Drivers of aggregation in a novel arboreal parasite: the influence of host size and infra-populations

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A B S T R A C T

As a novel arboreal parasite, New Zealand’s largest endemic moth, Aenetus virescens, is a biological oddity. With arguably the most unusual lepidopteran life history on earth, larvae grow to 100 mm, spending ~6 years as wood-boring parasites feeding on host tree phloem. Parasite fitness is a product of host suitability. Parasite discrimination between heterogeneous hosts in fragmented populations shapes parasite aggregation. We investigated whether A. virescens aggregation among hosts occurs randomly (target area effect), or if larvae select hosts based on host quality (ideal free distribution). Using long-term larval growth as an indicator of energy intake, we examined A. virescens aggregation in relation to host size and infra-population. Using a generalised linear model, the relationship between parasite intensity and host tree size was analysed. Reduced major axis regression was used to evaluate A. virescens growth after 1 year. Linear mixed-effects models inferred the influence of parasite infra-population on parasite growth, with host tree size as a random factor. Results indicate parasite intensity scaled positively with host size. Furthermore, parasite growth remained consistent throughout ontogeny regardless of host size or parasite infra-population. Aenetus virescens aggregation among hosts violates the ideal free distribution hypothesis, occurring instead as a result of host size, supporting the target area effect.

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1. Introduction

Host quality determines parasite growth, reproductive success, and survival (Théron et al., 1998; Poulin et al., 2003; Barber, 2005; Tschirren et al., 2007; Poulin and Forbes, 2011). Parasites aggregate, whereby few parasites infect many hosts and many parasites infect few hosts (Shaw et al., 1998; Tschirren et al., 2007; Calabrese et al., 2011; Poulin and Forbes, 2011; Poulin, 2013). Understanding parasite distribution patterns among hosts is crucial for accurate modelling of population dynamics (Poulin, 2000). Among hosts, exposure and susceptibility to parasites is heterogeneous, correlating with host size and age, driving parasite aggregation (Grutter and Poulin, 1998; Shaw et al., 1998; Poulin, 2013). Heterogeneity in host nutritional quality also influences parasite aggregation (Poulin et al., 2003; Tschirren et al., 2007). Endoparasites encounter finite resources (Poulin, 2007, 2013; Randhawa and Poulin, 2009; Daniels et al., 2013) and intensity-dependent resource competition reduces host quality, regulating parasite body size (Poulin, 1999; Barber, 2005). Furthermore, parasite dissemination and infection mode influence host selection (Lester, 2012; Poulin, 2013). Overall parasite success relies on discriminating between heterogeneous hosts in fragmented populations (Théron et al., 1998). Host selection is particularly significant for New Zealand’s largest endemic moth, Aenetus virescens (Doubleday) (Lepidoptera: Hepialidae), which has arguably the most unique lepidopteran life history on earth.

Aenetus virescens is a biological oddity. Male moths attain a wingspan of 150 mm (Fig. 1A) (Grehan, 1981, 1984). In flight, female moths oviposit eggs onto forest floors where first larval instars are mycophagous (Grehan, 1981, 1984; Tobi et al., 1993). Subsequent larvae select, ascend and parasitise a host tree, excavating a “7”-shaped tunnel into tree heartwood. Larvae remain enclosed for ~6 years, growing to 100 mm (Fig. 1B), feeding on phloem tissue at the tunnel entrance (Grehan, 1981, 1983, 1984; Tobi et al., 1993). A feeding scar is created, surrounding the tunnel opening (Fig. 1C). Constructions of silk and frass webbing cover feeding scars, behind which larvae feed nocturnally (Grehan, 1984; Tobi et al., 1993). Webbing potentially conceals larvae from predatory North Island kaka, Nestor meridionalis septentrionalis, or from invertebrates such as tree weta, Hemidenina spp., that shelter in vacated tunnels. Larvae remain solitary and concealed throughout arboreal development, with no direct conspecific interaction until mating after emergence. Larvae pupate within the tunnel entrance, emerging as moths in summer with no functioning
mouth parts and survive only 1–2 days (Grehan, 1981). Despite this remarkable parasitic life history, the ecological role of *A. virescens*, in particular the relationship with host trees, is poorly understood.

