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Research article

Aggregation of symbionts on hosts depends on interaction type and host traits

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Symbionts tend to be aggregated on their hosts, such that few hosts harbor the majority of symbionts. This ubiquitous pattern can result from stochastic processes, but aggregation patterns may also depend on the type of host–symbiont interaction, along with traits that affect host exposure and susceptibility to symbionts. Untangling how aggregation patterns both within and among populations depend on stochastic processes, interaction type and host traits remains an outstanding challenge. Here, we address this challenge by using null models to compare aggregation patterns in a neutral system of Balanomorpha barnacles attached to patellid limpets and a host–parasite system of *Gyrodactylus* spp. monogeneans and their Trinidadian guppy *Poecilia reticulata* hosts. We first used a model to predict patterns of symbiont–host aggregation due to random partitioning of symbionts to hosts. This null model accurately predicted the aggregation of barnacles on limpets, but the degree of aggregation varied across 303 quadrats. Quadrats with larger limpets had less aggregated barnacles, whereas aggregation increased with variation in limpet size. Across 84 guppy populations, *Gyrodactylus* spp. parasites were significantly less aggregated than predicted by the null model. As in the neutral limpet–barnacle system, aggregation decreased with mean host size. Parasites were significantly less aggregated on males than females because male guppies tended to have higher prevalence and lower parasite burdens than predicted by the null model. Together, these results suggest stochastic processes can explain aggregation patterns in neutral but not parasitic systems, though in both systems host traits affect aggregation patterns. Because the distribution of symbionts on hosts can affect symbiont evolution via intraspecific interactions, and host behavior and evolution via host–symbiont interactions, identifying the drivers of aggregation enriches our understanding of host–symbiont interactions.

Keywords: aggregation, distribution, host, parasite, symbiont, traits



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Introduction

Aggregation, where the majority of hosts harbor few or no symbionts and a minority harbor the majority of symbionts, is common in nature (Castro 1978, Hopkins et al. 2015), and considered one of the few 'laws' of host–parasite systems (Poulin 2007). The distribution of symbionts among hosts can influence ecological and evolutionary processes in host–symbiont systems through multiple mechanisms (Jaenike 1996). For example, symbiont aggregation means that hosts are differentially influenced by density-dependent effects in both mutualistic (Holland and DeAngelis 2010) and parasitic systems (Brown et al. 2012). Aggregation can also influence the strength of competitive interactions between symbionts, their chances of sexual reproduction, the chance of stochastic death of portions of the symbiont population, and thus the genotypes subject to natural selection (Jaenike 1996). Examining what drives aggregation both within and among populations is therefore critical to understanding the ecology and evolution of hosts and their symbionts.

Symbiont aggregation can result from both neutral, stochastic processes and ecological and evolutionary interactions within and between hosts and symbionts (Wilson et al. 2002, Poulin 2011). Theoretical models show that introducing stochastic variation in exposure can produce aggregated distributions in host–parasite systems (Gourbière et al. 2015). Furthermore, stochastic processes and statistical constraints explain the majority of variation in aggregation patterns observed across both host–parasite (Johnson and Wilber 2017, Wilber et al. 2017) and free-living taxa including mammals, plants, and invertebrates (Xiao et al. 2015, 2016). The relative importance of such neutral processes and biological interactions in driving aggregation likely depends on the type of host–symbiont interaction (organisms living in close relationships, sensu Pianka 2011). For example, in neutral host–symbiont systems in which neither host nor symbiont receive any benefit from the association even though they continue to live in close association (Pianka 2011), stochastic processes likely dominate, but host population demography may modify patterns of aggregation. In host–parasite systems defined as relationships in which there is a negative impact on host for positive benefit of symbiont (Pianka 2011), host age and size, density, behavior, immune response, and parasite-induced mortality may all affect patterns of parasite aggregation (Anderson and Gordon 1982, Morrill and Forbes 2012, Johnson and Hoverman 2014, Wilber et al. 2017): for example, as hosts clear infections or modify their behavior to avoid infection (Johnson and Hoverman 2014, Stephenson et al. 2018). Combining neutral model predictions with multi-population comparisons between different host–symbiont interaction types offers a promising approach for disentangling how aggregation depends on stochastic processes, interaction type and host traits.

Here, we use a stochastic model, a neutral patellid limpet host–*Balanomorpha* barnacle symbiont system, and the Trinidadian guppy *Poecilia reticulata*–*Gyrodactylus* spp. host–parasite system as a case study to explore how interaction

type and host traits affect aggregation of symbionts. In the neutralistic limpet–barnacle system, barnacles settle on the shells of limpets but have no known effects on the behavior or fitness of hosts and receive no benefit from living on the host shell. We therefore predicted that aggregation in this system results principally from stochastic settlement by barnacles and may be modified by host population demography (e.g. size/age), which affects both the duration of exposure and barnacle carrying capacity. In contrast, we expect aggregation of *Gyrodactylus* spp. parasites, which cause mortality and modify the behavior of guppy hosts, to depend more on ecological context and host traits. Particularly, we expect patterns of parasite aggregation to differ across guppy populations that differ in predation regime, which affects various guppy traits that mediate their interactions with parasites (Magurran 2005, Stephenson et al. 2015a, Walsman et al. 2022). We also expect parasite aggregation differences between the sexes because male and female guppies differ in behavior and their response to gyrodactylid parasites (Stephenson et al. 2015a).

