GREGARINE INFECTION OF ZYGOPTERA IN DIVERSE HABITATS

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Eugregarines inhabiting the midgut of zygopteran imagines usually appear as sublethal parasites; when present in large numbers, they enfeeble the host by damaging its gut wall. After the dragonfly adult season the gregarines survive in the habitat as extrahost oocysts. To become infective, they presumably have to pass the winter in the habitat; the infection occurs through ingestion. — In western Norway, *Pyrrhosoma nymphula* and *Enallagma cyathigerum* appear either in large, compact colonies, based on small, solitary tarns or ponds, or as marginal populations of low density, associated with streams and lakes. The intermingling of imagines from neighbouring breeding sites is insignificant. The dense colonies of small waters appear most prone to overinfection; the marginal populations harbour low numbers of gregarines and many are gregarine-free. — There is a gregarine-induced susceptibility to predation. The dense dragonfly colonies accumulate infective gregarine germs, maintaining a high infection potential for successive generations. In such populations the gregarine infection is endemic, and represents one of the selection agents.

INTRODUCTION

Dragonflies have long been known to harbour intestinal gregarines (GEUS, 1969). The hosts are usually believed to suffer only insignificant harm from their parasites. However, in cases of heavy infection gregarines have been shown to inflict severe injuries to the midgut of zygopteran imagines which impair the viability of the host (ÅBRO, 1971, 1974). Certain zygopteran species present different patterns of gregarine infection in different climatic regions; incidental observations indicate that gregarines inhabiting Odonata might occur sporadically and/or within rather restricted areas (FOERSTER, 1938a, 1938b; ÅBRO, 1974). To clarify whether the size and density of zygopteran populations or the features of their habitats interfere with the pattern of gregarine infection, several

populations of the same zygopteran species, situated fairly close to one another but living in rather different kinds of surroundings, were investigated.

The present paper, which forms part of extensive studies on gregarines in Odonata (ÅBRO, 1971, 1974, 1976) reports on the gregarine-host interrelationship in the individual zygopteran as well as on the population level under various environmental conditions, as seen during one season, and also under long-term surveillance.

STUDY SITES

The studies were performed in an area of dry and wet heath and boggy ground with bodies of oligotrophic waters, sheltered by coniferous woodland, situated near Bergen, western Norway. Within a few square kilometers, three aquatic habitats representing different kinds of breeding sites for both zygopteran species studied, were chosen for a quantification of gregarine inhabitants. For comparison several other suitable habitats of similar kinds as those especially selected for census were explored over several seasons. The three selected localities are:

(I)   A solitary tarn (110 x 40 m; alt. 150 m), surrounded by hills, mostly covered with pines and coppices of deciduous shrubs and trees, and with fringes of rush and sedge along its banks. The tarn, a breeding site for rather large colonies of zygopterans, is naturally formed as a trough with central depths of 7-8 m. It exhibits scarcely discernible changes from year to year and is presumed to be of ancient standing.

(II) A small, slow-flowing boggy stream, forming several shallow eddy pools and creeks with calm water, where the Zygoptera breed sparsely. It is situated in a narrow valley (alt. 50 m), and is surrounded by sedgy meadows and pine forest. Along the banks grow luxuriant grass and rushy strips; the fen vegetation extends into the water. The immediate surroundings have sustained rapid successions over a number of years.

(III) A lake (460 x 160 m; alt. 92 m), with marshy ground and sparse pine wood sourrounding the water on most sides. Along the edges of wide, shallow inlets, where Zygoptera breed, are broad rushy and sedgy swamps. The banks have undergone profound successions over the years; the vegetation extends into the water where peat accumulates.

The localities (I) and (II) are situated nearly 1 km apart, with broad woody hills and ridges between. The locality (III) is nearly 1.2 km away from (I) and (II) and separated from them by heath and cultivated fields.

MATERIAL AND METHODS

Zygopteran hosts

(1) *Pyrrhosoma nymphula* (Sulz), distinguished by a rather closely synchronized emergence, flies early in the year. It breeds in gently flowing waters of stream pools as well as in ponds and small lakes.

(2) *Enallagma cyathigerum* (Charp.), breeding in the same habitats as *Pyrrhosoma*, starts its emergence about the same time but has the period of emergence considerably spread out.

