Discussion

Through the ages, Parasitology has made rapid perceptable progress both as a pure and applied science. The invention and consequent application of electron microscopy has contributed to a great extent as a result of which our conception has been developed about the structure and physiology of most parasitic groups, especially the Protozoa. Moreover, in the field of two branches of modern science viz., Immunology and Biochemistry astonishingly spectacular progress has been achieved.

Though ultimately different scientists define parasitism uniformly, it has been difficult to describe the term parasitism simply because of the fact that according to many authors the characteristic feature of a parasite is that it must be harm to its host. Crofton (1971) is of opinion that the term parasite should be restricted to organisms which are potentially capable of killing their hosts. According to Sokoloff (1972) the host serves as an intermediary between the parasite and the external environment in which the host is living. However, in 1982, Smyth threw light on the term ‘parasitism’ beautifully. According to him a parasite is an organism which is not only in continuous, intimate association with another organism the host (usually of a different species), but is also metabolically dependent, directly or indirectly on it to a certain extent. Thus, if a hypothetical scale is drawn with an increasing degree of metabolic dependence on their hosts, at one end is zero dependence, i.e., a free living organism and at the another end is hundred percent dependence or total parasitism. In between these two extremes lies a range of organisms which satisfy their metabolic requirements to a varying extent at the expense of the host.

The history of parasites offers basic importance of taxonomic Information constituting a basis for predictability and exploitation of all biological control potentialities. Among others, the need to evaluate the possibility of using gregarines for biological control of insect pests has also been stressed (Levine, 1977, 1988).

During the present work, studies in morphology, life history and ecological factors influencing incidence of infection of twenty four new species from the insect pests of various economically important vegetable plants, flowering plants and stored grains have been made. However, a short discussion on some aspects of the bionomics of the cephaline gregarines has been made here.

Detachment of epimerite

Different opinions have been expressed by previous workers as regards the detachment of the epimerite from the protomerite at the time of transformation into sporadin. Frenzel (1885) concluded that the epimerite slowly degenerated and was absorbed in the same way as a tadpoles’ tail. Léger and Duboscq (1902) recorded vacuole formation in Pyxina mobussi. Düke (1910) believed that vacuole formation caused detachment of the epimerite. Misra (1941) noted in Stylocephalus bahli that contrary to the observation of Düke, a bubble was formed at the end of the detached epimerite and the front end of the protomerite and this was an after-effect of detachment rather than its cause, as advocated by her.
Gametocyst and Oocyst

The gametocysts of the cephaline gregarines described in this thesis are opaque-white, milky-white, orange or lemon yellowish-orange in colour and spherical, rounded or ovoidal with thin or thick cyst wall. Sometimes cysts with one layer of ectocyst are obtained from the mid gut as well as hind gut or faecal matter. The cyst wall in most cases is double-walled. The gametocysts contain two equal or unequal gametocytes.

The gametocysts dehisce by formation of sporoducts of various sizes and numbers or by simple rupture liberating oocysts also of various shapes and sizes. The oocysts are liberated either singly or in chains. This incidence takes place outside the alimentary tract of the host. The oocysts are mostly double-walled, ovoidal, barrel-shaped, spherical and spindle shaped with eight rounded, dot like, rod like and chain like sporozoites arranged peripherally, linearly or irregularly. Such variations in the shape of the oocysts are of great importance in the taxonomy of cephaline gregarines, at least where little variations are seen in the structure of the epimerite and other features.

It has been pointed out earlier in this thesis that according to Watson (1916a), parasitism in eugregarines is "accidental" because the oocysts liberating from cysts are scattered over the grass and ground and are eaten by some host along with its food. However, in one instance, it has been observed under the laboratory condition that the cysts of Phlaebum sp. II n. sp. dehisce through a single pore with a great force so that the oocysts are thrown at a greater distance instead of crowding around it. It is likely that such a "jet publication" mechanism has been evolved in at least some eugregarine species for a better dispersal of the infecting stage, i.e., the oocyst.

Gliding Movement

In earlier separate chapters dealing with "Introduction" and "Historcial Résumé", mention has been made on the studies using Scanning Electron Microscopy for understanding the mechanism of gliding movement in the eugregarines. This is a unique type of movement where both single and paired eugregarines glide in the surrounding media without any deformation in shape. Under the light microscope there is nothing visible to reveal the gliding mechanism. Fibrils, tubules and myonemes have been suggested to be involved in gregarine movement. SEM studies have led to a wide speculation of the role of longitudinal folds in the motility of eugregarines (Vávra and Small, 1969; Hoshide, 1975; Walker et al., 1979 and many others).