First, we use a random partitioning model to test whether stochastic processes and statistical constraints predict the distribution of symbionts among hosts in the two systems. If host–symbiont interactions affect aggregation, the model should better predict patterns of aggregation in the neutral system than in the host–parasite system (Anderson and May 1978, Anderson and Gordon 1982). Second, we test whether host traits (size and sex) and ecological context (predation regime) affect patterns of aggregation. Finally, we tested whether departures from model predictions observed in the guppy–*Gyrodactylus* spp. system resulted from changes in maximum parasite burdens or the prevalence of infection. By addressing these questions, we tested what factors affect within- and among-population variation in symbiont aggregation.

Material and methods

Limpet–barnacle natural history

Common *Patella vulgata* and black-footed *P. depressa* limpets are microphagous grazers of intertidal rocky shorelines across north-west Europe (Hawkins 1981, Jenkins and Hartnoll 2001, Moore et al. 2007). These gastropod molluscs form conical shells whose basal long-axis length can reach up to 60 mm in 15–20 years. The principal mortality threats to limpets are predation by shore birds (Coleman et al. 1999) and crabs (Silva et al. 2008), and being dislodged by waves (Hartnoll and Wright 1977).

Barnacles, *Semibalanus balanoides* and *Chthamalus montagui*, are common sessile filter feeders. Both species are hermaphroditic and release nauplius larvae which drift in currents before settling on hard surfaces in spring (*S. balanoides*) and autumn (*C. montagui*) (Southward et al. 1995). Cyprid larvae choose where they settle based on cues from conspecifics, predators, and surface topography (Crisp 1961,

Crisp et al. 1963). After settling, cyprids metamorphose into adults and grow up to 1.5 cm in diameter.

Barnacles settle on and attach to limpets, and we chose this putatively neutral symbiont–host system for a number of reasons. First, the number of barnacles that settle on a limpet is determined principally by the propagule pressure of barnacle larvae and the size of the limpet shell. Second, barnacles are sessile and do not transmit between limpets. Thus, host–host interactions do not affect the distribution of barnacles among limpets. Third, to our knowledge there is no evidence that the presence or abundance of barnacles on limpets affects limpet growth, reproduction, or survival.

Limpet–barnacle data collection

We quantified the distribution of barnacles on limpets at 11 rocky shores in Anglesey, UK. We chose sites around the island to maximize variation in barnacle larvae propagule pressure and wave exposure which, due to prevailing currents and winds, is higher on the west than the east side of the island (<https://rstudio.bangor.ac.uk/shiny/dispersal>). At each study site, we haphazardly placed 0.25-m² quadrats on bed-rock surfaces at midshore level while the tide was out. For every limpet in every quadrat, we measured the length and width at the base of the shell along with the shortest and longest distance from the base to the top of the shell. We then counted the number of adult barnacles on each limpet. At each study site we continued placing quadrats until 600 limpets were sampled. In total we sampled 7159 limpets and 25 235 barnacles in 339 quadrats. We did not identify limpets or barnacles to species level, but Anglesey is near the northern range limit of *P. depressa* (Southward et al. 1995), so the majority of limpets were likely *P. vulgata*. In our analyses, we treat each of the 339 quadrats as a sample of a limpet–barnacle host–symbiont community. This spatial scale is appropriate for this system: limpets may move less than 0.5 m over the course of 6 months (Bustamante et al. 1995) and microscale differences in wave action across rock faces can lead to differential barnacle recruitment (Bustamante et al. 1995, Southward et al. 1995).

Guppy–*Gyrodactylus* spp. natural history

Trinidadian guppies (*P. reticulata*) are small poeciliid fish that are native to freshwater streams in Trinidad, Tobago, and parts of South America (Magurran 2005). In Trinidadian rivers, waterfalls restrict the dispersal of fish predators, which has resulted in evolved differences in guppy behavior and life history in populations above (upper course, low predation pressure) and below waterfalls (lower course, high predation pressure, Reznick and Endler 1982). Guppies socially interact in loose groups, ‘shoal’, more, invest more in reproduction, and mature younger and at smaller sizes in lower course populations (Magurran 2005). Male and female guppies differ in behavior and life history: females are more social than males and have indeterminate growth, whereas males stop growing after maturity (Magurran 2005). These differences between

the sexes and ecological contexts affect how guppies interact with their monogenean parasites, principally *Gyrodactylus turnbulli* and *G. bullatarudis*. These predation regime and sex differences affect shoaling and immune investment which may impact host exposure, probability of transmission, and susceptibility to infection (Stephenson et al. 2015a). *Gyrodactylus* spp. are directly transmitted viviparous monogenean parasites that live on guppy skin, have a generation time of 24–36 h, and can complete their lifecycle (average 5.5 days) on a single host (Scott and Nokes 1984, Harris and Lyles 1992). Gyrodactylids experience exponential growth on hosts and display aggregation patterns similar to those observed in other macroparasites (Scott 1987). Indeed, the gyrodactylid Taylor power law slope (1.613 ± 0.079 SE) falls well within the range shown in other host–parasite systems (Shaw and Dobson 1995). Wild-caught female guppies typically have higher parasite burdens than males (Stephenson et al. 2015a).