Gregarine parasites

The eugregarines *Hoplorhynchus oligacanthus* (Sieb.) and *Menospora polyacantha* Léger (Actinocephalidae) have been recorded from zygopteran imagines within the study area, the former predominating. Both species could be present simultaneously in a host but usually *Hoplorhynchus* was found alone. No narrow host specificity could be demonstrated; each of the gregarines seems to thrive in several dragonfly species, and a host can harbour at least two gregarine species at the same time. A few cases of probably abortive infections by gregarine species associated with hosts of other insect orders have been recorded (ÅBRO, 1974).

Total host population counts

The size of the adult populations at the tarn (locality I) has been estimated by daily collecting and counting exuviae during the time of emergence. The collections were necessarily incomplete because the exuviae are small and cryptic and may escape attention. Moreover, the emergence might be restricted in time, as in *Pyrrhosoma*, or spread over a rather long span, as in *Enallagma*. Thus the estimates of the total population during the adult season represent the minimum figures.

Marking techniques

In order to evaluate the dispersal and intermingling of imagines bred and emerged in the three chosen habitats, extensive markings were undertaken in 1975 and during a period of several years around 1975. Insects, both teneral and fully coloured, flying near the respective breeding sites, were captured, marked with site- and date-specific spots of cellulose paint, and released to be captured on subsequent occasions. For both species, the 1975 markings in all three habitats summed up to a total of 862 which resulted in 52 recaptures (6.0%). In additional years, 1020 markings were undertaken in the same habitats, giving 69 recaptures (6.8%).

Microscopy

Harmful effects of parasitizing gregarines are not easily quantified. However, a close connection between the gregarine burden of the host and the harm inflicted to it has been demonstrated (ÅBRO, 1971, 1974). In the present study, the number of trophozoites is presumed to reflect the harmful effects. As to quantification of the gregarines, fully coloured zygopterans, sampled during the middle of the adult season 1975 at the chosen localities (I), (II), and (III), were taken to the laboratory and their alimentary canal examined in aceto-orcein squash preparations. The hosts were classified (with class limits arbitrarily chosen) according to the number of trophozoites they harbored. Only counts of trophozoites (attached and free) are allowed for; gametocysts and oocysts (= "spores") were omitted and so were free sporozoites, as reliable figures for these stages were much more difficult to procure. Over several additional seasons dragonflies have been processed by conventional histotechnical procedures for examination with light or electron microscope (ÅBRO, 1974).

The same localities have been surveyed for a number of years to ascertain any changes in the infection pattern in accordance with fluctuations of the host populations.

RESULTS

DIRECT FIELD OBSERVATIONS

As a place of encounter, the isolated tarn (I) attracts considerable numbers of reproductively mature Zygoptera which space out along its banks; on fine days at the peak of the season the populations may approximate maximum density. The occurrences of imagines at breeding sites (II) and (III) never reached a similar density. As shown by sampling the larval population at these same sites did not exhibit a density similar to that found in the tarn (I) either. Collection of exuviae over several years demonstrated that the total adult Zygoptera populations at the tarn (I) could amount to 800 - 1500 individuals. On the basis of the well-synchro-nized emergence of *Pyrrhosoma nymphula,* population estimates for this species are considered most reliable.

MARKINGS AND RECAPTURES

Immediately after emergence, the imagines fly away from the water. In hot weather they disperse on the heathery hills and into surrounding woodland where they shelter and feed spending nearly two weeks. After completing this prerepro-ductive, or maturation period of adult life, they return to that water from where they emerged, in the present study indicated by recaptures of individuals marked on their maiden flignt. After attained maturation, imagines will make several returns to the water for reproduction, alternating with retreat and dispersal into surrounding woodland for feeding and roosting. In late afternoon and evening, after ending their reproductive activities at the water and before repairing to nocturnal roosting sites, the dragonflies disperse in the wood close to the water, searching for food. Mostly, roosting takes place randomly in trees and dense bushes, rarely on high grasses. It is not clear how far from the water zygopterans usually retreat; however, markings and occasional recaptures indicate that the distance seldom exceeds 200 m.

No specimen marked in any one of the sampling areas was ever found in any of the others. Despite extensive marking over several years in all chosen habitats, recaptures did not reveal intermingling among populations bred at the respective sites.