In Stylocephalus sp. I n. sp., which was subjected to the SEM studies, epicyteal folds and ectoplasmic network have been revealed. Walker et al. (1979) believed that the lateral undulations of the epicyteal folds were involved in gliding movement. Further research is needed in case of Stylocephalus sp. I n. sp. to understand the mechanism of gliding movement in it.
Host-parasite specificity

It is generally believed that a particular parasite has its preference for a particular host and hence is host-specific. Fahrenholz (cited from Noble and Noble, 1982) formulated the known parasitological rule that indicates, when a parasite or related species of parasites, infests various hosts then we can conclude about the phylogeny and phylogenetic relationships between hosts and their parasites. Accordingly, the host specificity of parasites is very important in studying the evolution of their hosts.

The studies of gregarines cover only a few out of several categories of host specificities. These specificities are: ecological, physiological, intraspecific and topical (Sokoloff, 1972).

The cases of narrow host specificity of gregarines are quite numerous. This is apparently caused by the fact that not all host species are suitable for the development of gregarines. Léger and Duboscq observed that sporozoites of Porospora sp., which naturally parasitize Mollusca and Crustacea, become degenerated in an accidental host.

But the example of one species of eugregarines infecting more than one host is not very rare. Gregarina blattarum (von Siebold) has been reported by many workers from different insect hosts (Watson, 1916a). Didymophyes gigantea Stein also has been reported from different insects.

Foerster (1938b), while discussing the problem of host specificity among gregarines, stated that out of 245 gregarine species, 158 were recorded from one host, 44 species from two hosts and 43 from more than two.

Haldar et al. (1976) have recorded Quadruspinospora chakravartyi from a grasshopper Aiolopus tamulus. The parasite was earlier obtained from Spathosternum sp. by Chakraborty and Haldar (1974).

Hirmocystis victoris was reported from two coleopteran insects, belonging to two different families viz., Crypocephalus snarls Duvir. of the family Chrysomelidae and Brumus suturalis (F.) of the family Coccinellidae by Kundu and Haldar (1981b).

In a recent study by Clopton, Janovy and Percival (1992), the changes associated with halometabolic insect development make the adult gut unacceptable to gregarines that are characteristically parasites of the larval gut. In a like manner, parasites characteristic of the adult gut do not establish and grow in the larval gut.

It may be inferred from the above discussion that among gregarines there are species with narrow and broad specificity. There is no doubt that many examples of broad host specificity are the exceptional cases or due to incomplete knowledge of facts which would make them clear and understandable. A further research using cross infection and reinfection experiments can only prove decisively whether a particular species of cephaline gregarines is specific for a specific insect host. The situation has been nicely summarized by Levine (1979) who states, "One of the criteria that has been used extensively to differentiate species has been difference in host species. This criterion is justified because it recognizes failure of
transmission from one host species to another, and it has been employed in the present paper. As is true of many Protozoa, and especially of the Apicomplexa, all the stages in the life cycle are not known for many species, and even for some genera and higher taxa. As a result the names of some gregarine species are based on a best guess rather than on a solid foundation. The situation can be rectified only by future research."

**Mixed protozoan infections**

Simultaneous infections of one host by two or more gregarine parasites have been noted by some workers (Lipa, 1967). Such cases are found in *Tenebrio molitor* L. infected with *Gregarina cuneata* and *G. steini*. Similar results are observed during my studies. In six cases I have found host insects are infected with dual types of gregarine. It is observed that different types of grasshoppers are simultaneously infected with the gregarine of the genus *Quadruspinospora* and *Phlaeobum*, the tenebrionid beetle *Gonocephalus* is quite often found to be infected simultaneously with the genus *Stylocephalus* and *Gregarina* and *Tribolium castaneum* simultaneously infected with the genera *Steinina* and *Hirmocystis*.

Lipa (1967) reported that even protozoans belonging to different orders infect a particular host.

The mechanism of simultaneous infection with two or more parasites and the course of such infection are not well known and they are one of the main gaps in the knowledge on invertebrate pathology. The problem of interrelationship between various pathogens in one insect host is of main interest for invertebrate pathology.