Guppy–*Gyrodactylus* spp. data collection

We compiled data from 100 guppy–*Gyrodactylus* communities, comprising 5238 guppies and 7189 *Gyrodactylus* spp. parasites. Data from 80 communities were collected between 2003–2009 (44 lower course, 36 upper course, Stephenson et al. 2015a). We used similar field methods to collect data from 20 communities in March 2020 (15 lower course, 5 upper course). We used seine nets to collect whole shoals, then transported fish to the lab. Preliminary analysis suggests this transportation method does not impact the distribution of symbionts among their hosts compared to other studies in this system in which fish were either isolated after capture (Gotanda et al. 2012), or held together for shorter periods than our holding time (Martin and Johnsen 2007, Supporting information). This is in line with findings that under laboratory conditions, *Gyrodactylus* spp. can take several days to transmit between guppies, particularly at infection intensities comparable to those on wild-caught fish (Stephenson et al. 2017). At the field station, we anesthetized fish using 0.02% tricaine methanesulfonate (Tricaine-S; Syndel Laboratories) and counted the number of *Gyrodactylus* spp. using a dissecting microscope. For each fish, we recorded sex, standard length (nearest mm), and weight (nearest mg). We calculated the scaled mass index (Peig and Green 2009) as a measure of host condition separately for males and females (because the sexes have different body shapes) and watercourses (because of guppy life history differences between the courses, Magurran 2005), therefore each host’s condition is relative to individuals with similar sex and course (Stephenson et al. 2015b). Additionally, we applied our null model to males and females separately to test whether parasite aggregation patterns differ between the sexes.

Feasible set modeling

We used a random partitioning model to produce null distributions (feasible sets) of symbionts on hosts (Fig. 1, Xiao et al. 2015, 2016, Johnson and Wilber 2017, Wilber et al. 2017).

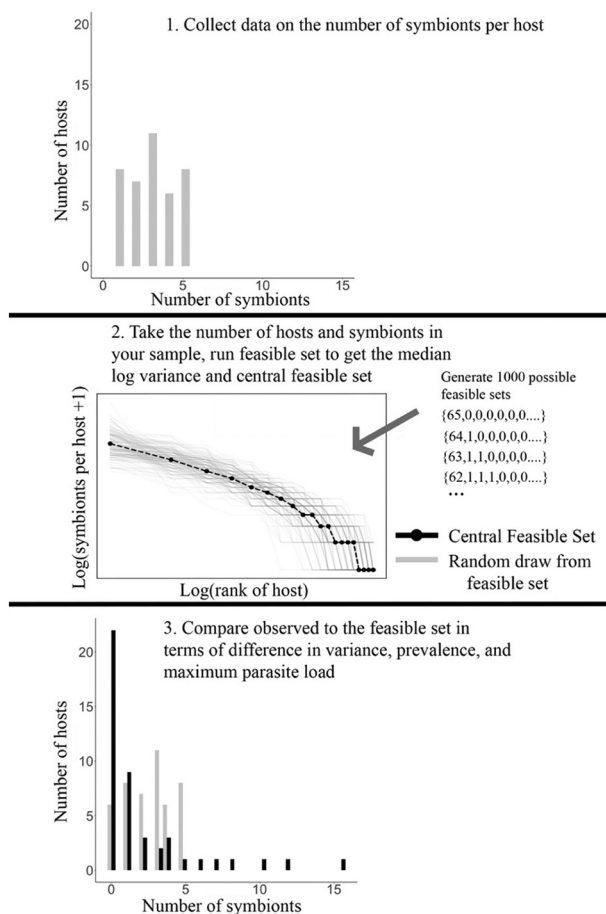


Figure 1. Using random feasible sets to explore aggregation of symbionts on hosts. 1) First, we quantify the distribution of symbionts among hosts in a natural community, illustrated by the histogram. Provided with two input variables, the total number of hosts and the total number of symbionts, the model randomly assigns symbionts to hosts. 2) For each community we ran the model 1000 times, to create a feasible set of distributions resulting from the random allocation of symbionts to hosts. These runs will create a possible macrostate of symbionts distributed among their hosts represented by the possible feasible sets. Symbionts will be unlabelled and every possible feasible set is equally likely. For each run, we take the variance in the number of symbionts per host. From the feasible set of 1000 runs, we extract: the median of the 1000 variances as a measure of expected aggregation; and the central distribution of the feasible set constructed from the median symbiont values for each host rank across all simulations. The difference between a community's observed \log_{10} (variance) and the median \log_{10} (variance) provides a measure of whether the community's symbionts are more (positive) or less (negative) aggregated than expected by the null model predictions. 3) We used the median \log_{10} (variance) calculated from the feasible set (black bars) as the null model to compare to the observed distribution (gray bars) and calculate the three response variables used in our linear mixed models: 1) the overall aggregation between the feasible set and observed distributions; 2) the difference in prevalence metric compares the proportion of infected individuals; and 3) the maximum parasite ratio compares the parasite burden of the most heavily infected hosts. The feasible set (black bars) shows a higher level of aggregation (less uniform with high peak) while the observed data (gray bars) show lower aggregation (more uniform with lower peak).

