Cryptic host specialisation within *Poecilochirus carabi* mites explains population differences in the extent of co-adaptation with their burying beetle *Nicrophorus vespilloides* hosts

Authors: Syuan-Jyun Sun[1*] and Rebecca M. Kilner[1]

Affiliations: [1]Department of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ, UK

Keywords: burying beetles, mites, local adaptation, host-parasite interactions, population divergence, reciprocal transplant.

Corresponding author: Syuan-Jyun Sun; sjs243@cam.ac.uk; +44-1223 (3)34466

Abstract: Symbiotic organisms adapt to one another but the extent of co-adaptation commonly varies among pairs of the same symbiotic species drawn from different populations. Here we identify some ecological causes of such differences between populations. We analysed the extent of local co-adaptation between burying beetles *Nicrophorus vespilloides* and their phoretic mites *Poecilochirus carabi* in Gamlingay and Waresley Woods, in Cambridgeshire, UK. Burying beetles transport mites to small vertebrate carrion upon which they both reproduce. We conducted reciprocal transplant experiments to test for evidence of local co-adaptation during reproduction. We found variation in the extent of local co-adaptation that was explained by cryptic host specialisation within *P. carabi* mites. *P. carabi* is a species complex, within which races of mites are specialised to associate with different species of burying beetle. We found that *N. vespilloides* from Gamlingay Wood carries a mixture of mite races, from each of the four *Nicrophorus* species that inhabits this wood. This mixture of races makes *P.carabi* harmful to Gamlingay *N. vespilloides*: together, they reduce beetle reproductive success. Experimentally purifying mites, so that Gamlingay *N. vespilloides* is associated only with the vespilloides mite race, improves beetle reproductive success. Waresley *N. vespilloides*, by contrast, carry a near pure race of vespilloides mites, which cause negligible damage to Waresley *N. vespilloides* reproductive success. This is probably because Waresley Wood harbours only two burying beetle species, which differ markedly in their reproductive biology. Cryptic host specialisation with *P. carabi* mites, combined with differences in the *Nicrophorus* guild between Gamlingay and Waresley Woods, therefore explain population differences in the extent of local adaptation between *N. vespilloides* and *P. carabi*.

Introduction

Ever since Darwin (1859), evidence has been gathering that natural selection causes populations to adapt in different ways to their different local environments. More recent work suggests that adaptation can happen on very local scales, even when there is still gene flow between populations (Thompson 2013; S.-J. Sun et al. unpublished manuscript). Nevertheless, the extent of local adaptation varies across landscapes, between populations of the same species (Thompson 1999, 2005). This is especially true for adaptations that arise from co-evolutionary interactions between symbiotic species, which are engaged in reciprocal selection. In specialist antagonistic interactions, or obligate mutualisms, each party exerts such strong selection on the other that there can be little differentiation between populations (Lively and Dybdahl 2000; Forde et al. 2004; Johnson et al. 2010). For many other pairwise species interactions, however, the outcome of these reciprocal interactions varies with local conditions (Chamberlain et al. 2014), and this can have a profound effect on the extent and pattern of local co-evolution and co-adaptation (Thompson 1999, Gandon and Michalakis 2002, 2005*b*). A key challenge is to understand the ecological factors that generate such geographical mosaics of coevolution and co-adaptation (Thompson 2013).

Environmental differences among populations are known to cause variation in the outcome of any species interaction. These might arise from variation in the availability of an essential resource (Johnson et al. 2010), or the presence of a common enemy species (Hopkins et al. 2017), or differences in the abiotic environment (Kersch and Fonseca 2005). Theoretical analyses suggest that this variation, in turn, can cause dramatic differences in the trajectory of local co-evolution (Nuismer et al. 2000). Suppose, for example, that one species has little effect on the fitness of another i.e. it is commensal. Here we might expect relatively little selection for reciprocal co-adaptation. By contrast, if the same species reduces (or enhances) the fitness of its partner species in a different population then we might expect greater reciprocal selection according to the strength of the fitness effect (Thompson et al. 2002). Therefore, determining ecological correlates of variation among populations in the extent of reciprocal selection between the same two partner species can potentially explain why there is geographical variation in the pattern of local adaptation (Johnson et al. 2010; Garrido et al. 2012; Gorter et al. 2016).

Interactions between species seldom partition neatly into discrete dyads. For example, one cactus species is in a protective mutualism with multiple species of ants

(Ness et al. 2006). One insect species commonly pollinates more than one plant species, while each plant species can be pollinated by more than one insect species (Thompson 2013). Multiple bumblebee species *Bombus* spp. commonly interact with multiple mite species (Haas et al. 2019). Likewise, host species richness and abundance is positively correlated with parasite species richness (Hechinger and Lafferty 2005). Multispecies associations are likely to generate variation in the strength of reciprocal selection. For example, multiple infection of different parasite species or strains can differentially influence the fitness among different host species interacting in the same community, depending on the susceptibility and tolerance of each host species (Friesen et al. 2017). Therefore understanding how interactions with multiple species cause variation in selection can help account for additional geographical variation in the pattern of local co-adaptation.

Here we determine the extent of local co-adaptation between burying beetles *Nicrophorus vespilloides* (Coleoptera: Silphidae) and their phoretic mites *Poecilochirus carabi* (Mesostigmata: Parasitidae) in two neighbouring yet geographically isolated populations (Gamlingay and Waresley Woods) in Cambridgeshire, UK. Mites are termed "phoretic" because they are benign passengers when travelling onboard burying beetles. However interactions between mites and beetles while they breed on the same carrion are highly variable, ranging from parasitic (De Gasperin and Kilner 2015) to commensal to mutualistic (Sun et al. 2019; S.-J. Sun & R. M. Kilner, unpublished manuscript). This means it is likely that the extent of local reciprocal adaptation between burying beetles and their phoretic mites will vary accordingly, from population to population.

Although Gamlingay and Waresley Woods are no more than 2.5 km apart, we have discovered differences in the *Nicrophorus* guild inhabiting each woodland (S.-J. Sun et al. unpublished manuscript). Both woods contained the smallest burying beetles *N. vespilloides* and largest burying beetle *N. humator*. However, only Gamlingay Wood is additionally routinely inhabited by intermediate-sized *N. interruptus* and *N. investigator*. As a result, *N. vespilloides* in Waresley Wood has adapted to breed on larger carrion, whereas *N. vespilloides* in Gamlingay Wood is confined to breeding on smaller carrion (S.-J. Sun et al. unpublished manuscript).