In the sparsely populated habitats, a few recaptures of markings from the maiden flight revealed the longevity of 5-6 weeks for *Pyrrhosoma,* and 3-4 weeks for *Enallagma.*

HISTOPATHOLOGY

Gregarines were usually confined to the midgut but in heavily loaded indivi-

duals, they could sometimes be found also in the fore- and hindgut. Alterations of the midgut wall have been recorded from scarcely visible to massive deteriorations of the epithelium brought about by bending movements of the crowding, detached gamonts about to associate in syzygy. With heavy infection the epithelial damages far exceed restoration by the groups of regenerative cells at the epithelial basement lamina. Apparently, a threshold of deterioration exists beyond which recovery does not occur. Even a moderate infection might cause a disappearance of the epithelial brush border and a disorganization of the peritrophic membrane, which constitutes a mechanical barrier or filter lining of the midgut. In some cases, local ulceration or ruptures of the midgut wall through which gregarines could enter the haemocoele, were recorded. Such individuals, recognized by their reduced agility and the occurrence of discoloration spots on the abdomen, were readily captured in the field. As the gregarine load increases with time, hosts of somewhat advanced age demonstrated defects most clearly.

Electron micrographs of the midgut revealed the presence of numerous short rod-shaped bacteria of an appearance conform to the *Pseudomonas* type. In healthy individuals the bacteria are confined to the gut lumen and do not seem to cause any harm. The bacteria together with fungus spores might be found among midgut content or in faeces of the hindgut; they are kept off the midgut epithelium by the peritrophic membrane. Examination of feeble and discolored dragonflies, taken in the field, revealed ulcerated areas of the midgut epithelium and sometimes local ruptures of the wall; similar bacterial rods could be found in the haemocoele next to the gut, usually associated with decomposing cells. Gram staining of the bacteria appeared variable, though mostly negative.

CENSUS OF GREGARINES

Table I presents the material from localities (I), (II) and (III), examined during the middle of the 1975 season. The hosts are classified according to the number of trophozoites they harboured. The rather low total of hosts sampled in each habitat (30–40 approx.) is due to the difficulty of obtaining ample specimens within an observation period of two weeks in the sparsely populated habitats (II) and (III). As to the infection, no significant difference between males and females could be demonstrated. Gregarines were never found in the larvae. Generally, the incidence of infected dragonflies and the average number of gregarines present in individual hosts increased with the advancement of the season and with the increased post-emergence age (ÅBRO, 1971, 1974). Consequently, the percentage of individuals without gregarines tends to diminish towards the end of the season. The presence of gregarines of different developmental stages in hosts of advanced age reflects the occurrence of reinfection in the course of adult life. There is no evidence of autoinfection (ÅBRO, 1974). With the season fading, a decrease in the average number of gregarines was recorded. A spontaneous

Table I
Gregarine infection of adult Zygoptera in three different habitats
(16-30 June 1975)

Habitat	Individuals examined	Number (%) of individuals infected by trophozoites							Individuals (%) not infected
		1-10	11-20	21-50	51-100	101-300	301-500	501-900	
Pyrrhosoma nymphula									
Solitary tarn	42	—	—	2 (4.8)	5 (11.9)	17 (40.5)	15 (35.7)	2 (4.8)	1 (2.3)
Pools & creeks of a stream	34	8 (23.5)	6 (17.7)	9 (26.5)	—	—	—	—	11 (32.3)
Inlets of a lake	31	5 (16.1)	21 (67.7)	4 (12.9)	1 (3.3)	—	—	—	—
Enallagma cyathigerum									
Solitary tarn	43	—	3 (7.0)	4 (9.3)	9 (20.9)	15 (34.9)	10 (23.3)	—	2 (4.6)
Pools & creeks of a stream	36	2 (5.6)	6 (16.6)	—	2 (5.6)	—	—	—	26 (72.2)
Inlets of a lake	35	—	13 (37.1)	5 (14.3)	—	3 (8.6)	—	—	14 (40.0)

defaunation of gregarines has not been proved.

In Table I, the figures for *Enallagma* are to be interpreted against the background of time-spread emergence, which leads to a population displaying, at any time, considerable age variation. This contrasts the situation in *Pyrrhosoma*, with its well-synchronized emergence, resulting in a more homogeneous age structure of the population. For both species the table shows that heavy infections appear at the ancient solitary tarn (habitat I), where the adult populations are large and attain high density.