The feasible set approach overcomes the constraints of imposing Poisson or negative binomial distributions when exploring patterns in aggregation (Shaw and Dobson 1995, McVinish and Lester 2020). Previous work has robustly shown that the feasible set fits host–symbiont aggregation patterns better than the Poisson (Wilber et al. 2017) which almost universally fails at fitting host–parasite distributions (Shaw and Dobson 1995). The alternative approach of fitting a negative binomial distribution to model aggregation allows for a descriptive measure of aggregation but cannot itself demonstrate when the aggregation of a community is abnormal or what host traits may be driving it (Wilber et al. 2016, Johnson and Wilber 2017).

The feasible set produces null distributions constrained by only two values: the total number of symbionts and the total number of hosts in a sampled community. Symbionts are randomly distributed among hosts to yield one possible configuration of symbionts on hosts (Xiao et al. 2016). The feasible set is process independent and acknowledges that many different mechanisms can work together to produce similar levels of aggregation. Philosophically, the feasible set is agnostic to which mechanisms may be at play, assuming that any mechanisms that are present are working only to generate the constraints (total hosts and symbiont, Locey and White 2013).

For each limpet–barnacle and guppy–gyrodactylid community, we created a feasible set of 1000 of the possible configurations. For each configuration we calculated the \log_{10} of the variance of the number of symbionts on hosts, then extracted the median value of these log-variances. We used the difference between the \log_{10} variance observed in a community and the median \log_{10} variance of the feasible sets (observed minus feasible) as a metric of symbiont aggregation relative to that expected based on random processes and statistical constraints.

We then calculated the central feasible set to compare the two tails of the symbiont distribution from the feasible set to those in observed populations (Fig. 1). To create a central feasible set, we sampled the 1000 configurations, plotted them in the log rank curve, and took the median value at each rank. We used the tails of the central feasible set to compare how parasite prevalence and the number of parasites on the most heavily infected individual in the population differ between the central feasible set and the observed data. Therefore, the initial feasible set analysis determines whether symbionts are more or less aggregated than expected by the null model predictions, whereas the central feasible set analysis is used to identify whether departures from expectation are caused by differences in prevalence or maximum burden.

Analysis

Do feasible sets predict aggregation of barnacles on limpets?

We limited our analysis to communities with four or more hosts and five or more symbionts (avoiding the ‘forbidden

zone' Johnson and Wilber 2017). We thus analyzed data from 303 quadrats containing 6267 limpets and 24 947 barnacles. For each limpet–barnacle community, we calculated the \log_{10} variance of the number of barnacles per limpet. We then subtracted the median \log_{10} variance from the community's feasible sets (Xiao et al. 2016, Johnson and Wilber 2017), and used this difference as an aggregation metric. A positive value indicates that barnacles are more aggregated on limpets than predicted by the null model, whereas a negative value indicates the opposite.

We first applied an intercept only linear mixed model (hereafter LMM); all using the 'lme4' package in R (www.r-project.org, Bates et al. 2015) to run a t-test using Satterthwaite's method for degrees of freedom on our aggregation metric ($n=303$; $H_0=0$) to compare the distribution of barnacles on limpets in natural communities to that predicted by the feasible sets. To test whether limpet length explained variation in aggregation among the 303 communities, we used a LMM with our aggregation metric as the response variable and the mean and variance of limpet length and the mean of limpet width in a community as explanatory variables. We centered and scaled the predictor variables using the scale function for ease of comparisons between our two focal systems given the need for scaling in the guppy model. We used a random effect for site in all analyses to control for potential site effects. Collinearity of predictor variables was assessed using the pairs function (Zuur et al. 2009) as well as the 'car' (Fox and Weisberg 2019) and 'performance' (Lüdecke et al. 2021) packages. We validated this model using the 'performance' and 'DHARMA' (Hartig 2021) packages. We used conditional F-test in the Anova function in the 'car' package to test for significance.

Do feasible sets predict aggregation of *Gyrodactylus* spp. on guppies?

As above, we removed communities not satisfying the abundance criteria. The remaining 53 communities, some split by sex, yielded 86 samples with 2316 guppies and 5844 *Gyrodactylus* spp. parasites. We used the intercept only LMM to run a t-test using Satterthwaite's method for degrees of freedom with a site random effect to determine whether parasites were more or less aggregated than expected by the null model predictions ($n=86$, $H_0=0$).

We next tested whether ecological context and host traits were associated with variation in parasite aggregation using an LMM with the aggregation metric as the response variable. The model included the community's river course (upper/lower) and host sex class variables, and the mean length and variance in length of host guppies, the residual of body condition regressed against mean length of individuals to account for differences in condition based solely on mean length, and the \log_{10} of the mean parasite burden as continuous variables. We included a random effect term for community to control for non-independence of males and females within the same community. Preliminary analysis showed that the difference in \log_{10} variance (our measure of aggregation) was not

significantly correlated between communities of males and females from the same site ($t=1.644$, $df=31$, $p=0.1103$, $r=0.2832$). All continuous variables were centered and scaled using the scale function in R (www.r-project.org) due to differences in the measurement units between them. We then checked for collinearity and validated the models using the methods described above.

Do host traits affect aggregation of *Gyrodactylus* spp. by influencing prevalence or maximum parasite burden?

We asked whether the host traits identified through the analysis above as influencing parasite aggregation did so through changes in the proportion of infected individuals (prevalence), or in the number of parasites on the most heavily infected hosts (maximum burden). We first calculated the difference in prevalence between the observed and the median feasible set configuration. Second, we calculated the log ratio of maximum parasite burden between the observed samples and the median feasible set configuration. We used these as response variables in models with the same explanatory variables and random effects as the overall aggregation model described above. We checked for collinearity and validated the models using the methods described above. We used conditional F-test in the Anova function in the 'stats' package in R to test for significance. All statistical analyses were performed in R, and we provide all code, output, and plots of the model fits and validation in the supplement.

