The evolution of intermediate castration virulence and ant coexistence in a spatially structured environment

András Szilágyi, István Scheuring, David P. Edwards, Jerome Orivel and Douglas W. Yu

1Department of Plant Taxonomy and Ecology, Eötvös University, Pázmány Péter sétány 1C, H-1117 Budapest, Hungary
2Research Group of Theoretical Biology and Ecology, Department of Plant Taxonomy and Ecology, Eötvös University and HAS, Pázmány Péter sétány 1C, H-1117 Budapest, Hungary
3School of Biological Sciences, University of East Anglia, Norwich NR47TJ, UK
4Laboratoire Evolution et Diversité Biologique, UMR-CNRS 5174, Université Toulouse III, 118 route de Narbonne, 31062 Toulouse cedex 9, France
5Ecology, Conservation, and Environment Center (ECEC), State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Science, Kunming, Yunnan 650223 China

*Correspondence: E-mail: dougwyu@gmail.com

Abstract

Theory suggests that spatial structuring should select for intermediate levels of virulence in parasites, but empirical tests are rare and have never been conducted with castration (sterilizing) parasites. To test this theory in a natural landscape, we construct a spatially explicit model of the symbiosis between the ant-plant Cordia nodosa and its two, protecting ant symbionts, Allomerus and Azteca. Allomerus is also a castration parasite, preventing fruiting to increase colony fecundity. Limiting the dispersal of Allomerus and host plant selects for intermediate castration virulence. Increasing the frequency of the mutualist, Azteca, selects for higher castration virulence in Allomerus, because seeds from Azteca-inhabited plants are a public good that Allomerus exploits. These results are consistent with field observations and, to our knowledge, provide the first empirical evidence supporting the hypothesis that spatial structure can reduce castration virulence and the first such evidence in a natural landscape for either mortality or castration virulence.

Keywords

Cooperation, evolution of virulence, host-pathogen, mutualism, myrmecophyte, parasite, rock-paper-scissors, spatial games, tolerance, trade-off.

INTRODUCTION

Castration parasites attack and destroy the sexual organs of their hosts, thereby preventing host reproduction and causing host resources to be reallocated to growth and/or survival, which directly benefits the parasite (Bonds 2006). Such parasites are known from a wide variety of taxa (Yu & Pierce 1998; Hurd 2001), and mean-field models find that such parasites should evolve total 'castration virulence' (Jaenike 1996; O’Keefe & Antonovics 2002; Bonds 2006) because the virulence-transmission trade-off, which selects for intermediate virulence when parasites kill their host (Alizon et al. 2009), does not apply to castration. Consistent with this, Jensen et al. (2006) found that a castrating bacterial parasite of Daphnia simultaneously displayed total castration virulence and intermediate mortality virulence, and Sloan et al. (2008) reported near-total sterilization of four plant species by host-specific anther-smut fungi.

However, other studies have observed less-than-total castration virulence (Jaenike 1996; Lopez-Villavicencio et al. 2005; Sloan et al. 2008), and so far, these observations have been explained as resulting from recent host shifts,
rendering the pathogens maladapted to their new host species. Alternatively, the hosts might exhibit tolerance (see below).

A third explanation for the evolution of less-than-total castration virulence is spatial structure. A considerable body of theory now shows that when parasite offspring dispersal is limited to local neighbourhoods, there is selection for lower mortality virulence (Lion & van Baalen 2008; Alizon et al. 2009) and lower castration virulence (Jaenike 1996; O’Keefe & Antonovics 2002). Locally dispersing parasites become surrounded by kin, and the resultant, countervailing kin selection selects for reduced virulence so that parasite kin, including offspring, are given host offspring to infect.

Correlative evidence supporting the spatial-structure hypothesis has existed for some time (Ewald 1983; Herre 1993), and, more recently, there have been successful experimental tests. Kerr et al. (2006) and Boots & Mealer (2007) used microcosm experiments to demonstrate that the mortality virulence of viruses declines when the movement of hosts and pathogens is restricted. In addition, in the mutualism literature, vertical transmission (spatial structuring in the limit) has long been accepted as a mechanism for aligning the interests of host and symbiont (Yamamura 1993; Ferdy & Godelle 2005; Foster & Wenseleers 2006; Sachs & Wilcox 2006).