We tested whether these ecological differences between the two woodlands, and the adaptations that have ensued, could potentially affect the extent of reciprocal local adaptation between burying beetles and their mites. We tested for local co-adaptation by exposing beetles from each woodland to mites from the alternative woodland. In

addition, we tested whether carcass size plays an important role in modulating the extent of local adaptation. Specifically, we predicted (1) that Gamlingay *N. vespilloides* and mites should be less adapted to each other on a larger carcass, because they are seldom exposed to this breeding resource (S.-J. Sun et al. unpublished manuscript). Second, we investigated the composition of *P. carabi* mite community carried by *N. vespilloides* from each woodland. *P. carabi* exists as a species complex, comprising distinct races of mites that are each specialised to breed on different species of burying beetle. The mite's lifecycle is timed to match the duration of parental care in the host burying beetle species, so that the new generation of mites can disperse on the burying beetle parents when they have finished looking after their young. However, each burying beetle species differs slightly in the time it spends tending its larvae. This has favoured local adaptation in the mite populations associated with each species of burying beetle, which in turn has generated distinct mite races (Wilson 1982; Müller and Schwarz 1990, Brown & Wilson 1992). Nevertheless, mite races can still interbreed and cannot be told apart phenotypically except behaviourally, through their relative preference for different burying beetle species (Wilson 1982; Müller and Schwarz 1990, Brown & Wilson 1992). Previous work suggests that when sympatric *Nicrophorus* species do not differ much in their duration of care, then each species of burying beetles carries a mixture of the different mite races associated with each of the sympatric beetles and the mites hybridise across races (Brown and Wilson 1992). Mixing happens when *Nicrophorus* species gather to feed, for example on larger carrion (Brown and Wilson 1992). However, in populations where *Nicrophorus* species differ considerably in their duration of care, each species is more likely to carry its own specialist mite race and mites are more likely to be reproductively isolated (Brown and Wilson 1992). Accordingly, we predicted: (2) that *N. vespilloides* from Gamlingay Wood should be more likely to carry a mixture of mite races, whereas *N. vespilloides* from Waresley Wood should be more likely to carry the *N. vespilloides* race of mites. We further predicted (3) that this should affect the extent of local adaptation. Specifically, *P. carabi* mites and *N. vespilloides* from Gamlingay Wood should show greater levels of reciprocal local adaptation than those from Waresley Wood.

Methods

Study species

Burying beetles transport phoretic mites to small carrion, upon which both species then reproduce. When a beetle discovers a carcass, mite deutonymphs disembark from the host beetle, moult into the adult stage, mate, and reproduce (Schwarz and Müller 1992). *N. vespilloides* are the most abundant burying beetles at our study sites (80.6% in Gamlingay Wood and 93.9% in Waresley Wood; S.-J. Sun et al. unpublished manuscript), and the *P. carabi* mite species complex is the most commonly found mite species associating with *Nicrophorus* beetles (Schwarz et al. 1998).

Field observations

Surveys of burying beetle communities were conducted in Gamlingay (Latitude: 52.15555°; Longitude: −0.19286°) and Waresley (Latitude: 52.17487°; Longitude: −0.17354°) Woods in Cambridgeshire, UK. From June to October in 2016-1017, five traps at each site were baited with mouse carcasses and hung in the same location, separated by at least 150m (S.-J. Sun et al. unpublished manuscript). We checked the traps every 2-3 weeks and collected all *Nicrophorus* spp. Traps were then refilled with fresh mice at each collection. Beetles and mites were then brought to the lab (Department of Zoology, University of Cambridge). The number and sex of each beetle species were recorded at each location for each trapping event. Beetle's pronotum width was measured to the nearest 0.01 mm as a standardised measurement of body size (Jarrett et al. 2017). We separated mites from their beetle hosts using CO_2 anaesthetisation, and counted the number of mites per beetle.

Origin and maintenance of burying beetles and mites

Both species were kept under laboratory conditions at 21 ± 2°C and on a 16:8 light to dark cycle.

<u>Beetles</u> were kept individually in plastic boxes (12cm x 8cm x 2cm) filled with moist soil. Field-collected beetles were kept for at least two weeks before they were subjected to experimentation to even out any differences in sexual maturity and nutritional status. To breed beetles, *N. vespilloides* collected from the field sites were paired on a mouse in a breeding box lined with damp soil. All breeding boxes were then placed into cupboards to mimic underground environments. After eight days, we collected the dispersing larvae and transferred them to eclosion boxes (10 x 10 x 2 cm, 25 compartments) filled with moist soil. At eclosion, each emerging beetle was

183 moved to a plastic container (12 x 8 x 2 cm) with moist soil. We fed beetles twice a
184 week with minced beef for 2-3 weeks until they were sexually mature.
185 Mites were maintained as distinct populations, according to their woodland of origin,
186 and apart from burying beetles. To breed mites, each month we transferred 15 mites
187 deutonymphs chosen at random, and a pair of beetles from the same population, to a
188 new breeding box (17 x 12 x 6 cm with 2 cm of soil) furnished with a fresh mouse
189 carcass (n = 10 for each population). After breeding, beetle parents and third-instar
190 larvae were removed from the box. The mites remained and were given another adult
191 beetle, and thereafter supplied with minced beef twice a week.
192
193 *Prediction 1: Carcass size affects local adaptation*
194 To assess the extent of local adaptation on carcasses of different sizes, we adopted a
195 fully factorial design of experimental reciprocal mite infestation (Nuismer and
196 Gandon 2008; Garrido et al. 2012; Blanquart et al. 2013). These experiments were
197 carried out in four blocks. Each beetle population (Gamlingay/Waresley) was infested
198 with either 10 Gamlingay mite deutonymphs or 10 Waresley mite deutonymphs, on
199 25-30 g (26.84 ± 0.18 g) large carcasses or 15-20 g (16.94 ± 0.12 g) small carcasses,
200 thereby generating eight treatments in total (2 x 2 x 2). Mites were introduced directly
201 onto the carcass, when beetles were paired. Pairs of beetles were unrelated, to prevent
202 inbreeding. At larval dispersal, 8 days after pairing, we counted all larvae and
203 weighed the whole brood (to the nearest 0.1mg). We calculated the average larval
204 mass for each brood (total brood mass divided by the number of larvae). To determine
205 the reproductive success of mites, we used CO_2 to detach dispersing mite
206 deutonymphs from adult beetles, at the end of the breeding event.
207 In parallel, we also bred Gamlingay and Waresley *N. vespilloides* without mites
208 on large (26.31 ± 0.20 g) and small (16.48 ± 0.12 g) carcasses, to test whether they
209 performed better or worse than the beetles exposed to mites in the local adaptation
210 experiment. We made the same measurements of reproductive success as described
211 above. We also bred mites in the absence of beetles by allowing ten mite
212 deutonymphs to breed on a large (28.04 ± 0.14 g) or small (17.31 ± 0.24 g) mouse
213 carcass. Seven days later, we introduced a pair of beetles from the same population to
214 associate with mites, and counted the dispersing mite offspring attaching to beetles
215 the next day (day 8) so that mites had the same duration of reproduction as in the
216 treatments with beetles.
217

Prediction 2: Gamlingay N. vespilloides carry a mixture of P. carabi races

To investigate population differences in the number of mite races present, we used consecutive choice experiments. *P. carabi* from either Gamlingay or Waresley Woods were allowed to choose between one of the following field-collected burying beetle species: *N. vespilloides*, *N. humator*, *N. interruptus*, *and N. investigator*. Each species was represented by one individual, drawn at random from a pool of field beetles (184 *N. vespilloides*, 98 *N. humator*, 129 *N. interruptus* and 100 *N. investigator*). Burying beetles in this pool were haphazardly chosen from four field populations (Gamlingay Wood, Waresley Wood, Madingley Wood (Latitude: 52.22658°; Longitude: 0.04303°), and Thetford Forest (Latitude: 52.41286°; Longitude: 0.75167°). This allowed us to remove any population specific effect on mite preferences, and to focus entirely on the species effect.

We used mites that had been bred for one generation in the lab without burying beetles and field-caught beetles. The four burying beetles (of the same sex, one from each species) were introduced into a plastic container (17 x 12 x 6 cm), around which they could move freely. At the same time 50 mites were also introduced – either from the Gamlingay population or from the Waresley population. The container held moist soil to a depth of 2 cm and minced beef in *ad libitum* quantities to prevent intraguild predation. The number of mites carried by each beetle species was recorded 24 h later, and used to assess the mixing of different mite races in each population. Mites that chose *N. vespilloides*, *N. humator*, *N. interruptus*, and *N. investigator* were defined as P-ves, P-hum, P-int, and P-inv, respectively. We then bred these mites separately on a fresh mouse carcass, with one mouse carcass for each race of mite identified in the first experiment. The offspring of these breedings were then tested again for their burying beetle preferences, as a further test of extent to which the mite races were mixed in each woodland. Just as before, we introduced 10 mites and one beetle from each of the four species in a plastic container, and counted the number of mites on each beetle after 24 h.