Host trees parallel islands, comprising large, complex, ecological entities surrounded by uninhabitable space (Kuris et al., 1980; Southwood and Kennedy, 1983). The Ideal Free Distribution theory (IFD) predicts free and mobile organisms select habitat “islands” providing the greatest reward (Tregenza, 1995; Tyler and Gilliam, 1995; Stewart and Komers, 2012; Williams et al., 2013). Beneath tree outer bark, phloem carries photosynthates from the canopy to the roots (Högberg et al., 2001; Zwieniecki et al., 2004; Pompon et al., 2011). Phloem thickness scales strongly with tree diameter (Ammann, 1969; Speights and Conway, 2010; Hölttä et al., 2013; Davis and Hofstetter, 2014), and the sole foodstuff of *A. virescens*. Conversely, the hydrostatic conductance of phloem is reduced in older, larger trees, indicating a decreased flow of photosynthates (Yoder et al., 1994; Bond, 2000; Hölttä et al., 2013). Whilst larger trees present a greater quantity of food, nutritional quality is likely reduced. If *A. virescens* discriminate between hosts with varying nutritional benefits, smaller trees will have a greater parasite intensity. However, the Target Area Effect (TAE) states larger habitable “islands” merely represent larger targets more easily intercepted by randomly dispersing individuals (MacArthur and Wilson, 1967; Lomolino, 1990; Matter and Roland, 2003). For example, larger hosts present larger surface areas, and thus an increased chance of discovery by parasites (Poulin, 2013). Moreover, larger hosts tend to be older and have had longer to accrue parasites (Poulin, 2013). If *A. virescens* disperse randomly, larger trees will have greater parasite intensity than smaller trees.

Energy intake scales with body size and growth (see Lindstedt et al., 1986; Keeley and Grant, 1995; Greenleaf et al., 2007). When *A. virescens* feed, phloem tissue is masticated and the contents ingested. The resulting feeding scar is indicative of the phloem quantity consumed by an individual larva. If larvae can discern host quality, we examine how host size and parasite intensity drive *A. virescens* aggregation. We answer the following three questions. (i) Does parasite intensity scale positively with host size? (ii) Does parasite body size scale positively with feeding scar size? (iii) Is parasite growth intensity-dependent?

2. Materials and methods

2.1. Study site

Data were collected in Zealandia, a mainland island reserve at the southern tip of the North Island, New Zealand (41°28’S, 174°74’W). The climate is mild and temperate, with elevations ranging from 160 to 380 m above sea level (m.a.s.l.) (Blick et al., 2008). Primary forest was cleared for agriculture in the late 1800s (Blick et al., 2008; Burns et al., 2012; Burns, 2012) and Zealandia now comprises 225 ha of successional broadleaf-conifer forest enclosed by a mammal-resistant fence. Introduced mammalian predators were eradicated in 2000, aiding the re-introduction of native fauna and flora, in particular native birds (Burns, 2012). Dominant broadleaf evergreen trees such as *Coprosma* spp. (Rubieaeae), *Melicytus ramiflorus* (Violaceae), *Aristotelia serrata* (Elaeocarpaceae), *Pseudopanax arboreus* (Araliaceae), *Dysoxylum spectabile* (Meliaceae) and *Scheflera digitata* (Araliaceae) are common. The dense understorey comprises tree ferns, *Cythea* spp. (Cytheaceae), shrubs including *Brachythecium repandum* (Asteraceae), *Geniostoma rupestre var. languioliium* (Loganiaceae), *Piper excelsum* (Piperaceae), and vines such as *Rhipogonum scandens* (Ripogonaceae).

