Cascading effects of defaunation on the coexistence of two specialized insect seed predators

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Summary

1. Identification of the mechanisms enabling stable coexistence of species with similar resource requirements is a central challenge in ecology. Such coexistence can be facilitated by species at higher trophic levels through complex multi-trophic interactions, a mechanism that could be compromised by ongoing defaunation.

2. We investigated cascading effects of defaunation on *Pachymerus cardo* and *Speciomerus giganteus*, the specialized insect seed predators of the Neotropical palm *Attalea butyracea*, testing the hypothesis that vertebrate frugivores and granivores facilitate their coexistence.

3. Laboratory experiments showed that the two seed parasitoid species differed strongly in their reproductive ecology. *Pachymerus* produced many small eggs that it deposited exclusively on the fruit exocarp (exterior). *Speciomerus* produced few large eggs that it deposited exclusively on the endocarp, which is normally exposed only after a vertebrate handles the fruit. When eggs of the two species were deposited on the same fruit, *Pachymerus* triumphed only when it had a long head start, and the loser always succumbed to intraguild predation.

4. We collected field data on the fates of 6569 *Attalea* seeds across sites in central Panama with contrasting degrees of defaunation and wide variation in the abundance of vertebrate frugivores and granivores. *Speciomerus* dominated where vertebrate communities were intact, whereas *Pachymerus* dominated in defaunated sites. Variation in the relative abundance of *Speciomerus* across all 84 sampling sites was strongly positively related to the proportion of seeds attacked by rodents, an indicator of local vertebrate abundance.

5. *Synthesis.* We show that two species of insect seed predators relying on the same host plant species are niche differentiated in their reproductive strategies such that one species has the advantage when fruits are handled promptly by vertebrates and the other when they are not. Defaunation disrupts this mediating influence of vertebrates and strongly favours one species at the expense of the other, providing a case study of the cascading effects of defaunation and its potential to disrupt coexistence of non-target species, including the hyperdiverse phytophagous insects of tropical forests.

Key-words: *Attalea butyracea*, facilitation, intraguild predation, multi-trophic interactions, Panama, seed beetles, seed fate, top-down control, trophic cascades

Introduction

Identification of the mechanisms that enable the coexistence of species with similar resource requirements is

fundamental for understanding the organization and maintenance of biodiversity. This challenge has traditionally been addressed by focusing on antagonistic interactions in just one (competition) or two (predator–prey) trophic levels (Gurevitch, Morrison & Hedges 2000; Chase *et al.* 2002). However, the recognition of intraguild predation, that is competitors killing and eating each other (Arim & Marquet 2004), and facilitative interactions as important community structuring forces (Bruno,

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Stachowicz & Bertness 2003) has fostered the adoption of more holistic multi-trophic approaches (Tscharntke & Hawkins 2002; Smith, Mooney & Agrawal 2008; Visser *et al.* 2011). Further, interspecific interactions may show conditionality when their net outcome varies with the ecological context (Bronstein 1994). The heterogeneity of natural communities thus requires studying species coexistence in contrasting multi-trophic contexts to disentangle the effects of the antagonistic and facilitative mechanisms (Amarasekare 2008).

Phytophagous insects provide paradigmatic cases of species that coexist despite similar resource requirements. Seed predators in particular are a diversified guild of insects that often show high degrees of niche overlap due to their high levels of host specificity (Janzen 1980; Novotny & Basset 2005; Delobel & Delobel 2006). Competing seed parasitoid species are typically differentiated in their adult phenologies and oviposition behaviours (Mitchell 1975; Messina 1991; Johnson & Romero 2004; Espelta, Bonal & Sánchez-Humanes 2009). Yet, despite these differences, a single seed may end up with larvae of more than one species (Espelta, Bonal & Sánchez-Humanes 2009). Seeds are discrete resources, which sometimes cannot sustain the development of more than one individual, and thus, larval competition within a seed is intense. One of the usual outcomes of such competition is intraguild predation among larvae leaving a single survivor (Wilson & Janzen 1972; Janzen 1975; Traveset 1991; Peguero, Bonal & Espelta 2014). Though such direct interspecific competition is clearly important, a community-wide perspective is needed to account for indirect, enemy-mediated and facilitative effects across different trophic levels (Kaplan & Denno 2007).

Coexistence between species that share the same key resource could be stabilized if the outcome of their competition varies with the community context (Smith, Mooney & Agrawal 2008). In the case of insect seed predators sharing the same host plant, variation in the abundance of another seed consumer or shared enemy can modulate their intraguild competition if one of the insects is more vulnerable to or favoured by this third agent. For instance, one insect could dominate its competitor in the presence of a facilitative species, but be subordinate in its absence. Variation in relative dominance would then result from this third species disproportionately favouring the inferior competitor and/or disadvantaging the superior competitor. If the abundance of the third species is spatially heterogeneous, the coexistence of competing species at a regional level could be stabilized (see Gurevitch, Morrison & Hedges 2000 and Chase *et al.* 2002 for a similar reasoning for competitors under variable predation pressure). However, to our knowledge, the hypothesis that multi-trophic interactions may mediate the coexistence of specialized seed predators has never been empirically tested.

Vertebrates are key candidates for mediating the coexistence of seed predators, as they may feed on the fruits and seeds as well as on larvae inside fruits and seeds (Sallabanks & Courtney 1992). Examples of top-down control by reptiles, birds and mammals of insect species at lower trophic levels are many (Terborgh *et al.* 2001; Böhm, Wells & Kalko 2011; Visser *et al.* 2011), but we know of no study that has considered whether vertebrates facilitate the coexistence of competing insects such as specialized seed predators. The question of whether or not vertebrates mediate insect species coexistence is particularly important given the diversity of insects combined with global defaunation trends, that is the extirpation and population decline of native vertebrate species, which can increase the variability of local abundances producing many unanticipated cascade effects of biodiversity loss (Dirzo *et al.* 2014).

Here, we study the consequences of defaunation for species coexistence by assessing whether and how vertebrate frugivores and granivores affect the competitive balance of two specialized bruchid beetles (Bruchinae: Pachymerini) whose larvae are seed predators of the tropical palm *Attalea butyracea* (hereafter '*Attalea*'). Previous studies suggested that these bruchids differ in oviposition strategy. The larger species – *Speciomerus giganteus* (hereafter '*Speciomerus*') – lays its eggs on exposed endocarps on the forest floor, and is thus a post-dispersal seed parasitoid (Wright 1983). The smaller species – *Pachymerus cardo* (hereafter '*Pachymerus*') – is thought to lay its eggs on the exocarp of the fruits while they are developing in the canopy, and would thus be a pre-dispersal seed parasitoid, like its local sister species *Pachymerus bactris*, the seed parasitoid of the palm *Astrocaryum standleyanum* (Jansen *et al.* 2010). Though multiple larvae usually infest the same seed, only one adult bruchid can emerge, suggesting that intraguild predation occurs among bruchid larvae (Wilson & Janzen 1972; Wright 1983).

