LETTER

Colonisation and competition dynamics can explain incomplete sterilisation parasitism in ant–plant symbioses

Corina E. Tarnita,[1,2]* Todd M. Palmer,[2,3] and Robert M. Pringle[1,2]

[1]*Department of Ecology & Evolutionary Biology, Princeton University, Princeton, NJ, 08544, USA*
[2]*Mpala Research Centre, Box 555, Nanyuki, Kenya*
[3]*Department of Biology, University of Florida, Gainesville, FL, 32611, USA*

*Correspondence: E-mail: ctarnita@princeton.edu

Abstract

Sterilisation of parasites prevents host reproduction, thereby diverting host resources to their own benefit. Previous theory predicts that parasites should evolve maximum virulence, yet hosts are often incompletely sterilised. Whereas prior attempts to resolve this paradox have sought evolutionary explanations, we present theory and experiments showing that incomplete sterilisation can arise from ecologically driven fluctuations in parasite load. The African ant–plant *Acacia drepanolobium* reproduced more when occupied by small colonies of the sterilising symbiont *Crematogaster nigriceps*. In nature, small colonies result from interference competition between ant colonies; these territorial conflicts thus provide intermittent windows of opportunity for host reproduction. Our mean-field model shows that numerical insufficiency of parasites can produce partial sterilisation of host populations, creating the appearance of reduced virulence even if ants have evolved to sterilise completely. This general framework helps explain both the apparent ubiquity of partial sterilisation parasitism and the ability of these symbiotic associations to persist.

INTRODUCTION

Parasitic sterilisation, or castration, entails 'destruction or alteration of gonad tissue, reproductive behaviour, hormonal balance, or other modification' that reduces host reproduction (Baudoin 1975). Sterilisation parasitism has been identified in a wide variety of taxa and ecosystems, including among plants and ants, insects and *Nosema* fungi, snails and trematodes, fish and tapeworms, and crustaceans parasitized by both bacteria and dinoflagellates (Hall *et al.* 2007). The reduction of host reproduction by sterilisation parasites causes host resources to be reallocated to other functions, such as growth and maintenance, that more directly increase parasite fitness (reviewed in Lafferty & Kuris 2009; see also Bonds 2006; Hall *et al.* 2007; Frederickson 2009).

Assuming transmission is mostly horizontal, mean-field models predict that sterilisation of parasites should evolve total virulence (Jaenike 1996; O'Keefe & Antonovics 2002; Bonds 2006). However, empirical studies often report intermediate levels of sterilisation efficacy (Jaenike 1996; Lopez-Villavicencio *et al.* 2005; Sloan *et al.* 2008; Szilágy *et al.* 2009, Malé *et al.* 2012). Various explanations have been proposed to account for this mismatch between theory and data, including recent host shifts that render parasites maladapted to their new hosts (Jaenike 1996); evolution of host tolerance strategies (Izzo & Vasconcelos 2002; Edwards & Yu 2008); the ability of hosts to sanction parasites (Malé *et al.* 2014); and the evolution of intermediate virulence (Szilágy *et al.* 2009).

Myrmecophytes (ant plants) are excellent model systems in which to study sterilisation parasitism, because the partners are macroscopic and can be experimentally manipulated. Most ant–plant symbioses are mutualistic, with plants providing nesting space (domatia) and/or food (nectar or food bodies) in return for protection against enemies; however, some plant–ants have evolved parasitic behaviours (Yu 2001). In at least four ant species across three continents, ants sterilise their hosts by damaging or destroying floral structures (Yu & Pierce 1998; Stanton *et al.* 1999; Izzo & Vasconcelos 2002; Gaume *et al.* 2005; Frederickson 2009; Malé *et al.* 2012). As in other sterilisation parasite-host associations (Hall *et al.* 2007), sterilisation parasitism by plant–ants can enhance host growth by diverting resources from reproduction (Frederickson 2009; Malé *et al.* 2012). Greater growth directly benefits ants by increasing nesting space, enabling colonies to produce more workers and reproductives. Thus, there should be strong selection for ants to sterilise their hosts completely. Yet in all of the myrmecophyte systems studied to date, some fraction of trees occupied by sterilisation parasites do reproduce (i.e. sterilisation is not 100% effective: Yu & Pierce 1998; Edwards & Yu 2008; Izzo & Vasconcelos 2002; Gaume *et al.* 2005; Palmer *et al.* 2010; Malé *et al.* 2012). Previous efforts to account for this phenomenon have focused on *Allomerus* spp. and their association with *Cordia* and *Hirtella* host plants in South America, and have emphasised the importance of host tolerance (Izzo & Vasconcelos 2002; Edwards & Yu 2008) and sanctioning strategies (Malé *et al.* 2014). In the only prior theoretical treatment of this issue, Szilágy *et al.* (2009) built a spatially explicit model showing that sterilisation virulence can evolutionarily cycle between high and low levels in structured environments, assuming asexual symbionts that are weak dispersers.

Such explanations are rooted in evolutionary mechanisms. However, we propose that the theoretical prediction that sterilisation parasites should evolve maximal virulence is not incompatible with the occurrence of incomplete sterilisation at

the host-population level, and that in many cases, ecological processes such as colonisation, competition, population dynamics, and local extinction may be sufficient to explain the widespread occurrence of incomplete sterilisation. We start with the observation from several sterilisation-parasite systems (e.g. Ebert *et al*. 2004; Heins et al. 2010) that reproduction does not cease immediately following infection, but instead attenuates as parasite load increases. This suggests that although parasites may have evolved to sterilise completely, total sterilisation is only possible once the parasite load has exceeded a critical threshold. We hypothesise that hosts experience intermittent periods of low parasite load – due, for example, to the lag between initial infection and the threshold required for total sterilisation, or to factors that reduce the load of a previously infected host below that threshold (e.g. competition with other parasites, host immune response, fire, drought, and other abiotic stresses). These periods provide windows of opportunity for host reproduction that can (1) account for incomplete sterilisation at the host-population level and (2) enable the persistence of host populations at stable equilibrium levels, even at high parasite prevalence.