A threshold host density for establishment of an infection could not be determined.

DISCUSSION

Gregarines are adapted to their hosts in the sense that the life cycle of the gregarine has to fit the dragonfly development. Different gregarine species have been found to inhabit respectively larval and imaginal stages of zygopteran species (FOERSTER, 1938a, 1938b). Unlike findings in zygopterans from other climatic regions (SCHNEIDER, 1875; FOERSTER, 1938a, 1938b; GEUS, 1969), the larvae in the present study were never found to harbour any gregarines. Thus, in the habitats studied, suitable zygopteran hosts are only temporarily available and do not survive the cold season. In the course of a zygopteran's adult life-span, gregarines could be found as oocysts (= "spores"), free sporozoites, trophozoites, attached and free gamonts, and gametocysts; occasionally all these

stages could be recognized at the same time within one and the same host (ÅBRO, 1974). Faecal pellets containing gametocysts are expelled by imagines mostly during nocturnal roosting in trees and shrubs, and probably also in daytime while roosting during intervals of cloudy or rainy weather (ÅBRO, 1976). Pellets, containing young gametocysts, drop to the damp woodland ground where the gregarine completes its extrahost development. There, the gametocysts undergo sporogony. Sooner or later they dehisce and a dispersal of oocysts, the infective stage, takes place. Outside the dragonfly flying season the gregarines persist as highly resistant free-living oocysts, presumably in a state of cryptobiosis, on the woodland ground or on watery surfaces at the zygopteran breeding sites, well suited to survive several months of exposure to the conditions of the temperate zone winter. The life-span of free oocycts must exceed the length of the interval between the successive dragonfly adult seasons. Viability tests indicate that sporozoites within oocysts have to undergo an obligate period of quiescence and freezing before excystation (ÅBRO, 1976). It is likely that extrahost oocysts have to spend a winter in the habitat to become infective.

Harmful effects of the gregarines are not always clearly defined or easily demonstrated and may depend on whether we are interpreting (1) local tissue effects of the gregarines, (2) the effects on the individual host organism, or (3) how gregarines and hosts interact on the population level.

Since zygopterans might ingest gregarine germs several times in the course of their adult life, infections tend to become more strenuous with increasing age of the hosts; the insects of advanced age will demonstrate harmful effects most markedly (ÅBRO, 1971, 1974). It should be remembered that a large output will develop from a relatively small input, as an ingested oocyst contains eight sporozoites, each of which becomes a trophozoite (ÅBRO, 1976).

In heavily infected zygopterans, gregarines act as primary agents bringing about lesions in the midgut wall through which adventitious bacteria may enter the haemocoele (ÅBRO, 1971, 1974). By entrance to the haemocoele proteolytic bacteria could give rise to septicaemia and subsequent invasion of susceptible tissues. According to DORN (1976, 1977), *Pseudomonas* bacteria, widely distributed in decomposing organic matter of soil and fresh water, appear as occasional pathogens in insects. In the present study, feeble discolored zygopterans with local damages of the gut wall, probably moribund, might be suffering from bacterial septicaemia of the *Pseudomonas* type. Circulating haemocytes appear unable to cope with the development of such sickness. *Pseudomonas aeruginosa,* the causative agent of an epidemic disease occurring in the laboratory breeds of the heteropteran *Oncopeltus fasciatus* was detected in gut content and faeces; in sick bugs the bacteria were present in the haemocoele and extra- and intracellularly in the fat body, obviously dissolving it (DORN, 1976).

As to the zygopteran species studied, the dispersal of imagines during the prereproductive maturation period appears to bring about only a slight intermix-

ture of neighbouring colonies; nor might the populations intermingle to any extent during subsequent reproductive and possible postreproductive periods, as shown by the marking experiments. The return of *Enallagma cyathigerum* and *Pyrrhosoma nymphula* to that breeding site from where they emerged, corresponds to the "homing" reported from other zygopteran species (UTZERI et al., 1976). Oviposition at the original breeding site might imply advantages for survival of the larvae.