Fig. 1. New Zealand’s largest endemic moth, *Aenetus virescens*. (A) Adult moth (female), (B) parasitic arboreal-phase larva, (C) parasite feeding scar on *Aristotelia serrata* host tree, covered with silk and frass webbing (webbing is outlined).
2.2. Data collection and analysis

2.2.1. Parasite intensity and host size

Parasite intensity, tree height and tree diameter at breast height (DBH = 1.25 m) were recorded for A. serrata host trees (n = 63). Host size was calculated as tree bole surface area. Tree boles were considered conical in shape, starting wide at the base, tapering towards the crown. We calculated the surface area of the cone-shaped tree bole as

\[ HS = \pi rs + \pi r^2 \]

where \( HS \) is host size, \( r \) is radius of tree bole, and \( s \) is slant of tree bole. Slant was calculated as

\[ s = \sqrt{r^2 + h^2} \]

where \( h \) is height. Tree canopies were discounted from host size calculations as parasites were observed in tree boles only. We used a generalised linear model (GLM, family = poisson) to analyse the influence of host size on parasite intensity.

2.2.2. Parasite size and feeding scar

Long-term growth of parasites was established via feeding scars. We measured the size (cm\(^2\)) of all feeding scars with larvae present in February 2013 (feeding scar, \( FA_{2013} \); n = 116) using image analysis software, imageJ (Abramoff et al., 2004). A random subset of parasites (n = 18) was selected to analyse the relationship between feeding scar and larval body size. Larvae were weighed in the field. Head width, tail width, body length and volume were measured in the laboratory using digital calipers. A pairwise correlation matrix deduced the allometric relationships between larval body parts. Principal component analysis (PCA) provided a representative parasite body size (PCA1). We used reduced major axis regression (RMA) to analyse the relationship between feeding scar size and PCA1, providing slope and intercept parameters (±95% confidence limits (CI)). The slope and intercept parameters estimated parasite body size for all feeding scars where larvae were not collected (n = 98). One year later (\( FA_{2014} \)), 57 feeding scars were randomly selected and their size (cm\(^2\)) measured. The RMA slope and intercept parameters were used to provide an estimated size for \( FA_{2014} \) based on calculated parasite sizes. A paired t-test compared estimated \( FA_{2014} \) with actual \( FA_{2014} \). A non-significant difference allowed the feeding scar to become a proxy for parasite body size. Larval growth was calculated across ontogeny as the difference between \( FA_{2013} \) and \( FA_{2014} \) (n = 57). Using RMA slope and intercept parameters (±95% CI) we established whether the relationship between \( FA_{2013} \) and \( FA_{2014} \) differed from isometry (1:1). A simple linear regression analysis of the influence of host size on parasite growth.

2.2.3. Parasite growth and intensity

Within an “island”, available energy is a function of competitor intensity and discrete resources (Tregenza, 1995; Randhawa and Poulin, 2009; Tseng and Myers, 2014), with host saturation reducing host quality (Kaplan and Denno, 2007). Accounting for non-independence of parasites sharing a host, the influence of “tree” was included as a random factor in linear mixed-effects models. Models analysed whether larval growth was influenced by (i) the sum of conspecific larvae in a host tree, (ii) the sum of conspecific larvae occurring above a focal individual in a host tree (upstream competitors), (iii) the summed size of feeding scars with larvae present in a host tree, and (iv) the summed size of feeding scars with larvae present, occurring above the focal individual in a host tree. For each model a likelihood ratio test was executed to elucidate the degree of influence each independent variable had on larval growth. All variables, excluding “tree”, were square root transformed to meet normality assumptions.

Data analysis was performed in R version 3.1.0. (R Core Team, 2014). The package “smart” was used to conduct RMA analyses (Warton et al., 2012). Linear mixed-effects models, including the likelihood ratio tests, were executed using the package “lme4” (CRAN.R-project.org/package = lme4). P < 0.05 was considered statistically significant.