Using observational and experimental field data, we tested two key predictions of this hypothesis in the myrmecophyte *Acacia drepanolobium*, in which the sterilising ant species (henceforth CAS, following Szilágy *et al*. 2009) *Crematogaster nigriceps* competes with its non-sterilising (NCAS) congener *Crematogaster mimosae* (along with two less-common species) for exclusive occupation of host trees (Young *et al*. 1997; Palmer *et al*. 2000). In this system, low ant abundance on reproductively mature host trees occurs frequently due to inter- and intraspecific territorial conflicts between colonies for possession of trees; these conflicts proceed as wars of attrition until the losing colony is extinguished and the winner is greatly reduced in size (Palmer 2004; Rudolph 2012). Trees can also be abandoned by ants and subsequently recolonised by either foundress queens or established colonies from nearby trees (Palmer *et al*. 2010). We predicted that (1) host reproduction would be negatively correlated with colony activity level; (2) experimental reduction of colony size would increase host reproduction; and (3) experimentally induced conflicts between ant colonies would increase host reproduction.

Motivated by these empirical results, we developed mean-field models for one- and two-ant species systems that incorporate effects of colony size on sterilisation efficacy. We show that although each ant species is assumed to have evolved maximal sterilisation virulence, colony-size effects can lead to a stable, non-zero, and incompletely sterilised host population at equilibrium.

MATERIALS AND METHODS

Study system

Fieldwork was conducted at the Mpala Research Centre in central Kenya (0˚17' N, 37˚52' E), where *A. drepanolobium* is the dominant tree on clay-rich vertisol soils (Young *et al*. 1997). Similar habitats occur across large swaths of East Africa. At Mpala, four species of obligate ant symbionts compete for exclusive occupation of host trees (Palmer, *et al*. 2000, 2002), with individual colonies occupying one-or-more trees. We conducted experiments in the 'secondary study site' described by Palmer (2004). At this site, CAS *C. nigriceps* and NCAS *C. mimosae* each occupy 40–55% of trees, with the additional NCAS *Crematogaster sjostedti* and *Tetraponera penzigi* each occupying fewer than 5% of trees. This area is homogeneous with respect to topography, vegetation structure, and species composition (Fig. 1) and is therefore ideal for experimental manipulations: *A. drepanolobium* occurs in near-monoculture (>95% canopy cover) with little variance around the mean height of 1.67 m (SD = 0.28, n = 215).

Conflicts occur whenever trees occupied by different colonies come into contact, or when expansionist colonies initiate ground-based takeover attempts, and they proceed with ∼ 1 : 1 mortality until the winning colony (re)assumes

Figure 1 Photograph of the study area at Mpala Research Centre in central Kenya, showing dense monospecific cover of *Acacia drepanolobium* trees between 1- and 2-m tall (see elephant for scale). Upwards of 90% of these trees are occupied by either the sterilizing ant symbiont *Crematogaster nigriceps* (top right) or the non-sterilising symbiont *C. mimosae* (bottom right).

exclusive control of the contested tree(s) (Palmer *et al.* 2000). Competitive takeovers occur on $\sim$ 8% of trees per year (Palmer *et al.* 2010). The $\sim$ 1 : 1 mortality ratio of these conflicts means that competitive outcomes are determined by colony size (Palmer 2004; importantly, this property allows us to manipulate the winners and losers of experimentally staged conflicts as described below). Experiments by Rudolph (2012) showed that winning colonies lose up to $\sim$ 66% of their workers; if the winning colony is expanding (as opposed to defending), then ant density on each individual tree decreases further, as a cohort of workers splits off to patrol the newly acquired tree (for experimental purposes, we estimated 75% to represent a realistic reduction of per-tree worker number following severe conflicts). Rates of host-tree abandonment differ among ant species and are a decreasing function of tree size, but can occur for $\sim$ 5–10% of trees per year in the 2-m size class examined in this study (Palmer *et al.* 2010).

Crematogaster nigriceps destroys the axillary buds that give rise to lateral branches and flowers (Stanton *et al.* 1999). Ants can only destroy young buds and not late-stage buds, flowers, or fruits. This behaviour is thought to reduce lateral canopy spread and thus the likelihood of contact with neighboring trees and hostile takeover by other colonies (Stanton *et al.* 1999) and to increase hosts' production of domatia. The negative correlation between fruit and domatium production (Fig. S1) is consistent with the latter possibility, which has been demonstrated experimentally in other myrmecophytes (Frederickson 2009). *Acacia drepanolobium* trees in other parts of East Africa are occupied by different subsets of the four ant species in our study population: in parts of southern Kenya and northern Tanzania, *C. nigriceps* is the only species present (Stapley 1998), whereas in south-central Kenya, *C. nigriceps* occurs together with *C. mimosae* and *T. penzigi* (Martins 2011). As at Mpala, *C. nigriceps* incompletely sterilises its host trees at these other sites (Martins 2011, TMP unpublished data), although potential differences among populations in this behaviour have not been systematically quantified.

Reproduction of unmanipulated *C. nigriceps* trees

In April 2014, we surveyed 215 *A. drepanolobium* trees occupied by CAS *C. nigriceps* to assess (1) the frequency of incomplete sterilisation and (2) whether host reproductive output is correlated with colony activity level, a proxy for worker abundance (adapting methods from Palmer & Brody 2007; see also Palmer *et al.* 2008). At each tree, one observer (same for all trees) randomly selected a live branch and shook it three times. A second observer, using a tally counter, recorded the number of workers recruiting to the disturbance over 30 s; observers then counted all flowers. We analysed the incidence of incomplete sterilisation (defined as trees with $\geq$ 1 flower) as a function of colony activity using binary logistic regression. Total number of flowers per tree was analysed using negative-binomial regression (glm.nb) in the MASS package of R 2.14 (Venables & Ripley 2002; R Development Core Team 2012). Here and throughout when using negative-binomial models, we verified that the dispersion parameter ϕ < 1.5, indicating an appropriate level of overdispersion (Zuur *et al.* 2009). Because our

empirical work tests specific, directional predictions motivated by our hypothesis, we use one-tailed P values throughout.

Experiment 1: reduction of CAS C. nigriceps colony size
We directly tested the central mechanism of our hypothesis: that reduction in colony size increases host reproduction. In October 2012, we selected 40 similarly sized trees occupied by *C. nigriceps*. Twenty of these trees were randomly assigned to 'colony-size reduction' treatment; the remainder served as procedural controls. On each colony-reduction tree, we used a syringe to inject 75% of the domatia with a short-lived insecticide (alphacypermethrin, diluted 1 : 20 in water) to approximate the upper range of post-conflict reduction (Rudolph 2012). We controlled for the effects of disturbance and fluid addition by injecting an equivalent volume of water into 75% of the domatia on the control trees. This pesticide has an outdoor half-life of 1–4 weeks, so although our insecticide treatment kills workers and brood inside domatia, thereby reducing colony size, it is unlikely to have lasting effects on insect herbivores (Stanton & Palmer 2011).

