



# Dose-independent virulence in phoretic mites that parasitize burying beetles

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## ABSTRACT

Virulence, the negative impact of parasites on their hosts, typically increases with parasite dose. Parasites and hosts often compete for host resources and more parasites will consume more resources. Depending on the mechanism of competition, increasing host resources can benefit the host. Additional resources can also be harmful when the parasites are the main beneficiaries. Then, the parasites will thrive and virulence increases. While parasite dose is often easy to manipulate, it is less trivial to experimentally scale host resources. Here, we study a system with external host resources that can be easily manipulated: *Nicrophorus* burying beetles reproduce on vertebrate carcasses, with larger carcasses yielding more beetle offspring. Phoretic *Poecilochirus* mites reproduce alongside the beetles and reduce beetle fitness. The negative effect of mites could be due to competition for the carrion between beetle and mite offspring. We manipulated mite dose and carcass size to better understand the competition between the symbionts. We found that mite dose itself was not a strong predictor of virulence. Instead, the number of mite offspring determined beetle fitness. At larger doses, there was strong competition among adult parental mites as well as mite offspring. While increasing the carcass size increased both host and parasite fitness, it did surprisingly little to alleviate the negative effect that mites had on beetles. Instead, relative virulence was stronger on large carcasses, indicating that the parasites appropriate more of the additional resources. Our results demonstrate an ecological influence on the selection of parasites on their hosts and suggest that virulence can be dose-independent in principle.

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## 1. Introduction

Parasite and host fitness are generally traded off: the more fitness the parasite gains by the interaction, the lower the fitness outcome for the host, i.e. the higher the virulence (Cressler et al., 2016). This is caused by parasites competing with the host for host resources (Timms et al., 2001; Brunner et al., 2005). Virulence also depends on parasite dose, the number of parasites a host is exposed to: more parasites mean more competitors, leaving fewer resources for the host. Higher parasite doses also often lead to increased infection success (Kinnula et al., 2015) in cases where parasites need to overcome host defences and can better do so with multiple simultaneous attacks (Regoes et al., 2002; Schmid-Hempel and Frank, 2007; Ben-Ami et al., 2008; Leggett et al., 2012). Parasites that reproduce sexually on the host require a minimum dose that allows them to find mating partners (May and Anderson, 1979; Nehring and Müller, 2009). While a high parasite dose may increase infection success and the overall number of

parasite offspring, it can also reduce the per capita fitness of parasites, because parasites compete amongst themselves for host resources as well (Ebert et al., 2000; Regoes et al., 2002). The abundance of host resources can significantly affect the virulence. Sometimes better fed hosts might be better at fending off parasites (Zanette and Clinchy, 2010). In other cases, parasites manage to monopolise most of the resources to produce more parasite offspring (Ebert et al., 2000; Zanette and Clinchy, 2010; Wale et al., 2017), so that more host resources can cause higher virulence.

While parasite dose is often easy to experimentally change, it is rather difficult in most host-parasite systems to manipulate the resources that hosts and parasites have available. The exact effect of resources on competition between parasites and hosts, competition among parasites, and ultimately virulence, are thus often impossible to unveil. Here, we use a system in which the resource needed for host and parasite reproduction is external and thus easily manipulated. The burying beetle *Nicrophorus* monopolises vertebrate carcasses for reproduction (Eggert and Müller, 1997). Beetle offspring compete for the carcass resource as their food source and the competition can be easily manipulated by changing carcass size (Bartlett and Ashworth, 1988). Parents manage

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competition among their offspring by regulating clutch size and the number of larvae they allow access to the resource, thereby presumably adjusting the eventual size of their offspring (Bartlett and Ashworth, 1988; Müller et al., 1990; Trumbo, 1990; De Gasperin and Kilner, 2015a). At larger carcasses, more offspring are raised.

Virtually all *Nicrophorus* adults carry phoretic *Poecilochirus* mites that also reproduce on the carcass. Mite transmission between host beetles appears to be mainly horizontal, occurring whenever adult beetles encounter each other at large carcasses that are used for feeding (Schwarz and Koulianos, 1998). After reproduction at a carcass, most individuals of the next mite generation disperse on the beetle parents once these finish brood care, while the remainder waits for dispersal with the beetle offspring (Eggert and Müller, 1997; Schwarz and Koulianos, 1998). The mites reduce the number and weight of beetle offspring, an effect that is stronger when more mite offspring develop (De Gasperin et al., 2015; De Gasperin and Kilner, 2015b; Nehring et al., 2017). The mites have been observed to attack eggs or newly eclosed larvae and might thus kill them directly (Beninger, 1993; Blackman and Evans, 1994; De Gasperin and Kilner, 2015b), or they might feed on the carcass and thus compete with beetles for this resource. Further, the mites seem to weaken the effect of brood regulation by beetle parents (De Gasperin and Kilner, 2016) and can reduce the male adult life span (De Gasperin and Kilner, 2015a). While many lines of evidence indicate moderate negative effects of mites on beetles under most conditions (Wilson and Fudge, 1984; Wilson and Knollenberg, 1987; De Gasperin et al., 2015; De Gasperin and Kilner, 2015b, 2016; Nehring et al., 2017), two studies report that mites can positively affect beetles because beetles hunt nematodes and eat fly eggs, both competitors of the beetles (Springett, 1968; Wilson and Knollenberg, 1987). When enough flies or nematodes are present, the beetles will not only be relieved of these competitors by the mites but also face less competition from mites because these might feed less on the carcass or beetle eggs (De Gasperin et al., 2015).