In contrast, to our knowledge, there are no tests of any sort of the prediction that spatial structuring reduces the virulence of castration parasites. We require an appropriate empirical system and a spatial model that incorporates the system’s key features, so as to generate relevant and testable predictions, thus following the trajectory taken in the literature on mortality virulence (Boots & Sasaki 2000; Boots & Mealer 2007). We take a first step by developing a spatially explicit model informed by previous work on the spatial ecology of an ant-plant symbiosis, and we compare the results to preliminary observations showing that castration virulence varies at a landscape scale in the ant Allomerus octarticularis var. demerarae Wheeler (Myrmicinae).

Ant-plant symbioses are one of the most tractable model systems for the study of mutualism and parasitism (Davidson & McKey 1993; Yu 2001; Heil & McKey 2003). Hundreds of species of tropical plants provide ants with food and housing (domatia) in the form of hollow swellings and/or pouches. In return, ant-symbionts protect their host plants from herbivores and competitors. However, some ant symbionts are parasitic, failing to protect their host plants (Janzen 1975; Gaume & McKey 1999; Edwards & Yu 2007; Edwards et al. 2007; Clement et al. 2008). Other ant species are castration parasites, protecting leaves but preventing host plant reproduction by destroying flowers and floral buds (Yu & Pierce 1998; Izzo & Vasconcelos 2002; Gaume et al. 2005; Frederickson 2009).

Allomerus is an obligate symbiont of the ant-plant Cordia nodosa L. (Boraginaceae), an understorey, perennial treelet found across Amazonia, and in Peru, it is principally inhabited by Allomerus and at least three species of Azteca (Dolichoderinae). Each plant hosts one ant colony (Yu & Pierce 1998; Yu et al. 2001, 2004). The plant provides its resident ant colony with housing in the form of swellings (domatia) at branch internodes (Yu & Pierce 1998) (Fig. S1a) and with food bodies (Solano et al. 2005). In return, Allomerus and Azteca workers protect new shoots from herbivory (Yu & Pierce 1998; Frederickson 2005; Edwards et al. 2006b, 2007). Allomerus workers also destroy flowers and thereby prevent fruiting (Fig. S1b), thus diverting resources from plant reproduction to growth, increasing colony fecundity (Yu & Pierce 1998; Frederickson 2009).

However, it has been observed that Allomerus can display less-than-total castration virulence, such that some colonies allow some flowers and fruit to escape destruction (Yu & Pierce 1998; Edwards & Yu 2008). In part, successful flowering can be explained by host tolerance strategies (Edwards & Yu 2008), because some flowers are produced on new shoots, which are hidden from the ant worker caste that normally destroys the flowers. However, tolerance is unlikely to be the full explanation for partial flowering in C. nodosa as total castration is observed in other subpopulations (this study), and we would expect tolerance to be observed across the entire population as all C. nodosa plants must generate new shoots. Also, a recent host shift is unlikely, as this species of Allomerus is found on C. nodosa throughout Amazonian Peru (Debout et al. 2009) and in French Guiana (J. Orivel, unpublished data) and has never been collected from other ant-plants, or elsewhere.

The Azteca symbionts do not castrate, and, thus, the system proximately relies on the persistence of Azteca, which compete with Allomerus for host plants (Yu & Pierce 1998; Yu et al. 2001, 2004). All the ant species are dependent on C. nodosa for colony establishment and survival; female alates (new, winged ant queens) disperse from their natal colonies, mate and search for a sapling (Edwards et al. 2006a). After arrival, the queen enters a domatium and attempts to found an ant colony. Despite apparently complete resource niche overlap, the two genera can coexist via a dispersal-fecundity trade-off (documented in Yu et al. 2001, 2004); Allomerus colonies are more fecund, but their female alates are poor flyers, whereas individual Azteca alates can fly much longer distances, but there are few of them as colony fecundity is low. Coexistence is obtained because the superior fecundity of Allomerus gives it a higher colonization rate in locations where C. nodosa is abundant, whereas the superior dispersal of Azteca ability allows alates to capture isolated saplings where saplings are rare. An important consequence is that the frequency of the better-dispersing
Azteca (higher-fecundity Allomerus) goes up as plant density decreases (increases) (Yu et al. 2001).

Thus, considerable evidence supports the assumption that Allomerus is dispersal-limited, which is bolstered by the observation that Allomerus exhibits significant genetic isolation-by-distance at < 400m, while Azteca does not (G. Debout and D. Yu, unpublished data). Thus, to interpret the flowering data, we build a spatially explicit model that is embedded in our understanding of the spatial ecology of this system and allows for both interspecific competition and for virulence evolution in Allomerus. It is necessary to build a specific model because the evolution of cooperation in spatial systems depends in “subtle” ways on the details of the system (Boots & Sasaki 2000; Lion & van Baalen 2008). Here, we find that the presence of the true mutualist, Azteca, causes virulence to evolve in a qualitatively different way than when the mutualist is absent and in a way that is consistent with field data.