**Hyperparasitism**

According to Manwell (1977) few opportunities to secure food and shelter are ignored in the world of living things, and this is, of course, the key to their survival. It should, therefore, come as no surprise that gregarines themselves have their protozoan parasites, and doubtless others. Indeed, there is an entire family of Microspora, the Metchnik-Kovelliidae, all of which are gregarine parasites. Hosts of the latter are usually annelids, which may be further evidence of the antiquity of both gregarines and their hyperparasites. However, some are known from other hosts. Codreanu (1967) cites a microsporan which parasitizes the gregarine *Enterocystis rithrogenae*, the host of which is the Mayfly, and Corbel (1968) mentions another microsporan which resides in a gregarine, *Gregarina causinae*, parasitic in the cricket, *Gryllus assimilis*. Parasitized gregarines from marine crabs and ascidians have also been reported, many others are probably still undiscovered. Very careful studies have, however, failed to obtain any hyperparasitic microsporan in the gregarines observed by me.

**Histopathological effects of gregarines**

Parasitologists believe that in association of host and parasite, the host plays the role of a peculiar living medium where part or complete life cycle of the parasite takes place. At the same time the host serves as an intermediary between the parasite and the external environment in which the host is living (Sokoloff, 1972).
Researchers on the parasitic infections in the insect pests have revealed that many of them are parasitized by members of the sub-kingdom Protozoa, particularly the eugregarines and microsporans. The pathogenicity of eugregarines for their hosts is not generally accepted. Since the gregarines never cause serious injury to their hosts, that is why, perhaps, they have not been studied as thoroughly as other pathogenic protozoans (Nelson, 1970). As they live in the gut lumen, many others assume that they are rather harmless commensals (Théodoridès, 1988). On the other hand, schizogregarines have long been recognized as lethal to their hosts.

According to Upa (1967), some of the protozoan parasites are not only injurious to their hosts but may also be used for the biological control of harmful and noxious insects. He does not believe in the non-pathogenicity of the eugregarines, especially for species inhabiting the gastric caeca of the hosts. By extensive studies he points out that the infection of eugregarines in caeca causes hypertrophy of the tissues involved and the rupture of the caecal wall, with subsequent entrance of bacteria that induce septicemia and consequent death of the host. The occurrence of numerous tiny cavities in the surrounding tissues are due to pinocytic action of the parasite.

Upa (1967) also suggests that mechanism of injury caused by eugregarines is the same as in the case of schizogregarines. This great similarity in the type of damage of host tissue might be due to same mechanism of food assimilation by gregarines. It is pinocytosis.

It also appears that under certain conditions eugregarine species, not ordinarily pathogenic, may become so. Such is the case with meal-worm larvae infected with Gregarina polymorpha. They are usually not significantly affected by the parasite, but are harmed when fed a suboptimal diet (Harry, 1967).

Lankesteria elarki, a parasite of the tree-hole species Aedes sierrensis, destroys epithelial cells of the mid gut and malpighian tubules of its host (Sanders and Poinar, 1973).

Upa (1967) has shown similar results in case of Ephestia kuheniella, and Amphimallon solstitialis L. infected with Leidyana ephestiae and Strictospora provincialis respectively where trophozoites almost completely destruct the epithelial cells.

Effect of internal parasite on the growth of the host

Leonova (1937) found no effect of G. polymorpha or G. minuta on Tribolium confusum. On the other hand, Sumner (1936) found that the development of Tenebrio molitor is much better when it is parasitized with gregarines. Harry (1967) states that larvae of Tenebrio molitor harbour considerable populations of gregarines in the mid gut. They are not necessary for the normal growth of the larvae and under optimal conditions of temperature, relative humidity, and diet, they do not prolong the larval period. The presence of the eugregarine, G. polymorpha (Hammerschmidt) has a considerable effect on the final pupal weight and on the ability of the larvae to complete their development.

Ashford (1970) showed that when T. castaneum larvae were parasitized with Lymphotropha triboli the larval growth rate was greatly reduced, but there was no visible effect on activity. The surviving
adults were somewhat smaller than non-parasitized adults. About 75% of the parasitized beetles had normal longevity and fecundity while the rest were almost sterile and died within 30 days.