We address the interaction between parasite dose, resource competition, and virulence, by manipulating parasite dose and resource availability of beetle reproduction events. We hypothesise that mites and beetles compete for resources, potentially the reproduction carcass, and that this competition should result in virulence. This led us to predict that larger parasite doses would lead to lower host fitness and lower per-capita parasite fitness, and that larger resources would reduce the competition overall, leading to higher host and parasite fitness with a lower impact of mites on beetles. We also tested the dose effect in a second experiment excluding beetles, hypothesising that mite-mite competition for resources limits per capita mite fitness (Nehring and Müller, 2009). Finally, since parasites often need to overcome host defences, which is more likely to be successful in larger doses, we also analysed our data to test whether a higher dose leads to greater “infection” success.

## 2. Materials and methods

All experiments were conducted with direct offspring of field caught beetles at 20 °C under a 16:8h light:dark cycle. *Nicrophorus vespilloides* (Herbst, 1783) beetles and the *Poecilochirus carabi* (Canestrini and Canestrini, 1882) mites they carried were anaesthetized with CO<sub>2</sub>, which caused the mites to fall off so we could remove and count them. We let the beetles mate and lay eggs in pairs on mouse carcasses of ca. 20 g and transferred the eggs to new carcasses where they were cared for by single nematode-free foster parents. Field caught beetles carry nematodes that harm beetle fitness (Wang and Rozen, 2019), an effect that might

obscure the effects of the mites we were focussing on here. The number of offspring per resource was adjusted to yield the largest offspring possible, which is a simple method to standardise beetle size. First generation beetles were used for the experiments, while mites came from laboratory cultures that were regularly supplemented with field-caught individuals. The mites were bred without beetles in groups of 10 deuteronymphs on cattle liver, which was freed from mould daily.

### 2.1. Mite dose and resource value experiments

We set up pairs of non-sibling adult beetles for copulation in boxes lined with moist tissue paper. On the following day, we provided them with a fresh mouse cadaver and moved the cadaver with the adherent beetles to a peat-filled bucket (diameter 18 cm, height 16 cm), and added a pre-defined number of mite deuteronymphs. Buckets were covered with a plexi glass lid so that we could observe whether the beetles buried the carcass. After 3 days, the lids were replaced with an inverted bucket that had an exit that would trap the parental beetles when they left the brood and walked on the surface. We checked for trapped beetles twice each day and counted the mites on the beetles. After 21 days, when both parental beetles had left, we opened the buckets carefully to remove the beetle offspring pupae, for which we recorded the weight and the number of mites they carried.

To test the effect of mite dose (i.e. the number of deuteronymphs that the parental beetles carry before reproduction), we set up 42 pairs of burying beetles, each with 10 g carcasses, either without any mites ( $n = 10$ ) or with 6, 10, 17, 30, or 50 mite deuteronymphs ( $n = 6-7$  per group). In the field, individual burying beetles carry on average 10–20 deuteronymphs with maximum numbers well over 200 (Springett, 1968; Korn, 1983; Schwarz et al., 1998) depending on species and season, so that the mite numbers we chose here are well within the natural range. We analysed the effect of mite dose on the general success of beetle (replicates with/without beetle offspring) and mite reproduction (replicates with more mite deuteronymphs after beetle reproduction than were initially added to the replicate) using generalised linear models (glm) with a binomial error family. For all successful breeding attempts (excluding those where either mites or beetles did not reproduce), we analysed the quantitative effect of mite dose on beetle fitness (number of pupae and total pupal weight), average beetle pupal size, the total number of mite deuteronymphs developed, and per capita mite fitness (number of offspring per deuteronymph initially added to the replicate). For all analyses presented here, we counted all mite offspring because we suspected that each developing mite would remove some of the resources, i.e. this is the relevant number to evaluate competition among mites and with beetle larvae. An analysis using only mite offspring that left the resource with the parental beetles yielded qualitatively and quantitatively similar results. For beetle fitness, we also used the total number of mites that developed as a predictor because we know from previous experiments that this variable is correlated with beetle fitness (Nehring et al., 2017). For count data (offspring number) we used the quasipoisson error family because all data were over-dispersed; for all other data (per capita fitness, weight) we used gaussian errors. We always included all possible interactions into the models and analysed the effect of each predictor by sequentially adding them to the model using the ANOVA function in R (R Development Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria) with F- (gaussian, quasipoisson) or  $\chi^2$ -tests (binomial) and considered error probabilities <5% as significant. We further disentangled the effects of the correlated variables (mite dose and total mite offspring number) in a principal component analysis based on beetle offspring

number, beetle brood weight, average pupal size, total mite offspring number, and mite per capita fitness.

To evaluate the effect of resource value (i.e. nutrition provided by the carcass) on the interaction of mite and beetle fitness, we set up 41 pairs of beetles with either 10, 30, or 50 mites, and carcasses weighing 5 g, 10 g, or 20 g, in a full-factorial design, with  $n = 4–5$  per combination. We analysed the data as described for the mite dose experiment, with carcass weight as an additional predictor. Again, we performed an analysis of the likelihood of mite and beetle failure (binomial model) based on the full dataset and then analysed the quantitative effects (gaussian/quasipoisson models) only for those replicates where both mite and beetle reproduction were successful.

## 2.2. Mite reproduction without beetles

To better be able to analyse mite reproduction and intraspecific mite competition, we set up mites to reproduce without beetles as for the initial mite breeding (see Section 2.1) in boxes of  $10 \times 10 \times 6$  cm with a 1 cm layer of peat. For food, we provided a cube of cattle liver (5–7 g). We took care that all pieces had the same dimensions so that the surface and thus the possibility for access was the same in all replicates. The liver was never entirely consumed. In total, there were 42 replicate groups that consisted of either 10 ( $n = 18$ ), 20 ( $n = 12$ ), or 40 ( $n = 12$ ) deuteronymphs. We checked the groups twice each day and counted the number of males, females, and deuteronymphs. When checking the groups, we removed mould from the diced liver pieces to slow down their deterioration and to keep them accessible for the mites (mites cannot cut through layers of mould before feeding). From day 8 onwards, when no adults were visible any more, we checked the boxes daily and removed all developing deuteronymphs until day 22, when no new deuteronymphs were appearing in any of the replicates. Data were analysed as described above, with dose as the only predictor and the number of males, females, and total developing deuteronymphs, as well as per capita mite fitness as dependent variables. We also correlated the number of offspring with the number of females that we observed.