Prediction 3: The extent of local adaptation is reduced when mite races are mixed

These experiments were focused on *N. vespilloides* and *P. carabi* mites drawn specifically from Gamlingay Wood. We experimentally manipulated the composition of the mite community associated with each burying beetle (n = 10 deutonymphs), generating three treatments in all: a) pure *N. vespilloides* race of *P. carabi*; b) a mixture of all four races of *P. carabi*; and c) no mites (control). The mites used were

253 descendants of the second generation of *P. carabi* from the experiment above, and
254 races were determined from the preferences they exhibited in this experiment. They
255 were introduced at beetle pairing, directly onto the carcass. Pairs of beetles were
256 sequentially assigned to one of the three mite treatments, introduced into a breeding
257 box (17 x 12 x 6 cm with 2 cm of soil) and given a 15-20 g (17.71 ± 0.16) mouse
258 carcass to breed upon. We took the same measurements of beetle reproductive success
259 as in the previous experiments, when larvae dispersed away from the carcass 8 days
260 after pairing.
261
262 **Statistical analyses**
263 We analysed the data using generalized linear mixed models (GLMM) with the glmer
264 function in the *lme4* package in R version 3.4.3 (R Development Core Team 2014).
265 To obtain minimal adequate models, we applied a stepwise approach to exclude non-
266 significant variables and interactions (Crawley 2007). We included block as a random
267 effect in all models. To test for significant interactions, *post-hoc* interaction contrasts
268 were conducted using the testInteractions function with a Holm's correction in the
269 *phia* package (de Rosario-Martinez 2015). Tukey HSD tests were used for *post-hoc*
270 pairwise comparisons, as necessary, using the *lsmeans* package (Lenth 2016).
271
272 *Field observation*
273 To determine whether mite abundance differed between *Nicrophorus* beetle species
274 and population, we used a negative binomial GLMM (using the function glmer.nb in
275 the *lme4* package to account for data overdispersion). We included as explanatory
276 variables the interaction between beetle species and population, sex, and body size of
277 beetles, while sampling year was included as a random factor.
278
279 *Prediction 1: Carcass size affects local adaptation*
280 *Local adaptation of beetles to mites* We analysed two measures of beetle reproductive
281 success when exposed to different mite populations using GLMMs: brood size (using
282 a Poisson distribution) and average larval mass (using a Gaussian distribution). Beetle
283 treatment (Gamlingay/Waresley), mite treatment (control/local/foreign), carcass size
284 (large/small) and their interaction were included as explanatory variables. For the
285 analysis of average larval mass, we also included larval density (brood size divided by
286 carcass mass) as a covariate. In all models, block was included as a random factor.

Local adaptation of mites to beetles We analysed the number of dispersing mite deutonymphs present at the end of each reproductive bout using a negative binomial GLMM. Beetle treatment (control/local/foreign), mite treatment (Gamlingay/Waresley), carcass size (large/small) and their interaction were included as explanatory variables. Block was included as a random factor.

Prediction 2: Gamlingay N. vespilloides carry a mixture of P. carabi races
We analysed variation in the number of mites carried by each beetle species using a Poisson model, with an offset of the log total number of mites allowed to make a choice in each trial. We included mite population (Gamlingay/Waresley), beetle species (*N. vespilloides*, *N. humator*, *N. interruptus*, *and N. investigator*), and their interaction as explanatory variables. Sex and body size of beetles were included as covariates. We also included as random factors the sampling year (2017 or 2018), and trial ID. We analysed the data in a similar manner for the second choice experiment, testing for consistency of beetle species preference among mites in the next generation. However, this time we analysed only those the mites that made the same choice as their parents.

Prediction 3: The extent of local adaptation is reduced when mite races are mixed
We used generalized linear models (GLM) to analyse two measures of beetle reproductive success when exposed to different mite populations: brood size (using a Poisson distribution) and average larval mass (using a Gaussian distribution). We included mite treatments and carcass mass as explanatory variables in each model. When analysing variation in average larval mass, we additionally included larval density as a covariate. We also analysed variation in mite reproductive success using a negative binomial GLM with mite treatments and carcass mass as explanatory variables.

Results

Field observation
In total, 1464 *Nicrophorus* individuals were caught over the two sampling years (779 and 685 for Gamlingay and Waresley Woods, respectively), carrying a total of 17,171 *P. carabi* mite deutonymphs on four beetle species (fig. A1). We found that the mite load on each *Nicrophorus* species varied differently between populations (beetle species x population interaction, $\chi^2 = 49.65$, d.f. = 3, $p < 0.001$; fig. A1). Specifically,

322 *N. vespilloides* from Gamlingay had an average lower number of mites compared to
323 *N. interruptus* (*post-hoc* comparison: $z = 2.82$, $p = 0.025$), but carried similar number
324 of mites compared to those of *N. humator* (*post-hoc* comparison: $z = 0.45$, $p = 0.970$)
325 and *N. investigator* (*post-hoc* comparison: $z = 1.30$, $p = 0.563$). In Waresley Wood,
326 however, *N. vespilloides* carried more mites than *N. humator* (*post-hoc* comparison: z
327 $= -7.23$, $p < 0.001$), but we could detect no difference in the mite load carried by *N.*
328 *vespilloides* and *N. interruptus* (*post-hoc* comparison: $z = -2.47$, $p = 0.064$), nor
329 between *N. vespilloides* and *N. investigator* (*post-hoc* comparison: $z = -1.17$, $p =$
330 0.645). Moreover, comparing mite abundance on the beetle species, between
331 woodlands, we found that *N. humator* from Gamlingay had higher mite abundance
332 than *N. humator* from Waresley (*post-hoc* comparison: $z = 4.72$, $p < 0.001$), and there
333 was a tendency for Gamlingay *N. interruptus* to carry more mites than those from
334 Waresley (*post-hoc* comparison: $z = 1.89$, $p = 0.060$). In contrast, Gamlingay *N.*
335 *vespilloides* had lower number of mites than Waresley *N. vespilloides* (*post-hoc*
336 comparison: $z = -7.43$, $p < 0.001$). We could detect no significant difference between
337 Gamlingay and Waresley in mite abundance on *N. investigator* (*post-hoc* comparison:
338 $z = 0.82$, $p = 0.414$).
339
340 *Prediction 1: Carcass size affects local adaptation*
341 *a) Local adaptation of beetles to mite populations*
342 In general, we found that the different mite populations affected burying beetle brood
343 size in different ways, depending on the size of the carcass used for reproduction and
344 the burying beetle's woodland of origin (table 1*A*).
345 *Brood size*
346 To understand the factors driving the significant three-way interaction (fig. 1*A*, 1*B*;
347 table 1*A*), we initially split the dataset by beetle population, to understand the separate
348 effects of the mites and carcass size on burying beetle brood size in each population.
349 In Gamlingay Wood, we found that burying beetles exposed to Gamlingay mites
350 produced smaller broods, but that the extent of reduction in brood size depended on
351 carcass size (mite origin x carcass size interaction, $\chi^2 = 20.02$, d.f. = 2, $p < 0.001$).
352 Gamlingay mites significantly reduced brood size when beetles bred on both small
353 carcasses (*post-hoc* comparison, no mites v. local mites: $z = 2.92$, $p = 0.010$), and
354 large carcasses (*post-hoc* comparison, no mites v. local mites: $z = 6.24$, $p < 0.001$),
355 but the magnitude of difference in brood size was greater when beetles bred on large
356 carcasses. On small carcasses, Gamlingay beetles produced broods of similar size