We resurveyed the ant colonies on these 40 trees in February, April, and July 2013. Two trees were excluded in April, and two more in July, after being killed by elephants or taken over by *C. mimosae*. We assayed colony activity levels (recruitment to standardised disturbance, as described above) and analysed the count data (square-root transformed for normality) using repeated-measures multivariate analysis of variance (MANOVA) in JMP 10.0 (SAS Institute, Cary, NC, USA). In April, we counted all flowers and fruits on each tree. We used logistic regression to analyse the incidence of incomplete sterilisation (trees with $\geq$ 1 flower *or* fruit) and negative-binomial generalised linear models (GLMs) to analyse reproductive output and the relationship between reproduction and colony-activity level in the preceding survey.

Experiment 2: staged takeovers of host trees by CAS C. nigriceps
We tested whether competitive takeovers by *C. nigriceps* lead to incomplete sterilisation. Specifically, we predicted that (1) host trees subjected to intraspecific takeovers by *C. nigriceps* would reproduce more than unmanipulated control *C. nigriceps* trees, because conflicts greatly reduce worker abundance; (2) trees on which *C. nigriceps* supplanted NCAS *C. mimosae* would reproduce more than either unmanipulated *C. nigriceps* trees or intraspecific-takeover trees, because trees occupied by NCAS ants prior to takeover should have more incipient reproductive structures and therefore require more CAS workers to destroy them; and (3) unmanipulated NCAS trees would reproduce more than any trees taken over by CAS colonies.

In March 2010, we selected 40 neighbouring pairs of similarly sized trees. In 20 of them, each tree was occupied by a different colony of CAS *C. nigriceps*. In another 20 pairs, one tree was occupied by *C. nigriceps* and the other by *C. mimosae*. For all 40 pairs, we selected one focal tree to be the 'loser' by placing tanglefoot barriers around the base of all nearby trees occupied by that colony, preventing workers on those trees from recruiting to the conflict. We then tied the trees together with steel wire to incite conflict. (These procedures are described in detail by Palmer 2004.) We also

selected 20 *C. nigriceps* and 20 *C. mimosae* focal trees to serve as unmanipulated controls (thus, 80 total focal trees). We resurveyed each focal tree six times over the subsequent two years (May and July 2010, January and July 2011, March and July 2012). Trees patrolled on more than one occasion by ant species other than the one intended by the treatment were thenceforth excluded from the experiment. For each tree, we summed flowers and fruits within each survey (to account for stochastic phenological variation among trees) and averaged these values across surveys; we then compared these means across treatments using negative-binomial GLMs with planned pairwise contrasts.

RESULTS

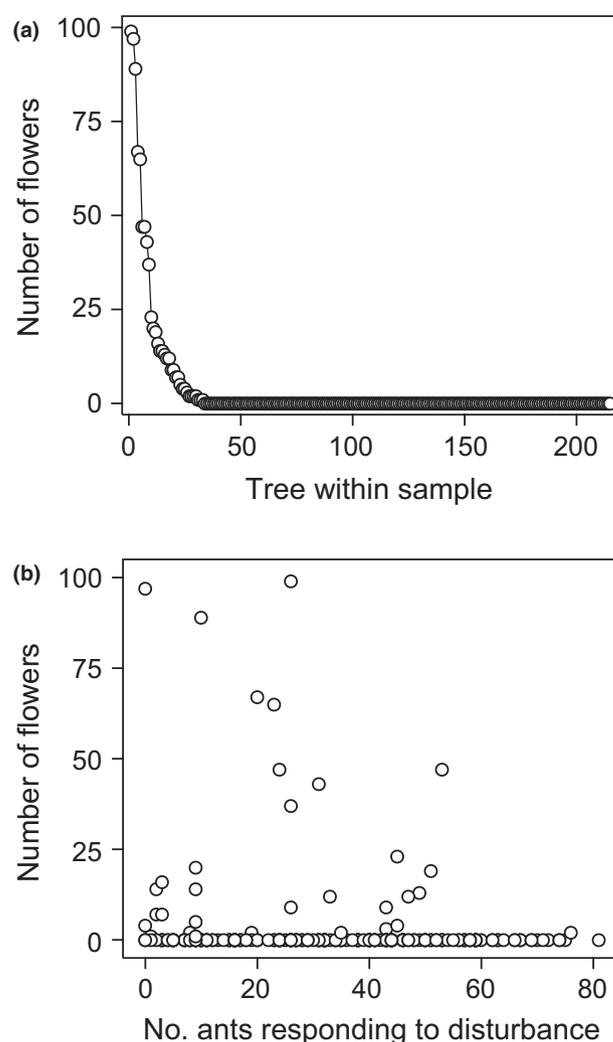### Reproduction of unmanipulated *C. nigriceps* trees

Thirty-three of 215 host trees (15.3%) occupied by *C. nigriceps* had at least one flower. On these, flower number ranged from 1 to 99 (mean 24.0, median 12; Fig. 2a). Incomplete sterilisation was significantly more frequent for trees with fewer ants recruiting to disturbance (logistic regression, $\chi^2 = 6.76$, df = 1, $P = 0.0047$). Similarly, there was a significant negative relationship between total number of flowers per tree and number of ants recruiting (Fig. 2b; negative-binomial regression, $P = 0.016$).

Experiment 1: colony-size reduction

Pesticide application did not extinguish any colonies, but decreased the number of workers recruiting to the experimental disturbance site by 60–70%, which persisted for at least nine months (Fig. 3a) (repeated-measures MANOVA, $F_{1,34} = 28.3$, $P < 0.0001$). Colony-size reduction significantly increased the incidence of incomplete sterilisation after six months, with 44% of trees (8 of 18) having either flowers or fruits, compared with 5% (1 of 20) in the control treatment (logistic regression, likelihood-ratio $\chi^2 = 8.93$, df = 1, $n = 38$, $P = 0.0014$). Flowers were present on six trees, all in the reduced-colony treatment (Fig. 3b). Fruits were present on six trees in the reduced-colony treatment (Fig. 3c). One tree in the control treatment produced two fruits; notably, however, this tree had the lowest mean colony-activity level within its treatment (three ants responding, 79% fewer than the next-lowest value of 14: Fig. 3d). Both flower and fruit production showed pronounced negative associations with a colony-activity level in the preceding survey (Fig. 3d).