MATERIALS AND METHODS

Censuses of C. nodosa were conducted in seasonal to perhumid rainforest in the Departments of Madre de Dios and Loreto, the southernmost and northernmost provinces of Amazonian Peru respectively. We present censuses from seven locations, conducted from 2002 to 2006 (Fig. 1, Supporting Information). Plants at each location were scored for reproductive status and ant inhabitant (Azteca or Allomerus). Host plant densities were estimated using strip censuses. Cordia nodosa produces one domatium per internode, and flowers develop on the domatia, allowing domatia number to be used as an index of plant size (Yu & Pierce 1998; Edwards et al. 2006b; Frederickson 2006) (Fig. S1a). Thus, reproductive status was quantified as the number of domatia with at least one sexual part (floral buds, flowers, or fruit; details in Fig. 1 and Supporting Information). Statistical tests were conducted with R 2.8.0 (R Development Core Team 2008).

RESULTS

Flowering and fruiting censuses

Surveys revealed that 62% and 42% of Allomerus-inhabited plants at Cocha Cashu Biological Station (EBCC) and Madre Selva Biological Station (MS), respectively, were producing at least one sexual part (Fig. 1a). At the remaining five locations, zero sexual parts were observed on Allomerus-inhabited plants (Fig. 1a), except for a single floral bud on a new shoot at Libertadores Tambopata Lodge (TPL), which was subsequently castrated (mean sexual parts at EBCC and MS vs. the other sites, two-sample Wilcoxon test, \( W = 38862, \) two-tailed, \( P < 0.0001 \)).
Ant densities and ratios

The locations at which *Allomerus*-inhabited plants successfully produced sexual parts (EBCC, MS) had significantly higher frequencies of *Allomerus* than did the other locations (generalized linear model, *Allomerus* : *Azteca* ratio $\sim$ FloweringObservedOn*Allomerus*, $n = 7$, d.f. = 1, $P = 0.020$, two-tailed, quasibinomial errors, logit link, variance explained = 70.2%, Fig. 1b, details in Supporting Information).

**MODEL**

**Two ant species**

*Definition of the model*

For simplicity, we introduce the acronyms NA (No Ant) for plants not yet inhabited by an ant colony (saplings), and CAS and NCAS for plants inhabited (and defended) by Castrating *Allomerus* and Non-castrating *Azteca* respectively. The dynamics of the ant-plant system play out on an $N \times N$ grid with periodic boundary conditions. Variation in host plant density is achieved by allowing plants to establish only in $h$ fraction of the grid points, which are distributed randomly on the grid. To start with we disperse some plants to suitable grid points and some NCAS and CAS-ants onto those plants with a uniform initial castration and dispersal level.

First, we describe the plant reproduction step. In every $T$ timestep the CAS and NCAS-plants die with probability $p_{\text{CAS}}$ and $p_{\text{NCAS}}$ respectively. NA-plants are attacked by herbivores, so their probability of death $p_{\text{NA}}$ is higher than $p_{\text{CAS}}$ and $p_{\text{NCAS}}$. In every $kT$ time step, where integer $k \geq 1$, the ant-defended plants reproduce. Mortality follows an asynchronous updating rule; in each timestep, we choose (number of all plants)/$T$ plants randomly, and they die according to the probability above. The update for reproduction is similar; in each timestep, (number of ant-defended plants)/(kT) plants are chosen for reproduction. This algorithm ensures that individual probabilities of death and reproduction are not dependent on density.

Ant-inhabited (CAS, NCAS) plants produce seeds. The number of seeds is negatively correlated with the castration level $C$ of the resident ant colony:

\[ N_{\text{seed}} = \lambda_0 (1 - C^\alpha)^{1/\alpha} \]  

(1)

where $\lambda_0$ is the number of seeds in a non-castrated plant, and $\alpha$ characterizes the strength of the effect of castration on seed production. In this formulation, lower values of $\alpha$ cause the effect of castration level $C$ on plant fecundity $N_{\text{seed}}$ to be stronger (Supporting Information). The castration level of NCAS-inhabited plants is obviously zero.

All seeds disperse according to a Gaussian distribution with $\sigma_d$ variance. If more than one seed arrives in the same time step at a given empty and suitable grid point, the winner is chosen randomly. There are no internal dynamics in either the plants or the ant colonies, so age structure is neglected, and a new colony can produce queens in the following time step, if it is chosen.