357 whether breeding with Gamlingay or Waresley mites (*post-hoc* comparison, local
358 mites v. foreign mites: $z = -0.53$, $p = 0.859$). On larger carcasses they produced fewer
359 larvae when breeding with Gamlingay mites compared with Waresley mites (*post-hoc*
360 comparison, local mites v. foreign mites: $z = -7.10$, $p < 0.001$).
361 For beetles from Waresley Wood, we found that mite populations consistently
362 reduced beetle brood size ($\chi^2 = 7.17$, d.f. = 2, $p = 0.028$), irrespective of carcass size
363 (mite origin x carcass size interaction, $\chi^2 = 0.40$, d.f. = 2, $p = 0.819$). Waresley beetles
364 produced fewer larvae when breeding alongside Waresley mites than when breeding
365 with no mites at all (*post-hoc* comparison, no mites v. local mites: $z = 2.68$, $p =$
366 0.020). Waresley beetles produced broods of similar size whether breeding with
367 Waresley or Gamlingay mites (*post-hoc* comparison, local mites v. foreign mites: $z =$
368 1.42, $p = 0.332$). Breeding on large carcasses always generated more offspring than
369 on small carcasses (carcass size effect: $\chi^2 = 48.48$, d.f. = 1, $p < 0.001$).
370
371 *Average larval mass*
372 We found that beetles produced smaller larvae when breeding on a smaller carcass,
373 and that the woodland origin of the mites breeding alongside them affected the
374 magnitude of decrease in larval mass (fig. 1*C*, 1*D*; table 1*B*). The woodland origin of
375 the beetles did not additionally affect this interaction (beetle population x mite origin
376 x carcass size interaction, $\chi^2 = 0.81$, d.f. = 2, $p = 0.668$). When beetles bred on a small
377 carcass alongside mites from the same woodland population, their larvae were smaller
378 than when they bred alongside mites from the other woodland (*post-hoc* comparison,
379 local mites v. foreign mites: $t = -4.57$, $p < 0.001$), and when they had no mites at all
380 (*post-hoc* comparison, local mites v. no mites: $t = -4.64$, $p < 0.001$). Neither effect
381 was seen on larger carcasses (*post-hoc* comparisons: local mites v. foreign mites: $t =$ -
382 0.77, $p = 0.721$; local mites v. no mites: $t = -1.34$, $p = 0.373$).
383
384 *b) Local adaptation of mites to beetle populations*
385 We found that the reproductive success of the mites depended on whether or not they
386 were breeding alongside beetles from the same woodland population, and that this
387 relationship varied with the size of the carcass (fig. 2; table 2). To understand the
388 factors driving this significant three-way interaction, we initially split the dataset by
389 mite population, to understand the separate effects of the burying beetles and carcass
390 size on mite reproductive success.

In Gamlingay Wood, we found that mites produced more offspring on larger carcasses, but that this effect was modulated by the presence of beetles on the carcass and their woodland of origin (beetle origin x carcass size interaction, $\chi^2 = 8.80$, d.f. = 2, $p = 0.012$; fig. 2*A*). In general, Gamlingay mites had greater reproductive success when they bred alongside beetles from their own woodland than when there were no beetles present at all, whether they bred on a small (*post-hoc* comparison, no beetles v. local beetles: $z = -8.82$, $p < 0.001$) or large carcass (*post-hoc* comparison, no beetles v. local beetles: $z = -12.45$, $p < 0.001$). On smaller carcasses, Gamlingay mite reproductive success was enhanced to a similar extent, whether beetles were from Gamlingay or Waresley Woods (*post-hoc* comparison, local beetles v. foreign beetles: $z = -2.02$, $p = 0.106$). On larger carcasses, mites had even greater reproductive success when breeding alongside Gamlingay beetles rather than Waresley beetles (*post-hoc* comparison, local beetles v. foreign beetles: $z = 2.42$, $p = 0.041$).

Similarly, we found that Waresley mites produced more offspring on larger carcasses, but that the magnitude of this effect depended on the presence of beetles on the carcass and their woodland of origin (carcass size x beetle origin interaction, $\chi^2 = 6.93$, d.f. = 2, $p = 0.031$; fig. 2*B*). This time, we found that mites produced a similar number of offspring when breeding on a small carcass regardless of whether beetles were present or not (*post-hoc* comparison, no beetles v. local beetles: $z = -1.69$, $p = 0.208$) and regardless of the beetles' woodland of origin (*post-hoc* comparison, local beetles v. foreign beetles: $z = -1.07$, $p = 0.531$). However, on larger carcasses, mites tended to produce more offspring when breeding alongside Waresley beetles than when breeding alongside Gamlingay beetles (*post-hoc* comparison, local beetles v. foreign beetles: $z = 2.22$, $p = 0.067$). They produced a similar number of offspring with Waresley beetles as when they did breeding with no beetles at all (*post-hoc* comparison, no beetles v. local beetles: $z = -1.69$, $p = 0.209$).

Prediction 2: Gamlingay N. vespilloides carry a mixture of P. carabi races

The first lab-bred generation of *P. carabi* differed significantly from each other in their relative preference for the different *Nicrophorus* species, depending on whether they were derived originally from Gamlingay or Waresley Woods (mite population x beetle species interaction, $\chi^2 = 151.61$, d.f. = 3, $p < 0.001$; fig. 3*A*; table A1). *P. carabi* derived from each woodland showed clear preferences for the different *Nicrophorus* species (Gamlingay: $\chi^2 = 293.06$, d.f. = 3, $p < 0.001$; Waresley: $\chi^2 = 459.69$, d.f. = 3, $p < 0.001$). However, Gamlingay mites were similarly likely to favour *N. interruptus*