Experiment 2: staged takeovers by C. nigriceps

We succeeded in forcing takeovers in the intended direction in 39 of the 40 experimentally induced conflicts, although only 22 of focal trees remained exclusively occupied by the intended ant species for the 28-month duration of the experiment. As predicted, mean reproductive output was greatest for unmanipulated NCAS *C. mimosae* focal trees and least for unmanipulated CAS *C. nigriceps* trees (Fig. 4). Intraspecific takeovers of *C. nigriceps* trees by other *C. nigriceps* colonies increased reproductive output nearly three-fold relative to unmanipulated *C. nigriceps* trees, and interspecific takeovers of NCAS *C. mimosae* trees by CAS *C. nigriceps* colonies

Figure 2 Reproduction of 215 unmanipulated *Acacia drepanolobium* trees occupied by the sterilising ant symbiont (CAS) *C. nigriceps* in April 2014. (a) Number of flowers on each tree, in decreasing order from left to right. (b) Significant negative relationship between colony activity and flower number (negative-binomial regression, coefficient = −0.037, SE = 0.017, $P = 0.016$).

increased reproduction another three-fold relative to the intraspecific CAS takeover trees (Fig. 4, Fig. S2, Table S1).

THEORETICAL MODEL FOR ONE ANT SPECIES

We present a mean-field model with one host and one symbiont showing that when sterilisation completeness is contingent on parasite load, and parasite load is a function of various ecological processes, incomplete sterilisation occurs at equilibrium in well-mixed populations – even when parasite transmission is entirely horizontal, there is no energetic cost associated with the parasitic behavior, and the parasites are therefore assumed to have evolved maximal virulence (Jaenike 1996). This in turn may help explain another apparent paradox: the persistence of obligate symbioses in which a sterilisation parasite is the only possible symbiont, as occurs in some *A. drepanolobium* populations (Stapley 1998).
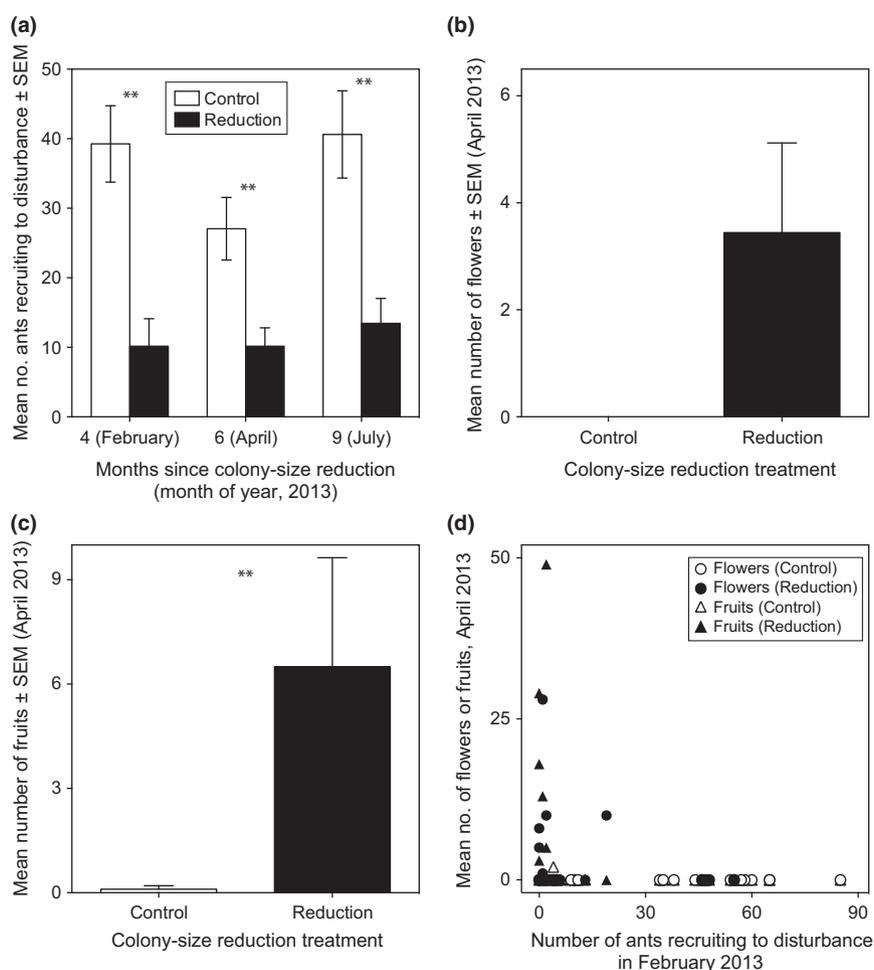
Figure 3 Results of colony-size reduction experiment. In October 2012, we injected pesticide into 75% of domatia of trees in the 'Reduction' treatment ($n = 20$) and water into 75% of domatia of trees in the 'Control' treatment ($n = 20$). (a) Pesticide application decreased the number of workers recruiting to experimental disturbances (a proxy for colony size) by 65%, on average, over nine months; asterisks indicate significant differences ($P \leq 0.0017$) between treatments in one-way ANOVAs for each survey (following significant MANOVA reported in the text). (b) Flower number in April 2013; flowers were present only on trees with reduced colonies. (c) Fruit number in April 2013; asterisks indicate significant difference between treatments (negative-binomial GLM, $P = 0.0005$). (d) Relationship between colony activity level in February 2013 and number of flowers and fruits in April, showing strong negative association between reproductive output and colony size in preceding months (negative-binomial regression, both $P < 0.007$).