Heavy gregarine infections contribute to a reduced average longevity (ÅBRO, 1971). In compact colonies associated with ancient, steady habitats, a gregarine--induced mortality seems to occur. Marking experiments have demonstrated that a few imagines of *Pyrrhosoma* approaching maximum life-span were either gregarine-free or only slightly infected or they appeared to have become infected late in their adult life (ÅBRO, 1971). It might also be that host individuals collected in the fading season are more tolerant to gregarine infection than the majority of those flying around the peak. Since damage from gregarines becomes discernible only in somewhat older imagines, it is uncertain to what extent the gregarine infection affects their ability to survive during the prereproductive phase and their reproductive rate. A decrease in average gregarine load recognized towards the end of the adult season (ÅBRO, 1971) could mean that heavily infected individuals, suffering high mortality, have been eliminated and slightly parasitized ones survive. On the other hand, a positive survival value appears to be correlated with the occurrence of less infected individuals of scattered marginal populations.

Since *Enallagma* and *Pyrrhosoma*, both breeding in gently flowing water, in western Norway appear in colonies of two main types, either as large compact populations centred around small and isolated aquatic habitats, or as sparse, more diffuse ones associated with wider bodies of water, the host populations seem to exhibit gregarine infection of two types. An isolated tarn or pond might produce large numbers of imagines, and such habitats tend to accumulate infective gregarine germs in the densities higher than found at localities sparsely and fortuitously populated, thus securing both high prevalence and incidence of gregarine infection and a high infection potential for successive dragonfly generations. Recurrent infections of the host seem to be a prerequisite to promote reproductive processes in the gregarines for eventually producing infective germs (ÅBRO, 1976). However, such habitats tend to produce overinfected hosts that exhibit illness. Overinfection of hosts implies a potential danger to the parasites. Although the adult dragonfly populations may vary in size from year to year, the pattern of gregarine infection is maintained in the particular habitat (ÅBRO, 1971, 1974). A dragonfly colony of a certain size and density in an ancient, imperceptibly-changing habitat seems to be essential for attaining an infection pattern of insignificant variability. Probably, in such cases gregarines and host population have had ample time to co-adapt, thus approaching a balanced state.

In such habitats gregarine infection is endemic. By way of comparison, more rapidly changing habitats, sparsely inhabited by zygopterans, hold fewer gregarine germs, dispersed over wider areas. As a result, the infective germs may encounter only a very limited range of possible hosts in succeeding generations. Diffuse, semi-isolated populations of the greater water bodies demonstrate a gregarine infection fluctuating annually with a clear prevalence of gregarine-free individuals, and when infected the hosts harbour only a few gregarines and are without discernible disease.

The age structure of the host populations represents a complicating factor. Because of the well-synchronized emergence in *Pyrrhosoma,* its adult populations are reasonably homogeneous as to the age structure, compared to those of *Enallagma.* Since the gregarine load within the lifespan of the host follows a cumulative trend, the influence on longevity appears most obvious in the *Pyrrhosoma* populations, with a reduction in the proportion of heavily infected individuals toward the end of the season. Survivors, similar to those occurring in the *Pyrrhosoma* colonies, are not so convincing in *Enallagma,* presumably because the average lifespan of adult *Enallagma* is shorter than that of *Pyrrhosoma* (ÅBRO, 1974).

Interpreting the viability of the gregarine-infected Zygoptera is difficult, since these often suffer also from mite-infestation, causing injuries to the host's cuticle/epidermis and adjoining tissues (ÅBRO, 1982). Feeble zygopterans taken in the field appeared to be of advanced age. Thus debility from normal aging processes also has to be considered. The effects of gregarines interact with those of other parasites, with weather, predators, and intra- and interspecific competitors. There is circumstantial evidence that predators selectively prey on the more heavily parasitized zygopterans which are often inferior competitors (ÅBRO, 1974). The incessant weather changes in western Norway act as stressing agents on hosts and can modify infection rates. If gregarines are capable of directly or indirectly increasing the mortality rate of the zygopteran populations, then for a stable host-gregarine relation to persist, overinfection has to be avoided. In the absence of such a regulation, the interrelationship can be stable only for short periods when a particular combination of factors controlling the density of both dragonfly and gregarine populations occurs fortuitously. The association between gregarines and zygopteran imagines lasts for a rather short period, up to a few weeks. In a context of several factors stressing the populations of adult Zygoptera, a gregarine-induced mortality appears almost entirely compensatory, not additive; hence its impact as a regulating agent on the population level is judged minimal except as an agent of selection.

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