Succinctus

Explaining parasite aggregation: more than one parasite species at a time

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A B S T R A C T

Studies generally have neglected parasite-centric views in explorations of whether the oft-seen patterns of parasite aggregation are adaptive. Using simulation models, we explored the effects of aggregation on coinfection with hetero- or conspecific parasite species characterised by different mean abundances. Increasing aggregation increased the probability of conspecific co-occurrence for parasites with low mean abundances, and increased median numbers of conspecifics for all species. In comparison, increasing aggregation generally decreased the probability, intensity and diversity of heterospecific co-occurrence, irrespective of mean abundance. Researchers should weigh the respective costs and benefits of increasing co-occurrence with conspecifics and decreasing coinfection with heterospecifics in explaining aggregation.

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Aggregation of macroparasites within host populations is a general law of parasite ecology: the majority of hosts are infected with few or no parasites of a given species, whereas a small proportion of hosts are infected with many parasites (Crofton, 1971; Shaw and Dobson, 1995; Poulin, 2007a). Most proposed mechanisms generating this near-universal pattern fall into two broad categories: environmental heterogeneity and heterogeneity in host susceptibility. That is, aggregation arises principally from variation in the distribution of hosts or infective parasite stages across space or time, or is due to variation in the potential of hosts to attract, defend against, and/or provide resources to parasites (Wilson et al., 2002; Poulin, 2007b). Additional factors suggested to influence the degree of macroparasite aggregation include direct reproduction in or on hosts, and parasite accumulation with host age or with trophic level (Poulin, 2007b; Lester and McVinish, 2016).

Among those studies exploring the causes of macroparasite aggregation, few consider adaptive benefits of aggregation. However, parasite aggregation lends stability to systems wherein a host species is attacked by multiple parasite species (Dobson, 1985; Dobson and Roberts, 1994; Morand et al., 1999; Krasnov et al., 2006) and might therefore be adaptive by reducing fitness costs to parasites. Additionally, aggregation of single-species infective stages in the environment, a cause of parasite aggregation among hosts, might be a means of overwhelming host defenses upon host encounter (Morrill and Forbes, 2016). Whether these adaptive

explanations of parasite aggregation are generalizable, remains unknown. However, cases of infective stage clumping to overcome host defenses might be evident for only some parasite-host associations or, while beneficial, might not explain entirely the degree of aggregation observed. Thus, efforts should focus on adaptive explanations for parasite aggregation that consider the potential for interactions and selection from both conspecifics and heterospecifics, and which might be candidates for universal explanations.

Such explanations will require due consideration of how distributions are experienced by parasites. Parasite distributions are typically described from a host-centric perspective using measures such as prevalence, mean intensity and various indices of aggregation, all of which are useful for describing the potential for selection on hosts (and indirectly, potential selection on parasites). However, such measures provide less insight into the infection intensities experienced by average parasites. Whereas a host may have only a low probability of being infected by many parasites when loads are aggregated, parasite individuals in those few high-intensity infections experience a high proportion of the parasite population (Poulin, 2007b).

We used simulation models to address how parasite aggregation among hosts, relative to random distributions, affects the probability and the degree of co-occurrence, or the potential for interactions, with conspecifics and heterospecifics. That is, we explored the significance of parasite aggregation in terms of its potential for heterospecific interactions among parasites within infracommunities, building on earlier perspectives and rationales (Dobson and Roberts, 1994; Krasnov et al., 2006). We did so by

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considering conspecific and heterospecific co-occurrences separately. These questions are important insofar as host individuals are generally infected by multiple parasite taxa (Petney and Andrews, 1998). From the individual parasite species' perspective, we can ask: what are the consequences of aggregation on the probability and the intensity of coinfection with one or more heterospecifics? This question implicitly ignores the direction and magnitude of potential interactions and selection imposed by conspecifics, so it is also important to ask how these heterospecific patterns compare with co-occurrence of conspecifics.