The model is general and can be adapted to many systems, but as it was inspired by the *A. drepanolobium* ant–plant system, we refer to trees and ant colonies instead of generic 'hosts' and 'parasites'. Because we are interested in trees, and not in the dynamics of ants *per se*, we capture the latter indirectly by modeling only the tree population and subdividing it into different classes according to their ant occupancy; transitions between classes account for colony dynamics. Although individual ant colonies can occupy multiple trees, this is irrelevant to our analysis, which is from the perspective of the tree; we therefore use the term 'colony' to mean the cohort of workers physically occupying a single tree. We consider an age-structured model with two classes of trees: adults that can reproduce and saplings that cannot. The death rates of both classes and the reproductive rates of adults depend on whether or not the trees are occupied, and by what colony sizes. The model describes the ecological dynamics of species interactions and is not an evolutionary model: all colonies are assumed to have evolved maximal

virulence, but their size determines whether they can actually achieve complete sterilisation. A colony is 'small' if it is unable to fully sterilise an adult host; otherwise it is 'large'. Adult trees are either fully sterilised (zero reproduction) or incompletely sterilised (≥ 1 flower or fruit). As in nature, parasite transmission occurs only horizontally via colonisation or intraspecific takeovers; thus reproduction of occupied trees results in unoccupied ('empty') trees. We have the following categories, where capital letters denote abundances and rates of adults and corresponding lower-case letters denote saplings (see Fig. 5 for the schematic description and Table S2 for parameter values):

Empty trees: Saplings (x) do not reproduce; they die at rate d_0. At rate h they grow into empty adults. *Adults* (X) produce empty saplings at rate R_0 and die at rate D_0. All empty trees can be colonised by queens to become occupied saplings or adults occupied by small colonies.

Occupied saplings (y) die at rate d_1 and do not reproduce, so no sterilisation is necessary. We assume that colony growth
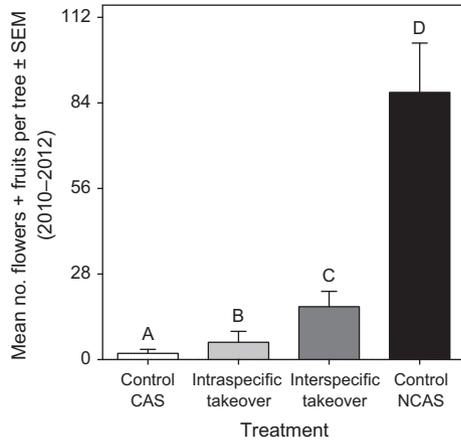
Figure 4 Results of the staged-takeover experiment. Control CAS: unmanipulated focal trees ($n = 20$) occupied by the sterilising ant species *C. nigriceps*; Intraspecific takeover: focal trees ($n = 20$) initially occupied by *C. nigriceps* and experimentally subjected to takeover by other *C. nigriceps* colonies; Interspecific takeover: focal trees ($n = 20$) initially occupied by the non-sterilising ant species *C. mimosae* and experimentally subjected to takeover by *C. nigriceps* colonies; Control NCAS: unmanipulated focal trees ($n = 20$) occupied by *C. mimosae*. Letters indicate significant differences ($P < 0.05$) among treatments in mean reproductive output across the six surveys, using one-tailed tests in planned pairwise contrasts of coefficients from a negative binomial GLM. See Table S1 for statistical details and Fig. S2 for flowers and fruits graphed separately.
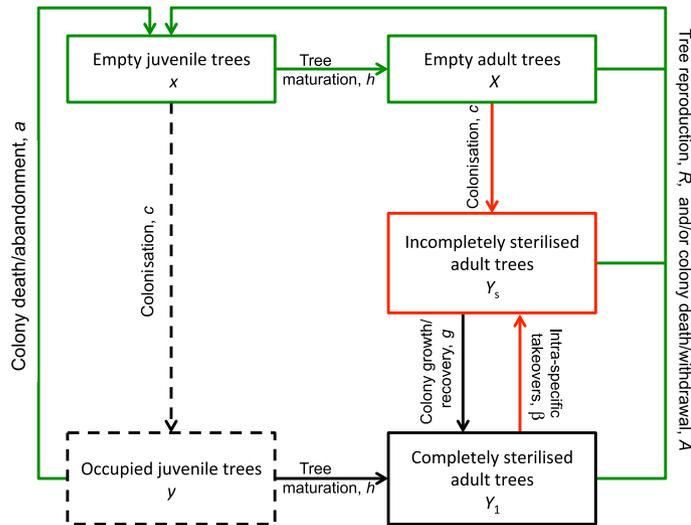
Figure 5 Schematic depiction of the theoretical model for the system with just one ant species (the sterilising symbiont *C. nigriceps*). Boxes represent different categories of trees; colors correspond to those used in Figure 6. Trees are either saplings or adults, with the former maturing at rate h. Saplings do not reproduce and are assumed to be either empty (x) or occupied by the sterilising ant symbiont (y). Adult trees are similarly either empty or occupied, but the latter can be occupied by a small colony and thus be incompletely sterilised (Y_s), or they can be occupied by a large colony and thus be fully sterilised (Y_1). The transition from the Y_s category to the Y_1 category is given by the growth/recovery rate g of the ant colony; the reverse transition can occur due to new colonisation events (rate c) and/or intraspecific takeovers (rate β), both of which result in small colonies.

rate is much faster than tree growth rate, such that occupied saplings transition into fully sterilised adults (Y) at rate h. Colonies on saplings produce queens at rate c_1, and die or withdraw at rate a_1.

Incompletely sterilised adult trees (Y_s) are adult trees occupied by 'small' colonies, resulting from recent colonisation or competitive conflict. They reproduce at rate $(1 - s)R_0$ and die at rate D_s (i.e. a Y_s tree has a fraction $0 \leq s < 1$ of its reproductive structures destroyed; this represents the average level of sterilisation incurred by a Y_s host). Their colonies produce queens at rate C_s and die or abandon at rate A_s. Y_s trees transition into completely sterilised adults (Y) at rate g, the average growth rate of small (incompletely sterilising) colonies into large (fully sterilising) ones.

Completely sterilised adult trees (Y) are occupied by 'large' colonies; they do not reproduce, and die at rate D_1. They produce queens at rate C_1 and die or abandon at rate A_1.