3. Results

3.1. Parasite intensity and host size

Data were obtained from 63 A. serrata host trees. Host size varied from 25.53 m\(^2\) to 247.20 m\(^2\) (median = 107.11 m\(^2\)). Parasite prevalence was high with 73.02% of hosts harbouring at least one parasite. The number of feeding scars per infected host varied from 1 to 64 (median = 4), with 306 feeding scars in total. Live parasites accounted for 116 feeding scars, varying from 1 to 19 (median = 2) per infected host. Furthermore, 190 feeding scars were post-parasite (the parasite had emerged), varying from 1 to 45 (median = 2.5) per infected host. Parasite intensity scaled positively with host size (GLM: \( F = 31.49, df = 55, P < 0.001 \), Fig. 2).

3.2. Parasite size and feeding scar

Parasite weight, head width, tail width, length and volume scaled positively with each other; parasite length being the principal component (PCA1) (Supplementary Fig. S1). Parasite body size scaled positively with \( FA_{2013} \) (\( R^2 = 0.75; F = 50.63, df = 16, P < 0.001 \)). Based on calculated parasite body size from \( FA_{2013} \), we found no significant difference between estimated and actual \( FA_{2014} \) (\( t = -0.0001, df = 17, P = 0.9999 \)). Henceforth, feeding scar was a proxy for parasite body size. The size of \( FA_{2013} \) scaled positively with the size of \( FA_{2014} \) (RMA regression: \( R^2 = 0.76; P < 0.001 \), Fig. 3). All feeding scars increased in size, differing from isometry. However, growth did not vary significantly with parasite size, remaining consistent throughout ontogeny (slope: 1.07, 95% CI = 0.94–1.22; intercept: 1.44, 95% CI = 0.24–2.63, Fig. 3).

3.3. Parasite growth and intensity

Host size did not significantly influence larval growth (\( R^2 = 0.02; F = 2.034, df = 55, P = 0.16 \)). Furthermore, when host tree was included as a random factor, parasite intensity did not influ-
parasite intensity commonly scales with host size (see Poulin, positively with host size. A prominent pattern in parasite ecology, correlations between host size and parasite intensity, although

(2000; Poulin and Morand, 2000). In particular, a meta-analysis of 76 different fish host–parasite relationships found positive correlations between host size and parasite intensity, although

ence parasite growth. Growth was not significantly influenced by the sum of conspecific larvae in a host tree ($\chi^2 = 0.0536$, df = 4, $P = 0.8169$, Fig. 4A), the sum of conspecific larvae occurring above a focal individual in a host tree (upstream competitors) ($\chi^2 = 2.614$, df = 4, $P = 0.1056$, Fig. 4B), the summed size of all feeding scars with larvae present per host tree ($\chi^2 = 0.2615$, df = 4, $P = 0.6091$, Fig. 4C), nor the summed size of all feeding scars with larvae present occurring above a focal individual per host tree ($\chi^2 = 2.3312$, df = 4, $P = 0.1268$, Fig. 4D).

