Infracommunity level aggregation in the monogenean communities of crucian carp (*Carassius carassius*)

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SUMMARY

Aggregation is one of the distinctive features in parasite-host relationships, which has generally been studied at the level of host communities. Parasite aggregation at the infracommunity level may nevertheless be important for intraspecific interactions such as parasite mating success and opportunities for cross-fertilization. In the present paper, we studied the infracommunity aggregation of 3 highly abundant *Dactylogyrus* (Monogenea) species occurring on the gills of crucian carp (*Carassius carassius*). In line with the previous work on monogenean communities, we observed no competition between the species. At the species level, parasites were distributed unevenly on the gills showing aggregation in the majority of infracommunities. However, aggregation decreased with increasing parasite abundance, which supports the hypothesis that less aggregation may be needed to ensure successful mating when the distance to a potential mate decreases with increasing number of conspecifics. Lack of interspecific interactions, species specific site selection and the importance of study scale for aggregation patterns in dactylogyrids are discussed.

Key words: parasite community ecology, infracommunity, interspecific interactions, competition, intraspecific interactions, *Dactylogyrus*, Monogenea, *Carassius carassius*.

INTRODUCTION

Aggregation, i.e. the tendency of parasites to accumulate to certain host individuals, is a distinctive feature in most parasite-host relationships (e.g. Shaw & Dobson, 1995; Shaw, Grenfell & Dobson, 1998; Wilson *et al.* 2002) and reasons for the aggregated distribution in hosts, as well as its ecological and evolutionary consequences, comprise one of the fundamental aspects in parasitology (Combes, 2000; Hudson *et al.* 2002). Because of aggregated distribution, parasites experience varying conditions in hosts with respect to intraspecific interactions such as competition with conspecifics. Aggregation may also be beneficial for parasites as high density of conspecifics could enhance mate finding and cross-fertilization (Rohde, 1977, 1979). Studies on parasite aggregation have generally been conducted at the level of host populations but they have rarely considered aggregation at infracommunity level i.e. within host individuals. However, the distribution of conspecifics within a host may have a profound influence for intraspecific interactions between parasites. This is particularly evident in systems where parasite niche is large in relation to size of

parasites and individuals are, for instance, forced to aggregate to ensure their mating success. In this study we explored the infracommunity level aggregation of 3 monogenean species on the gills of the 'pond type' crucian carp (*Carassius carassius*).

'Pond type' crucian carp live in dense populations (Bagge, Poulin & Valtonen, 2004), where conditions are ideal for the reproduction and transmission of directly transmitted parasites. Indeed, abundance of monogeneans of the genus *Dactylogyrus* on the gills of these fish is unusually high compared to other monogenean-fish systems (e.g. Rohde, 1977, 1979; Dzika & Szymanski, 1989; Hayward, Lakshmi Perera & Rohde, 1998; Simkova *et al.* 2001) and other parasite species are virtually absent (see Bagge *et al.* 2004). Thus, these communities are ideal for studies on intraspecific and interspecific interactions. Monogenean communities are generally characterized as non-interactive with respect to interspecific interactions (Rohde, 1977, 1979; Anderson *et al.* 1993; Rohde *et al.* 1994; Bagge & Valtonen, 1996, 1999; Geets, Coene & Ollevier, 1997; Hayward *et al.* 1998; Gutièrrez & Martorelli, 1999; Gutièrrez, 2001; Simkova *et al.* 2001; Morand *et al.* 2002). However, exceptionally high parasite abundances on the gills of the crucian carp may change this assumption and make the negative interspecific associations more likely. Furthermore, monogenean parasites are usually aggregated in host populations, one possible reason for this being higher probability