Results

Do feasible sets predict aggregation of barnacles on limpets?

The feasible sets accurately predicted the distribution of barnacles among their limpet hosts: the difference in the \log_{10} variance between the natural communities and the feasible sets was not significantly different from 0 ($t=0.323$, $df=7.988$, $p=0.755$, Fig. 2b). Across communities, barnacle aggregation decreased with the scaled mean length of limpets (-0.046 ± 0.021 SE, $F=4.544$, $df=1$, $p=0.035$, Fig. 2c), and increased with the scaled variance in limpet length (0.092 ± 0.019 SE, $F=22.515$, $df=1$, $p < 0.001$, Fig. 2d).

How do feasible sets and host traits explain the aggregation of parasitic *Gyrodactylus* spp. among their guppy hosts?

In contrast to the neutral limpet–barnacle system, *Gyrodactylus* spp. were less aggregated on their guppy hosts than predicted by the feasible sets ($t=-3.929$, $df=53.960$, $p < 0.001$, Fig. 3b). Similar to the limpet–barnacle system, parasites aggregation decreased with the mean host length (-0.113 ± 0.042 SE, $F=4.923$, $df=1$, $p=0.030$, Fig. 3c). *Gyrodactylus* spp. parasites were more aggregated among females than males (0.088 ± 0.037 SE, $F=5.4$, $df=1$, $p=0.024$, Fig. 3d), and one sample t-tests confirmed that parasites were less

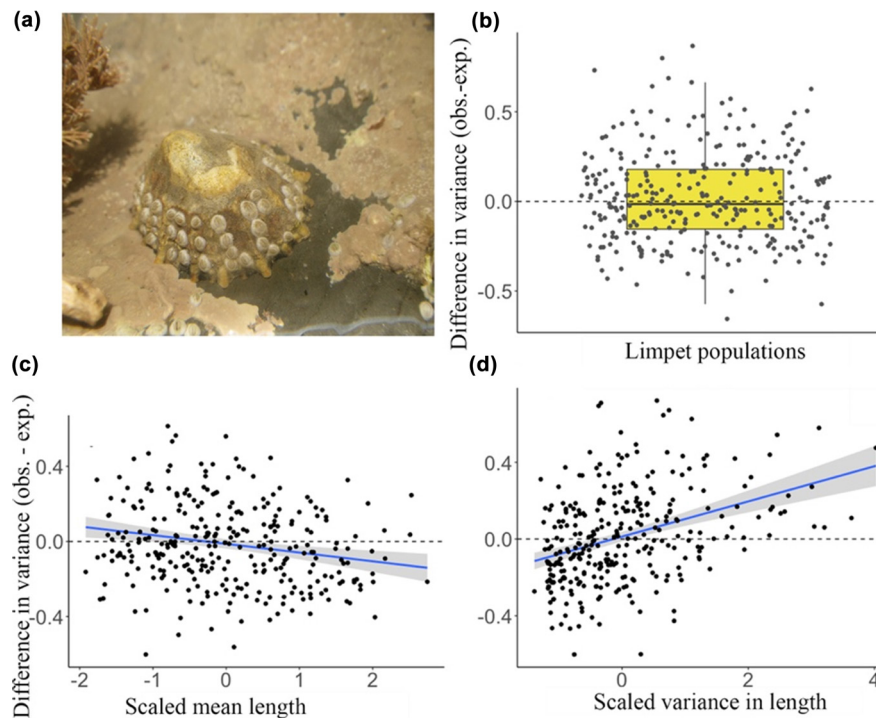


Figure 2. The feasible set accurately predicted barnacle distribution among their limpet hosts, and limpet size explained some of the variation in barnacle aggregation. (a) A photograph showing a limpet host shell encrusted with symbiotic barnacles. Photo credit: Ally Evans. (b) The difference in variance between the observed and predicted distributions did not significantly differ from zero. Points are the difference in variance for each population and are jittered horizontally for clarity. Box-plot gives the median (central line), interquartile range (box), and extremes of the data (whiskers). (c). Barnacle aggregation decreased with increasing scaled mean length of limpets within the population. (d) Barnacles were more aggregated in limpet populations with higher scaled variation in length. In (c) and (d) points are partial residuals of linear mixed models described in the main text, and the line and shading gives model fit \pm 95% confidence intervals.

aggregated on both females ($t = -2.63$, $df = 48$, $p = 0.0113$) and males ($t = -3.69$, $df = 36$, $p = 0.0007$) than predicted by the feasible set. Lastly, we found no differences in the aggregation of *Gyrodactylus* spp. between the different predation regimes (-0.031 ± 0.037 SE, $F = 0.694$, $df = 1$, $p = 0.409$).

Where does the distribution of gyrodactylids differ from the feasible sets: in prevalence or maximum parasite burden?