## 3. Results

### 3.1. Mite offspring number is the main predictor of beetle fitness

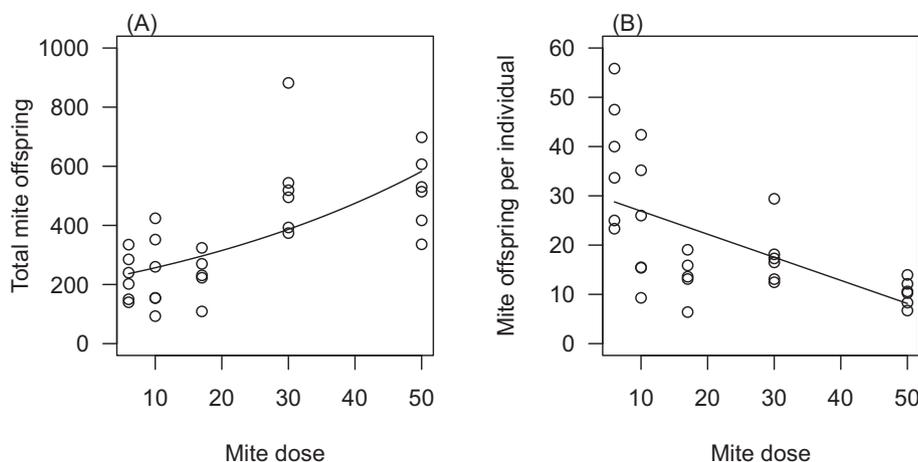
We set up 42 pairs of burying beetles, each with 10 g carcasses, and with between zero and 50 mites per pair. There was no signif-

icant effect of mite dose on beetle failure to reproduce (binomial glm  $\chi^2_1 = 1.4$ ,  $P = 0.24$ ). Beetle failure to reproduce ( $\chi^2_1 = 19.9$ ,  $P < 0.001$ ), but not mite dose ( $\chi^2_1 = 0$ ,  $P > 0.99$ ; interaction beetle failure  $\times$  dose  $\chi^2_1 = 0$ ,  $P > 0.99$ ), predicted whether or not mite reproduction was successful. Overall, four beetle pairs from the treatments of 0–17 mites had no offspring and in all these cases we found one of the parental beetles dead in the peat after the experiment. In replicates where mites were added, mite reproduction was always successful unless beetle reproduction failed as well: in three of the failed beetle replicates we recovered fewer mites than we had added.

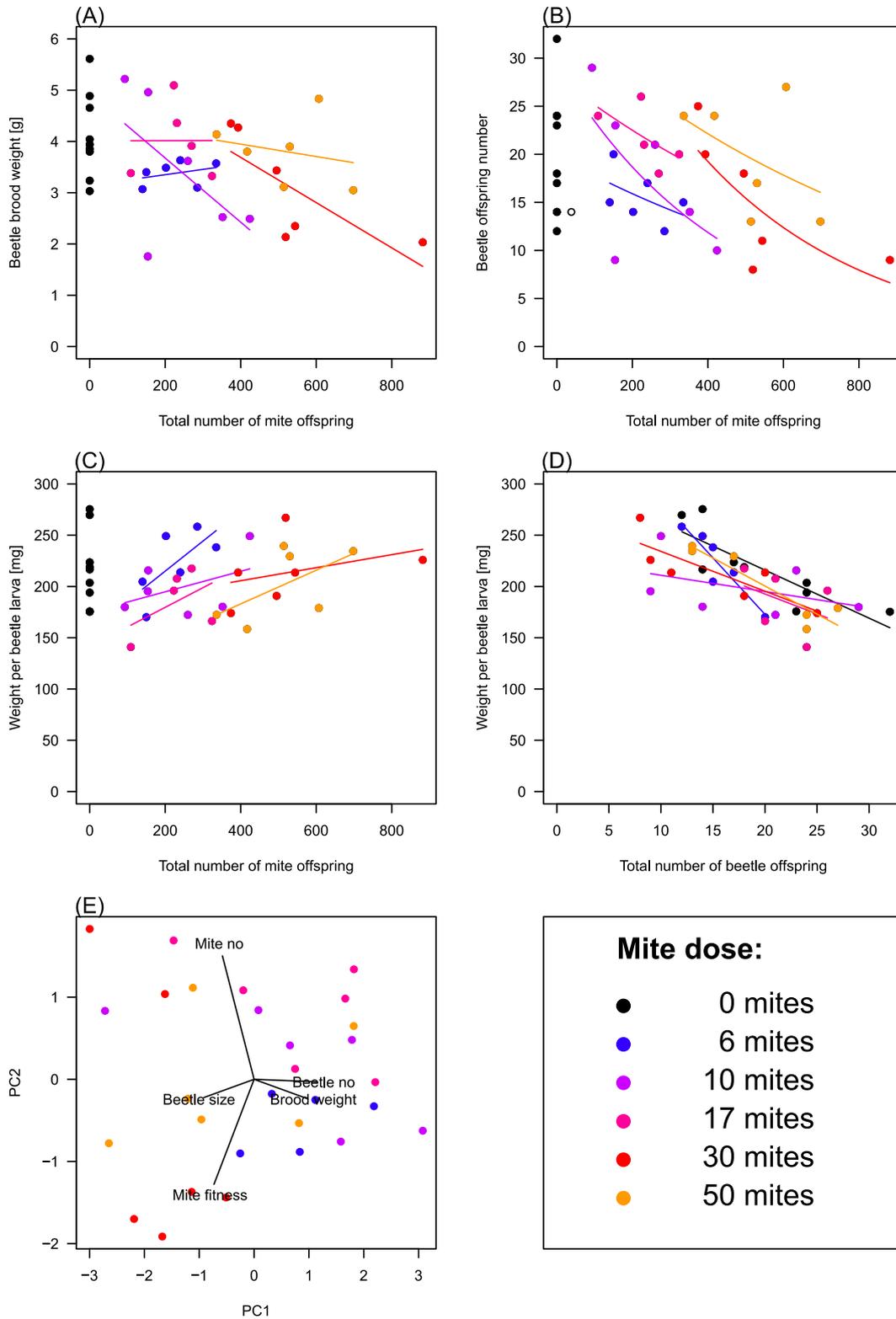
When both mite and beetle reproduction was successful, the total number of mite offspring increased with mite dose, the number of deuteronymphs that were initially added to the reproduction event (Fig. 1A; ANOVA quasipoisson glm  $F_{1,27} = 21.4$ ,  $P < 0.001$ ). In turn, per capita mite fitness decreased with dose (Fig. 1B; glm  $F_{1,27} = 15.0$ ,  $P < 0.001$ ).