We let β be the rate of intraspecific conflict and we reasonably assume that only colonies sufficiently large to completely sterilise their current trees (i.e. Y) are likely to initiate an expansionist conflict. When a large colony on an adult tree encounters a colony on a sapling, it takes over without sustaining major losses:

$$Y + y \xrightarrow{\beta} Y + y \qquad (1)$$

We conservatively assume that when a large colony (Y tree) meets a small colony (Y_s tree), the conflict does not reduce the size of the large colony below the total-sterilisation threshold; consequently, the large colony is able to fully sterilise both its original and the newly acquired tree. However, because the latter was previously occupied by a small colony, it is not completely sterilised when the conflict occurs; if this happens after floral buds have matured, the expanding large colony cannot affect its new host's reproduction until the next reproductive event. This occurs a fraction f_I of the time, and we call it a 'type I legacy effect'. The remaining takeovers will occur during or just prior to budding and the expanding large colony will be able to completely sterilise its new host:

$$Y + Y_s \xrightarrow{\beta} (1 - f_I)(Y + Y) + f_I \underbrace{(Y + Y_s)}_{\text{type I legacy}} \qquad (2)$$

When two large colonies (Y) meet, we assume that the winner is sufficiently diminished by the conflict that it becomes small. However, because the newly acquired tree was previously occupied by a large colony, it is already completely sterilised and the new colony has time to grow large before the next budding event. We call this a 'type II legacy effect', and it occurs a fraction f_{II} of the time:

$$Y + Y \xrightarrow{\beta} (1 - f_{II})(Y + Y_s) + f_{II} \underbrace{(Y + Y)}_{\text{type II legacy}} \qquad (3)$$

The rate g of transition from being incompletely to completely sterilised depends on the frequency of intraspecific takeovers: small colonies resulting from takeovers are larger than incipient colonies, and coupled with type II legacy effects they yield a higher g. Thus, the higher the conflict rate, the greater the

average transition rate. Let g_β be the transition rate in single-ant-species systems having intraspecific takeovers at rate β; if no intraspecific takeovers occur (as in the *Cordia-Allomerus* system: Szilágy *et al.* 2009), $\beta = 0$. Then we expect that $g_0 < g_\beta$. Similarly, the average amount of sterilisation incurred by a tree also depends on the rate of intraspecific takeovers. If most incompletely sterilising colonies result from takeovers, an average tree will be more thoroughly sterilised than if most small colonies result from colonisation by foundresses; thus, $s_0 < s_\beta < 1$.

We assume that ants reproduce continuously (consistent, for example, with the year-round reproduction of *Crematogaster* ants in our field system: Stanton *et al.* 2002). Furthermore, we assume that the trees either reproduce continuously or that reproductive structures can be found throughout the year (the latter being the case for the trees in our study population). Empty trees are produced continuously due (1) to recruitment of saplings from soil seed-banks when old trees die and space is produced, and (2) to episodic abandonment or stochastic death of ant colonies. These assumptions are not necessary to yield incomplete sterilisation, but they are useful in enabling us to write a simple and tractable continuous-time model:

$$\frac{dx}{dt} = R_0(X + (1 - s_\beta)Y_s)(1 - (x + X + y + Y_s + Y)/K)$$
$$- x(c_1 y + C_s Y_s + C_1 Y) + a_1 y - (h + d_0)x$$

$$\frac{dX}{dt} = hx - X(c_1 y + C_s Y_s + C_1 Y) + A_s Y_s + A_1 Y - D_0 X$$

$$\frac{dy}{dt} = x(c_1 y + C_s Y_s + C_1 Y) - (h + a_1 + d_1)y$$

$$\frac{dY_s}{dt} = X(c_1 y + C_s Y_s + C_1 Y) - (g_\beta + A_s + D_s)Y_s$$
$$- (1 - f_I)\beta Y Y_s + (1 - f_{II})\beta Y^2$$

$$\frac{dY}{dt} = hy + g_\beta Y_s - (A_1 + D_1)Y - (1 - f_{II})\beta Y^2 + (1 - f_I)\beta Y Y_s$$

$$(4)$$

Here K imposes a density limitation on the recruitment of new saplings – the more trees in the population, the fewer saplings will recruit. We explore whether this system can have an equilibrium with no incomplete sterilisation (i.e. $Y_s^* = 0$) but with ants still present (i.e. either $y^* \neq 0$ or $Y^* \neq 0$). If there are no takeovers (i.e. $\beta = 0$) and $Y_s^* = 0$, then from $dY_s/dt = 0$ either $X^* = 0$ or $y^* = Y^* = 0$; the latter is the no-ants case which is not of interest. If $X^* = 0$, then from $dX/dt = 0$ follows $x^* = 0$ and $Y^* = 0$ yielding at equilibrium $dy/dt < 0$, so $y^* = 0$ as well. This gives the trivial equilibrium (i.e. extinction of both plants and ants). A similar argument works when takeovers occur. This analysis is not a proof that an equilibrium with ants present exists; only that both with and without takeovers, as long as the sterilising ants are still present in the system at equilibrium, some non-zero level of incomplete sterilisation will exist. It is difficult to solve analytically for mixed equilibria in this nonlinear system. However, numerical analysis shows that at least one stable mixed equilibrium exists (Fig. 6, Fig. S5).

This analysis shows that the existence of incomplete sterilisation is independent of parameter values, provided that ants still persist in the system. However, the degree of incomplete
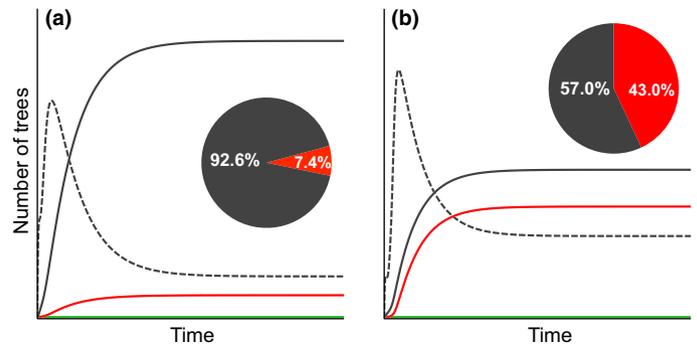
Figure 6 Number of trees in the different categories depicted in Fig. 5, as predicted by the theoretical model with one sterilising ant species. In (a), intraspecific takeovers do not occur; in (b), intraspecific takeovers do occur. Green = unoccupied adults and saplings; red = incompletely sterilised adults; dashed black = occupied saplings; solid black = completely sterilised adults. Pie charts in each panel show the percentage of incompletely sterilised (red) and completely sterilised (black) trees at equilibrium. See Table S2 for a full list of parameters.

sterilisation does depend on the parameters: greater colony growth/recovery rates shorten the window of opportunity for tree reproduction and reduce the incidence of incomplete sterilisation; greater colony death/abandonment rates increase production of empty, ready-to-be-colonised trees and increase the incidence of incomplete sterilisation; lower death rates of occupied trees (e.g. if the sterilising ant defends well against herbivores) generate larger equilibrium tree populations and more incomplete sterilisation, because longer-lived hosts are more likely to clear infection and be recolonised. Importantly, however, although Fig. 6 shows results for a symbiont that protects its host (as appropriate for ant–plant systems), the proof above shows existence of incomplete sterilisation for any sterilising symbiont. Finally, the higher the takeover rate, the greater the incidence of incomplete sterilisation, because colony sizes will be reduced more frequently (see Figs. S3 and S4 for sensitivity analyses). If the rate of encounter between colonies is an increasing function of tree density, as we expect, then the frequency of incomplete sterilisation should be greater at high tree densities (as also found by Szilagyi et al. 2009, via a different mechanism).