In exploring this series of questions, we could assume first that co-occurring conspecifics show a low net cost from intraspecific competition, or show a net benefit from aggregation. This could occur even if the growth, fecundity and/or survival of parasites is negatively affected at high intensities of infection if aggregation also favours the overcoming of host defenses or favours mate finding and outbreeding, given that some degree of outbreeding is likely optimal even for species capable of self-fertilisation (Vázquez-Prieto et al., 2015). We could assume, secondly, that more heterospecifics per host have a greater negative effect on the fitness of hosts and the focal parasites that are infecting them, than do fewer parasite species and parasite individuals per host. While this is likely true for hosts, it need not always be the case for parasites as some parasite species might well facilitate others in nature (Ewing et al., 1982). However, beyond such intricate interactions, net negative effects attributable to coinfection by heterospecifics are expected due to other direct and negative parasite-parasite interactions (e.g. competition for similar host resources) or due to indirect interactions through debilitation of the host (e.g. eliciting cross-immunity).

Our model simulates four parasite species, each with a different mean abundance ($\mu = 1, 2, 5$ and 10), infecting a single host population. Coinfecting parasites do not interact; the presence of one parasite species does not change the probability or degree of infection by any additional species. Rather, 1000 hosts are randomly assigned parasites from each of these species based on negative binomial distributions, which as a rule fit well to macroparasite distributions (Shaw and Dobson, 1995; Poulin, 2007b). Parasite species distributions are similarly aggregated in each trial: the parameter k, which varies inversely with aggregation, was set across all four simulated distributions at either 0.1 (highly aggregated), 1.0 (moderately aggregated), or 10 (approximately random; convergence with a Poisson distribution occurs at $k > 8$ (Southwood and Henderson, 2000)). As such, we compared the more frequently observed natural patterns of aggregation with the rarer instance of parasite species being randomly distributed among hosts to test the effects of parasite distributions on species co-occurrences and overall measures of parasitism (Shaw and Dobson, 1995). All simulations were performed using R (v. 3.1.2; R Development Core Team, 2014. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation or Statistical Computing. http://www.R-project.org/).

In every trial, conspecific and heterospecific co-occurrence was assessed from the perspective of each of four focal parasite species. For each individual parasite of each species, we recorded the total number of heterospecifics coinfecting the same host, as well as the number of co-occurring conspecifics. For example, if a single host is infected by five *A* parasites, one *B* and one *C* parasite and no *D* parasites, then for the *A* parasite each of five individuals co-occurs with four conspecifics and two heterospecifics, whereas for each *B* and *C* parasite one individual co-occurs with six heterospecifics and no conspecifics. Probabilities and median numbers of co-occurring conspecifics and heterospecifics were tallied for each parasite and averaged over all parasites within species over 1000 runs of the model for each degree of aggregation. We could then assess, from the perspective of the parasite, whether a given level

of aggregation resulted in higher or lower numbers of co-infecting conspecifics and heterospecifics than others.