We use the superscript $(C)$ for the parameters of castrating (CAS) ants. There are two heritable traits of a foundress queen: the level of castration exhibited by her eventual colony $0 < C < 1$ and her maximum dispersal distance $0 < d^{(C)}$. As castration increases colony fecundity (Yu & Pierce 1998; Frederickson 2009), whenever a CAS-inhabited plant reproduces, the number of ant queens that disperses is equal to

\[ q_0^{(C)} + e^{(C)} C \]  

(2)

$q_0^{(C)}$ is the number of dispersing individuals if there is no castration, $e^{(C)}$ is a positive parameter (the strength of the effect of the castration on the number of dispersing queen) and $C$ is the level of castration. $C$ therefore encapsulates the conflict in this system: high values of $C$ increase colony fecundity but decrease plant fecundity (Fig. S1; Table 1).

Dispersing queens search for colonizable plants (NA) via correlated random walks (Benhamou 2006). That is, it is more likely (with probability 0.8 in our implementation) for a dispersing queen to choose the same direction (up, down, left, right) in this time step that was chosen previously. If the dispersing queen successfully finds an NA plant, it establishes a new colony. The probability of dying before finding an NA plant is

\[ q_1^{(C)} + f^{(C)} d^{(C)} \]  

(3)

where $q_1^{(C)}$ is the death rate of the new queen independent of dispersal ability, $f^{(C)}$ is a positive constant measuring the effect of dispersal ability on death and $d^{(C)}$ is the maximum distance that is attainable by a dispersing queen. $f^{(C)}$ is inversely proportional to the energy cost of active searching.

Every founder carries her heritable traits $C$ and $d^{(C)}$. We assume asexual reproduction, and the traits mutate according to a Gaussian random distribution with $\sigma_C$ and $\sigma_d^{(C)}$ variance respectively.

The dynamics of the non-castrating (NCAS) ants are similar, but these ants have only one heritable trait, maximum dispersal ability $d^{(N)}$. All the other assumptions are the same as the castration ants with $C = 0$. We use the superscript $(N)$ to distinguish NCAS parameters.
Table 1 Parameter definitions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
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<tbody>
<tr>
<td>$N$</td>
<td>Length of the grid side</td>
</tr>
<tr>
<td>$b$</td>
<td>Fraction of grid points suitable for plant establishment</td>
</tr>
<tr>
<td>$p_{NA}$</td>
<td>Death probability of NA plants</td>
</tr>
<tr>
<td>$p_{CAS}$</td>
<td>Death probability of CAS plants</td>
</tr>
<tr>
<td>$p_{NCAS}$</td>
<td>Death probability of NCAS plants</td>
</tr>
<tr>
<td>$k$</td>
<td>Time multiplier, plant reproduction step</td>
</tr>
<tr>
<td>$S_0$</td>
<td>Number of seeds of a non-castrated plant</td>
</tr>
<tr>
<td>$\sigma_S$</td>
<td>Variance of seed dispersal distance</td>
</tr>
<tr>
<td>$(N)$</td>
<td>CAS ant parameters</td>
</tr>
<tr>
<td>$C_0$, $d^{(C)}_0$, $d^{(N)}_0$</td>
<td>NCAS ant parameters</td>
</tr>
<tr>
<td>$\sigma_{(C)}$, $\sigma_{(N)}$, $q^{(C)}$, $q^{(N)}$, $\phi^{(C)}<em>\text{max}$, $\phi^{(N)}</em>\text{max}$</td>
<td>Effect of dispersal ability on the death rate of a new queen</td>
</tr>
<tr>
<td>$d^{(C)}$, $d^{(N)}$</td>
<td>Maximum dispersal distance of ant queens</td>
</tr>
<tr>
<td>$q^{(C)}$, $q^{(N)}$, $\phi^{(C)}$, $\phi^{(N)}$</td>
<td>Effect of dispersal ability on the death rate of a new queen</td>
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**Results**

In this subsection, we analyse the results of our numerical simulations, which were conducted on a $500 \times 500$ grid. Ant-inhabited plants (CAS and NCAS) die with probability $p_{CAS} = p_{NCAS} = 0.04$. Ant-free saplings (NA) die with the higher probability $p_{NA} = 0.4$. For plant reproduction, we used the following parameters: $k = 5$, $S_0 = 100$, $\sigma_S = 5$.