A few workers were successful in working out in an artificial reinfection of gregarines to their natural insect pests. For example, Allegre (1948) was successful in artificially reinfecting *Gregarina rigida* (Hall) Ellis to its grasshopper host, *Malnoplus differentialis*.

A unique habitat is stored product where the insect food is evenly distributed within definite boundaries. The insects populate small foci which gradually expand. If the host does not move between foci the pathogens will not spread. The stored product is a prime media to use protozoans as preinfestation prophylactic by distributing infective qualities of the pathogens.

In their investigations on *Tribolium castaneum*, Haldar et al. (1988) have obtained gregarines infecting different food items. Pathogens infective in several species differ in pathogenicity and virulence from host to host. A dual infection in a single host may result in eventual dominance of one pathogen. Some hosts yield greater number of oocysts, while the pathogens have varying effects on longevity of hosts.

Infected larvae of *T. castaneum* with the gregarine become lethargic, the colour of their body becomes blackish with a few dark spots and these are unable to pupate, ultimately dying within one month. Both larvae and adults of *T. castaneum* became infected in the laboratory conditions by ingestion of massive doses of oocysts by later instars prevented formation of adults. Infected larvae developed at a slower rate and their ability to infest stored grains reduced to a great extent. Diseased adults died sooner or later, mated less often and laid fewer eggs than healthy ones as was also been observed by Lipa (1967).

Almost similar results were obtained during my study in the larvae and adults of *T. castaneum* infected with the gregarines of various genera.

**Environment of the insect life and Conoidasidan infections**

Foerster (1938b), Semans (1941) and others have given information on the relations between the environments of arthropod's life and infection with gregarines. Semans (1941) observed that orthopterans inhabiting environments with rich vegetations were more frequently infected than those inhabiting other biotopes. Foerster, in the same year, stressed that the insects inhabiting humid environments showed heavy infections due to the fact that under such circumstances the cysts of eugregarines had better chances for maturation and sporulation.

During my observation on insect pests of economically important plants of fruit, vegetables and also of stored grains, I have observed especially high infection of gregarines at certain particular periods. This may be due to high density of insects and individuals of various gregarines living together, and to good conditions for maturation of cysts and oocysts. However, though living under same conditions, some insects are not infected with gregarines.
Many workers (Foerster, 1938b; Upa, 1967) also noticed the same phenomenon. The occurrence of gregarines or their absence in some insects, if this does not fit the general rule, must be considered as a result of several factors, e.g., kind of food, phylogeny, pH of gut, etc.

**Food of arthropods and gregarine infections**

Food of insects, somehow, has direct effect on the nature of gregarine infection. It has already been pointed out by Lipa (1967), Sengupta and Haldar (1986) and Ghosh et al. (1987) that the food habits of insects have some role in the distribution as well as morphological variations of gregarine fauna in their guts.

During the present investigation, it has been noted with interest that the same host insect (i.e., *Tribolium castaneum*) has been found to harbour two distinctly different species of the genus *Hirmocystis* viz., *Hirmocystis* sp. I n. sp. and *Hirmocystis* sp. II n. sp. from wheat flour and gram respectively. These findings confirm the contentions of the above-mentioned authors.

Ghosh et al. (1986) reported that infected hosts exhibited the presence of gregarines belonging to not only different species but also to different genera and families. For example, they obtained *Gregarina basiconstrictonea* and *Hirmocystis oxeata* belonging the families *Gregarinidae* and *Hirmocystidae* respectively.

In course of my investigation, *T. castaneum* collected from different food sources is found to be infected with three different genera of gregarines, viz., *Hirmocystis*, *Gregarina* and *Steinina*.

Lipa (1967) observed that the insects of both larvae and adults living in the same environment and consuming the same food consequently had the identical gregarine fauna.

**Role of pH on gregarine infection**

Scarce information is available on the relationship between the occurrence of gregarines and the role of pH in the host's gut. Semans (1941) explained that due to acid reaction in their gut (pH 5.6), no gregarines were observed in Tettigonidae, but Acrididae which had pH 6.0 were heavily infected with gregarines. Ghose (1943) found that location of various gregarine species in the gut of *Tenebrio molitor* depends on pH. *G. cuneata* Stein inhabits pars cardiaca of the host where pH ranges from 4.4 to 5.8; *G. polymorpha* inhabits the ventriculus part where pH is from 6.3 to 7.5 and *G. steini* Bernadt parasitizes the part where malpighian tubules have their exits and where pH is from 5.5 to 8.2. In the present study the maximum concentration of trophozoites and sporadins have been found in the mid gut portion of the host gut where the pH generally ranges between 4.0 to 6.5.