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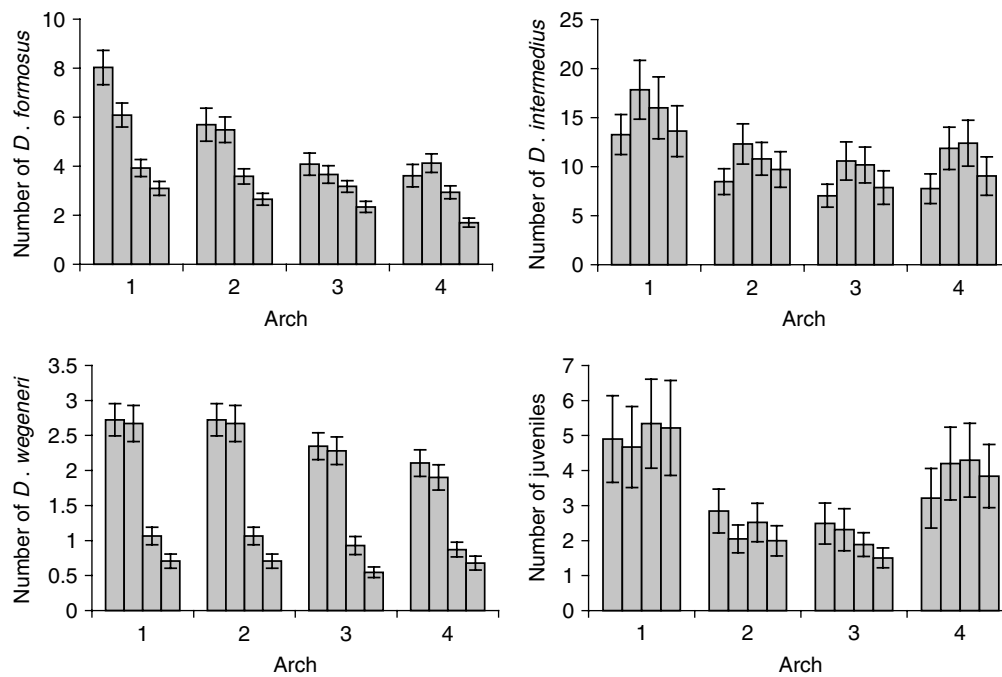
Fig. 1. Mean abundances ($\pm$ S.E.) of dactylogyrids on the gill arches (1–4) and sectors (1–4, from left to right) within each arch of the crucian carp (*Carassius carassius*). Note differences in the abundance between species.

of mate finding (Morand *et al.* 2002). Again, high parasite abundances may change this scenario as less aggregation might be needed for interactions between conspecifics.

In this study, we first explored if negative associations (competitive exclusion) occurred between parasite species on the gills of the crucian carp. Second, if no such interactions were observed, we analysed the intraspecific aggregation at the level of infracommunities in relation to the abundance of conspecific parasites. We expected that at low abundances parasites would show higher aggregation to ensure cross-fertilization whereas in higher abundances they would be less aggregated (i.e. more widely dispersed on the gills) since the distance to a potential mate is shorter.

MATERIALS AND METHODS

Parasitological analyses

During the summer of 1998, 170 'pond type' crucian carp were studied from 9 populations in Finland (Bagge *et al.* 2004). The fish were collected with fish traps and killed in the laboratory immediately prior to examination. The fish were measured for length and weight and studied for metazoan parasites from eyes, inner organs and gills. Gill arches were dissected from the left side of the fish. Each arch was divided into 4 sectors (numbered 1–4 from the outermost to the innermost) and a separate slide was prepared for each, totalling 16 sectors and slides per fish. All adult monogeneans were identified to the species level with a compound microscope

(100–400× magnification) and all post-larval forms were counted. Identification was based on the sclerified parts of the parasites (Gusev, 1985) and was performed on fresh slides. Prevalence and mean abundance (Bush *et al.* 1997) were then calculated for each parasite species.

Statistical analyses

Differences in mean parasite abundances between gill arches and sections were analysed using two-factor nested ANOVA. The analysis was performed on pooled log-transformed data excluding such ponds where the parasite species in question was absent. Significance levels were Bonferroni corrected when needed.

To explore if negative interactions occurred between the 3 *Dactylogyrus* species, we calculated Spearman correlations for all species pairs (*D. formosus*-*D. intermedius*, *D. formosus*-*D. wegeneri* and *D. intermedius*-*D. wegeneri*), which is a common method for studying interspecific interactions in parasites (e.g. Poulin & Valtonen, 2002; Vidal-Martínez & Poulin, 2003). Correlations were calculated separately within each of the 9 crucian carp populations. We analysed the interactions using data only from the first sector of the first gill arch, which provided a study scale small enough for interactions to occur and on which the abundance of all 3 species was high (see Fig. 1). Parasite numbers within this sector were also strongly correlated with the total parasite abundance on the gills in all parasite species (Spearman correlations: *D. formosus*, $r^2 = 0.871$, $P < 0.001$; *D. intermedius*, $r^2 = 0.927$, $P < 0.001$;

D. wegeneri, $r^2 = 0.550$, $P < 0.001$; juveniles, $r^2 = 0.794$, $P < 0.001$) thus providing a reasonable estimate of variance in parasite abundance. Cases where one of the species was absent from a population or where both species were absent from the sector (i.e. double-zeros), were excluded. Resulting one-tailed *P*-values for each species interaction were then analysed using the inverse chi-square method by Fisher in which a combination *P*-value is calculated from multiple independent tests (see Hedges & Olkin, 1985). In the case of positive correlation between species, the *P*-value for negative interaction was calculated as $1 - P$.