Ant parameters are set so that the NCAS and CAS ants exhibit a dispersal-fecundity trade-off, as described above (Introduction). The castrating CAS ants are given a higher fecundity: $q_0^{(C)} = 4$, $\phi^{(C)} = 8$, thus giving them a maximum offspring number of $q^{(C)}_\text{max} = 12$, in contrast to the constant $q^{(N)}_\text{max} = 6$ for the non-castrating NCAS ants. However, the NCAS ants are given a greater dispersal ability by granting them a smaller energetic penalty of dispersal $f^{(N)} = 0.03 < f^{(C)} = 0.05$, so that they have the advantage in finding and thus capturing isolated NA plants (saplings). The basic death rate of new queens is the same across species $q_1^{(C)} = q_1^{(N)} = 0.3$. This parameterization leads to stable coexistence over a wide range of $b$. Note that by including $d^{(C,N)}$ in eqn 3, we penalize the evolution of better dispersal ability, reflecting the cost of producing larger queens, which are the better dispersers (Yu et al. 2004). NCAS ants can evolve a higher value of $d^{(N)}$, given their lower penalty of dispersal $f^{(N)}$.

The castration strength parameter is set to $\alpha = 0.8$ (eqn 1), which results in a steeper-than-linear reduction in seed number, as the castration parameter $C$ increases (Fig. S2); $\sigma_C = 0.00065$ and $\sigma_f^{(C,N)} = 0.005$. In all simulations the initial values of the traits were $C_0 = 0.5$, $d_0^{(N,C)} = 5$. The initial number of each species was $0.1 \times b \times N \times N$. A typical time evolution is shown in Fig. S3.

To describe the behaviour of the system as a function of plant density $b$, we searched the whole region of $b$ from 0 to 1. When $b < 0.4$, fluctuations in the densities of NCAS and CAS plants were high, and all ant colonies eventually went extinct, due to demographic stochasticity. At $b > 0.5$, we never observed extinction in the ant-plant system (at least using this or similar parameter sets). As expected, given the dispersal-fecundity trade-off between CAS and NCAS ants (Yu & Wilson 2001), the frequency of the better-dispersing NCAS ant drops with plant density $b$ (Fig. 2a).

We found that the achieved dispersal distances of dispersing queens (spatial average in each timestep) are only ~60% of the maximal dispersal distances, $d^{(C)}$ and $d^{(N)}$, and almost independent of $b$. This tells us that the dispersal kernel is right skewed and that long-distance dispersal to isolated plants is only an occasional occurrence.

Most importantly, we also found that the equilibrium level of castration $C_{eq}$ (and the variance around the mean) declines monotonically from 0.93 to 0.76 as plant density increases from $b = 0.6$ to 1.0 (Fig. 2b). At lower values of $\alpha$ (such that the effect of castration on ant fecundity is stronger, Fig. S2), the achieved $C_{eq}$ values are lower for each value of $b$ (data not shown).

**Detailed analysis**

To gain insight into why these results obtain, we now analyse the system from snapshots of ant densities and castration level at high plant density (here, $b = 1$), with all other parameter values the same as above. The density plots are patchy (Fig. 3a), but the distribution of the castration level is almost homogeneous and unimodal (Fig. 3b,c).

The frequency of castrating CAS ants is also high (Fig. 2a), which reduces overall seed production. As a result,
holes (no plants) occasionally appear in the array, which are then re-colonized in a predictable and informative sequence (Fig. 3a). Seeds (mainly from NCAS plants) disperse into the hole and grow into isolated NA saplings that are then mostly colonized by NCAS queens, which are the superior dispersers. NCAS-inhabited plants then seed many local saplings, and this new batch of plants eventually allows high-fecundity CAS ants to invade. The local production of saplings by NCAS-inhabited plants also allows a relatively high local castration value, $C_{eq} > 0.8$, to evolve in the CAS ants (Fig. 3b). Eventually, NCAS ants decline, and the local level of castration returns to the overall spatial equilibrium $C_{eq} = 0.77$ (Fig. 3b).

Similar dynamics can be observed more frequently at low plant density ($h = 0.7$, Fig. S4), with the result that the mean and variance in $C_{eq}$ are greater than at high plant density (Fig. 2b). A higher proportion of CAS ants evolve high castration levels ($C = 0.92$, Figs 3c and S4) at low plant density because there are more low-density patches where the above re-colonization dynamics takes place: an increase in NCAS-inhabited plants leads to an increase in saplings, followed by CAS invasion and the evolution of high castration levels (URLs to movies are in Table S1).