Guppies had a higher number of infected individuals than predicted by the central feasible set ($t = 7.06$, $df = 51.247$, $p < 0.001$, Fig. 4a). The difference between observed and predicted prevalence increased significantly with the mean length of guppy hosts (0.040 ± 0.015 SE, $F = 6.98$, $df = 1$, $p = 0.010$, Fig. 4b). Both males ($t = 4.82$, $df = 36$, $p < 0.001$) and females ($t = 5.623$, $df = 48$, $p < 0.001$) had a higher proportion of infected individuals than predicted by the central feasible set, but did not differ from each other (-0.18 ± 0.013 SE, $F = 1.78$, $df = 1$, $p = 0.187$, Fig. 4c).

Guppy hosts had a lower maximum parasite burden than predicted by the central feasible set ($t = -3.01$, $df = 53.282$, $p < 0.004$, Fig. 4d). Guppy populations with large mean body lengths tended to have lower than expected maximum parasite burdens (-0.075 ± 0.028 SE, $F = 6.733$, $df = 1$,

$p = 0.0113$, Fig. 4e). We found that the host sexes differed in how well the feasible set predicted their maximum parasite burdens (0.062 ± 0.023 SE, $F = 7.514$, $df = 1$, $p = 0.008$, Fig. 4f). The most heavily infected males had significantly fewer parasites than expected from the feasible set (post hoc t -test: $t = -3.090$, $df = 36$, $p = 0.004$), whereas the maximum parasite burden of females did not significantly differ from the feasible set ($t = -1.87$, $df = 48$, $p = 0.068$).

Discussion

We found that feasible sets accurately predicted the aggregation of symbionts on their hosts in the neutral limpet–barnacle, but not in the parasitic guppy–*Gyrodactylus* spp. system. In addition to this, the feasible set was able to capture similar levels of variation in our study relative to other host–symbiont systems (Johnson and Wilber 2017). This suggests both that the feasible set approach provides a suitable null model for predicting symbiont aggregation driven by stochastic processes, and that direct biological interactions can affect aggregation of symbionts on hosts (Anderson and May 1978, Anderson and Gordon 1982). We further found that host traits can explain some of the variation we see between populations and can affect aggregation through multiple ways. For

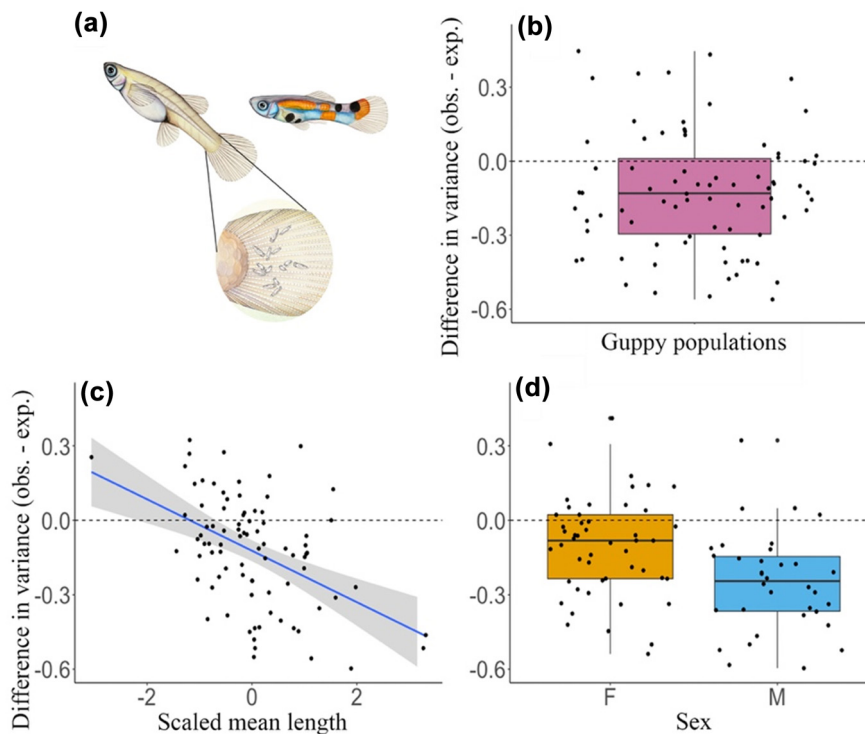


Figure 3. *Gyrodactylus* spp. were less aggregated than predicted by the feasible set, and host mean length and sex significantly predicted aggregation. (a) Illustration of a male (colourful, with modified anal fin) and female (less colourful, full anal fin). Inset shows the female tail infected with gyrodactylids. Credit: Julie Johnson, Life Science Studios. (b) *Gyrodactylus* spp. parasites were less aggregated than expected given the feasible set. (c) Aggregation decreased as guppy scaled mean length increased. Points are the partial residuals from the linear mixed model described in the main text, the line and shading give the model fit $\pm 95\%$ confidence intervals. (d) *Gyrodactylus* spp. were significantly less aggregated on both the male and female guppies than predicted by the feasible set, with aggregation significantly lower on males than females. In (b) and (d), points show the difference in variance between the observed distribution and that predicted by the feasible set for each community, and are horizontally jittered for clarity. Boxplots give the median (central line), interquartile range (box), and extremes of the data (whiskers).

example, host size often mediates interactions between hosts and symbionts (Poulin 2000, Hopkins et al. 2015), and in both systems we found that symbiont aggregation decreased with mean length of hosts in a population. Further, in the parasitic system we found that prevalence was higher than expected and increased with the mean size of hosts in a population. Host sex also appeared to affect patterns of aggregation: male, but not female, guppies had lower maximum parasite burdens than predicted by the central feasible sets. Here we discuss each of these results in turn.