The results of our simulations demonstrated that effects of aggregation on coinfecting heterospecifics were much more consistent than its effects on coinfecting conspecifics. We started first with the effects of aggregation on conspecifics. Aggregation did not have a pronounced effect on the mean probability of coinfection with conspecifics for parasite species with a high mean abundance
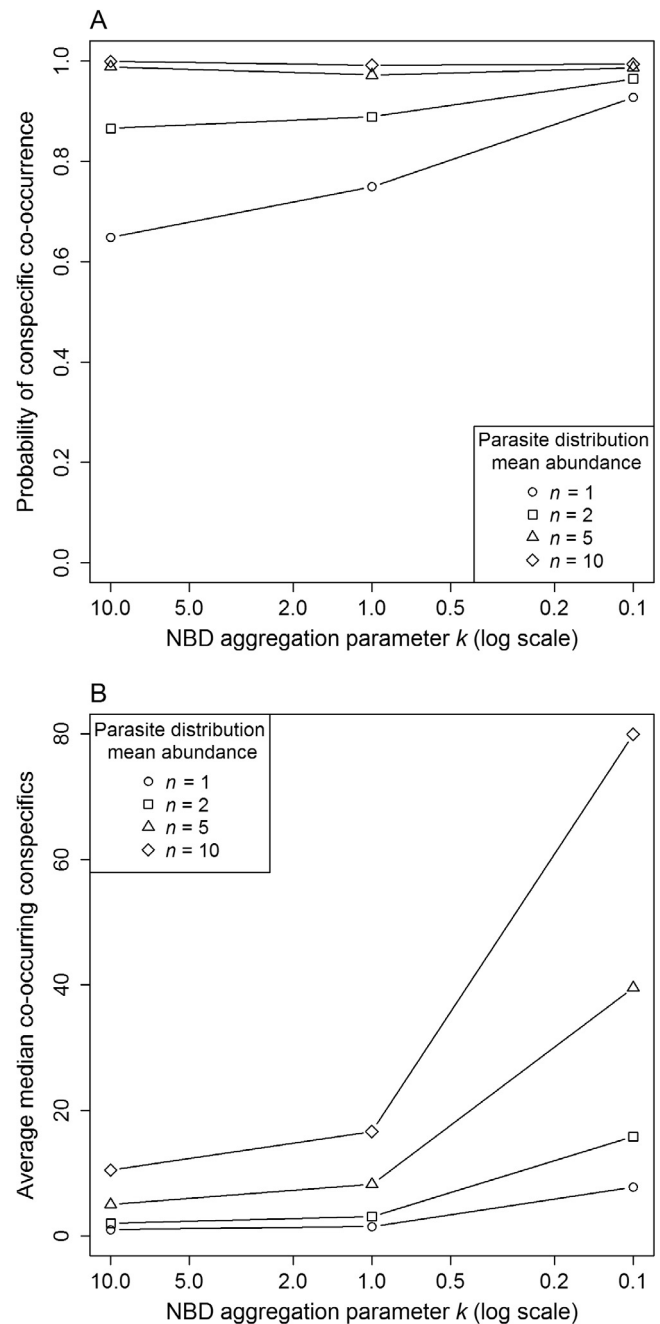

Fig. 1. Parasite perspective of the effect of macroparasite aggregation on conspecific co-occurrence for four species of macroparasites of varying mean abundances. Macroparasite distributions are simulated from negative binomial distributions (NBDs), with mean abundances ranging from $n = 1–10$ and aggregation parameters (k; inversely related to degree of aggregation) ranging from 0.1 to 10. (A) Probability of focal parasite co-occurrence with conspecifics. (B) Average median number of co-occurring conspecifics. Results are averaged over 1000 trials. Bootstrap 95% confidence intervals were small enough to fit within the plotted symbols; vertical non-overlap of points therefore indicates significant differences at $\alpha = 0.05$.

of 5 or 10 (Fig. 1A). Across all k values, the mean probability of coinfection with conspecifics for those parasite species approached 1, or absolute certainty. For parasite species with a mean abundance of 1 to 2, there was a pronounced effect of k (particularly for $k = 0.1$) on the probability of coinfection with conspecifics. At the lowest mean abundance, the parasite significantly increased its chances of co-infecting with conspecifics from 64.9% to 92.7%, for $k = 10$ versus 0.1, respectively. There was a less pronounced, but still significant, effect (from 86.6–96.5% for $k = 10$ versus 0.1, respectively) for the parasite species with a mean abundance of 2.

The effect of increasing aggregation on the mean median number of coinfecting conspecific individuals was stronger (Fig. 1B). Here, all graphs showed an upswing in numbers of coinfecting conspecific individuals at the highest aggregation ($k = 0.1$). The degree of upswing increased with mean parasite abundance. Taken collectively, the increasing probability of coinfection with conspecifics for species with low mean parasite abundance can be achieved by increasing aggregation (reducing k to 0.1). In comparison, species with high mean abundance do not have an appreciably greater probability of coinfection with conspecifics at lower values of k, but do have much greater numbers of coinfecting conspecific individuals at $k = 0.1$. In summary, aggregation generally increased the potential for conspecific interactions.

Aggregation had a pronounced and qualitatively contrasting effect on the mean probability of coinfection with parasites of a different species, for all parasite species (Fig. 2A). Here, parasites had a high probability of coinfection at $k = 10$ (always >97%) and a much lower probability of coinfection with heterospecifics (between 60% and 70%) at $k = 0.1$. Whereas the probability of coinfection with heterospecifics was dictated largely by k at its lowest value, the mean median number of coinfecting heterospecifics decreased with decreasing k and, to a lesser extent, with increasing mean abundance of the focal parasite (Fig. 2B).