Vertebrates may affect these bruchids in at least three ways (Fig. 1). First, many vertebrate species feed on the fruit pulp, thus exposing the endocarp to oviposition by *Speciomerus*. Secondly, three species of rodents remove and store seeds as food supplies in shallow soil surface caches, thus moving seeds out of reach of bruchids (Jansen *et al.* 2010, 2012, 2014). Thirdly, at least two rodent species also consume bruchid larvae (Silvius & Fragoso 2002; Gálvez & Jansen 2007) and may exert a strong top-down control over bruchid populations (Wright & Duber 2001; Visser *et al.* 2011). Previous studies monitoring fruit falling into traps showed that 63% of the fruits have their endocarps partly exposed ($n = 2468$, 21 palms) on Barro Colorado Island (BCI), which has a complete vertebrate community, vs. just 18% ($n = 5270$, 27 palms) in Soberania National Park (SNP) which has high levels of poaching (P.A. Jansen, unpubl. data). This suggests that the accessibility of *Speciomerus* to the endocarps is lower
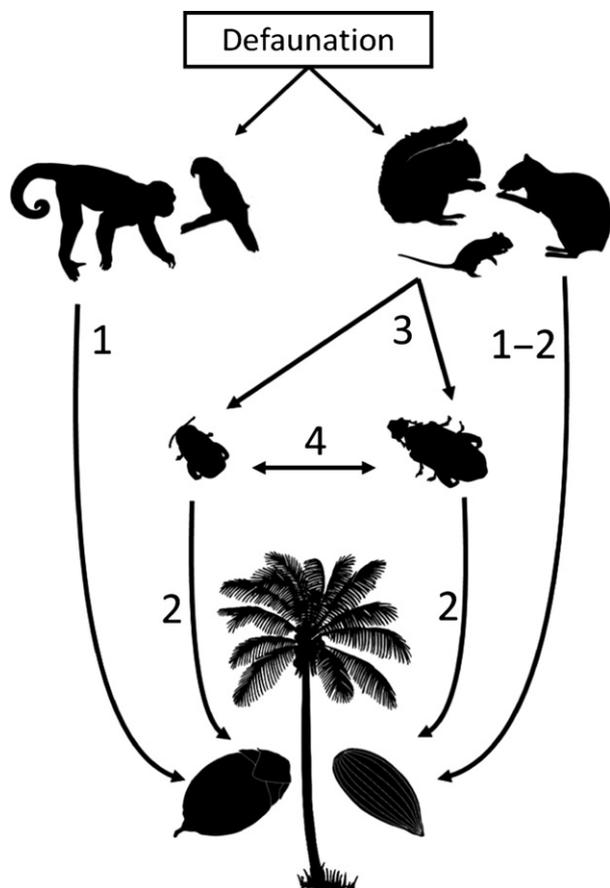
Fig. 1. Food web associated with the fruits and seeds of the palm *Attalea butyracea* in central Panama. Numbers denote different trophic interactions: 1. Fruit pulp feeding; 2. seed feeding; 3. predation of bruchid larvae by rodents; 4. intraguild predation of larvae. Defaunation cascades down the web to affect the fate of the palm seeds.

where frugivore populations are smaller, allowing more *Pachymerus* larvae to develop. Therefore, our overarching hypothesis was that vertebrates mediate the coexistence of these beetles and that defaunation could favour one parasitoid at the expense of the other.

We tested the following specific predictions: (i) *Pachymerus* and *Speciomerus* differ importantly in reproductive ecology; (ii) *Speciomerus* larvae kill *Pachymerus* larvae in the same seed, unless *Pachymerus* has a sufficiently long head start; and (iii) relative abundances of the two species will depend on vertebrate abundance, with *Speciomerus* dominating where vertebrate frugivores are common, because the latter facilitate its oviposition and reduce the head start of *Pachymerus*. We tested these hypotheses through oviposition trials and competition experiments, combined with sampling at sites with contrasting vertebrate abundance. Overall, our results indicate that vertebrates indeed mediate the coexistence of the specialized seed predators in this system by modifying their competitive balance. Thus, we demonstrate that multi-trophic interactions can contribute to the coexistence of insect species with similar resource requirements.

Materials and methods

STUDY SYSTEM

This study was carried out in central Panama, at BCI, in SNP, and at small (<1·5 ha) islands in Gatun Lake (hereafter referred to as islets). Annual rainfall averages 2600 mm with a distinct 4-month dry season from January to April. The vegetation is semi-deciduous tropical moist forest. Because BCI is well protected from poaching while SNP is not, vertebrates are much more abundant on BCI than in SNP (Wright *et al.* 2000). The islets are virtually defaunated because they are too small to sustain populations of vertebrates larger than rats (Adler & Seamon 1991; Wright & Duber 2001). Thus, these three types of site form a gradient of defaunation.

Attalea butyracea (Mutis ex L.f.) Wess. Boer (formerly *Scheelea zonensis*) is a monoecious arborescent canopy or sub-canopy palm that is abundant in Panama (Wright 1983). Mature individuals annually produce one to three infructescences with 100–600 ovoid drupes (3–6 cm length) that ripen during the first half of the wet season (Wright 1983). These fruits have a tough exocarp, a soft oily mesocarp and a stone, that is a hard woody endocarp that usually encloses one seed, although around 2% contain two or even three seeds (Bradford & Smith 1977). The woody endocarps remain on the forest floor more than 3 years as they slowly decompose (Wright 1983). The mesocarp is eaten by many vertebrate species including Baird's tapir (*Tapirus bairdii* Gill), white-faced monkey (*Cebus capucinus* L.), howler monkey (*Alouatta palliata* L.), kinkajou (*Potos flavus* Schreber) and red-lored parrot (*Amazona autumnalis* L.), all of which are able to open the tough exocarp (Wright *et al.* 2000; G. Peguero, pers. obs.). Of these, only Baird's tapir ingests the endocarps (Jansen *et al.* 2014). Three rodent species – red-tailed squirrel (*Sciurus granatensis* Humboldt), Central American agouti (*Dasyprocta punctata* Gray) and Central American spiny rat (*Proechimys semispinosus* Tomes) – are also able to open the endocarps to eat the seeds or the bruchid larvae inside (Wright & Duber 2001; Gálvez & Jansen 2007; Jansen *et al.* 2014).

Two bruchid beetles prey on *Attalea* seeds in this area: *S. giganteus* Chevrolat and *P. cardo* Fåhareus (henceforth referred to by their genus names). They have no other local hosts (S. Gripenberg, pers. comm.) and no known larval predators (S.J. Wright, unpubl. data). *Speciomerus* is larger and is a post-dispersal seed parasitoid that only lays eggs on defleshed endocarps, that is after opening or removal of the exocarp by a frugivore (Wilson & Janzen 1972; Wright 1983). In contrast, *Pachymerus* can lay its eggs directly on intact and partially defleshed fruits (Silvius & Fragoso 2002; D. Gálvez, unpubl. data) hence could be a pre-dispersal seed parasitoid. Previous observations also suggest differences in fecundity traits like egg number and size (D. Gálvez, unpubl. data). Relative abundance differs among sites. *Speciomerus* is dominant in BCI (Visser *et al.* 2011) whereas *Pachymerus* accounts for more than half of bruchid emergences in the SNP (S.J. Wright, unpubl. data).

REPRODUCTIVE ECOLOGY

We quantified differences between the two bruchids in the timing of adult emergence, egg number and size, and oviposition site preferences. We collected 750 endocarps beneath approximately 15 haphazardly selected trees along Pipeline Road in SNP during

the dry season of 2013 and incubated them in 15 20-litre buckets sealed off with insect screening at ambient temperature but protected from rain and direct sunlight. Buckets were checked daily, the timing of emergence was recorded and each bruchid was identified to species. We evaluated interspecific differences in the timing of emergence using a generalized estimating equation model (GEE) with a Poisson log-link function that included the interaction between species and emergence time (in ordinal days), treated each incubation bucket as a blocking factor and included an autoregressive correlation structure for each bucket (block) to account for the longitudinal nature of the data.