In the replicates with successful beetle reproduction, mite dose on its own affected neither the number of beetle offspring (quasipoisson glm, ANOVA  $F_{1,36} = 0.0003$ ,  $P = 0.99$ ) nor the total beetle brood weight ( $F_{1,36} = 0.34$ ,  $P = 0.56$ ). Mite offspring number negatively affected both beetle offspring number (Fig. 2A; quasipoisson glm  $F_{1,36} = 6.0$ ,  $P < 0.05$ ) and beetle brood weight (Fig. 2B;  $F_{1,36} = 8.1$ ,  $P < 0.01$ ). Since initial mite dose and mite offspring number are correlated (Fig. 1), separating the effects of these variables is difficult and whether or not a factor can be found to be significant depends on whether or not the model already accounts for the other factor. After accounting for the effect of mite offspring number, we found a positive effect of mite dose on beetle fitness (Fig. 2; beetle offspring number  $F_{1,35} = 11.7$ ,  $P < 0.01$ ; beetle brood weight  $F_{1,35} = 7.0$ ,  $P < 0.05$ ), but no interaction (beetle offspring number  $F_{1,34} = 0.5$ ,  $P = 0.47$ ; beetle brood weight  $F_{1,36} = 0.1$ ,  $P = 0.75$ ). This means that the beetles are better off when the same number of mite offspring is produced by more mites that are initially added (regression lines for higher doses in Fig. 2A, B are higher than those for smaller doses), i.e. beetles seem to profit from mite-mite competition. The results do not change qualitatively when we exclude replicates in which no mites were added (data not shown).

Interestingly, the average size per beetle larva appears to depend on mite dose and total mite offspring number (Fig. 2C). While the interaction is not significant ( $F_{1,34} = 3.2$ ,  $P = 0.08$ ), both mite dose and mite offspring number become significant when we first account for the other factor (see Supplementary Table S1 for details), an artefact that is caused by the correlation between



**Fig. 1.** Competition among mites. The total number of mite offspring at a 10 g carcass increases with mite dose (mites added before reproduction, (A)), while per capita mite fitness (total mite offspring divided by mite dose) decreases (B). Lines are regression lines from models with Quasipoisson (A) and Gaussian (B) errors.



**Fig. 2.** Dose and virulence. The total number of developing mites negatively affects the total weight (A) and number (B) of beetle offspring. In turn, individual larvae grow larger when the dose is small but more mite offspring develop (C), indicating that mites affect beetle brood regulation early in the process. Further, there is a trade-off between beetle larval size and number (D) that is not affected by the mites. In a principal component analysis (E), the opposing syndromes become apparent. Each dot is a replicate, and the lines originating in the middle indicate in which direction the different fitness measurements increase: individual beetle offspring size (beetle size), per capita mite fitness (mite fitness) and total mite offspring number (mite no) on one side, and beetle offspring number (beetle no) and total beetle brood weight (brood weight) on the other.

dose and mite offspring number (Fig. 1). Individual larvae grow larger when mites produce many offspring relative to their initial dose. When we add the number of beetle larvae as a third predictor, we find that the average larval size strongly declines with the number of beetle larvae (Fig. 2D;  $F_{1,36} = 33.4$ ,  $P < 0.001$ ) but that this factor does not interact with mite dose ( $F_{1,32} = 0.48$ ,  $P = 0.49$ ) or the number of mite offspring ( $F_{1,33} = 0.11$ ,  $P = 0.74$ ), i.e. in our experiments the mites do not affect the trade-off between larval size and number.

In a principal component analysis based on data with both successful mite and beetle reproduction, the first two components had eigenvectors larger than 1 and explained 56% and 21% of the total variance, respectively. The first principle component (PC) represented beetle fitness (beetle offspring number and brood weight, which were highly correlated) traded off with individual beetle larval size (Fig. 2E). The second PC represented overall mite offspring number, which was traded off with per capita mite fitness. However, mite offspring number and per capita fitness were not entirely orthogonal to the beetle fitness parameters, but high overall beetle fitness reduced both mite offspring number and per capita mite fitness. Mite dose did not affect beetle fitness (PC1, glm  $F_{1,27} = 0.22$ ,  $P = 0.64$ ), but did increase overall mite offspring number and decreased per capita mite fitness (PC2, glm  $F_{1,27} = 87.3$ ,  $P < 0.001$ ).

### 3.2. Stronger negative interaction between symbionts on larger resources

We set up 41 pairs of beetles with either 10, 30, or 50 mites and carcasses weighing 5 g, 10 g, or 20 g ( $n = 4-5$  per combination). The likelihood of beetle broods failing was independent of mite dose (binomial glm  $\chi^2_1 = 0.29$ ,  $P = 0.59$ ), but higher when the resources were smaller (binomial glm,  $\chi^2_1 = 6.61$ ,  $P = 0.01$ ). Five replicates with 5 g carcasses and three pairs with 10 g carcasses did not produce beetle offspring. Mite reproduction was successful in two replicates with failed beetle reproduction. In both, the beetle parents behaved as usual, i.e. they spent the same amount of time at the carcass as they would with live offspring, and during this time also prepared the carcass and kept it from deteriorating (10 g carcass, 50 mites added, 338 mites produced in one replicate; 5 g carcass, five mites added and eight produced in the other).