The mean number of other species coinfecting with the focal parasite decreased with increasing aggregation: the average number of co-occurring heterospecific species across 1000 trials ranged from 0.80 to 0.96 (± 0.02 S.D.) for $k = 0.1$, from 2.00 to 2.41 (± 0.04) for $k = 1.0$, and from 2.43 (± 0.08) to 2.82 (± 0.09) for $k = 10$. Within each tested aggregation level, the average number of coinfecting species always decreased across these ranges with increasing mean abundance of the focal parasite. In summary, aggregation consistently decreased the potential for heterospecific interactions.

To reiterate, our approach was to consider the effect of aggregation across multiple species on patterns of coinfection while treating conspecifics separately from heterospecifics. Not surprisingly, at higher mean abundances, sharing hosts with conspecifics was likely regardless of underlying distributions, and as aggregation increased, individual parasites experienced higher average numbers of co-occurring conspecifics across all focal parasites. Morrill and Forbes (2016) demonstrated that environmental aggregation of infective stages might be expected if it increases parasite fitness by helping them overwhelm host defenses upon infection. This adaptive benefit likely would be limited by intraspecific competition, which increases with aggregation. Our results suggest that aggregation also can affect parasites by decreasing heterospecific interactions and the potential for their net negative effects. Such consequences may generally bolster the advantages of aggregation beyond what might otherwise be expected in adaptive arguments based principally on considerations of intraspecific effects.

In fact, the effects of aggregation on coinfections with heterospecifics were generalizable. Regardless of the focal parasite's mean abundance, macroparasite aggregation decreased the probability, intensity and diversity of heterospecific interactions. As such, aggregation may confer advantages with respect to the frequency or strength of interspecific interactions. Thus, there might be an advantage (or slight disadvantage) of aggregation in
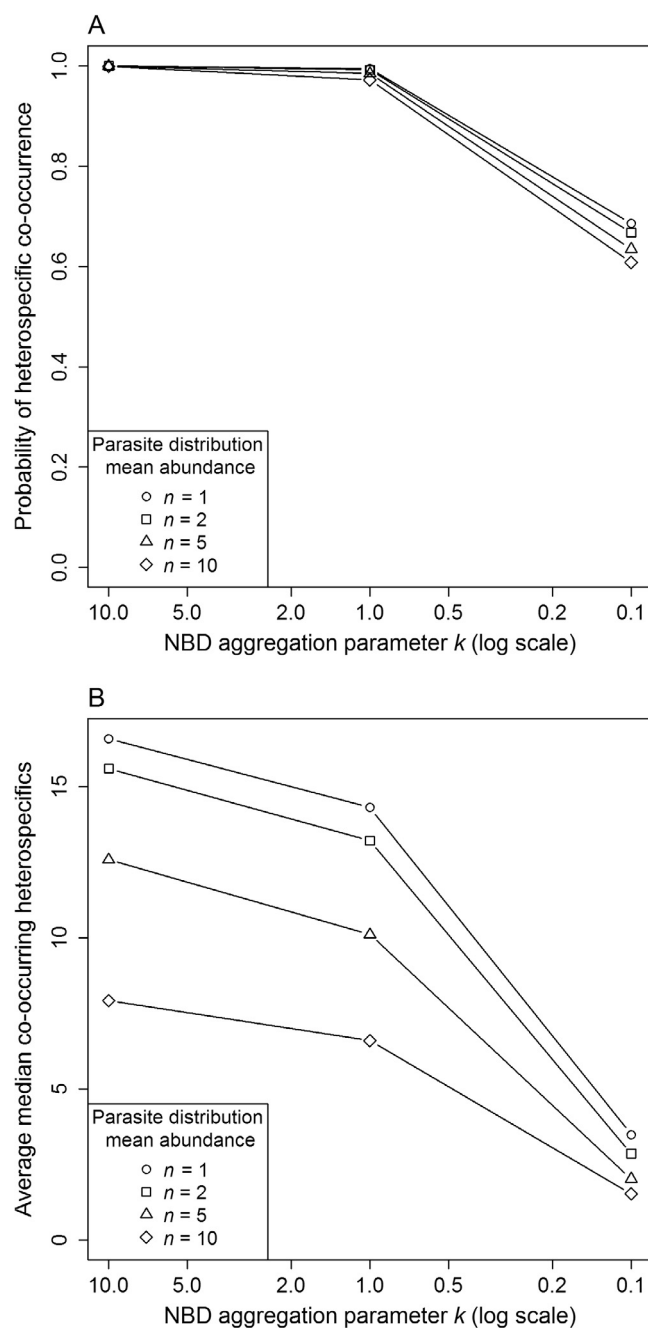