The emerged bruchids were mixed and placed in groups of 10–20 conspecifics in 25 × 20 × 8 cm plastic containers with dry leaves and water in glass vials. After allowing for mating, 17 females of each species were placed individually in plastic containers with one intact fruit, one partially defleshed endocarp (i.e. a gashed fruit conserving half of the exo- and mesocarp) and a bare endocarp (i.e. a fruit with exo- and mesocarp completely removed). These treatments mimic the three most common levels of fruit handling by vertebrates: a non-handled fruit, an endocarp partially defleshed by a canopy-dwelling pulp feeder and an endocarp entirely defleshed, as a scatter-hoarding rodent would do (Silvius & Fragoso 2002; Gálvez & Jansen 2007; Jansen *et al.* 2010). Fruits were mixed and randomly assigned to treatments to ensure that there were no differences in size or maturation stage.

Every 72 h, the fruits were replaced by new ones (i.e. fruits collected and manipulated the same day). The number of eggs laid in each fruit treatment and whether they were attached to the exocarp or to the endocarp in the partially defleshed fruits was monitored daily until the beetles died. Interspecific differences in oviposition preference were inferred from the proportion of eggs laid on each fruit type and the proportion of eggs attached to endo- vs. exocarp. We fitted generalized linear models (GLMs) with binomial or, if overdispersed, quasibinomial errors, and with species as factor and lifetime as covariate, to assess potential behavioural differences related to maternal lifetime.

A random subsample of the eggs of six females per species (35 ± 6 eggs per female, mean ± SE, $N = 418$) were photographed and their maximum length and width measured to the nearest 0·001 mm using the software IMAGEJ (Schneider, Rasband & Eliceiri 2012). Egg volume was calculated from these measurements under the assumption that the eggs were prolate spheroids, that is as $(4\pi/3)a^2c$, where a is the equatorial diameter and c is the polar diameter. Generalized linear mixed models (GLMMs) were used to assess interspecific differences in egg volume and fecundity (egg number), including species as factor, mother beetle as a random effect nested within species and maternal lifetime (days) as covariate.

INTRAGUILD INTERACTION TRIALS

We conducted a series of incubation trials to test the hypothesis that the outcome of the intraguild interaction among the larvae of *Pachymerus* and *Speciomerus* depends on the timing of infestation. In July 2013, we collected 257 ripe intact fruits directly from the canopy of five haphazardly selected palms along Pipeline Road in SNP, using a pole pruner. The exo- and mesocarp of each fruit were completely removed with a knife, and fresh unhatched eggs (i.e. laid <24 h before) of both species were placed onto the bare endocarps to create the following six experimental treatments: (i) one egg of *Pachymerus*; (ii) one egg of

Speciomerus; (iii) one egg of each species simultaneously ('P0' hereafter); (iv) one egg of each species with a 10-day head start for *Pachymerus* ('P10' hereafter); (v) one egg of each species with a 30-day head start for *Pachymerus* ('P30'); and (vi) a control group without egg addition. The endocarps were incubated individually in transparent plastic cups with pierced lids to allow air movement, and adult emergences were monitored during the next wet season until they ceased. Each endocarp was then classified by three possible outcomes: emergence of a *Pachymerus* adult, a *Speciomerus* adult or neither.

We used three contingency analyses to evaluate differences in beetle emergence patterns among egg addition treatments. The first compared the control, *Pachymerus*-only and *Speciomerus*-only treatments to assess the effectiveness of the egg addition treatments. This identified bruchid infestations prior to fruit collection (emergence from the control treatment) and the viability of the experimental eggs added to the endocarps. The second contingency analysis compared the *Pachymerus*-only, *Speciomerus*-only and P0 treatments to assess the impact of interspecific interaction between the two bruchid species. Significantly lower emergence of either species in the simultaneous infestation treatment compared with the appropriate single species treatment would indicate that the interspecific interaction is costly. The final contingency analysis compared the P0, P10 and P30 treatments to assess the effect of the timing of oviposition on the outcome of the interspecific interaction.

SEED FATES IN THE FIELD

We conducted a field study during July 2013 to quantify amongst-site differences in the relative abundance of the two bruchids and to determine whether this variation was correlated with differences in rodent abundance and associated levels of feeding on *Attalea* seeds. We investigated the fate of endocarps collected from the forest floor and top soil (5 cm depth). In BCI and SNP, we selected six and five 50-m radius circular plots, respectively. The plots were separated by more than 250 m and located in secondary forests avoiding steep slopes and streams. In Gatun Lake, we selected 11 small (<1·5 ha) islets known to be almost defaunated (see Adler & Seamon 1991). At each plot/islet, we collected all the endocarps under focal *Attalea* individuals (4–6 trees per plot in BCI and SNP, and 1–6 trees per islet upon availability). We defined the area under a palm as the area within 3 m of the trunk (i.e. *c*. 28 m²). We avoided fruiting individuals in order to avoid collecting endocarps from the current season. All endocarps sampled were between 1 and 3 years of age, so that any surviving beetle would already have emerged before the seeds were collected.

We examined the endocarps to determine the fates of their seeds (cf. Wright *et al.* 2000; Visser *et al.* 2011). Emerging bruchids leave distinct circular exit holes that differ in diameter between *Speciomerus* (6·1 ± 0·53 mm) and *Pachymerus* (4·9 ± 0·54 mm, mean ± SD, $n = 16$ per species). The three rodent species leave different tooth marks: agoutis gnaw holes in the sides of the endocarp, red-tailed squirrels leave long gashed tooth marks and triangular openings at one end of the endocarp (Gálvez & Jansen 2007), and spiny rats split the endocarps in half leaving a clean and polished cut (S.J. Wright, pers. obs.). Agoutis and squirrels make smaller openings when extracting bruchid larvae (38·1 ± 17·5 mm², $n = 12$) than when extracting seeds (116 ± 31·8 mm², $n = 10$, mean ± SD; data from Gálvez &

Jansen 2007); hence, we could distinguish seed predation from larval predation (Visser *et al.* 2011).

Thus, all collected endocarps were classified into one of eight distinct seed fates: intact endocarp (I), endocarp opened by a spiny rat (R), endocarp opened by an agouti to extract a bruchid larva (Abr) or the seed (Asd), endocarp opened by a squirrel to extract a bruchid larva (Sbr) or the seed (Ssd), endocarp with a *Speciomerus* emergence hole (Spec) and endocarp with a *Pachymerus* emergence hole (Pach). We calculated the proportions of each seed fate for each focal tree. We also measured the maximum width and length for a representative subsample (*c.* 50%) of the collected endocarps at each focal tree and calculated their volume assuming equivalence with a prolate spheroid.