When beetles were successful, mites were more likely to fail in small doses (binomial glm  $\chi^2_1 = 5.1$ ,  $P < 0.01$ ) but were not affected by the resource size or the interaction ( $\chi^2_1 < 1.4$ ,  $P > 0.24$  for both). Three of the four replicates with successful beetle reproduction

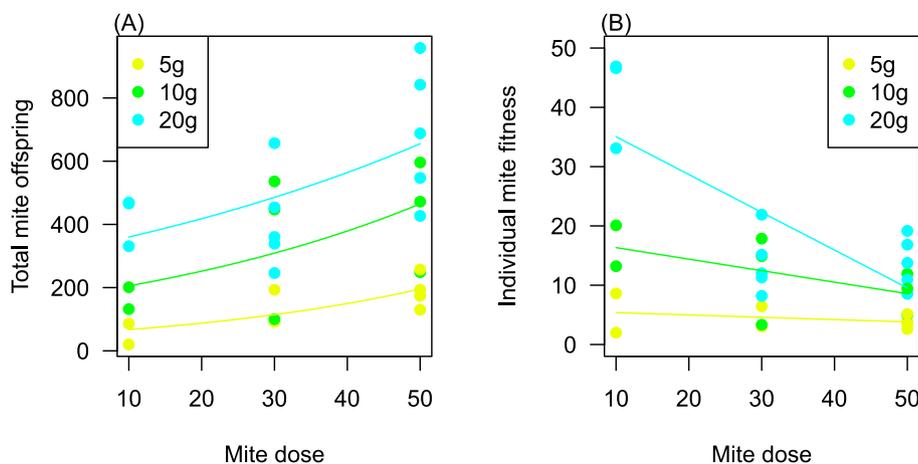
but mite failure were with 10 mites and the fourth at a dose of 30 mite individuals.

In the replicates with successful mite and beetle reproduction, both mite dose ( $F_{1,27} = 9.0$ ,  $P < 0.01$ ) and carcass size (quasipoisson glm  $F_{1,26} = 29.8$ ,  $P < 0.001$ ) independently increased the number of mite offspring produced (Fig. 3A; interaction  $F_{1,25} = 0.24$ ,  $P = 0.62$ ). Per capita mite fitness increased with resource size ( $F_{1,27} = 27.5$ ,  $P < 0.001$ ) and decreased with increasing mite dose (Fig. 3B, glm  $F_{1,26} = 18.1$ ,  $P < 0.001$ ). However, the effect of a large carcasses was strong only when the mite dose was low, and a large carcass could not rescue per capita mite fitness at a high mite dose (interaction term  $F_{1,25} = 10.9$ ,  $P < 0.01$ ). Overall, a mite dose of circa 10 deutonymphs already saturates a 5 g carcass, while 50 or more mites will saturate carcasses of 20 g, indicating that there is competition among mites for the carcass and that doses of more than three mites would already exceed the carrying capacity of a gram of carcass.

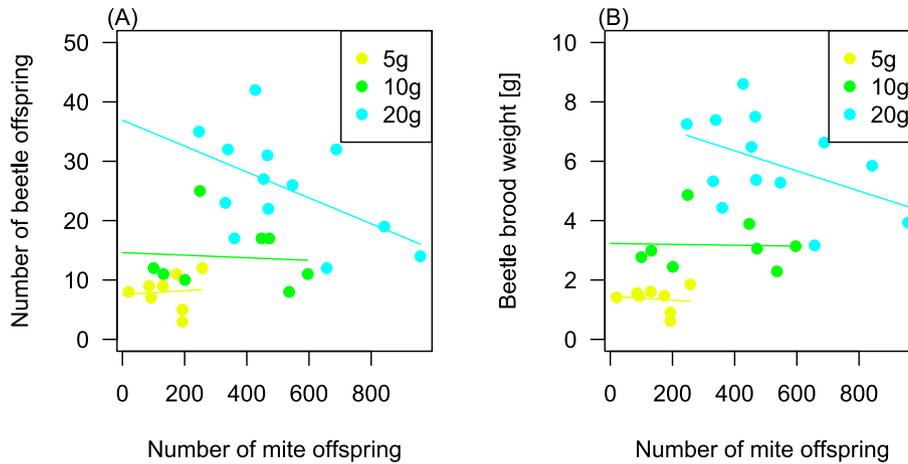
The number of beetle offspring strongly depended on carcass size (Fig. 4A; quasipoisson glm  $F_{1,27} = 43.6$ ,  $P < 0.001$ ). Mite dose did not have an effect ( $F_{1,26} = 0.9$ ,  $P = 0.34$ ) unless we previously accounted for the negative effect of the total number of mite offspring ( $F_{1,26} = 4.4$ ,  $P < 0.05$ ). Then, increasing mite dose slightly reduced the number of beetle offspring ( $F_{1,25} = 7.0$ ,  $P < 0.05$ ). The interaction between mite offspring number and carcass size was not significant ( $F_{1,24} = 2.4$ ,  $P = 0.14$ ) but it had a considerable effect size (partial  $\eta^2 = 0.10$ ) and visual inspection of the data suggests that the negative effect of mite offspring number on beetle offspring number is stronger when the carcasses are larger (Fig. 4B). Other interactions were negligible (all  $P > 0.5$ ).