For the analysis of the relationship between aggregation and parasite abundance, pooled data from the 9 crucian carp populations were used to generate variability in parasite numbers between individual fish. We used $\mathcal{J}$ (Ives & May, 1985; Ives, 1991 *a*, *b*) as a measure of intraspecific aggregation, which is the proportional increase in the number of conspecific competitors experienced by a random individual relative to a random distribution. $\mathcal{J}$ was calculated for each parasite species at infracommunity level using parasitological data from the 16 gill sectors of each fish as:

$$\mathcal{J}_1 = \frac{\sum_{i=1}^{p} \frac{n_{1i}(n_{1i}-1)}{m_1} - m_1}{m_1} = \frac{\frac{V_1}{m_1} - 1}{m_1}$$

where n_{1i} is the number of parasites on sector *i*, m_1 is the mean number of parasites on all 16 sectors and V_1 is the variance in the number of parasites between the 16 sectors in host 1. Sectors were treated as 'independent' samples and, although this is an oversimplification because of possibility of parasite movement, it represents a snapshot of the location of parasites on the gills at one particular time. Furthermore, parasites are probably more likely to switch locations within an arch, but possibility of between-arch switches cannot be excluded and therefore we used data from 16 sectors, instead of calculating $\mathcal{J}$ separately within each arch. When $\mathcal{J} > 0$ parasites are aggregated and when $\mathcal{J} = 0$ they are randomly dispersed. Negative values of $\mathcal{J}$ indicate a tendency towards uniform distribution. The relationship between aggregation and parasite abundance was analysed for each parasite species using linear regression analysis, including cases where the parasite abundance was more than 1. All analyses were conducted in SPSS statistical package.

RESULTS

Four monogenean species were found on the gills of crucian carp: *Dactylogyrus formosus*, *D. intermedius*, *D. wegeneri* and *Gyrodactylus carassii*. The mean abundance of *G. carassii* was very low [1.6 ± 0.2 (all figures indicate mean $\pm$ S.E.)] and for the purpose of this study, we subsequently focused on the 3

Table 1. Result of the nested ANOVA on the number of dactylogyrids on the gill arches and sectors of the crucian carp (log-transformed data)

	Factor	MS	F	df	P
D. formosus	Arch	3·40	22·7	3	<0·001
	Sector	1·52	10·1	12	<0·001
	Error	0·15			
D. intermedius	Arch	1·01	2·5	3	0·056
	Sector	0·48	1·2	12	0·282
	Error	0·40			
D. wegeneri	Arch	0·62	8·3	3	<0·001
	Sector	2·48	32·9	12	<0·001
	Error	0·07			
Juveniles	Arch	1·17	6·4	3	<0·001
	Sector	0·10	0·6	12	0·865
	Error	0·18			

Dactylogyrus species. The most common species were *D. formosus* (prevalence 99%) and *D. wegeneri* (97%), which were found also in high numbers (abundances $65·0 \pm 4·5$ and $24·3 \pm 1·4$, respectively). However, the most abundant species, *D. intermedius* (mean abundance $223·0 \pm 36·1$), was not found from all populations and had a prevalence of 78% in those ponds where it was observed. *Dactylogyrus* juveniles were found on 88% of the fish, their mean abundance being $60·4 \pm 13·3$. The abundance of *D. formosus* differed between the arches and sectors (Fig. 1, Table 1) and the highest parasite numbers were observed on the first arch and on the first 2 sectors within each arch. The pattern was similar for *D. wegeneri* although the parasite abundance was lower compared to *D. formosus* (Fig. 1, Table 1). In *D. intermedius*, parasite numbers tended to be higher on the first gill arch, but this was not significant at the 5% level (Fig. 1, Table 1). Moreover, mean abundances of *D. intermedius* were higher on the second and third sector although the difference was not statistically significant. Numbers of juveniles were highest on the first and fourth arch, but no difference was observed between sectors (Fig. 1, Table 1).