In summary, the castration level $C$ appears to be driven by the density of saplings (NA) produced mainly by NCAS-inhabited plants. CAS ants can evolve locally higher values of $C$ (and thereby increase colony fecundity) when NCAS-inhabited plants (and, thus, NA saplings) are locally abundant. This dynamics occurs during the re-colonization of the hole in the high-density simulations (Fig. 3, Table S1a) and in the ‘moving’ patches in low-density simulations (Fig. S4, Table S1b). Conversely, $C$ evolves to lower values, at a cost to colony fecundity, when saplings are rare, as holds for the remainder of the array (Figs 3 and S4). We therefore interpret the array-wide effects of plant density on castration level ($C$) as being driven by the frequency of non-castrating NCAS ants. In short, $C$ evolves to a lower mean value when the mutualistic NCAS ants are rare.

**One ant species**

*Definition of the model and results*

If the presence of the non-castrating NCAS ant allows the castrating CAS ant to evolve a higher equilibrium castration level $C$, then removal of the NCAS ant altogether from the model should result in even lower equilibrium values of $C$, assuming that the system can persist in the first place. Thus, we ran a model in which the reproduction and dispersal steps of CAS ants and the plants are the same, but NCAS ants are absent. We used the same $500 \times 500$ grid with periodic boundary conditions, with ant parameters $q_1^{(C)} = 0.3$, $f^{(C)} = 0.05$, $q_0^{(C)} = 4$, $\varepsilon = 8$, $a = 0.8$,

![Figure 2](image-url)
\( r_C = 0.00065 \) and \( r_d = 0.005 \). The CAS and NA plants die with probabilities \( p_{\text{CAS}} = 0.04 \) and \( p_{\text{NA}} = 0.4 \). For plant reproduction, \( k = 5, S_0 = 100 \) and \( \sigma_S = 5 \), all as before. We ran a second simulation in which we decreased local seedfall by increasing dispersal variance: \( \sigma_S = 10 \).

In our simulations, ant density (number of colonies)/(\( N \times N \times b \)) and the equilibrium level of dispersal ability \( d_C \) were independent of \( b \). However, and as expected from the previous results, \( C_{\text{eq}} \) values were lower than in all the simulations containing NCAS ants (Fig. 2b vs. 2c).

Moreover, and interestingly, \( C_{\text{eq}} \) declined as \( h \) declines (Fig. 2c), which is the opposite relationship observed in the two-ant system (Fig. 2b). This is because there are no longer any non-castrating NCAS ants, so decreasing \( h \) does not increase NCAS frequency (as it does in the two-ant model, Fig. 2a). In the one-ant system, reducing \( h \) results only in more isolated ant colonies, causing a higher frequency of saplings to be derived from local seed production rather than from seeds produced by plants elsewhere. This favours the evolution of lower castration levels, as the queens require saplings in which to establish.

The importance of local production of saplings is emphasized by the effect of reducing local seedfall (higher \( \sigma_S \), Fig. 2c), which results in the evolution of higher castration levels, \( ceteris paribus \). We interpret this to mean that as seeds are dispersed more widely, the system becomes more mean-field-like as local saplings are derived from a larger part of the landscape. This penalizes the evolution of castration less, and \( C \) evolves to higher levels. In the Supporting Information, we show that castration evolves to the maximum value in a mean-field, one-ant model, underlining the importance of spatial structure in selecting for intermediate values of \( C \).

**DISCUSSION**

Motivated by the *Allomerus–Azteca–Cordia* ant-plant symbiosis, we investigated a spatially structured model for the evolution of ant dispersal and castration behaviour. Female NCAS alates are superior dispersers, while CAS ants castrate host plants and thereby enjoy higher colony fecundities. Our model recovers previous results showing that this dispersal-fecundity trade-off allows the stable partitioning of variation in patch density (Yu et al. 2001, 2004). We also find that the opposing effects of CAS and NCAS ants on local plant density (Figs 3 and S4) are enough to produce spatial variation in patch density and, thus, coexistence, even when overall host plant density \( h \) is very high (Fig. 2a). This result likely relies on the fact that low-pathogenicity castration parasites can cause deterministic host extinction (Boots & Sasaki 2002). In contrast, the spatially implicit metacommunity model of Yu et al. (2001), in which spatial variation in plant density is exogenous, is not able to achieve.
coexistence when patch density was high, as mass immigration from high-density subpopulations allows the high-fecundity ant to drive the superior disperser extinct in the low-density subpopulations. Thus, a spatially explicit formulation, in which the symbiont endogenously causes the density of patches to vary, increases the robustness of coexistence and, therefore, of the persistence of the Azteca-Cordia mutualism. Using a range of other parameter values (A. Szilágyi, I. Scheuring, unpublished data), we have observed opposing and long-term oscillations of NCAS and CAS ants, which means that each ant can invade when rare, further underscoring the robustness of coexistence.