Total beetle brood weight was influenced by the same predictors as the number of beetle offspring (Fig. 4B). Carcass weight had a clear effect increasing brood weight (Fig. 4, glm  $F_{1,27} = 77.2$ ,  $P < 0.001$ ) while the effects of total mite offspring number ( $F_{1,26} = 3.0$ ,  $P = 0.10$ ) and mite dose ( $F_{1,26} = 0.2$ ,  $P = 0.26$ ) were negligible on their own. When we accounted for mite dose before testing for the effect of mite offspring number, the latter became significant (see Supplementary Table S2 for details) and there was a trend of stronger negative effects of the number of mite offspring on larger carcasses (interaction offspring number  $\times$  carcass size  $F_{1,23} = 3.2$ ,  $P = 0.09$ ).

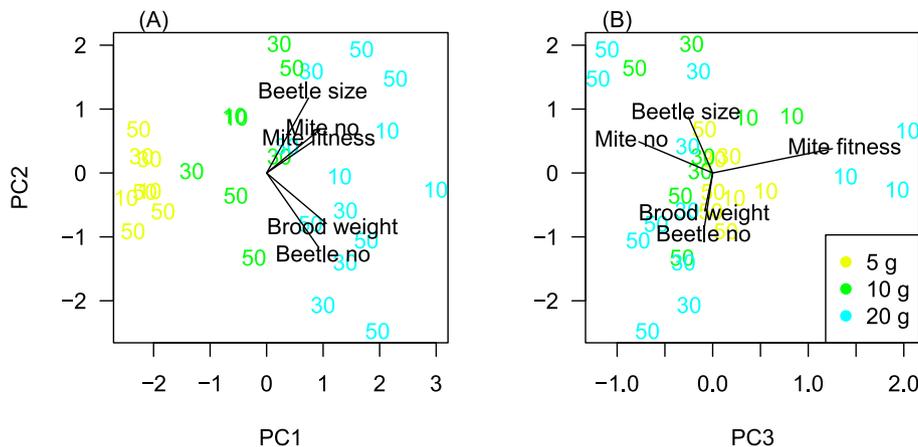
The size of individual pupae was strongly predicted by the size of the carcass ( $F_{1,27} = 18.1$ ,  $P < 0.001$ ), with larger pupae on larger carcasses. As in the first experiment, pupal size was positively correlated with the total number of mite offspring ( $F_{1,26} = 7.6$ ,  $P < 0.05$ ), and negatively with mite dose ( $F_{1,25} = 10.1$ ,  $P < 0.01$ )



**Fig. 3.** Mite fitness and resource availability. Total mite offspring number (A) increased with mite dose and resource size; individual mite fitness (B) decreased with dose but at low doses increased with resource size.



**Fig. 4.** Beetle fitness and resource availability. Beetle offspring number (A) increased with resource size, but this gain was obliterated when mite offspring production was successful. Effects on beetle brood weight (B) were comparable to those on beetle offspring number.



**Fig. 5.** Overview over the fitness effects. Principal component analysis disentangling the effects of carcass size (colour-coded), mite dose (numbers), and mite fitness (total number of mite offspring, per capita mite fitness) on beetle fitness (total brood weight, no. of beetle offspring, individual beetle larva size). Lines point from the origin into the direction in which the respective fitness measure increases.

and the number of beetle offspring ( $F_{1,24} = 5.2$ ,  $P < 0.05$ ). However, note that these factors are confounded (see Figs. 3 and 4).

Again, the effects of the confounding factors are best illustrated in a principal component analysis (Fig. 5). Carcass weight is the main influence on the first principle component (Fig. 5A; glm on PC 1  $F_{1,26} = 151.8$ ,  $P < 0.001$ ) and increases the fitness of individual mites, beetle parents (beetle offspring number and total brood weight), and beetle offspring (individual pupal size), while mite dose has no effect here ( $F_{1,27} = 0.18$ ,  $P = 0.67$ ). There are no significant effects on the second principal component (all  $P > 0.58$ ) and the third PC had an eigenvalue lower than one. However, the picture on the second and third principal components (Fig. 5B) resembles that on the first two principle components from the dose experiment (Fig. 2E): Mite dose ( $F_{1,27} = 87.8$ ,  $P < 0.001$ ) and its interaction with carcass weight ( $F_{1,25} = 2.7$ ,  $P < 0.001$ ), but not carcass weight alone ( $F_{1,26} = 1.0$ ,  $P = 0.33$ ), predicted the third principle component, showing that the per capita fitness of a few mites on large carcasses is disproportionately high.