While we are cautious to generalize our results as we only compare between two systems, our results suggest host–symbiont interactions can affect symbiont aggregation. In addition to interaction type, the life-histories of neutral barnacles and *Gyrodactylus* spp. parasites may also affect aggregation and differences we see between the two systems. *Gyrodactylus* spp. are direct developers, transmit through social contact between hosts, and can establish large populations from a single exposure and successful infection (Harris and Lyles 1992). Barnacles release propagules into the environment which subsequently colonize hard substrates, with some individuals settling on limpet hosts: heavily infected individuals need multiple exposures and successful infection events. However,

somewhat analogous to *Gyrodactylus* spp., barnacle colonization increases the rugosity of the limpet’s shell, promoting other barnacles to colonize more easily. This could potentially increase aggregation by making already-colonized limpet hosts more likely to obtain more symbionts and reducing the chance they establish on other individuals (Crisp 1961, Crisp et al. 1963). Previous work suggests that within-host reproduction can increase aggregation in host–parasite systems (Gear and Hudson 2011) and that aggregation may be determined by immigration relative to births on hosts (Bailey et al. 1962, Kitzes 2019). However, gyrodactylids transmitting between socially interacting hosts decreases the overall abundance and aggregation of parasites in laboratory conditions (Tadiri et al. 2018). Therefore, the differences in aggregation between our systems may be the result of their different transmission modes. Symbiont life histories, regardless of the nature of their interaction with their hosts, can have large impacts on aggregation: for example, theoretical work suggests that parasites that reproduce continuously are less aggregated than populations that reproduce or establish infections in waves (Janovy and Kutish 1988), and trophically transmitted parasites are more aggregated than those that stay at one trophic level (Lester and McVinish 2016).

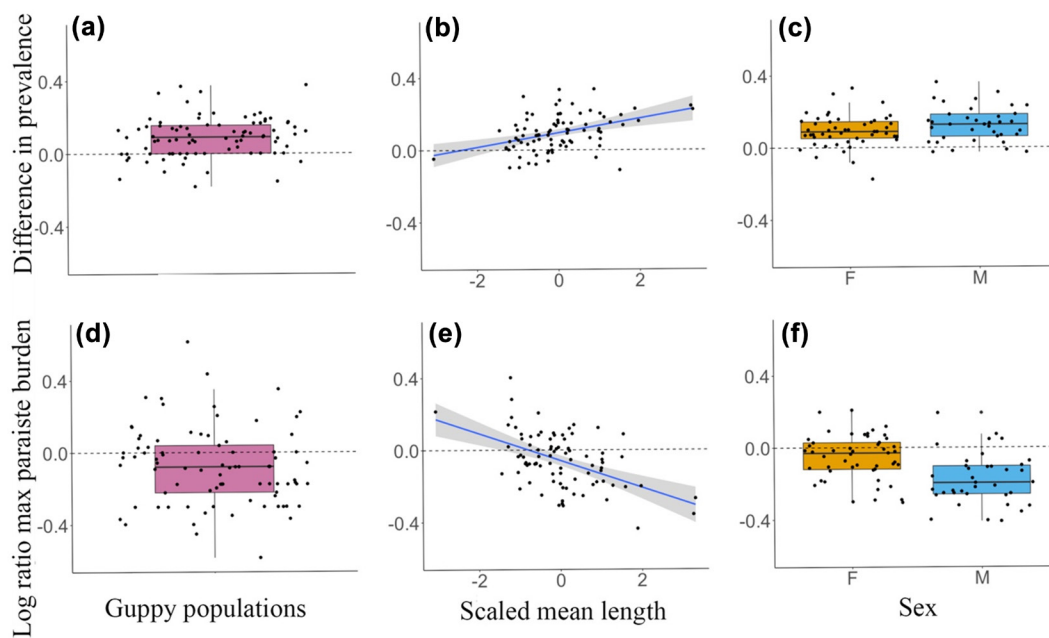


Figure 4. Host traits can drive aggregation patterns through influencing the proportion of infected individuals (prevalence) and the parasite intensities of the heaviest infected individuals. (a) *Gyrodactylus* spp. prevalence was higher than predicted by the feasible set. (b) Prevalence increased as guppy scaled mean length increased. Points are the partial residuals from a linear mixed model and the gray area around the line is the 95% confidence intervals. (c) Prevalence was higher among both male and female guppies than expected from the feasible set. (d) There were fewer parasites on the most heavily infected individual than expected from the feasible set, (e) particularly in populations of larger guppies. (f) The most heavily infected hosts had fewer parasites compared to the feasible set, among males but not females. In (a) and (d), points are the difference between observed populations and the feasible set and are jittered horizontally for clarity. In (b), (c), (e) and (f), points are the partial residuals from the linear mixed model described in the main text. In (b) and (e), the line and shading give the model fit and 95% confidence interval. In (c) and (f), points are jittered horizontally for clarity. In (a), (c), (d), and (f), box-plots give the median (central line), interquartile range (box), and extremes of the data (whiskers).

Future work could usefully apply our approach to data from a broader range of host–symbiont interactions to identify symbiont traits and interaction type that explain variation in aggregation.