426 and *N. vespilloides*. They favoured *N. investigator* less frequently and *N. humator*
427 even less (fig. 3*A*; table A1). In contrast, Waresley *P. carabi* showed a clear
428 preference for *N. vespilloides*. Their next-favoured beetle species was *N. interruptus*,
429 followed by *N. investigator*, and then *N. humator* (fig. 3*A*; table A1). We used *post-*
430 *hoc* comparisons to compare the strength of the mite preference for each *Nicrophorus*
431 species between populations. We found that Gamlingay mites showed stronger
432 preference for *N. humator* ($z = 5.99$, $p < 0.001$) and *N. interruptus* ($z = 5.45$, $p <$
433 0.001) than mites from Waresley. They also tended to have a stronger preference for
434 *N. investigator* ($z = 1.93$, $p = 0.054$). By contrast, Waresley mites showed a higher
435 preference for *N. vespilloides* than Gamlingay mites ($z = -9.04$, $p < 0.001$).
436 We paired mites that showed the same preference for burying beetle species, and
437 tested whether the preferences of the offspring matched those of their parents, to test
438 for indirect evidence that mites were segregating into genetic races. The extent to
439 which the beetle preferences aligned between the generations varied by woodland
440 (mite population x beetle species interaction, $\chi^2 = 50.42$, d.f. = 3, $p < 0.001$). We
441 found that Waresley P-ves mites consistently had stronger preference for *N.*
442 *vespilloides* than Gamlingay mites ($t = -2.17$, $p = 0.030$; fig. 3*B*). In contrast, both P-
443 hum and P-int mites from Gamlingay Wood showed stronger consistence in their
444 choice of *N. humator* ($t = 5.48$, $p < 0.001$; fig. 3*B*) and *N. interruptus* ($t = 4.92$, $p <$
445 0.001; fig. 3*B*), respectively, compared to those from Waresley Wood. Gamlingay P-
446 inv mites also preferred *N. investigator* compared to Waresley P-inv mites ($t = 1.78$, p
447 $= 0.075$; fig. 3*B*), although the effect was relatively weak.
448
449 *Prediction 3: The extent of local adaptation is reduced when mite races are mixed*
450 We tested whether the variation caused by mites in Gamlingay beetle reproductive
451 success (fig. 1) could be explained by the differential mixing of mite races within
452 woodlands. We created experimental mite communities, manipulated to different
453 degrees to contain mites with different beetle preferences. We showed that mite
454 community had an effect on beetle's brood size ($\chi^2 = 36.02$, d.f. = 2, $p < 0.001$; fig.
455 4*A*). Beetles produced a similar number of larvae whether they were breeding without
456 mites or with a pure population of *N. vespilloides*-specific mites (*post-hoc*
457 comparison, $z = -1.38$, $p = 0.351$). Beetles that bred alongside mites that varied in
458 their preference for different beetle species produced fewer larvae than beetles that
459 had no mites at all (*post-hoc* comparison, $z = 4.50$, $p < 0.001$). We found no effect of
460 the mite treatments on average larval mass (mite treatment: $\chi^2 = 4.11$, d.f. = 2, $p =$

461 0.128; fig. 4*B*). The beetle preferences of the mites also explained variation in mite
462 reproductive success (fig. 4*C*). Mites produced more offspring in a pure population of
463 *N. vespilloides*-specific *P. carabi* than when in a mixture of different *P. carabi* 'races'
464 ($\chi^2 = 18.13$, d.f. = 1, $p < 0.001$).
465
466 **Discussion**
467 Spatially-structured interactions between hosts and symbionts can result in population
468 differences in the strength of selection that interacting species exert upon one another.
469 This, in turn, can lead to geographical variation in the pattern of co-evolution and co-
470 adaptation between partner species (Thompson 2013). Our experiments show that this
471 is true for interactions between *N. vespilloides* and *P. carabi* mites from Gamlingay
472 and Waresley Woods, and they identify the ecological factors that cause these
473 differences in selection.
474 In our first experiment, we tested whether the extent of co-adaptation varies
475 between woodlands, with resource availability. From the mites' perspective, we found
476 evidence of adaptation to the local *N. vespilloides* population that varied in strength
477 with the size of the carcass upon which both species bred (fig. 2). Regardless of
478 carrion size, Gamlingay mites were locally adapted to breed alongside Gamlingay *N.*
479 *vespilloides*. By contrast, Waresley mites were more weakly adapted to their local
480 hosts, and this was evident only when they bred on a larger carcass. One explanation
481 is that Waresley mites more frequently breed on larger carrion than smaller carrion
482 (S.-J. Sun et al. unpublished manuscript).
483 Turning to the beetles' perspective, at first sight we seemingly found no
484 equivalent evidence of adaptation in beetles to their local mite population (fig. 1). In
485 general, mites appear to be parasitic because they reduced beetle reproductive
486 success. For Gamlingay beetles breeding on a large carcass, the local mite population
487 seems to be even more parasitic than the foreign mite population.
488 However, to fully understand these findings, we need to consider the results of
489 the subsequent experiments. Here we found that mites substantially depress burying
490 beetle fitness only when multiple mite races coexist within the same *N. vespilloides*
491 breeding event. Gamlingay beetles carried a mixture of mite races, including some
492 hybrids (we infer their existence through the discovery that mite offspring show lower
493 fidelity than their parents to a particular beetle species; fig. 3). Furthermore,
494 experimentally mixing the mite races carried by Gamlingay *N. vespilloides* was
495 sufficient to cause reduced beetle reproductive success, and also reduced mite

496 reproductive success (fig. 4). By contrast, Waresley mites displayed a marked
497 preference for associating with *N. vespilloides*, and this preference persisted between
498 generations. Therefore, we conclude that Waresley beetles carry, almost exclusively,
499 a near pure-bred race of P-ves mites.

500 These results suggest that *N. vespilloides* beetles are locally adapted to mites
501 after all, but only to mites of the P-ves race. The adaptation involves a form of
502 tolerance to parasitism rather than a defence against parasitism (Svensson and Råberg
503 2010). Consequently, when exposed to mites of the P-ves race, beetles suffer
504 relatively little, or no, reduction in reproductive success. Furthermore, beetles from
505 both Gamlingay and Waresley Woods are similarly tolerant of the P-ves race of mites
506 (fig. 1, 4*A*).

507 However, Gamlingay *N. vespilloides* are not as well-adapted to mites from
508 other races, with whom they presumably share a less specialist co-evolutionary
509 history. These other mite races are also apparently more dependent on the beetle than
510 the P-ves race, even after they have arrived on the carcass (fig. 2). This might explain
511 how they are able to reduce beetle reproductive success to a greater extent than the P-
512 ves race. Interestingly, Waresley *N. vespilloides* were better able than Gamlingay *N.*
513 *vespilloides* to tolerate the presence of mites from other races. We suggest that this is
514 because Waresley females routinely lay more eggs than Gamlingay females, and so
515 can better withstand any offspring mortality caused by mites from other races (S.-J.
516 Sun et al. unpublished manuscript). What remains to be clarified is the extent of co-
517 adaptation between other *Nicrophorus* spp. and each race of mites.

518 Geographic mosaic theory suggests that populations differ in the extent of co-
519 adaptation between interacting species because the structure of selection varies
520 between populations; because the strength of reciprocal selection varies between
521 populations; or because genetic variation influences the capacity for co-adaptation in
522 different populations; or some combination of all three of these factors. This study is
523 relatively rare in showing how the first two factors contribute to variation in the
524 extent of local co-adaptation between *N. vespilloides* and its *P. carabi* mites. The
525 structure of selection differs between populations as a consequence of differences in
526 the *Nicrophorus* guild between the two woods (S.-J. Sun et al. unpublished
527 manuscript). In Gamlingay Wood, where four *Nicrophorus* species live, *N.*
528 *vespilloides* carry a mixture of mite races from all four *Nicrophorus* species. The non-
529 *vespilloides* mite races are more dependent on *N. vespilloides* for reproductive
530 success, reduce *N. vespilloides* brood size to a greater degree, and also impair the

reproductive success of P-ves mites. Consequently, they impose stronger selection on *N. vespilloides* than the P-ves mites. Yet any counter-selection by *N. vespilloides* is bound to be weaker because the mites' descendants are likely to be carried by other *Nicrophorus* species. Hence Gamlingay *N. vespilloides* is less suitable as a host because P-ves mites routinely share these beetles with other mite races, which essentially dilute any adaptation that can occur between *N. vespilloides* and P-ves.

By contrast, in Waresley Wood, where there are routinely only two burying beetle species (S.-J. Sun et al. unpublished manuscript), *N. vespilloides* carries an almost pure population of P-ves mites. Perhaps *N. vespilloides* and *N. humator* differ too much in their duration of parental care for mixed races, and hybrids, to persist (Brown & Wilson 1992). P-ves mites are more self-sufficient on the carcass and consequently less costly both to *N. vespilloides* beetles and other P-ves mites. As a result, co-evolution between mites and *N. vespilloides* in Warelsey means that mites now impose only weak selection on *N. vespilloides*. In addition, the loss of intermediate-sized rivals for carrion, means that Waresley *N. vespilloides* routinely lays more eggs than Gamlingay *N. vespilloides* (Sun et al. unpublished manuscript). Coincidentally, this means Waresley *N. vespilloides* can now better endure the costs of breeding with mites from other races, when exposed to them experimentally.