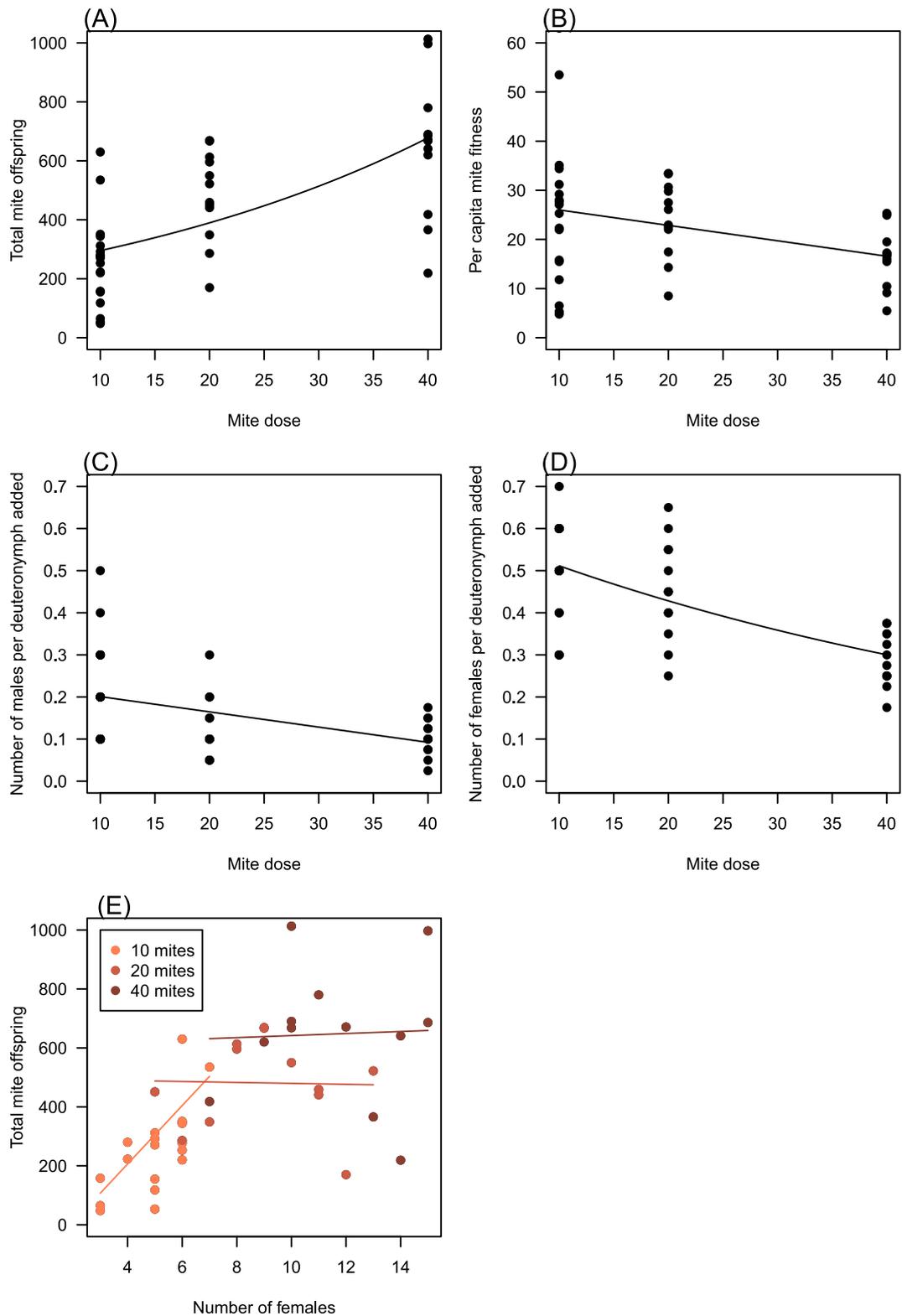
### 3.3. Intraspecific mite competition among both adults and offspring

When we bred mites without beetles in groups of 10–40 deuteronymphs, the total number of offspring that were produced

increased with dose (Fig. 6A; quasipoisson glm  $F_{1,40} = 28.7$ ,  $P < 0.001$ ), and per capita mite fitness decreased at the same time (Fig. 6B; glm  $F_{1,40} = 5.1$ ,  $P < 0.05$ ). The numbers of males (quasipoisson glm  $F_{1,40} = 14.1$ ,  $P < 0.001$ ) and females (glm  $F_{1,40} = 56.6$ ,  $P < 0.001$ ) that moulted also increased with dose. Per deuteronymph added, fewer males (Fig. 6C; glm  $F_{1,40} = 10.3$ ,  $P < 0.01$ ) and females (Fig. 6D; glm  $F_{1,40} = 30.7$ ,  $P < 0.001$ ) were observed at a larger mite dose, indicating that some of the competition already occurred between the mite deuteronymphs that arrived at a carcass, or between the adults. We observed more females than males (paired Wilcoxon test  $V = 8145.5$ ,  $P < 0.001$ ). The number of females that we observed predicted the number of offspring (quasipoisson glm  $F_{1,40} = 29.5$ ,  $P < 0.001$ ), but there was an interaction with dose (factor dose  $F_{1,39} = 5.5$ ,  $P < 0.05$ ; interaction  $F_{1,38} = 5.2$ ,  $P < 0.05$ ), indicating that the effect of increased numbers of females was absent in larger groups (Fig. 6E).

## 4. Discussion

Host and parasite fitness are typically traded off due to resource competition amongst the symbionts. We conducted experiments with burying beetles and their mites to elucidate whether and how parasite dose and resource availability can affect the fitness



**Fig. 6.** Mite competition in the absence of beetles. In the absence of beetles, increasing mite dose increased the number of mite offspring (A) while decreasing per capita mite fitness (B). The probability of males and females moulting into adults decreased with dose (C, D), and the number of mite offspring increased with female number only in the small groups (E).

outcome of the interaction. In our experiments, mite dose did not influence the likelihood of mite reproduction, nor did it predict beetle fitness directly. Instead, beetle fitness depended on the number of mite offspring produced at the resource, which suggests competition between mite and beetle offspring. This competition

could not be reduced by increasing resource value: the mite effect on beetle fitness was stronger on larger resources, presumably caused by the larger number of mite offspring developing on large resources. While low mite doses may limit the total number of mite offspring, and hence the fitness effect on beetles, there was

also significant intraspecific competition in mites already at relatively low densities, with competition taking place both between mite adults and among their offspring.

The mites were generally able to reproduce even at a low dose, indicating that the beetles do not mount a “first line of defence” that mites can overcome only in high numbers, as is known for many pathogens (Regoes et al., 2002; Leggett et al., 2012). There will be a minimal number of mite individuals required to ensure mating partners are available (May and Anderson, 1979; Nehring and Müller, 2009). However, these effects will be significant only in lower doses than the ones we have tested here. The few cases in which mite reproduction was not successful were mostly linked to beetles failing to reproduce. When the beetles prepare the carcass for their own offspring to feed on, they cut through the skin, which makes the resource available for the mites. This observation indicates that mites depend on the beetles and their manipulation of the carcass to be able to successfully reproduce. Delineating parasitism from competition is sometimes difficult when parasites do not eat parts of the host (Stewart and Schnitzer, 2017). The fitness interaction we typically observe between mites and beetles resembles competition, i.e. on beetle-tended carcasses both species appear to suffer from the interaction with the other symbiont. However, our results show that the mites require breeding beetles for their own reproduction and thus have a net positive fitness effect from the interaction, while the effect on the beetles is overall negative (De Gasperin and Kilner, 2015b; Nehring et al., 2017). Hence, the mites are beetle parasites.