In both systems, we found that symbiont aggregation decreased with increasing average host size. This relationship may partially be driven by the fact that size is an indicator of host age across both systems, and therefore provides an estimate of exposure to symbionts: populations with larger individuals may have more individuals who have been exposed for longer periods of time, accumulating symbionts, reducing aggregation. That barnacle aggregation increased with variation in limpet size is consistent with this interpretation: populations with more variation in symbiont exposure times should have more aggregated symbionts. Previous work has suggested that variation in host size could drive aggregation across many systems due to both exposure (Poulin 2013) and space available for colonization (Poulin 2000, Johnson and Hoverman 2014). While this could be a driver for both our systems, we did not find a difference between the guppy sexes in how size predicted aggregation: as females grow indeterminate and males do not, this perhaps indicates that age is not the only factor driving aggregation. Previous work in the guppy–*Gyrodactylus* spp. system suggests that larger female hosts may be able to hold larger parasite burdens

(Cable and van Oosterhout 2007, van Oosterhout et al. 2008, Stephenson et al. 2015a) without suffering as much body condition loss as males (Stephenson et al. 2015b), so larger individuals may be more tolerant of parasite infection. In both systems, populations with larger individuals may contain more individuals housing larger populations of symbionts and fewer individuals remaining uninfected due to cumulative exposure, which would decrease aggregation. This result highlights the need for further studies to disentangle the relative importance of host size, and variation in exposure, susceptibility and tolerance, in generating patterns of aggregation.

We did not find any differences in *Gyrodactylus* spp. aggregation between upper and lower course sites despite the fact that river course, and the associated difference in predation regime, is a key driver of variation in guppy size, investment in immune parameters, and social interaction, which impacts their interactions with *Gyrodactylus* spp. (van Oosterhout et al. 2003, Walsman et al. 2022). It is highly unlikely that our result reflects that course is an inadequate proxy for predation pressure: it is well-established and often-validated in this system (Reznick and Endler 1982, Magurran 2005, Stephenson et al. 2015a, Walsman et al. 2022). The lack of course differences could be driven by the fact that predation may not impact aggregation, or hosts

from these environments have many differences in their ecology not tied to predation (Magurran 2005) that could decrease aggregation. In lower course populations, individuals may face increased mortality from more virulent parasites (Walsman et al. 2022), which would decrease aggregation by eliminating the most heavily infected individuals from the population. In upper course populations, higher levels of parasite resistance (van Oosterhout et al. 2003) may result in lower maximum parasite burdens. Different mechanisms may therefore be acting to decrease aggregation across the courses, and examining differences in these traits among populations in the same course may help disentangle specific drivers of aggregation.

Host sex affected parasite aggregation in the guppy–gyrodactylid system, perhaps because of differences in parasite tolerance between sexes. Our results suggest that, even though parasites were less aggregated than expected overall, parasites were more aggregated among female than male guppies. This result was driven by males having lower maximum parasite burdens than expected, potentially because the most heavily infected males are more likely to suffer from parasite-induced host mortality (PIHM), and thus be missing from our surveys: heavily infected males are more likely to be swept downstream during flooding events and could suffer increased mortality from predators when infected (van Oosterhout et al. 2007, Stephenson et al. 2016). In contrast, we found that the maximum parasite burden was not significantly different than that predicted given the feasible set among females, supporting previous suggestions that female guppies are more tolerant of *Gyrodactylus* spp. infection than males (Stephenson et al. 2015a,b, 2016, Stephenson 2019, Jog et al. 2022).

PIHM can decrease aggregation when the host with the heaviest parasite burdens die off before they can be sampled, therefore truncating the tail of the distribution (Anderson and Gordon 1982). PIHM could therefore partially explain our aggregation and maximum parasite burden results: we found that the limpet–barnacle system, which should have the highest tolerance and lowest PIHM (as barnacles cause no harm to limpets), had the most aggregated symbionts, and male guppies, the putatively less tolerant of the sexes (Stephenson et al. 2015a, 2016), had the least aggregated symbionts, and appear to be ‘missing’ the most heavily infected individuals. These results are consistent with theoretical models that suggest the distribution and impact of PIHM can vary based on the relationship between mean parasite burden and mortality (Rousset et al. 1996). Overall, our results indicate that further exploration into how sex differences in host–parasite interactions affect population level disease dynamics is warranted.

We found that aggregation was lower than predicted because a higher proportion of guppies was infected than in the feasible set, potentially due to the transmission mode of the parasite. *Gyrodactylus* spp. rely on contact between hosts for transmission (Johnson et al. 2011), a common occurrence between socially-interacting guppies (Archard et al. 2009). Once a host becomes infected, direct parasite reproduction

can lead to heavy parasite burdens, increased transmission, and thus high prevalence, lowering parasite aggregation. Both host and parasite traits therefore likely explain why we observed lower aggregation than expected in our host–parasite system: comparisons across more systems with different traits will help to elucidate their relative importance.

Conclusion

Here, our analyses suggest that the aggregation of symbionts among their hosts differs between neutral and parasitic host–symbiont systems. These findings support previous suggestions that the type of interaction between hosts and their symbionts can shape symbiont aggregation, and we hope this will stimulate further work generalizing these patterns across interaction types. Our findings also suggest that biological and ecological differences both within and among host populations can change parasite aggregation. Elucidating the mechanisms driving aggregation can help us illuminate the role hosts and their symbionts play in driving eco-evolutionary processes.

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Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.4b8gthjx> (Clark et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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