Most of the interactions (Spearman correlations) between the 3 *Dactylogyrus* species were positive. Combination *P*-values for the species interactions indicated that there were no significant negative interactions between the species (tests for negative interactions: *D. formosus*-*D. intermedius*: $P = 1·57$, D.F. $= 10$, $P > 0·99$; *D. formosus*-*D. wegeneri*: $P = 2·07$, D.F. $= 18$, $P > 0·99$; *D. intermedius*-*D. wegeneri*: $P = 3·88$, D.F. $= 10$, $P > 0·95$).

Mean $\mathcal{J}$ values for *D. formosus*, *D. intermedius* and *D. wegeneri* were $0·50 \pm 0·06$, $0·42 \pm 0·07$ and $0·73 \pm 0·07$, respectively, the value for *D. wegeneri* being significantly higher compared to other species (ANOVA on log-transformed data: $F_{2,430} = 4·26$, $P < 0·05$). Mean $\mathcal{J}$ for the juvenile *Dactylogyrus* was $0·33 \pm 0·10$. Most of the individual values were positive indicating that parasites were aggregated
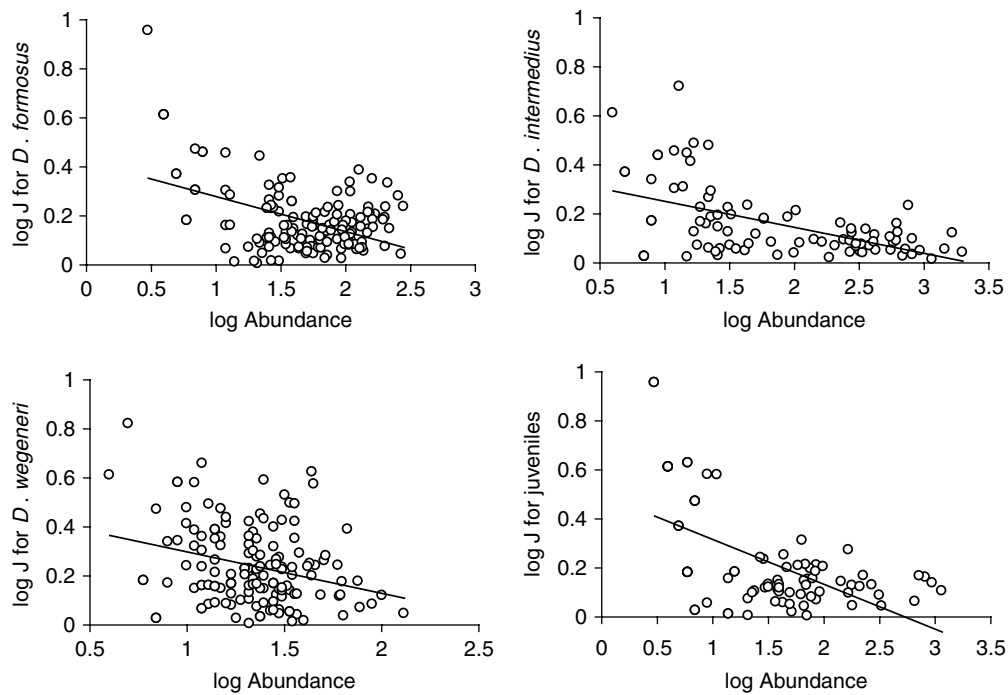
Fig. 2. Infracommunity level aggregation of dactylogyrids (J) in relation to parasite abundance. Linear regression: $r^2 = 0.21$, 0.29, 0.08 and 0.32 for *Dactylogyrus formosus*, *D. intermedius*, *D. wegeneri* and *Dactylogyrus* juveniles, respectively. Note differences in the abundance between species.

within their hosts. The proportion of negative J values was 9·5%, 10·9% and 11·0% for *D. formosus*, *D. intermedius* and *D. wegeneri*, respectively. In juveniles, however, the proportion was higher (30·7%). Negative J values were mainly associated with low parasite abundances in all parasite species and in juveniles. For the analysis of the relationships between infracommunity aggregation and parasite abundance, we excluded negative J values and performed the analysis using the values equal to or higher than zero. Infracommunity level aggregation was significantly decreased with increasing abundance in all parasite species (linear regression: *D. formosus*, $F = 39·12$, $P < 0·001$; *D. intermedius*, $F = 34·90$, $P < 0·001$; *D. wegeneri* $F = 12·37$, $P < 0·001$) i.e. parasites were more randomly dispersed within their hosts in higher abundances (Fig. 2). A similar result was found also in juveniles (linear regression: $F = 38·37$, $P < 0·001$; Fig. 2).