Animals are commonly hosts to diverse communities of symbionts. This study suggests that the structure of that community can vary cryptically and geographically and this plays a key role in determining the extent of local co-adaptation between a host and any one of its many symbionts.

Acknowledgements

S.-J.S. was supported by the Taiwan Cambridge Scholarship from the Cambridge Commonwealth, European & International Trust. R.M.K. was supported by a European Research Council Consolidator grant 301785 BALDWINIAN_BEETLES and a Wolfson Merit Award from the Royal Society.

Literature Cited

Blanquart, F., O. Kaltz, S. L. Nuismer, and S. Gandon. 2013. A practical guide to measuring local adaptation. Ecology Letters 16:1195–1205.

Brown, J. M., and D. S. Wilson. 1992. Local specialization of phoretic mites on sympatric carrion beetle hosts. Ecology 73:463–478.

Chamberlain, S. A., J. L. Bronstein, and J. A. Rudgers. 2014. How context dependent

are species interactions? Ecology Letters 881–890.
Crawley, M. J. 2007. The R Book. John Wiley & Sons, Ltd, Chichester, UK.
De Gasperin, O., and R. M. Kilner. 2015. Friend or foe: inter-specific interactions and conflicts of interest within the family. Ecological Entomology 40:787–795.
de Rosario-Martinez, H. 2015. phia: Post-hoc interaction analysis.
Forde, S. E., J. N. Thompson, and B. J. M. Bohannan. 2004. Adaptation varies through space and time in a coevolving host–parasitoid interaction. Nature 431:841–844.
Friesen, O. C., R. Poulin, and C. Lagrue. 2017. Differential impacts of shared parasites on fitness components among competing hosts. Ecology and Evolution 7:4682–4693.
Garrido, E., G. Andraca-Gómez, and J. Fornoni. 2012. Local adaptation: simultaneously considering herbivores and their host plants. New Phytologist 193:445–453.
Gorter, F. A., P. D. Scanlan, and A. Buckling. 2016. Adaptation to abiotic conditions drives local adaptation in bacteria and viruses coevolving in heterogeneous environments. Biology letters 12:20150879.
Haas, S. M., S. Cardinal, F. Beaulieu, and J. R. K. Forrest. 2019. Mite-y bees: bumble bees (*Bombus* spp., Hymenoptera: Apidae) host a relatively homogeneous mite (Acari) community, shaped by bee species identity but not by geographic proximity. Ecological Entomology.
Hechinger, R. F., and K. D. Lafferty. 2005. Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. Proceedings of the Royal Society B: Biological Sciences 272:1059–1066.
Hopkins, S. R., J. M. Wojdak, and L. K. Belden. 2017. Defensive symbionts mediate host-parasite interactions at multiple scales. Trends in parasitology 33:53–64.
Jarrett, B. J. M., M. Schrader, D. Rebar, T. M. Houslay, and R. M. Kilner. 2017. Cooperative interactions within the family enhance the capacity for evolutionary change in body size. Nature Ecology & Evolution 1:0178.
Johnson, N. C., G. W. T. Wilson, M. Bowker, J. Wilson, and R. M. Miller. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proceedings of the National Academy of Sciences of the United States of America 107:2093–2098.
Kersch, M. F., and C. R. Fonseca. 2005. Abiotic factors and the conditional outcome of an ant–plant mutualism. Ecology 86:2117–2126.

Lenth, R. V. 2016. Least-squares means: the R package lsmeans. Journal of Statistical Software 69:1–33.

Lively, C. M., and M. F. Dybdahl. 2000. Parasite adaptation to locally common host genotypes. Nature 405:679–681.

Müller, J. K., and H. H. Schwarz. 1990. Differences in carrier-preference and evidence of reproductive isolation between mites of *Poecilochirus carabi* (Acari, Parasitidae) living phoretically on two sympatric *Necrophorus* species (Coleoptera, Silphidae). Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere 117:23–30.

Ness, J. H., W. F. Morris, and J. L. Bronstein. 2006. Integrating quality and quantity of mutualistic service to contrast ant species protecting *Ferocactus wislizeni*. Ecology 87:912–21.

Nuismer, S. L., and S. Gandon. 2008. Moving beyond common-garden and transplant designs: insight into the causes of local adaptation in species interactions. The American Naturalist 171:658–668.

Nuismer, S. L., J. N. Thompson, and R. Gomulkiewicz. 2000. Coevolutionary clines across selection mosaics. Evolution; international journal of organic evolution 54:1102–15.

R Development Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria.

Schwarz, H. H., and J. K. Müller. 1992. The dispersal behaviour of the phoretic mite *Poecilochirus carabi* (Mesostigmata, Parasitidae): adaptation to the breeding biology of its carrier *Necrophorus vespilloides* (Coleoptera, Silphidae). Oecologia 89:487–493.

Schwarz, H. H., M. Starrach, and S. Koulianos. 1998. Host specificity and permanence of associations between mesostigmatic mites (Acari: Anactinotrichida) and burying beetles (Coleoptera: Silphidae: *Nicrophorus*). Journal of Natural History 32:159–172.

Sun, S.-J., N. P. C. Horrocks, and R. M. Kilner. 2019. Conflict within species determines the value of a mutualism between species. Evolution Letters.