To quantify differences in seed fates among site types (protected BCI, hunted SNP and completely defaunated islets), we analysed variation among focal trees in the proportions of endocarps in different categories. We used GLMMs with binomial errors and included plot/islet as a random effect. We performed five analyses:

1 To determine whether rodent activity paralleled the defaunation gradient, we analysed the proportion of endocarps handled by rodents [(R + Abr + Asd + Sbr + Ssd)/Total].
2 To determine how bruchid abundance varied over the defaunation gradient, we analysed the proportion of endocarps with bruchid emergence [(Spec + Pach)/Total].
3 To determine how bruchid escape from agoutis and squirrels varied over the defaunation gradient, we analysed the proportion of bruchid-infested endocarps from which a bruchid emerged [(Spec + Pach)/(Spec + Pach + Abr + Sbr)].
4 To determine how the relative abundance of the two bruchids varied over the defaunation gradient, we analysed the proportion of bruchid emergences comprised by *Speciomerus* [Spec/(Spec + Pach)].
5 To evaluate potential consequences of defaunation-related variation in seed predation for *Attalea* population dynamics, we analysed the proportion of intact endocarps that escaped bruchid and rodent attack [I/Total].

According to the previous research (Wright *et al.* 2000), across central Panama the abundance of squirrels and agoutis combined explains 68% of the variation in the abundance of vertebrates at a community level, so that their abundance can be considered a reliable proxy for the level of defaunation more generally (see Fig. S1, Supporting Information). To further illuminate how the two bruchid species respond quantitatively to variation in rodent activity, we fit models for *Pachymerus* emergence [Pach/Total], *Speciomerus* emergence [Spec/Total] and *Speciomerus* relative abundance [Spec/(Spec + Pach)] as a function of the proportion of endocarps handled by rodents [(R + Abr + Asd + Sbr + Ssd)/Total]. We fit these three relationships using GLMMs or nonlinear generalized additive mixed models (GAMMs), with binomial error distributions and plot/islet as a random factor nested with site type (SNP, BCI or islets).

To test whether rodents prefer larvae to seeds, we investigated how the proportion of larvae predated by rodents [(Abr + Sbr)/(Abr + Sbr + Spec + Pach)] change with the proportion of seeds predated by rodents [(Asd + Ssd)/(Asd + Ssd + Intact)]. We fit a GAM for larval predation as a function of seed predation to quantify the relationship between the two. Because it is impossible to differentiate between larval and seed predation in the endocarps handled by rats, they were excluded from this analysis.

Finally, we assessed whether the two bruchid species differ in the sizes of the endocarps they exploit. We conducted a GLMM on log-transformed endocarp volume, with site, emerging bruchid species and their interaction as fixed factors and plot/islet nested within site as a random effect. *P*-values were obtained by normal and Kenward–Roger approximation of degrees of freedom with pbkrtest (Halekoh & Højsgaard 2012).

All analyses were done with the R packages GEEPACK (Højsgaard & Halekoh 2006), GAMM4 (Wood 2011) and LME4 (Bates *et al.* 2014). Best models were selected according to Akaike's information criteria (AIC) and model assumptions were validated graphically.

Results

REPRODUCTIVE ECOLOGY

The two bruchid species differed in their emergence phenology, reproductive traits and oviposition preferences. Adults of *Pachymerus*, the smaller species, emerged significantly earlier in the fruiting season (day*species interaction, Wald = 25·2, $P < 0·0001$) than *Speciomerus*, although the emergence times overlapped substantially (see Fig. S2). Egg volume was almost 20 times larger in *Speciomerus* than in *Pachymerus* ($F_{1,406} = 10171$, $P < 0·0001$, Fig. 2a), whereas *Pachymerus* females laid five times more eggs than *Speciomerus* ($F_{1,31} = 8·2$, $P < 0·01$, Fig. 2b). Maternal lifetime did not affect fecundity ($F_{1,31} = 0·7$, $P = 0·4$).

Speciomerus females overwhelmingly chose to attach their eggs to the endocarp, that is in areas where the flesh had been removed, whereas *Pachymerus* preferred the exocarp and specifically under the bracts ($Z_{1,29} = 8·9$, $P < 0·0001$, Fig. 2c). Consequently, given the choice of one fully defleshed, one partially defleshed and one intact fruit as potential sites for egg deposition, *Speciomerus* females laid almost no eggs on intact fruits (just 1 of 241), and laid 2.4 times more eggs on fully defleshed than on partially defleshed fruits, whereas *Pachymerus* laid almost no eggs on fully defleshed fruits (just 18 of 1676), and laid 1.5 times more eggs on intact than on partially defleshed fruits (Fig. 2d). Thus, the two species showed significant differences in the proportions of eggs laid on each fruit type (fully defleshed $Z_{1,31} = 5·5$, $P < 0·0001$; partially defleshed $Z_{1,28} = −2·6$, $P < 0·05$; intact: $Z_{1,28} = −2·5$, $P < 0·05$). Maternal lifetime did not affect the probability of laying an egg on an endocarp ($Z_{1,30} = 0·7$, $P = 0·5$).

INTRAGUILD INTERACTIONS

Patterns of beetle emergence differed significantly between egg addition treatments (Fig. 3a; $\chi^2 = 44$, d.f. = 10, $P < 0·0001$). *Pachymerus* adults emerged from 30% of the endocarps in the control group, which received no experimental egg addition, implying that this species infested seeds directly in the canopy, before fruit collection (Fig. 3a). Adding a *Speciomerus* egg increased emergence of *Speciomerus* adults from zero to 25% ($\chi^2 = 18·5$, d.f. = 2, $P < 0·0001$, Fig. S3b), whereas the increase of
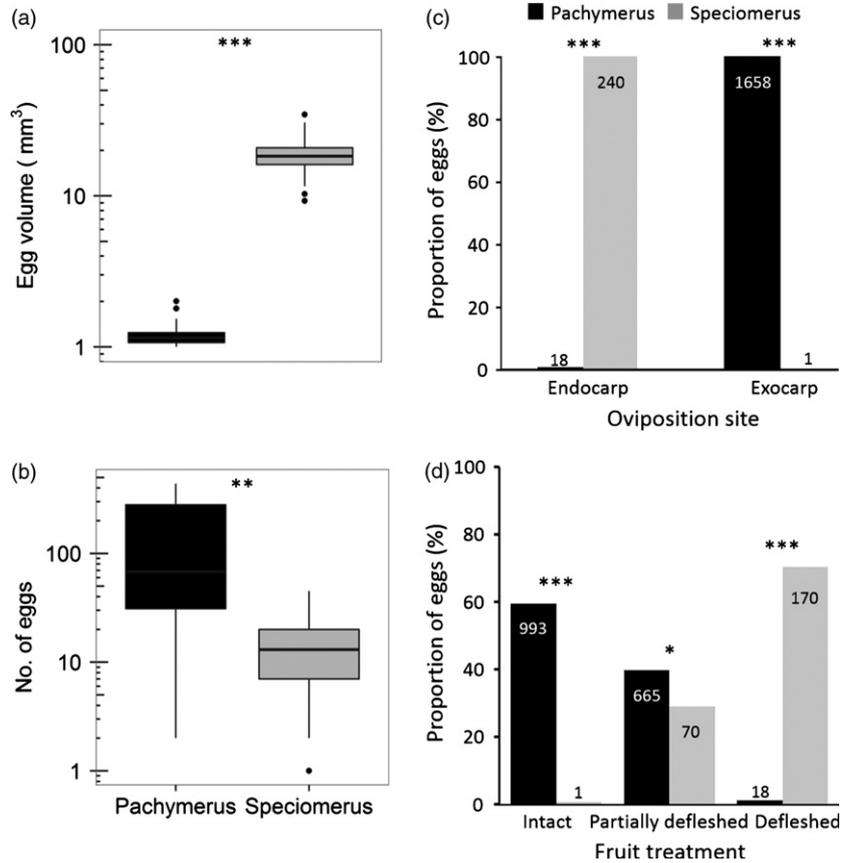
Fig. 2. Differences between the *Attalea* seed predators *Pachymerus cardo* and *Speciomerus giganteus* in egg size (a), egg number (b) and oviposition preferences in terms of preferred fruit tissue (c) and level of endocarp defleshing (d). Central lines are medians, boxes are 1st and 3rd quartile and whiskers show min and max values. Note log-scale in panels a and b. Overlaid numbers in panels c and d show the number of eggs. Significance levels (from generalized linear mixed models) are *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$.