4. Discussion

To our knowledge, drivers of aggregation in the novel arboreal parasite, A. virescens has not been empirically tested. In particular, we investigated whether parasites discriminate between heterogeneous host trees in a fragmented population (IFD), or if parasites randomly discover hosts (TAE). Furthermore, we assessed the influence of host size and parasite intensity on parasite aggregation. Despite their unique life history, A. virescens followed the common parasite distribution pattern of few hosts harbouring many parasites, and many hosts harbouring few parasites (Shaw et al., 1998; Tschirren et al., 2007; Calabrese et al., 2011; Poulin and Forbes, 2011; Poulin, 2013). We found parasite intensity scaled positively with host size. A prominent pattern in parasite ecology, parasite intensity commonly scales with host size (see Poulin, 2000, 2005; Poulin and Morand, 2000). In particular, a meta-analysis of 76 different fish host–parasite relationships found positive correlations between host size and parasite intensity, although relationship significance varied (Poulin, 2000). Larger hosts are predominantly older individuals with larger external surface areas and accrue greater parasite intensities through time and space (see Poulin and Morand, 2000; Poulin, 2013). Tree size is strongly correlated with tree age (Bond, 2000), whilst phloem thickness strongly scales with tree diameter (Amman, 1969; Speights and Conway, 2010; Hölttä et al., 2013; Davis and Hofstetter, 2014). Phloem translocates nutritionally-rich photosynthates from the canopy to the roots (Högberg et al., 2001; Zwieniecki et al., 2004; Pompon et al., 2011) and is the sole foodstuff for A. virescens. However, decreased phloem hydraulic conductance in older, larger trees denotes a reduced flow of photosynthates (Yoder et al., 1994; Bond, 2000; Hölttä et al., 2013). From the perspective of a parasite, larger trees offer a greater quantity, but a reduced quality of food, while smaller trees offer greater available energy per unit of phloem. Aenetus virescens are 10–35 mm in length when commencing the parasitic arboreal stage (Grehan, 1983), and parasites were recorded in host trees as small as 10 mm DBH. Parasites in smaller host trees negate the energetic cost required to ascend and parasitise larger hosts. If A. virescens discriminate between heterogeneous hosts, smaller trees should host greater parasite intensities than larger trees due to increased nutritional quality and lower energetic costs. With A. virescens intensity being greater on larger hosts, we conclude that host choice is not based on host quality, but on random discovery. This supports the TAE assumptions that larger “islands” support a greater number of individuals when dispersal is random (MacArthur and Wilson, 1967; Lomolino, 1990; Matter and Roland, 2003).

Significant scaling relationships were identified between A. virescens weight, head width, tail width, length and volume. Concurrently, A. virescens body size scaled positively with feeding scar size, allowing feeding scars to become proxies for parasite body sizes. Host tree size did not significantly influence feeding scar size, indicating that larvae consumed equivalent phloem quantities regardless of the host tree size. In all organisms, growth is a result of energy intake (see Lindstedt et al., 1986; Keeley and Grant, 1995; Greenleaf et al., 2007). The parasite growth rate should increase in nutritionally advantageous hosts (Barber, 2005). Conversely, we found that A. virescens growth rate remained consistent throughout ontogeny regardless of parasite size or host tree size. However, phloem nutritional quality may not solely influence parasite aggregation and growth. Competitive hosts generally have improved immune responses and may minimise nutritional availability to parasites (Barber, 2005). Aristotelia serrata are considered competitive owing to a fast-growing life strategy, particularly in early ontogeny (Dawson and Lucas, 2011). Moreover, factors such as temperature likely play a significant role in A. virescens development. Temperature is vital to ectotherm growth, influencing physiological and morphological characteristics (Poulin and Latham, 2003; Kingsolver et al., 2006). Smaller trees may provide less insu-

![Fig. 3. Growth of arboreal parasite Aenetus virescens feeding scar (proxy for parasite body size) between year 1, February 2013 (feeding scar, FA2013) and year 2, February 2014 (FA2014). Dashed line shows isometry (1:1). Solid line shows reduced major axis regression.](image)

![Fig. 4. Influence of infra-population on Aenetus virescens arboreal parasite growth on (A) the sum of conspecific larva in an Aristotelia serrata host tree, (B) the sum of conspecific larva occurring above a focal individual in a host tree (upstream competitors), (C) the summed size of all feeding scars with larvae present in a host tree, and (D) the summed size of feeding scars with larvae present, occurring above the focal individual in a host tree.](image)
vation from temperature extremes, potentially fostering disadvantageous microclimates in parasite tunnels. Nevertheless our results suggest that larger A. serrata size does not equate to increased host quality for parasites. The parasites are therefore not aggregating as a response to increased host quality, further supporting random dispersal as suggested by the TAE.

