulating signals and fulfill the demand of a reliable signal.

Developmental Lysis

During the sporulation process on solid media many of the myxobacteria cells lyse; the survivors have all of the enzymatic machinery necessary to feed on the dying cells and hence can exploit them as substrates (Chapter 5).

Myxobacteria aggregate as a response to signals generated by starvation even though most of them will lyse as a consequence of this response. The response is still reasonable if the likelihood of surviving as a resistant spore is even smaller when the cell remains outside the mass of cells. It is important to emphasize that during development, cell death is followed by lysis that can provide nutrients for the survivors.

Using group selection theory, it is easy to suggest an hypothesis to explain the lysis during sporulation. It is better for the group as a whole to have some cells lyse and feed others under starvation conditions then to have the entire population suffer. If a mass of cells can either make many deficient or incomplete spores of fewer mature and resistant spores, a smaller mass of mature and resistant spores has a clear advantage. Thus, a group in which some members lyse to nourish the survivors has an advantage over a group in which all cells sporulate independently when nutrients are limiting.

As pointed out earlier, group cooperation is always endangered by individual exploitation, that is, a lysis-resistant mutant will have a clear advantage during the sporulation stage. Hence it would be more logical to assume that individuals actually benefit from their adaptation to lyse upon death. Lysis is tightly coupled both in time and position with sporulation (Chapter 9). Complementation of SpoC mutants deficient in lysis and sporulation with other Spo mutants or wild-type cells restores both activities (Janssen and Dworkin 1982; Shimkets and Kaiser 1982a); so far no one has reported successful complementation of sporulation mutants that does not also result in their lysis. These observations are consistent with individual adaptation theory that suggests that the ability to lyse is correlated with the ability to sporulate.

One explanation may be that the lysis process is simply a by-product of the attempt to sporulate. Those cells that fail to sporulate, die, and their lysis is just a consequence of the specific biochemical reactions of the myxobacteria.
The assumption that individuals benefit in the cooperation may suggest an alternate explanation for the specific lytic process. The following is such a speculative hypothesis.

The lytic process is an individual adaptation that serves to attract other cells to the sporulating bacterium. Under severe starvation conditions some cells die and their lysis products can serve to nourish the others; their ability to lyse might serve as a signal to other bacteria to collaborate with it. Other things being equal, a lytic genotype should be more attractive as a collaborator for sporulation than another genotype that does not lyse when dead; a cell that would survive may benefit more from such a collaborator. We suggest that myxobacteria signal their commitment to lyse if they die and thus attract other cells to sporulate with them. A nonlytic mutant that may arise occasionally cannot signal its commitment to lyse and thus would be left out of the sporulating mass. The signal could be an altered cell wall that breaks more easily. It is interesting to note that while sporulating, rearrangements in cell wall are necessary, and the cell goes through a stage in which the cell wall sensitivity to antibiotics is altered (White et al. 1968). Moreover, cell wall debris have been reported to restore both lysis and sporulation in the deficient mutant C (Janssen and Dworkin 1982). Alternately, the occurrence of adenosine among sporulating cells (Shinkets and Dworkin 1981) might indicate, for example, a hyperpermeability of the cell envelope, serving as a signal to indicate that such a cell would lyse easily upon death. Other signals are obviously possible as long as they are reliable, that is, the signal has to be linked to the "message" in such a way that false signals could not benefit the signaler.

Conclusion

We hope that the highly abridged and speculative ideas presented in this chapter will be helpful in stimulating research on the social behavior of myxobacteria. We have attempted to understand some of the mechanisms responsible for the social interactions of myxobacteria in terms of evolution theories of social adaptation by individual selection. They were not intended to be post hoc explanations to facts that have already been established by experiments but rather to serve as a logic construction based on general assumptions concerned with the evolution of social interaction (Zahavi 1981). Such a theoretical construction may help to suggest new approaches to experimentation with myxobacteria.

Most evolutionary theories have arisen as a result of logic or mathematical models based on ideas generated from comparative observation; thus we believe that the use of microorganisms to test experimentally those theories would be most fruitful. It will be hard to consider any better candidate among bacteria than myxobacteria for testing theories concerning the evolution of social adaptation.