Pachymerus emergences in the *Pachymerus*-only treatment was not significant compared with those in the control group ($\chi^2 = 2.2$, d.f. = 2, $P = 0.14$, Fig. S3a).

The reduction in the expected number of adult emergences in the P0 treatment (i.e. simultaneous egg addition of both species) compared with the *Pachymerus*-only treatment ($\chi^2 = 11.6$, d.f. = 2, $P < 0.01$, Fig. S3c) and with the *Speciomerus*-only treatment ($\chi^2 = 7.6$, d.f. = 2, $P < 0.05$, Fig. S3d) suggests that interspecific competition is costly for both species. When in direct competition for the same seed, the relative success of the two species depended on the relative timing of egg addition ($\chi^2 = 10.3$, d.f. = 4, $P < 0.05$, Fig. 3b). When *Pachymerus* was given a head start of 30 days, there was no emergence whatsoever of *Speciomerus*, compared with 42% for *Pachymerus*. In contrast, when both eggs were added at the same time or when *Pachymerus* had a head start of just 10 days, *Speciomerus* emerged from 14% to 15% of all endocarps, whereas *Pachymerus* emerged at rates similar to the control.

SEED FATE IN THE FIELD

A total of 6569 *Attalea* endocarps were collected from beneath 84 focal palms (BCI: mean 67 ± 12 SE endocarps per palm; SNP: 70 ± 13; islets: 100 ± 20). At each site, around a third of the recovered endocarps were intact

(Fig. 4a). The frequency with which seeds were attacked by rodents varied strongly between the three site types as predicted (Fig. 4a: $Z_{SNP} = -4.5$, $P < 0.0001$, $Z_{Islets} = -2.8$, $P < 0.001$, contrasts respective to BCI which was the intercept level of the models, see Table S1 for parameter estimates). In BCI, 64.7% of the endocarps were opened by rodents, compared with 17.7% in SNP and 11.6% in the islets. In BCI and SNP, the most abundant rodent scars were from agoutis and squirrels, whereas on the islets most scars were made by spiny rats (Fig. 4b).

The proportion of seeds from which bruchids emerged showed the opposite pattern of variation among site types (Fig. 4a: $Z_{SNP} = 4.8$, $P < 0.0001$, $Z_{Islets} = 4.4$, $P < 0.0001$): just 6.3% of seeds on BCI had bruchid emergence holes, compared to more than half of endocarps in SNP and the islets. The proportion of bruchid infestations that resulted in adult emergences was lower on BCI than SNP and the islets ($Z_{SNP} = 3.4$, $P < 0.0001$, $Z_{Islets} = 6.7$, $P < 0.0001$), reflecting higher levels of larval predation by rodents on BCI (Fig. 4c). There was a significant difference between sites in the relative abundance of the two bruchid species (Fig. 4d: $Z_{SNP} = -6.7$, $P < 0.001$, $Z_{Islets} = -7.9$, $P < 0.001$). *Speciomerus* was overwhelmingly dominant on BCI, accounting for 95% of bruchid emergences whereas *Pachymerus* dominated on the islets with 70 % of the emergences, and the two species had similar abundances at SNP (Fig. 4d).
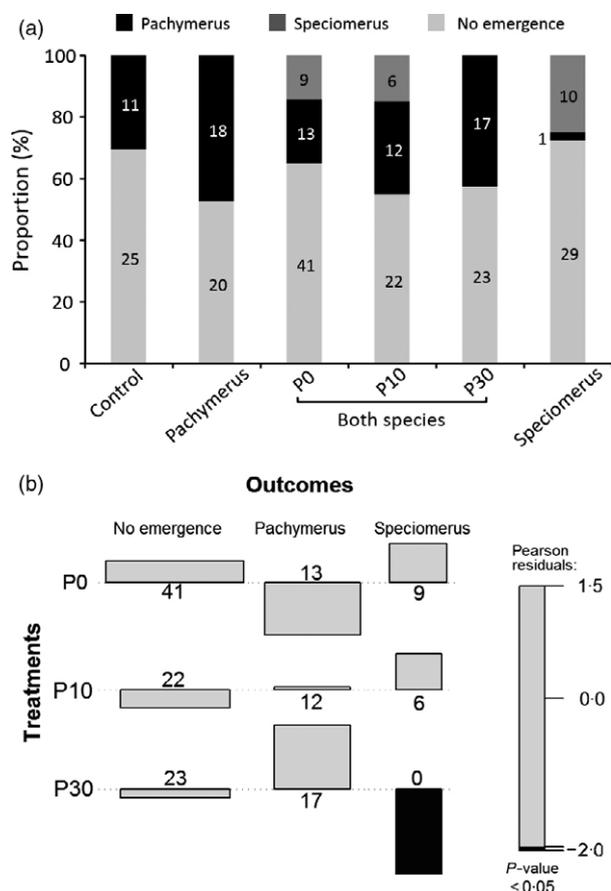
Fig. 3. Results of the trials in which *Attalea* seeds were incubated with and without addition of eggs of one or both species of the bruchid seed parasitoids *Pachymerus* and *Speciomerus*. Treatments were a control group of fruits directly collected from palms incubated without egg addition, and experimental additions of eggs of *Pachymerus* only, of both species with a head start of 0, 10 or 30 days for *Pachymerus* (P0, P10 and P30, respectively), and of *Speciomerus* only. (a) Differences among treatments in bruchid emergence. Overlaid numbers show counts for each outcome. (b) Association plot for the contingency analysis of treatments P0, P10 and P30 ($\chi^2 = 10.3$, d.f. = 4, $P < 0.05$). Numbers above and below the bars show the frequencies of each outcome for each treatment. The height of the bars is proportional to the corresponding Pearson residual. Within each treatment, the width of the bars is proportional to the frequency of the outcome (with frequency 0 assigned an arbitrary width). The significance level is for the overall analysis with black indicating the strongest deviation from the null hypothesis of independence between treatments and outcomes.

Variation in rodent attack among focal trees was strongly and differentially related to variation in the success of the two bruchid species (Fig. 5). The emergence of *Pachymerus* decreased as the levels of rodent attack increased ($Z_{1,84} = -14.2$, $P < 0.0001$, Fig. 5a). In contrast, *Speciomerus* emergences peaked at intermediate levels of rodent attack ($\chi^2 = 66.1$, estimated d.f. = 4.2, $P < 0.0001$, $r^2 = 0.35$, Fig. 5b) and its relative abundance reached almost total dominance when rodents were abundant ($Z_{1,84} = 15.2$, $P < 0.0001$, Fig. 5c). Larval predation risk

was higher than rodent seed predation risk at the vast majority of focal trees, with the fitted relationship far above the 1 : 1 line for most of the range of the data (estimated d.f. = 4.6; $F = 50.3$; $P = 0.0001$, $r^2 = 0.78$, Fig. S4).