The IFD suggests that the available energy of an “island” is a function of infra-population size (Tregenza, 1995; Stewart and Komers, 2012; Williams et al., 2013). “Islands”, in particular, have finite resources and conspecific interactions are exacerbated by more individuals vying for the same resources (Tregenza, 1995; Randhawa and Poulin, 2009; Tseng and Myers, 2014). In turn, host saturation reduces host quality (Kaplan and Denno, 2007). Infra-population size scales negatively with parasite body size in most host–parasite interactions (see Ikeda, 1979; Rankin and Borden, 1991; Poulin, 1999, 2007; Barber, 2005; Lagrue and Poulin, 2008; Neuhäuser et al., 2010; Duan et al., 2013; Blasco-Costa and Poulin, 2013). We found A. virescens intensity varied remarkably between hosts, ranging from 1 to 19 (median = 2) feeding scars with parasites present. Additionally, post-parasite feeding scars remained as wounds in phloem and ranged from 1 to 45 (median = 2.5) per host. Feeding scars are substantial wounds in trees. Cumulative wounding from multiple feeding scars per host produces large-scale phloem disruption. Phloem girdling often results from A. virescens parasitism, whereby feeding scars cover the full circumference of the tree, cutting off any downwards flow of photosynthates. Intensive phloem wounding, particularly phloem girdling, reduces xylem water transportation, altering water-use efficiency and decreasing tree fitness (Zwieniecki et al., 2004). Multiple feeding scars may therefore dramatically reduce host quality. Furthermore, any parasite above another conspecific in a tree bole interrupts the flow of energy travelling downwards. Parasite nutrient supply is potentially limited by competitors, thus growth is expected to decrease with an increasing infra-population. Contrarily, our results indicate that A. virescens growth did not significantly correlate with parasite intensity. Furthermore, growth of an individual parasite did not significantly correlate with the number of parasites directly above that individual. Additionally, the summation of all feeding scars per host did not significantly correlate with parasite growth. Moreover, feeding scars directly above a focal individual did not significantly influence its growth. Consequently, A. virescens growth, representative of energy intake, is not intensity-dependent. In fish host–parasite relationships, low parasite intensities do not constrain parasite growth, whereas large parasite intensities produce resource competition and intensity-dependent growth (Poulin and Morand, 2000; Poulin, 2005; Saldanha et al., 2009). Although A. virescens are small bodied compared with host trees and occur in relatively low numbers (post- and present-feeding scars, median = 4), the sizeable feeding scars were expected to decrease host quality. Interestingly, some tree species are tolerant of consumer attacks, inducing adaptive responses regulating nutrient availability and internal resource allocation (Haukioja and Koricheva, 2000; Stowe et al., 2000). These adaptations potentially mitigate fitness reductions in host trees from increased feeding scars. Therefore trees with greater parasite intensities may be no less advantageous for parasites if trees are tolerant of such damage. We conclude that the A. virescens relationship with their host trees contradicts the IFD, which predicts “island” resources are a function of conspecific competition. The IFD proposes that individuals actively select “islands” providing greater rewards. However, our results indicate that larger hosts provide greater available space but no increase in obtainable energy for parasites, as shown by parasite growth. Therefore, it is unlikely that A. virescens are aware of available resources when disseminating to a host tree. More likely, as stated by the TAE, larger hosts provide a larger target more easily intercepted by larvae, leading to greater infra-populations. Furthermore, parasite growth was not influenced by any level of parasite intensity. Thus, increasing infra-population does not reduce host quality, further violating assumptions of the IFD. In conclusion, A. virescens aggregation among hosts supports the TAE. Larger trees randomly accumulate greater parasite intensities due to being larger, older presences in the landscape.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jipara.2014.10.007.

References


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