Most importantly, we find that local dispersal by CAS ants (and host plants) causes castration virulence to evolve to an intermediate value (Fig. 2b,c). The evolution of virulence is additionally affected by the overall density of host plants and the consequent frequency of the mutualistic NCAS ants. As host plant density increases, NCAS frequency decreases (Fig. 2a), and the equilibrium level of castration C declines (Fig. 2b). This effect is driven by the frequency of NCAS ants, not host plant density, as in the one-ant model, increasing host plant density increases castration virulence (Fig. 2c). Seeds from NCAS plants are a public good that CAS alates can take advantage of (Fig. 3), which shifts the equilibrium away from the increased cost of dispersal to alate offspring and towards the fecundity benefit of castration.

The system resembles a four-state Rock-Paper-(Match-)Scissors game. Empty patches are colonized by saplings (NA), and better-dispersing NCAS ants are more likely to colonize these saplings, which leads to a build-up of NA plants and invasion by the more fecund CAS ants, finally resulting in the local depletion of susceptible hosts (Table S1). Thus, the cycle is Empty patch → NA → NCAS → CAS.

From a general theoretical standpoint, in the absence of trade-offs and limited dispersal (see O’Keefe & Antonovics 2002 and the present study) castration parasites find themselves in a tragedy of the commons. If any individual parasite fails to castrate its host fully, the benefit of the host offspring to the parasite is distributed amongst all parasite individuals, while the opportunity cost of foregone castration is borne only by the less virulent parasite(s). However, when dispersal is constrained, the game is transformed into the Rock-Paper-Scissors game, as described above, allowing the system to persist and castration polymorphism to be maintained.

Our model results are qualitatively consistent with the two observed features in the C. nodosa system: (1) there is landscape-scale variation in the inferred level of castration virulence (Fig. 1a), and (2) this variation can be explained in part by the ratio of Azteca to Allomerus (Fig. 1b). Note that host plant density is high at the MWC location (Fig. 1b), although Allomerus frequency is towards the low end (i.e. the relationship between host plant density and ant frequency is not perfect). Consistent with the model results, we observed no sexual parts on Allomerus-inhabited plants at MWC.

We conclude that spatial structuring is a viable explanation for the evolution of landscape-scale variation in castration levels in the C. nodosa system. To our knowledge, this is the first empirical evidence consistent with the hypothesis that spatial structure can affect castration virulence and the first such evidence in a natural landscape for either mortality or castration virulence.

The one-ant model resembles the standard Rock-Paper-Scissors game. An overly virulent castrating parasite suffers local depletion of susceptible hosts, enabling the spread of a less virulent castrator, and overall castration virulence evolves to a value that is less than in the two-ant model. Thus, the cycle is More virulent castrator → Empty patch → Less virulent castrator. As a result, increasing host plant density selects for higher values of C (Fig 2b,c) because a higher density of saplings allows CAS ants to exploit the fecundity benefits of castration without suffering increased dispersal costs to their alate offspring (there is no countervailing effect of increased mutualistic NCAS ants, Table S1c).

Interestingly, the ant-plant Hirtella physophora Mart & Zucc. (Chrysobalanaceae) might represent the one-ant case. Hirtella physophora hosts only Allomerus decemarticulatus Mayr throughout much of French Guiana, and the ant protects its host plant against herbivory. Consistent with our model (Fig. 2c), A. decemarticulatus castrates, but at a lower virulence; exclusion experiments have found that the ant castrates only about half of total flower production, and flowering has been observed in all populations censused (n = 8; P.J. Malé, A. Quilichini and J. Orivel, unpublished results). We do not yet know how or whether virulence varies with density.

The fact that the one-ant and two-ant models give opposite predictions for the effect of host plant density (Fig. 2b,c) underlines the importance of studying virulence within the correct context, informed by the spatial ecology of the system (Lion & van Baalen 2008). For instance, the one-ant model predicts that flowering and fruiting in Allomerus-inhabited plants should be more frequently observed in the low-density locations (i.e. locations TPL–S, Fig. 1), the opposite of our observations.