Measurements of endocarp size in 1728 endocarps provided no clear indication of differences in endocarp size preferences between the bruchids (Table S2 and Fig. S5). *Speciomerus* emerged from slightly larger endocarps than *Pachymerus* in BCI (8.4 ± 0.2 vs. 6.5 ± 0.6, respectively; mean $\pm$ 1 SE in cm^3) and the islets (6.9 ± 0.1 and 6.6 ± 0.06), but there were no differences in SNP (8.1 ± 0.1 vs. 8.2 ± 0.1).

Discussion

We investigated how vertebrates affected competitive interactions between two specialized seed predators of the palm *A. butyracea*. We found that the larger bruchid *Speciomerus* was the superior competitor in direct competition for a given seed but required the removal of the exo- and mesocarp of the fruits by a vertebrate frugivore before oviposition. The smaller but more fecund *Pachymerus* infested seeds inside intact fruits directly in the canopy, gaining a head start on its post-dispersal competitor. *Pachymerus* emergence dominated in the defaunated sites, but its dominance decreased as the proportion of seeds attacked by rodents increased. Rodent abundance is well correlated with the abundances of other mammals and serves as proxy for defaunation (Fig. S1). These results provide evidence for vertebrates influencing competitive interactions between seed predators, as well as for cascading effects of defaunation on species coexistence.

NICHE DIFFERENTIATION

The two bruchids showed pronounced differences in their reproductive ecology. The differences in egg volume and fecundity are consistent with a classical evolutionary trade-off between offspring size and number (Smith & Fretwell 1974). The finding that *Pachymerus* females preferred to oviposit on intact fruits along with the observation that incubated seeds collected directly from palms and subsequently stripped of exo- and mesocarp frequently already contained *Pachymerus* larvae indicates that this species oviposits on infructescences in the canopy. The same pre-dispersal oviposition behaviour has been observed in the congener *P. bactris,* which infests seeds of the palm *A. standleyanum* (Jansen *et al.* 2010). *Speciomerus* females, in contrast, exclusively laid their eggs on defleshed or partially defleshed endocarps that are typically available below parent palms (Wilson & Janzen 1972; Wright 1983). Several rodents cache these endocarps as food supplies out of reach of *Speciomerus* (Jansen *et al.* 2010, 2012, 2014), shortening the temporal window to lay their eggs. This, along with the uncertainty about prior infestation by its pre-dispersal competitor, may have
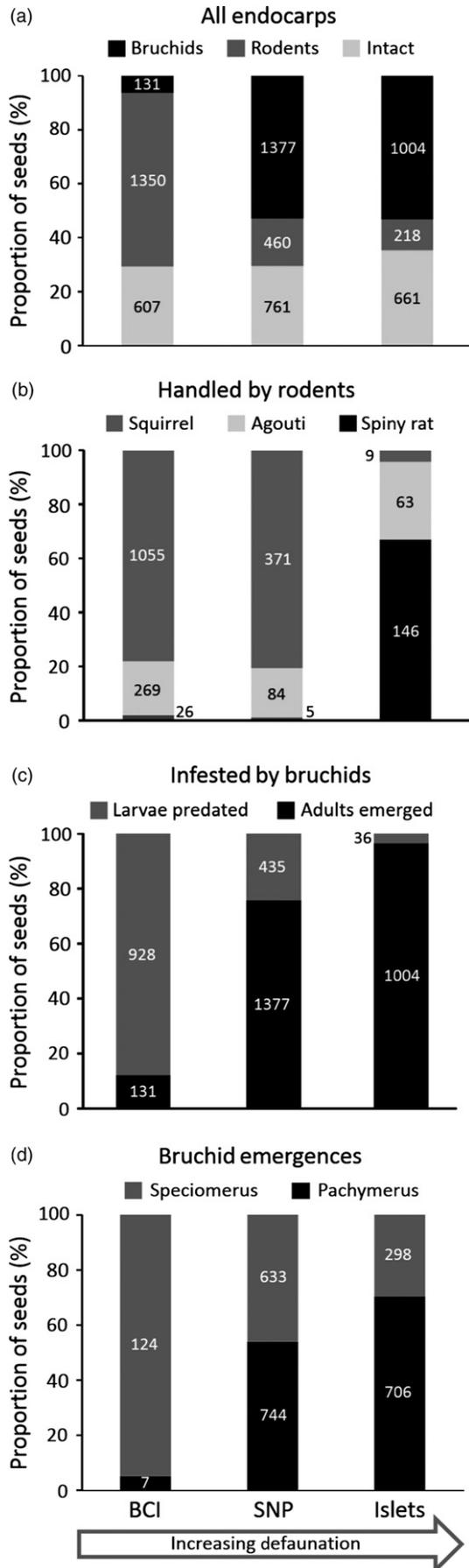
Fig. 4. Fates of *Attalea* seeds collected from sites with different levels of defaunation in central Panama. (a) Proportion of seeds with a bruchid emergence hole, opened by rodents, and intact; (b) proportion of seeds opened by squirrels, agouties or spiny rats relative to all seeds handled by rodents; (c) proportion of seeds with signs of larval predation by a rodent and with a bruchid emergence hole relative to all bruchid-infested seeds; (d) proportion of endocarps with bruchid emergence holes classified by bruchid species. Overlaid numbers show counts for each seed fate. Associated *P*-values are given in the text.

favoured greater larval competitive ability and the almost 20-fold larger energy investment per egg.

This interpretation of a more opportunistic vs. a more conservative reproductive strategy fits with bet-hedging theory predictions on optimal progeny size (Fox & Czesak 2000). A harsh environment (e.g. low density of oviposition sites and highly variable seed quality) is expected to select for adaptations that increase offspring survival such as larger egg sizes at the cost of lower fecundity (Czesak & Fox 2003), whereas an abundance of aggregated oviposition sites should select for a lower parental investment per egg and greater fecundities (Fox & Czesak 2000).

Pachymerus emerges earlier than *Speciomerus* at the onset of the wet season (Fig. S2), consistent with its pre-dispersal oviposition behaviour. However, the substantial overlap in adult activity of the two bruchid species precludes a resource partitioning based on phenological differences (Espelta, Bonal & Sánchez-Humanes 2009). The larvae of *Attalea* seed predators exhibited intraguild predation, with only one adult emerging per seed despite multiple infestations, consistent with previous observations (Wilson & Janzen 1972; Wright 1983). The incubation trials demonstrated that the outcome of this larval predation depends on the duration of the head start of the pre-dispersal seed parasitoid vs. its post-dispersal competitor. Similar results have been found with the bruchid *Callosobruchus maculatus* in which just 2 days of head start allowed the larvae of a less competitive strain to equal its more aggressive competitor (Messina 1991). These results highlight that oviposition strategy, that is seed infestation before or after primary dispersal, is a main axis of niche divergence for insect seed predators and is associated with several other life-history traits, such as adult phenology, egg size/number and oviposition preferences (Messina 1991; Traveset 1991; Johnson & Romero 2004).