Increasing mite dose increased the total number of mite offspring produced, which may in part be a direct effect of more females being present at the carcass: in the experiments without beetles, we observed the number of moulting females to be limiting offspring production in small groups. However, the increase in total offspring number does not match the increase in dose. Instead, per mite added to the experiment, fewer offspring were produced at larger doses. Such saturation indicates competition between the mites. As we observed fewer mite adults per mite added in larger groups, it is likely that these effects partly occurred before or during the adult moult. There is interference competition between mite males (Nehring and Müller, 2009), but the experiments presented here also demonstrate competition among females. In addition, increased numbers of mite females in larger groups do not translate into more mite offspring. This suggests that mite offspring also compete among each other, e.g. for food or undisturbed soil crevices that are suitable for moulting (Korn, 1982). These effects explain the observation that mites tend to avoid crowded beetles in favour of beetles carrying fewer mites (Schwarz and Müller, 1992).

As in previous studies, there was a negative correlation between the total number of mites and beetle fitness (De Gasperin and Kilner, 2015b; Nehring et al., 2017). While increasing mite dose increased total mite offspring number, this effect did not directly translate into stronger fitness effects on the beetles. If anything, it was the opposite. When large mite doses produced relatively few mite offspring, beetle fitness was higher; i.e. efficient mite reproduction harmed the beetles (De Gasperin and Kilner, 2016).

It is well known that beetle offspring compete for food (Bartlett and Ashworth, 1988), which caused individual beetle offspring size and total beetle offspring numbers to be negatively correlated in our experiments. Increasing the resource size increased beetle offspring numbers and brood weights, and the total number of mite offspring. In particular in small mite doses, larger resources softened mite-mite competition and increased per capita mite fitness. These effects are most easily explained by mite offspring competing for food among themselves and with the beetles. However, there are alternative explanations. It is possible that the deciding factor is not necessarily the amount but rather the access to food,

assuming larger carcasses have a larger surface that mites can feed on. Alternatively, crowded resources may also mean that mites disturb each other during the moult, leading to a higher juvenile death rate.

We did not find mite dose to affect the trade-off between the number of beetle larvae and average larval size (De Gasperin and Kilner, 2016). However, average larval size increased with the number of mite offspring, which is in line with previous findings that the presence of mites can lead to larger beetle offspring, and with the idea that mites might kill eggs and larvae and thus reduce larval numbers to lower levels than was intended by the beetle parents (Beninger, 1993; De Gasperin et al., 2015; De Gasperin and Kilner, 2016). Mites killing beetle eggs or larvae is unlikely to be the only negative impact of mites on beetles since eggs would be eaten by adult mites, so that the initial mite dose should affect the predation pressure. In any case, the total beetle brood weight suffers less from mites than the number of beetle offspring, and the interference between mites and beetles seems to happen in the early stages of each species' development. An alternative could be that beetles regulate to lower larval numbers sometimes (independent of the mites) and then, due to increased resource availability, more mites develop. With the data at hand, we are left to speculate about the mechanism behind resource dependency of mites and the interaction between mites and beetles.

Interestingly, the negative correlation between mite offspring number and beetle fitness was stronger on large carcasses. If mite-beetle competition were purely for nutrition, the opposite would appear more plausible; adding more resources should reduce the conflict. Beetles regulate their brood, i.e. they keep more larvae alive on large carcasses. If they overcompensated on large carcasses, more beetle larvae might be present per gram of carcass and thus competition might be stronger at larger carcasses. However, individual beetle larvae grew larger on larger than on smaller resources in our experiments and previous studies (Bartlett and Ashworth, 1988), so that there is no indication of overcompensation. If anything, competition for food should thus be relaxed on larger carcasses, and thus food does not appear to be the only resource beetles and mites compete for. The burying beetles regulate brood size and thus average offspring size by adjusting clutch size (number of eggs laid) to the size of the carcass. They typically lay more eggs than they allow larvae at the carcass (surplus larvae are killed), presumably allowing for some loss due to parasitized or unfertilized eggs (Bartlett and Ashworth, 1988; Müller et al., 1990; Trumbo, 1990). However, clutch size regulation according to carcass weight does not continue beyond carcass weights over 15 g, where average clutch size is around 30 eggs (Müller et al., 1990). It is thus possible that the number of surplus eggs (number of eggs laid minus the number of larvae allowed) was smaller at larger carcasses in our experiment, so that mite predation on eggs and larvae would have a more severe impact on beetle fitness because the beetles cannot compensate with larger clutches.

The mite-beetle system follows the same trade-off between host and parasite fitness that is observed in many other parasites, with symbionts competing for host resources both between and within species (Cressler et al., 2016). Dose effects, however, are surprisingly insignificant in predicting the outcome of competition in the mite-beetle system. This may be typical for systems where hosts mount no first line of defence and where intraspecific parasite competition is strong. We found that there is competition among mite adults as well as mite offspring, and that mites and beetles also interfere directly. The initial mite dose decreases per capita mite fitness, so that mite deuteronymphs ought to avoid crowded beetles. Individual beetle offspring appear to benefit from the competition among mite offspring and grow larger when the mite dose is high relative to resource size, although the fitness of

the beetle parents suffers. The exact mode of competition remains unclear, but may well be a mixture of direct interference in early stages of offspring development and exploitation of the same resource. Our results demonstrate that the selection of parasites on their hosts can strongly depend on the environment (e.g. carcass size) and suggest that virulence can be dose-independent in principle.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpara.2019.05.011>.

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