Fig. 2. Parasite perspective of the effect of macroparasite aggregation on heterospecific co-occurrence for four coinfecting species of macroparasites of varying mean abundances. Macroparasite distributions are simulated from negative binomial distributions (NBDs), with mean abundances ranging from $n = 1$–10 and aggregation parameters (k; inversely related to degree of aggregation) ranging from 0.1 to 10. (A) Probability of coinfection with non-focal parasite. (B) Average median number of coinfecting heterospecifics. Results are averaged over 1000 trials. Bootstrap 95% confidence intervals were small enough to fit within the plotted symbols; vertical non-overlap of points therefore indicates significant differences at $\alpha = 0.05$.

increasing co-occurrence of conspecifics, coupled with an advantage of reducing the potential for costly heterospecific interactions. Even if there is an overall disadvantage to co-occurring with conspecifics, aggregation could still be favoured if it lessens the costly impact from heterospecific interactions to a greater degree.

For a given parasite species, aggregation would be particularly beneficial whenever increasing coinfection with one or more parasite species quickly results in negative fitness consequences. Such

is expected if parasites are relatively large in size or numbers and similarly site-specific, and/or infection with one species elicits cross-immunity (Lello et al., 2004; Bush and Malenke, 2008). Because aggregation should decrease heterospecific encounter, aggregation may increase with higher similarity in site specificity or overlap between coinfecting species, or decrease with lower site-specificity as the parasites are freer to avoid direct competition for resources (see Lo and Morand, 2001). Selection on aggregation is likely affected both by the magnitude and directions of heterospecific and conspecific interactions, operating simultaneously. How labile aggregation is and whether it is context-dependent (e.g., dependent on whether or not other coexisting parasites share similar host resources) has not been studied.

We recognise that some parasite species might be facilitative, i.e. cause increases in individual fitness of other parasites during coinfection. Such recipient species might show less parasite aggregation than would otherwise be expected. If parasites experience mutually increased fitness through coinfection, then these species should demonstrate less aggregation among hosts, but would require a paucity of more competitively interacting heterospecifics in infracommunities. Selection toward less aggregation also assumes increasing coinfection does not reduce host fitness substantially. The consequences of coinfection from the host's perspective, which in turn can mediate consequences of coinfection for parasites through upregulated immunity or parasite-induced host mortality, could curb the adaptive benefits of random parasite distributions, even given facilitation.

Importantly, we simulated four coinfecting parasite species with mean abundances varying from 1 to 10, a realistic range when considering prevalent parasites in a given system (Karvonen et al., 2015; Loxton et al., 2016). However, we expect that our simulations would produce qualitatively similar results were the numbers and types of coinfecting species changed. Aggregation of macroparasites increases the probability of conspecific encounter from the perspective of the parasite, while decreasing the probability, intensity and diversity of heterospecific interactions more or less independently of species' specific traits (i.e. mean abundances).

The effects of aggregation on conspecific and heterospecific interactions (Figs. 1 and 2) are most significant at the highest tested degree of aggregation, $k = 0.1$. The biological relevance of these findings is contingent on how regularly this level of aggregation is observed in natural parasite systems. In a review of macroparasite distributions across a range of parasite taxa, Shaw and Dobson (1995) demonstrated that the vast majority of estimated k values fell within the 0–0.5 range, with even that subsample being highly positively skewed (over-represented towards $k = 0$). We therefore suggest that the results demonstrated herein represent real consequences of higher aggregation and are relevant in nature.

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