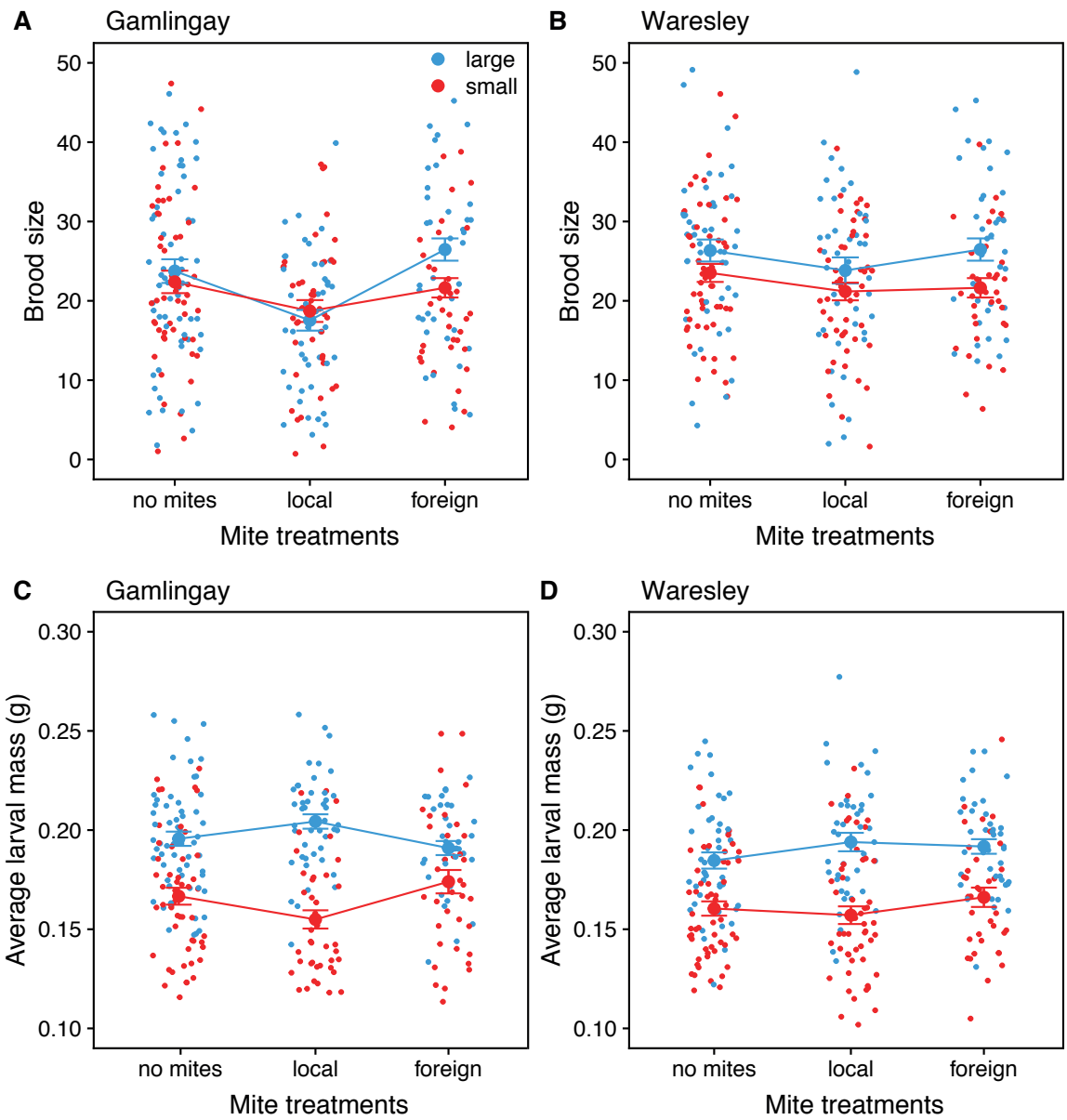
Svensson, E. I., and L. Råberg. 2010. Resistance and tolerance in animal enemy-victim coevolution. Trends in ecology & evolution 25:267–74.

Thompson, J. N. 1999. Specific hypotheses on the geographic mosaic of coevolution. The American Naturalist 153:S1–S14.

Thompson, J. N. 2005. The geographic mosaic of coevolution. University of Chicago

Press.
Thompson, J. N. 2013. Relentless evolution. University of Chicago Press, Chicago.
Thompson, J., S. Nuismer, and R. Gomulkiewicz. 2002. Coevolution and maladaptation. Integrative and Comparative Biology 42:381–387.
Wilson, D. S. 1982. Genetic polymorphism for carrier preference in a phoretic mite. Annals of the Entomological Society of America 75:293–296.

645 **Figures:**



646

647 **Figure 1:** Burying beetle reproductive success (mean ± SEM) in relation to woodland
648 of origin, carcass size and the three mite treatments. Data are shown for brood size of
649 beetles from (A) Gamlingay and (B) Waresley Woods and average larval mass from
650 (C) Gamlingay and (D) Waresley Woods. In the mite treatments, 'local' means that
651 beetles bred alongside 10 mite deutonymphs from the same woodland and 'foreign'
652 means that beetles bred alongside 10 mite deutonymphs from the other woodland. Each
653 point represents one brood.

654

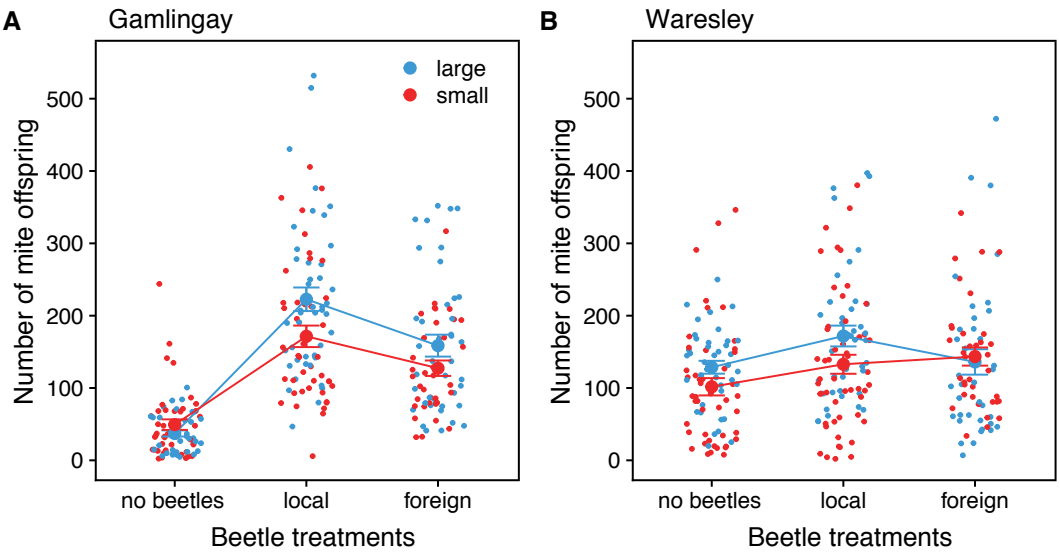
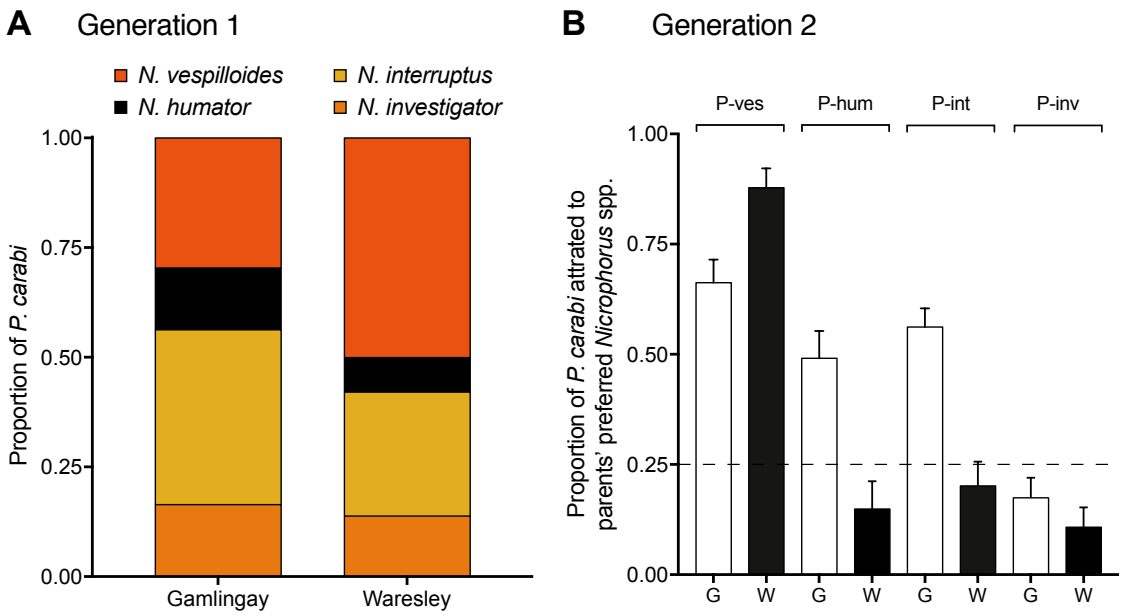


Figure 2: Mite reproductive success (mean ± SEM) in relation to woodland of origin, carcass size and the three beetle treatments. The number of mite offspring (deutonymphs) dispersing with adult beetles at the end of reproduction are shown for (A) Gamlingay mites and (B) Waresley mites. In the beetle treatments, 'local' means that mites bred alongside beetles from the same woodland and 'foreign' means that mites bred alongside beetles from the other woodland. Each point represents one brood.