CASCADING EFFECTS OF DEFAUNATION

Our study sites represented a sharp defaunation gradient (Adler & Seamon 1991; Wright *et al.* 2000), as shown by striking differences in the proportion of endocarps handled by rodents. Bruchid emergence relative to bruchid infestation was much higher in defaunated sites, probably due to the lower rates of larval predation by rodents. Rodents preferentially prey upon bruchid larvae (Fig. S4) in accordance with previous studies suggesting that
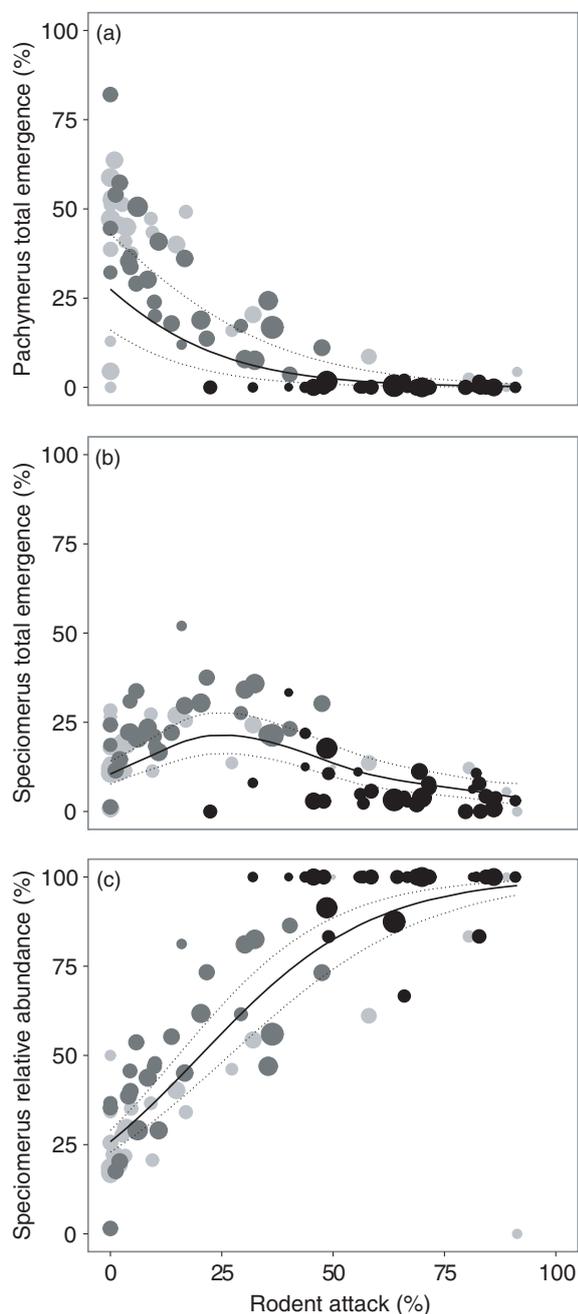
Fig. 5. Relationship of bruchid emergence with the frequency of rodent attack on seeds along a defaunation gradient in central Panama. Percentages of (a) *Pachymerus* emergence relative to all seeds [Pach/Total]; (b) *Speciomerus* emergences relative to all seeds [Spec/Total]; and (c) *Speciomerus* emergences relative to all bruchid emergences [Spec/(Pach + Spec)]. Rodent attack is defined by the proportion of seeds opened by rodents [(Sbr + Ssd + Abr + Asd + R)/Total]. Solid lines show regression fits according to generalized linear (a, c) or additive (b) mixed models with binomial errors and with plot nested within site as a random factor. Dotted lines show 95% confidence envelopes. Each point corresponds to a single focal tree, with point size proportional to sample size (number of seeds) and colours indicating sampling site types [Barro Colorado Island (BCI) = black, Soberania National Park (SNP) = grey, and Islets = light grey].

rodents can exert a strong top-down control over insect populations (Wright & Duber 2001; Gálvez & Jansen 2007; Visser *et al.* 2011). This top-down pressure had differential effects on the two competing seed predators. The relative abundance of *Speciomerus* increased with the levels of rodent attack, with *Speciomerus* being almost absolutely dominant on Barro Colorado where the level of larval predation by rodents was highest, while *Pachymerus* dominated at the most defaunated sites. One plausible reason is that *Speciomerus* is favoured by the higher abundance of vertebrate frugivores which expose endocarps as soon as fruits are ripe, thus reducing the head start of *Pachymerus*. In addition to rodents, many other vertebrate species feed upon *Attalea* fruits and there are remarkable differences in frugivory rates across central Panama linked with defaunation levels (Wright *et al.* 2000). Therefore, vertebrate frugivores and granivores may play a dual mutualistic and antagonistic role and modify the outcome of the intraguild predation of bruchids competing for *Attalea* seeds even at rather local scales (Fig. 5). If so, natural variation in vertebrate abundance and consumption of *Attalea* fruits at a landscape level would ultimately enable the coexistence of both bruchid species. A similar mechanism has been hypothesized to facilitate coexistence of specialized host-sharing milkweed aphids via spatial variation in the abundance of ants that promote their tended species by suppressing their superior non-tended competitors (Smith, Mooney & Agrawal 2008).

CONCLUSIONS

Natural food webs are spatially dynamic but empirical field-based evidence of how spatial variation in multi-trophic interactions affects species coexistence is scant (Amarasekare 2008). Vertebrates such as reptiles, birds and mammals are keystone ecological agents known to exert top-down control on the populations of insects at lower trophic levels (Böhm, Wells & Kalko 2011; Visser *et al.* 2011), and whose disappearance thus has cascade effects reverberating through entire food webs (Terborgh *et al.* 2001). Our results show that vertebrates may also modify the competitive balance of phytophagous insects sharing the same key resource, and can determine their relative abundances. Given the unprecedented rates of defaunation globally (Dirzo *et al.* 2014), this insight warns of potential unanticipated effects on biodiversity loss in hyperdiverse groups such as phytophagous insects.

Acknowledgements

Data accessibility

Data available from the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.gc850 (Peguero *et al.* 2016).

References

Adler, G.H. & Seamon, J.O. (1991) Distribution and abundance of a tropical rodent, the spiny rat, on islands in Panama. *Journal of Tropical Ecology*, **7**, 349–360.

Amarasekare, P. (2008) Spatial dynamics of foodwebs. *Annual Review of Ecology, Evolution, and Systematics*, **39**, 479–500.

Arim, M. & Marquet, P.A. (2004) Intraguild predation: a widespread interaction related to species biology. *Ecology Letters*, **7**, 557–564.

Bates, D., Mächler, M., Bolker, B. & Walker, S. (2014) Fitting linear mixed-effects models using lme4. *ArXiv pre-print*.

Böhm, S.M., Wells, K. & Kalko, E.K.V. (2011) Top-down control of herbivory by birds and bats in the canopy of temperate broad-leaved oaks (*Quercus robur*). *PLoS ONE*, **6**, e17857.

Bradford, D. & Smith, C. (1977) Seed predation and seed number in Scheelea palm fruits. *Ecology*, **58**, 667–673.

Bronstein, J.L. (1994) Conditional outcomes in mutualistic interactions. *Trends in Ecology & Evolution*, **9**, 214–217.

Bruno, J.F., Stachowicz, J.J. & Bertness, M.D. (2003) Inclusion of facilitation into ecological theory. *Trends in Ecology & Evolution*, **18**, 119–125.