DISCUSSION

This study explored the patterns in monogenean community organization on the gills of the crucian carp. We focused especially on the interspecific interactions and infracommunity level aggregation in this system, which is characterized by unusually high parasite abundances compared to other monogenean systems (e.g. Rohde, 1977, 1979; Dzika & Szymanski, 1989; Hayward *et al*. 1998; Simkova *et al*. 2001). Although monogenean communities are generally characterized as non-interactive (reviewed

by Morand *et al*. 2002; Rohde, 2002), we expected that the high abundances could result as competition between species for limited space and resources. However, no evidence of competitive exclusion was observed, which supports the previous findings and indicates that gills are not likely to be saturated, but species may coexist even in high abundances. Given that no competition was observed, we subsequently focused on the intraspecific interactions, which are generally considered more important in monogenean communities (Rohde, 1979, 1991; Morand *et al*. 2002).

At component community level, we observed differences in parasite distribution on the gills both within and between species. All species preferred the first gill arch regardless of parasite abundance and fewer individuals were found on the other arches. This may be simply because of the greater area of the first arch, which probably provides higher contact area for establishing parasites and more resources to sustain a higher number of individuals (see also Geets *et al*. 1997). The preference of monogeneans for the largest arch has been described also in roach (Koskivaara, Valtonen & Vuori, 1992; Bagge & Valtonen, 1996). We also found differences between the sectors within each arch as parasites preferred the first and the second sector. However, *D. intermedius* tended to deviate from this pattern to some extent as more individuals were found on the second and third sector. This may be because of factors such as specific habitat requirements or details in the transmission process, which require further work. We also

expected that the distribution of juveniles would reflect that of the adults in proportion to their abundances (assuming equal output of offspring from the adult populations). In fact, the distribution resembled best that of the most abundant species, *D. intermedius*, although some differences were evident, which may reflect detailed mechanisms in juvenile establishment on the gills (see below).

The distribution of individuals of one species, particularly at infracommunity level, is important in determining the opportunities for mating and cross-fertilization. Parasite aggregation has generally been considered on the level of host populations (e.g. Shaw & Dobson, 1995; Shaw *et al*. 1998; Morand *et al*. 1999) and the present work represents one of the few examples conducted at the level of individual hosts. We observed that in the majority of infra-populations of each *Dactylogyrus* species, parasites showed the tendency to aggregate, which is consistent with the previous results from monogenean communities (Poulin, 1998; Morand *et al*. 2002). However, in support of our hypothesis, parasites were less aggregated at higher abundances, which may be because less aggregation is needed as the distance to a potential mate decreases with increasing number of conspecifics (see also Rohde, 1979, 1991; Geets *et al*. 1997). In such circumstances parasites could also minimize their movement on the gills and thus minimize the risk of detachment and death. However, this may not apply to juveniles, which tended to be less aggregated than adults and showed also the greatest tendency towards uniform distribution (30·7% of the infracommunities). This suggests that in order to become more aggregated in the adult phase, juveniles should be able to change their position after establishment. This would seem reasonable since the transmission of these parasites is probably a stochastic event where the establishing parasites end up in different locations randomly in relation to location of conspecifics. Thus, to find a mate, juveniles would have to move within gill arches, or even between arches, although this could be traded-off against the risk of detachment. However, processes related to parasite movement and location in respect to conspecifics in finer scale could not be analysed from the present data.

Indeed, aggregation particularly at infra-community level is likely to be highly sensitive to study scale. Dactylogyrids are very small in relation to the size of their habitat and interactions between individuals are likely to take place even at the scale of individual gill lamellae. In this study we used the aggregation measure $\mathcal{J}$ to describe the distribution of conspecifics on individual hosts by dividing the gill area into 16 sections. Although this method allowed us to obtain a reasonable estimate of parasite aggregation for the purpose of this study, it is still too coarse to reveal patterns taking place between individual parasites. Therefore, more detailed evaluation of the importance of aggregation for parasite mating opportunities in the present system, as well as in other monogenean systems, would require studies considering the location of parasites in increasingly detailed scale.

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