664

665 **Figure 3:** Population differences of mite preferences between Gamlingay (G) and
666 Waresley (W) Woods. (A) Proportion of mites that were attracted to each *Nicrophorus*
667 spp. in the first generation and (B) proportion of mites that were attracted to their
668 parents' preferred *Nicrophorus* spp. P-ves, P-hum, P-int, and P-inv represent mites that
669 chose *N. vespilloides*, *N. humator*, *N. interruptus*, and *N. investigator*, respectively. The
670 dashed line at 25% represents the proportion of *P.carabi* associating simply by chance
671 with one of the *Nicrophorus* species.

672

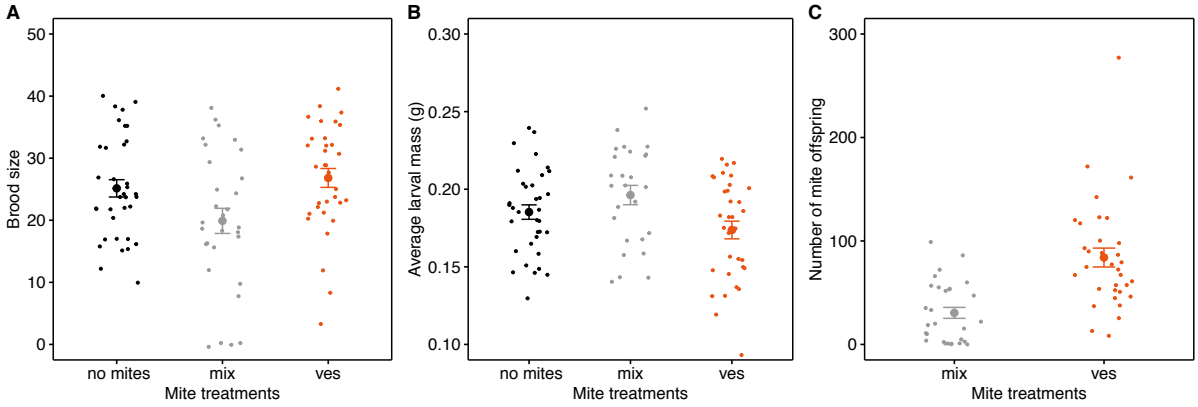


Figure 4: Reproductive success of beetles and mites from Gamlingay Wood, following experimental manipulations of mites on each carcass. Reproductive success of beetles were measured as (A) brood size, (B) average larval mass, whereas mite reproductive success was measured as (C) the number of deutonymphs dispersing with adult beetles. In the mite treatments, 'mix' means that beetles bred alongside 10 mites as a mixture of all four 'races' and 'ves' means that beetles bred alongside 10 mites from pure *N. vespilloides* race. Each point represents one brood. Means and standard error bars are shown.

Table 1: Results from the final models analysing the fitness components of beetle local adaptation

Dependent variable	Explanatory variables	X^2	d.f.	*p* value
A. Brood size	beetle population	5.49	1	0.019
	mite origin	59.33	2	<0.001
	carcass size	8.09	1	0.004
	beetle population*mite origin	23.73	2	<0.001
	beetle population*carcass size	0.66	1	0.418
	mite origin*carcass size	19.59	2	<0.001
	beetle population*mite origin*carcass size	11.00	2	0.004
B. Average larval mass	beetle population	0.019	1	0.890
	mite origin	1.80	2	0.406
	carcass size	8.30	1	0.004
	larval density	380.72	1	<0.001
	mite origin*carcass size	21.55	2	<0.001

697

698

Table 2: Results from the final models analysing the fitness components of mite local adaptation

Dependent variable	Explanatory variables	X^2	d.f.	*p* value
Number of mite offspring	beetle origin	152.61	2	<0.001
	mite population	66.31	1	<0.001
	carcass size	2.15	1	0.143
	beetle origin*mite population	65.29	2	<0.001
	beetle origin*carcass size	5.51	2	0.064
	mite population*carcass size	5.95	1	0.015
	beetle origin*mite population*carcass size	7.18	2	0.028

699

700

Appendix:

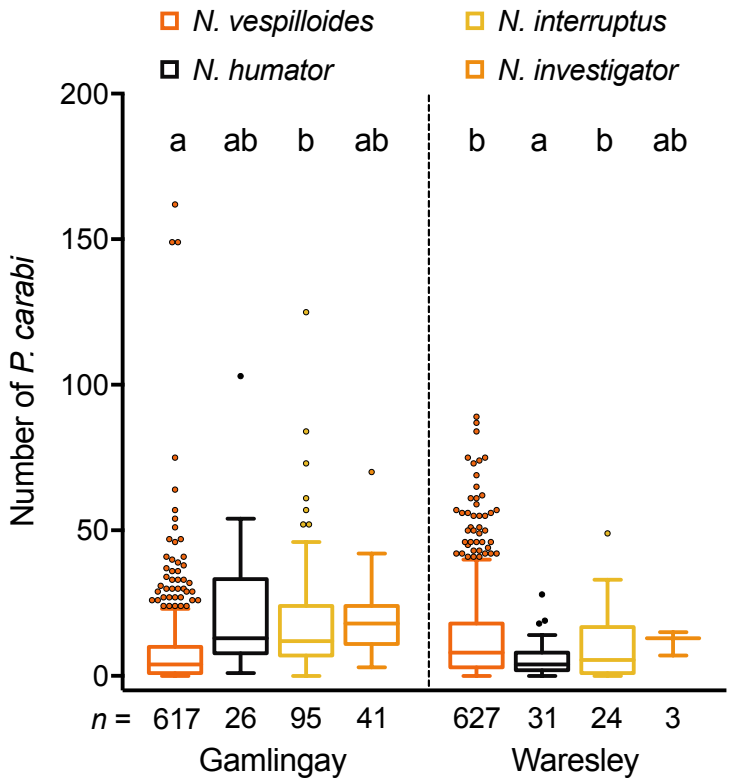


Figure A1: Number of *P. carabi* carried by each *Nicrophorus* spp. in Gamlingay and Waresley Woods. The box plot show median values, the 25th and 75th percentiles, interquartile ranges, and outliers as pointed. Letters indicate significant differences among groups in *Post-hoc* pairwise comparisons. Sample sizes are as indicated.

Table A1: Results of Tukey's post hoc comparisons for beetle species x population interaction in the first choice experiment

Gamlingay

Groups compared	**Estimate**	**SE**	**z-ratio**	***P*-value**
hum to int	-1.22	0.08	-15.54	<.0001
hum to inv	-0.29	0.09	-3.24	0.007
hum to ves	-1.10	0.09	-11.57	<.0001
int to inv	0.93	0.07	13.39	<.0001
int to ves	0.12	0.06	1.89	0.233
inv to ves	-0.81	0.08	-9.98	<.0001

Waresley

Groups compared	**Estimate**	**SE**	**z-ratio**	***P*-value**
hum to int	-1.53	0.10	-15.56	<.0001
hum to inv	-0.75	0.11	-6.93	<.0001
hum to ves	-2.21	0.11	-20.57	<.0001
int to inv	0.79	0.08	10.48	<.0001
int to ves	-0.68	0.06	-11.36	<.0001
inv to ves	-1.47	0.08	-19.23	<.0001

710