Chase, J.M., Abrams, P.A., Grover, J.P., Diehl, S., Chesson, P., Holt, R.D. *et al.* (2002) The interaction between predation and competition: a review and synthesis. *Ecology Letters*, **5**, 302–315.

Czesak, M. & Fox, C. (2003) Evolutionary ecology of egg size and number in a seed beetle: genetic trade-off differs between environments. *Evolution*, **57**, 1121–1132.

Delobel, B. & Delobel, A. (2006) Dietary specialization in European species groups of seed beetles (Coleoptera: Bruchidae: Bruchinae). *Oecologia*, **149**, 428–443.

Dirzo, R., Young, H.S., Galetti, M., Ceballos, G., Isaac, N.J.B. & Collen, B. (2014) Defaunation in the Anthropocene. *Science*, **345**, 401–406.

Espelta, J.M., Bonal, R. & Sánchez-Humanes, B. (2009) Pre-dispersal acorn predation in mixed oak forests: interspecific differences are driven by the interplay among seed phenology, seed size and predator size. *Journal of Ecology*, **97**, 1416–1423.

Fox, C. & Czesak, M. (2000) Evolutionary ecology of progeny size in arthropods. *Annual Review of Entomology*, **45**, 341–369.

Gálvez, D. & Jansen, P.A. (2007) Bruchid beetle infestation and the value of *Attalea butyracea* endocarps for neotropical rodents. *Journal of Tropical Ecology*, **23**, 381–384.

Gurevitch, J., Morrison, J. & Hedges, L. (2000) The interaction between competition and predation: a meta-analysis of field experiments. *The American Naturalist*, **155**, 435–453.

Halekoh, U. & Højsgaard, S. (2012) A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models–the R package pbkrtest. *Journal of Statistical Software*, **59**, 1–32.

Højsgaard, S. & Halekoh, U. (2006) The R package geepack for generalized estimating equations. *Journal of Statistical Software*, **15**, 1–11.

Jansen, P.A., Elschot, K., Verkerk, P.J. & Wright, S.J. (2010) Seed predation and defleshing in the agouti-dispersed palm *Astrocaryum standleyanum*. *Journal of Tropical Ecology*, **26**, 473–480.

Jansen, P.A., Hirsch, B.T., Emsens, W.-J., Zamora-Gutierrez, V., Wikelski, M. & Kays, R. (2012) Thieving rodents as substitute dispersers of megafaunal seeds. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 12610–12615.

Jansen, P.A., Visser, M.D., Wright, S.J., Rutten, G. & Muller-Landau, H.C. (2014) Negative density dependence of seed dispersal and seedling recruitment in a Neotropical palm. *Ecology Letters*, **17**, 1111–1120.

Janzen, D.H. (1975) Intra- and interhabitat variations in *Guazuma ulmifolia* (Sterculiaceae) seed predation by Amblycerus Cistelinus (Bruchidae) in Costa Rica. *Ecology*, **56**, 1009–1013.

Janzen, D.H. (1980) Specificity of seed-attacking beetles in a Costa Rican deciduous forest. *Journal of Ecology*, **68**, 929–952.

Johnson, C.D. & Romero, J. (2004) A review of evolution of oviposition guilds in the Bruchidae (Coleoptera). *Revista Brasileira de Entomologia*, **48**, 401–408.

Kaplan, I. & Denno, R.F. (2007) Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecology Letters*, **10**, 977–994.

Messina, F. (1991) Life-history variation in a seed beetle: adult egg-laying vs. larval competitive ability. *Oecologia*, **85**, 447–455.

Mitchell, R. (1975) The evolution of oviposition tactics in the bean weevil, *Callosobruchus maculatus* (F.). *Ecology*, **56**, 696–702.

Novotny, V. & Basset, Y. (2005) Host specificity of insect herbivores in tropical forests. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **272**, 1083–1090.

Peguero, G., Bonal, R. & Espelta, J.M. (2014) Variation of predator satiation and seed abortion as seed defense mechanisms across an altitudinal range. *Basic and Applied Ecology*, **15**, 269–276.

Peguero, G., Muller-Landau, H., Jansen, P. & Wright, J. (2016) Data from: Cascading effects of defaunation on the coexistence of two specialized insect seed parasitoids. *Dryad Digital Repository*, http://dx.doi.org/10.5061/dryad.gc850.

Sallabanks, R. & Courtney, S.P. (1992) Frugivory, seed predation, and insect-vertebrate interactions. *Annual Review of Entomology*, **37**, 377–400.

Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, **9**, 671–675.

Silvius, K. & Fragoso, J. (2002) Pulp handling by vertebrate seed dispersers increases palm seed predation by bruchid beetles in the northern Amazon. *Journal of Ecology*, **90**, 1024–1032.

Smith, C.C. & Fretwell, S.D. (1974) The optimal balance between size and number of offspring. *The American Naturalist*, **108**, 499.

Smith, R., Mooney, K. & Agrawal, A. (2008) Coexistence of three specialist aphids on common milkweed, *Asclepias syriaca*. *Ecology*, **89**, 2187–2196.

Terborgh, J., Lopez, L., Nuñez, P., Rao, M., Shahabuddin, G., Orihuela, G. *et al.* (2001) Ecological meltdown in predator-free forest fragments. *Science*, **294**, 1923–1926.

Traveset, A. (1991) Pre-dispersal seed predation in Central American *Acacia farnesiana*: factors affecting the abundance of co-occurring bruchid beetles. *Oecologia*, **87**, 570–576.

Tscharntke, T. & Hawkins, B.A. (2002) Multitrophic level interactions: an introduction. *Multitrophic Level Interactions* (eds T. Tscharntke & B.A. Hawkins), pp. 1–7. Cambridge University Press, Cambridge, UK.

Visser, M.D., Muller-Landau, H.C., Wright, S.J., Rutten, G. & Jansen, P.A. (2011) Tri-trophic interactions affect density dependence of seed fate in a tropical forest palm. *Ecology Letters*, **14**, 1093–1100.

Wilson, D. & Janzen, D. (1972) Predation on Scheelea palm seeds by bruchid beetles: seed density and distance from the parent palm. *Ecology*, **53**, 954–959.

Wood, S.N. (2011) Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, **73**, 3–36.

Wright, S.J. (1983) The dispersion of eggs by a bruchid beetle among Scheelea palm seeds and the effect of distance to the parent palm. *Ecology*, **64**, 1016–1021.

Wright, S.J. & Duber, H.C. (2001) Fragmentation alter seed dispersal, seed survival, and seedling recruitment in the Palm *Attalea butyracea* with implications for tropical tree diversity. *Biotropica*, **33**, 583–595.

Wright, S.J., Zeballos, H., Domínguez, I., Gallardo, M.M., Moreno, M.C. & Ibáñez, R. (2000) Poachers alter mammal abundance, seed dispersal, and seed predation in a neotropical forest. *Conservation Biology*, **14**, 227–239.

Supporting Information

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

Fig. S1. Correlation between rodent and vertebrate abundance across central Panama.

Fig. S2. Phenology of *Pachymerus* and *Speciomerus* adult emergence.

Fig. S3. Association plots of egg incubation trials.

Fig. S4. Relationship between larval predation and seed predation by rodents.

Fig. S5. Variation in *Attalea* endocarp volume by insect species and across sites.

Table S1. Results of the analyses on *Attalea* seed fate differences across sites.

Table S2. Results of the analysis of *Attalea* variance in endocarp volume.