Our analysis further shows that colony-size effects enable stable persistence of the host population despite nearly all individuals being occupied by sterilisation parasites. In the absence of this mechanism, the tree population only persists under restricted parameter regimes, requiring rare colonisation by foundress queens and high colony death/abandonment rates. This is unlikely unless extrinsic factors are at play – for example, climatic events that precipitate colony abandonment or death.

This one-species model can be extended to systems with sterilising and non-sterilising symbionts competing for hosts. In the Supporting Information, we model a system with two symbionts (CAS and NCAS), in which coexistence is maintained via a colonisation-competition tradeoff. We show that any equilibrium in which both symbionts coexist must display non-zero incidence of incomplete sterilisation. Numerical solutions show the existence of at least one such equilibrium (Fig. S5).

DISCUSSION

We have shown experimentally that a simple mechanism – numerical insufficiency – can lead to incomplete sterilisation in an ant–plant, and that competitive conflicts among ant colonies provide one route to this outcome in nature. We have shown theoretically that this mechanism, coupled with ecologically driven fluctuations in parasite populations, can account for empirical observations that parasitic sterilisation is rarely (if ever) 100% effective at the host-population level. We further showed that these processes enable persistence of both host and parasite populations, even when the parasite occupies nearly all hosts. In this framework, the theoretical expectation that sterilisation parasites should evolve maximal virulence (Jaenike 1996) is consistent both with a non-zero equilibrium level of reproducing hosts and with the apparent stability of systems (including *A. drepanolobium* in southern Kenya/northern Tanzania) in which the sterilisation-parasite prevalence is extremely high.

Our finding that sterilisation efficacy decreases with reductions in parasite load is consistent with findings in other systems – for example, the sterilising bacterium *Pasteuria ramosa* in the crustacean *Daphnia magna* (Ebert *et al.* 2004) and the cestode *Schistocephalus solidus* in the fish *Gasterosteus aculeatus* (Heins *et al.* 2010). We suggest that this is a plausible expectation in any system in which there is sufficient lag between infection and growth to full sterilising capacity. Hall *et al.* (2007) included this assumption into dynamic-energy-budgets models of how hosts, their resources, and their parasites interact at the level of the individual host. To our knowledge, however, our study is the first to invoke numerical insufficiency to explain incomplete sterilisation at the host-population level.

Various proximate causes could lead to numerical insufficiency in nature, including disease, drought, colonisation-extinction dynamics, and competition. A major cause of numerical insufficiency in our system is interference competition between ant colonies for host trees (Fig. 4). Previous work in other systems has identified cases in which interference competition leads to decreased virulence (reviewed in Alizon *et al.* 2013), but most such cases involve antagonistic effects among parasites coinfecting the same host. In our system, different colonies do not coexist within the same host. Instead, inter-colony conflict leaves a legacy of temporarily reduced parasite load: as the size of the surviving colony subsequently recovers, so does sterilisation efficacy. Another plausible manifestation of numerical insufficiency in this system is suggested by prior work (Stanton & Palmer 2011) on the energetic cost of mutualism: plants with small ant colonies have more resources to invest in reproduction and produce more flowers, which small colonies cannot eliminate.

Importantly, our conclusions emerge from simple ecological dynamics in mean-field models: they do not require the evolution of reduced virulence, host tolerance or sanctioning, spatial structure, or complex collective behaviors (e.g. precise regulation of the degree of host sterilisation by the colony, as required under reduced-virulence scenarios). We emphasise, however, that these mechanisms are not incompatible with our model and may still be important in governing the dynamics of sterilisation-parasite associations, perhaps in ways that are synergistic with colony-size effects. For example, previous studies have suggested that some myrmecophytes have evolved strategies for minimising damage by sterilisation parasites. *Cordia nodosa* tolerates sterilisation by increasing floral growth on new shoots patrolled primarily by a non-sterilising caste of *Allomerus octoarticulatus* (Edwards & Yu 2008), and *Hirtella* spp. host plants sanction sterilising *Allomerus* ants by reducing domatium and nectar production (Malé *et al.* 2014) or selectively aborting domatia (Izzo & Vasconcelos 2002). In systems like *Cordia* and *Hirtella*, interference competition for hosts is rare after the founding stage, meaning that reductions in CAS colony size below the total-sterilisation threshold are less frequent than in our field system. However, colony-size effects similar to those described in this paper could still operate due to colonisation-extinction dynamics and/or environmental factors that might episodically reduce colony size.

Although we do not attempt to rule out the evolution of intermediate virulence as an explanation for incomplete sterilisation, we do show that, contrary to prior claims, reduced virulence is not a *necessary* condition for its occurrence. Because ours is an easily tested ecological explanation, we suggest that it is an important hypothesis to consider and a relevant first principle to incorporate into future models of sterilisation parasite-host associations. Evaluating the likely interplay between ecological and evolutionary determinants of this phenomenon, perhaps via comparative study of *A. drepanolobium* populations throughout Kenya, represents an exciting avenue for future research.

AUTHORSHIP

CET conceived the study. CET and RMP developed the theory. CET, RMP, and TMP designed the experiments. RMP performed the experiments and analyzed data. CET and RMP wrote the manuscript with input from TMP.

REFERENCES

Alizon, S., de Roode, C. & Michalakis, Y. (2013). Multiple infections and the evolution of virulence. *Ecol. Lett.*, 16, 556–567.
Baudoin, M. (1975). Host sterilization as a parasitic strategy. *Evolution*, 29, 335–52.
Bonds, M.H. (2006). Host life-history strategy explains pathogen- induced sterility. *Am. Nat.*, 168, 281–293.

Ebert, D., Carius, H.J., Little, T. & Decaestecker, E. (2004). The evolution of virulence when parasites cause host sterilization and gigantism. *Am. Nat.*, 164, S19–32.