More generally, two-species spatial game models have shown that localized dispersal can prevent continuous Iterated Prisoner’s Dilemma games from evolving to mutual defection (Doebeli & Knowlton 1998). In fact, multiple factors generating strategy polymorphism (localized dispersal, spatial heterogeneity, high mutation rate and a limited number of interactants) can all result in the evolution and stability of mutualistic strategies (Boza & Scheuring 2004;
Scheuring 2005). Thus, even though general models (Jaenike 1996; O’Keefe & Antonovics 2002) provide insight and (once they are interpreted correctly) are consistent with our observations, it can be problematic to use such models to guide empirical testing. All host-parasite systems are embedded in a community. Relevant complications include multiple host species, multiple vectors and transmission modes, competing symbionts (both parasitic and mutualistic) and the nature of competition or cooperation amongst those symbionts, host immunity, and, of course, spatial variation in these factors (Lion & van Baalen 2008; Alizon et al. 2009; Antonovics 2009; Ferdy 2009).

Finally, we found that for the parasite to evolve intermediate castration virulence, the host plant need also be dispersal limited. Increasing host plant dispersal \( \sigma_S \) selects for higher castration virulence (Fig. 2c) because a greater proportion of local host plant saplings is derived from other plants, approaching a mean-field like system (mimicking the effect of NCAS ants). Similarly, Boots & Sasaki (2000) found that increasing host dispersal increases virulence. Neither prediction has yet been tested experimentally as logistical limitations in existing tests (Kerr et al. 2006; Boots & Mealer 2007) have meant that the dispersal of both hosts and parasites was restricted. Previous spatially explicit models of castration virulence (O’Keefe & Antonovics 2002) and mutualism (Doebeli & Knowlton 1998) also limited the dispersal of both host and symbionts. Dispersal is limited in our case as well, but we allow a non-zero probability of long-distance dispersal, which produces a distribution of distances, and we allow dispersal to evolve. Previous models have exogenously limited dispersal, often to nearest-neighbour patches.

We note that reproduction in the model is asexual, whereas ants reproduce sexually. Thus, we are assuming that male alates, which we did not model explicitly, do not regularly disperse longer distances than do the female alates. We also assumed that our snapshot flowering censuses reflect long-term differences and that the observed variation in flowering success (Fig. 1a) reflects variation in *Allomerus* behaviour, not variation in a host plant resistance strategy. Supporting evidence for the latter two assumptions is in Supporting Information.

A fourth assumption, common to all models of spatially structured virulence evolution, is that the timescales of microevolutionary and ecological change are similar. Given that castration intensity is a quantitative trait and one that we know varies across colonies within locations, the assumption seems reasonable (see also Hairston et al. 2005). We would prefer to document temporal change in castration intensity, but for now, we have used space as a proxy for time (Fig. 1).

Conducting spatial tests in natural landscapes requires us to quantify the characteristic spatial scales of the system of interest. In this work, we used independent trail systems covering areas on the order of 500–1500 ha as our spatial replicates (Yu et al. 2001). The implicit assumption is that each such replicate can be taken as a representative of a run on our model grid. That is, the spatial scale of each data point (Fig. 1) should be on the same order as the spatial scale of our model. In partial support, we find that coexistence obtains on the model grid, and previous results (Yu et al. 2004) suggest that the minimum size of a *C. nodosa* metacommunity sufficient to support coexistence is near the area covered by a large trail system, meaning that *Allomerus* and *Azteca* should be able to coexist in a landscape of this size (we gloss over caveats regarding stochasticity). Ideally, we would estimate the dispersal kernels of the symbionts and host in order to parameterize a spatially explicit model. This would give us a quantitative understanding of the appropriate size of a spatial replicate.

Finally, ant-plants continue to challenge us with difficult questions about the coexistence of species and the evolution of virulence. How do the multiple *Azteca* species coexist on *C. nodosa*? Why are their colony fecundities so low, when increasing fecundity would lead to the capture of host plants and habitat from *Allomerus*? Why are the *Azteca* species not castrators? For that matter, why is not every plant-ant a castration parasite? These questions strongly suggest that we still have much to learn about the evolution of virulence and cooperation and the ecology of coexistence.

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**REFERENCES**


SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1** (a) A domatium of a *Cordia nodosa* ant-plant, with floral buds. Here, the leaves are still expanding. (b) An *Allomerus* worker attacking a *C. nodosa* floral bud. (c) *Allomerus* alate larvae in a domatium with workers and brood, demonstrating space limitation. Photograph credits Mark Moffett and Douglas Yu.

**Figure S2** $N_{seed}$ as a function of $C$ with different values of $z$.

**Figure S3** The time evolution of traits.

**Figure S4** Snapshot analysis at low plant density, $b = 0.7$, $t = 500000$.

**Table S1** Ant densities and the evolution of castration level $C$.

**Text S1** Census locations.

**Text S2** Mean-field model of the one ant-one plant system.

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