**Evolution of gregarines**

Schrével and Philippe (1993) have put forward a possible explanation for the limitation in evolution of gregarines which, according to them, could be the type of gamogony, with its unusual cell association or syzygy between two gamonts. The definite information on evolution of major groups along with phylogenetic
framework for the Conoidasida might be obtained from the analysis of subunit ribosomal RNA (16s and 28s) of these apicomplexan parasites.

**Ecological factors influencing incidence of gregarine infection**

The successful use of eugregarines as agents for the biological control of insect pests is possible only when the nature of their incidence is known in details. It is a well-known fact that for the protozoan infection a suitable climatic condition is required. Eugregarines are mostly observed during the monsoon and post-monsoon months. In my present study I have found a close correlation between the environmental factors and infection of gregarines. It has been observed that a particular species requires a particular range of temperature, humidity and rainfall at which maximum percentage of infection is found. This leads to the obvious conclusions that the gregarines require favourable conditions of rainfall, humidity as well as temperature for their sporulation and subsequent growth in their hosts. The oocysts of the gregarines, as a rule, undergo development (i.e., sporulation) outside the host’s gut. During high temperature in summer or low temperature in winter when there is a minimum rainfall, the oocysts are subjected to desiccation and hence the incidence of infection is at a low level in these months. During the rainy seasons the average daily temperature ranges between 31.5°C to 33.7°C, but since there is sufficient daily rainfall (6.1mm to 20.5mm) and relative humidity (94.5% to 98.3%), particularly in the Gangetic West Bengal, the oocysts are subjected to least environment hazards and sporulate normally. In 1985, Patil et al., in an *in vitro* study showed that temperature of 30°C to 40°C are optimum for sporocyst formation and their maturity, while 35°C and below are most favourable for sporocyst viability. The present study also confirms their study *in vivo*.

It has been observed in the present study that a particular species of the gregarines has a peak of incidence in a particular month of a year depending upon the optimum favourable environmental conditions for the sporulation and subsequent growth in the host insect. Thus as already pointed out by Ghose et al. (1986) the incidence of infection of gregarines may be considered as an additional distinguishing feature at the species level along with morphological and morphometrical variations.

Taxonomy acts as an information storage as well as retrieval system which is a data processing science. Every biologist uses taxonomic nomenclature and description on molecular aspect of the species or parts of the species. It is now widely accepted that the major aim of the taxonomy is to produce a general purpose or natural system of classification that can be used to make inductive generalisation not only by taxonomists and phylogenists but also by geneticists, ecologists, physiologists and biochemists. The ideal taxonomic classification for them would be the one with maximum content of ecological information and maximum predictive potential in ecology. Ecological utility has to be partly sacrificed in the interest of other disciplines.

The taxonomic practice is essential for valuable and productive morphological criteria. In taxonomic practice species is treated as a random aggregate of individuals, which agrees with the diagnosis by having essential properties of the type or the specimen on which the nomenclature is based. The taxonomic species is thus a unit of convenience for the purpose of identification (Mukherjee, 1999).
Discussion

The ecologist's attention is centred on environmental interactions concerning individual, population and community. The ecological value of species depends upon the ecological information it conveys.

Concluding Remarks

In their paper on "The Gregarines", Schrével and Philippe (1993) have concluded that despite their limited distribution among animals, gregarines have realized a fantastic diversification of their cell organization. They have further stressed the need for future investigation on several aspects of gregarine biology as follows: "As shown by numerous ultrastructural studies, the trophozoites of gregarines are unique cell types in regards to many aspects of their motility, cortex organization and polarity. In contrast, their physiology and biochemistry are poorly documented, and it may be expected that new mechano-chemical mechanisms will be discovered to explain gliding motility and that new aspects of membrane permeability will be found to explain the resistance of trophozoites to the high degree of ionic variation in their environments. Many aspects of gregarine nuclear division and cytoskeleton organization must be investigated by immunological and affinity probes in order to explain the cascades of events that occur during gregarine gamogony and sporogony."