Edwards, D.P. & Yu, D.W. (2008). Tolerating sterilization by hiding flowers in plain sight. *Behav. Ecol. Sociobiol.*, 63, 95–102.

Frederickson, M.E. (2009). Conflict over reproduction in ant-plant symbiosis: why Allomerus octocarticulatus ants sterilize Cordia nodosa trees. *Am. Nat.*, 173, 675–681.

Gaume, L., Zacharias, M. & Borges, R.M. (2005). Ant-plant conflicts and a novel case of sterilization parasitism in a myrmecophyte. *Evol. Ecol. Res.*, 7, 435–452.

Hall, S.R., Becker, C. & Cáceres, C.E. (2007). Parasitic sterilization: a perspective from a model of dynamic energy budgets. *Integr. Comp. Biol.*, 47, 295–309.

Heins, D.C., Baker, J.A., Toups, M.A. & Birden, E.L. (2010). Evolutionary significance of fecundity reduction in threespine stickleback infected by the diphyllobothriidean cestode Schistocephalus solidus. *Biological Journal of the Linnean Society*, 100, 835–846.

Izzo, T.J. & Vasconcelos, H.L. (2002). Cheating the cheater: domatia loss minimizes the effects of ant sterilization in an Amazonian ant-plant. *Oecologia*, 133, 200–205.

Jaenike, J. (1996). Suboptimal virulence of an insect-parasitic nematode. *Evolution*, 50, 2241–2247.

Lafferty, K.D. & Kuris, A.M. (2009). Parasitic sterilization: the evolution and ecology of body snatchers. *Trends Parasitol.*, 25, 564–572.

Lopez-Villavicencio, M., Enjalbert, E., Hood, M.E., Shykoff, J.A., Raquin, C. & Giraud, T. (2005). The anther smut disease on *Gypsophila repens*: a case of parasite sub-optimal performance following a recent host shift? *J. Evol. Biol.*, 18, 1293–1303.

Malé, P.J.G., Leroy, C., Dejean A., Quilichini, A. & Orivel, J. (2012). An ant symbiont directly and indirectly limits its host plants reproductive success. *Evol. Ecol.* 26, 55–63.

Malé, P.J.G., Ferdy, J.B., Leroy, C., Roux, O., Lauth, J., Avilez, A.*et al.* (2014). Retaliation in response to sterilization promotes a low level of virulence in an antplant mutualism. *Evol. Biol.* 41, 22–28.

Martins, D.J. (2011). Multiple-species interactions in African ant-acacias. PhD Thesis, Harvard University, Cambridge, MA.

O'Keefe K.J. & Antonovics, J. (2002). Playing by different rules: the evolution of virulence in sterilizing pathogens. *Am. Nat.*, 159, 597–605.

Palmer, T.M., Young, T.P., Stanton, M.L. & Wenk E. (2000). Short-term dynamics of an acacia ant community in Laikipia, Kenya. *Oecologia*, 123, 425–435.

Palmer, T.M., Young, T.P. & Stanton, M.L. (2002). Burning bridges: priority effects and the persistence of a competitively subordinate acacia-ant in Laikipia, Kenya. *Oecologia*, 133, 372–379.

Palmer, T.M. (2004). Wars of attrition colony size determines competitive outcomes in a guild of African acacia ants. *Anim. Behav.*, 68, 993–1004.

Palmer, T.M. & Brody, A.K. (2007). Mutualism as reciprocal exploitation: African plant-ants defend foliar but not reproductive structures. *Ecology*, 88, 3004–3011.

Palmer, T.M., Stanton, M.L., Young, T.P., Goheen, J.R., Pringle, R.M., & Karban, R. (2008). Breakdown of an ant-plant mutualism follows the loss of large herbivores from an African savanna. *Science*, 319, 192–195.

Palmer, T.M., Doak, D.F., Stanton, M.L., Bronstein, J.L., Kiers, E.T., Young, T.P.*et al.* (2010). Synergy of multiple partners, including freeloaders, increases host fitness in a multispecies mutualism. *Proc. Natl. Acad. Sci.*, 107, 17234–17239.

R Development Core Team (2012). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rudolph, K. (2012). Consequences of obligate partnership for hosts and defenders in an African Acacia-ant mutualism. PhD Thesis, University of Florida, Gainesville, FL.

Sloan, D.B., Giraud, T. & Hood, M.E. (2008). Maximized virulence in a sterilizing pathogen: the anther-smut fungus and its co-evolved hosts. *J. Evol. Biol.*, 21, 1544–1554.

Stanton, M.L., Palmer, T.M., Young, T.P., Evans, A. & Turner, M.L. (1999). Sterilization and canopy modification of a swollen thorn acacia tree by a plant-ant. *Nature*, 401, 578–581.

Stanton, M.L., Palmer, T.M. & Young, T.P. (2002). Competition-colonization trade-offs in a guild of African Acacia-ants. *Ecol. Monogr.*, 72, 347–363.

Stanton, M.L. & Palmer, T.M. (2011). The high cost of mutualism: effects of four species of East African ant symbionts on their myrmecophyte host tree. *Ecology*, 92, 1073–1082.

Stapley, L. (1998). The interaction of thorns and symbiotic ants as an effective defence mechanism of swollen-thorn acacias. *Oecologia*, 115, 401–405.

Szilágy, A., Scheuring, I., Edwards, D.P., Orivel, J. & Yu, D.W. (2009). The evolution of intermediate sterilization virulence and ant coexistence in a spatially structured environment. *Ecol. Lett.*, 12, 1306–1316.

Venables, W.N. & Ripley, B.D. (2002). *Modern Applied Statistics with S. Fourth Edition*. Springer, New York.

Young, T.P., Stubblefield, C.H. & Isbell, L.A. (1997). Ants on swollen-thorn acacias: species coexistence in a simple system. *Oecologia*, 109, 98–107.

Yu, D.W. (2001). Parasites of mutualisms. *Biological Journal of the Linnean Society*, 72, 529–546.

Yu, D.W. & Pierce, N.E. (1998). A sterilization parasite of an ant-plant mutualism. *Proc. R. Soc. Lond. B*, 265, 375–382.

Zuur, A.F., Leno, E.N., Walker, N., Saveliev, A.A., & Smith, G.M. (2009) *Mixed-effects Models and Extensions in Ecology with R*. Springer, New York.

SUPPORTING INFORMATION

Additional Supporting Information may be downloaded via the online version of this article at Wiley Online Library (www.ecologyletters.com).