1    **Cryptic host specialisation within *Poecilochirus carabi* mites explains**

2    **population differences in the extent of co-adaptation with their**

3    **burying beetle *Nicrophorus vespilloides* hosts**

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17  **Abstract:** Symbiotic organisms adapt to one another but the extent of co-adaptation

18  commonly varies among pairs of the same symbiotic species drawn from different

19  populations. Here we identify some ecological causes of such differences between

20  populations. We analysed the extent of local co-adaptation between burying beetles

21  *Nicrophorus vespilloides* and their phoretic mites *Poecilochirus carabi* in Gamlingay

22  and Waresley Woods, in Cambridgeshire, UK. Burying beetles transport mites to

23  small vertebrate carrion upon which they both reproduce. We conducted reciprocal

24  transplant experiments to test for evidence of local co-adaptation during reproduction.

25  We found variation in the extent of local co-adaptation that was explained by cryptic

26  host specialisation within *P. carabi* mites. *P. carabi* is a species complex, within

27  which races of mites are specialised to associate with different species of burying

28  beetle. We found that *N. vespilloides* from Gamlingay Wood carries a mixture of mite

29  races, from each of the four *Nicrophorus* species that inhabits this wood. This mixture

30  of races makes *P.carabi* harmful to Gamlingay *N. vespilloides*: together, they reduce

31  beetle reproductive success. Experimentally purifying mites, so that Gamlingay *N.*

32  *vespilloides* is associated only with the vespilloides mite race, improves beetle

33  reproductive success. Waresley *N. vespilloides*, by contrast, carry a near pure race of

34  vespilloides mites, which cause negligible damage to Waresley *N. vespilloides*

35  reproductive success. This is probably because Waresley Wood harbours only two

36  burying beetle species, which differ markedly in their reproductive biology. Cryptic

37  host specialisation with *P. carabi* mites, combined with differences in the

38  *Nicrophorus* guild between Gamlingay and Waresley Woods, therefore explain

39  population differences in the extent of local adaptation between *N. vespilloides* and *P.*

40  *carabi*.

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**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

80    (Ness et al. 2006). One insect species commonly pollinates more than one plant

81    species, while each plant species can be pollinated by more than one insect species

82    (Thompson 2013). Multiple bumblebee species *Bombus* spp. commonly interact with

83    multiple mite species (Haas et al. 2019). Likewise, host species richness and

84    abundance is positively correlated with parasite species richness (Hechinger and

85    Lafferty 2005). Multispecies associations are likely to generate variation in the

86    strength of reciprocal selection. For example, multiple infection of different parasite

87    species or strains can differentially influence the fitness among different host species

88    interacting in the same community, depending on the susceptibility and tolerance of

89    each host species (Friesen et al. 2017). Therefore understanding how interactions with

90    multiple species cause variation in selection can help account for additional

91    geographical variation in the pattern of local co-adaptation.

92         Here we determine the extent of local co-adaptation between burying beetles

93    *Nicrophorus vespilloides* (Coleoptera: Silphidae) and their phoretic mites

94    *Poecilochirus carabi* (Mesostigmata: Parasitidae) in two neighbouring yet

95    geographically isolated populations (Gamlingay and Waresley Woods) in

96    Cambridgeshire, UK. Mites are termed "phoretic" because they are benign passengers

97    when travelling onboard burying beetles. However interactions between mites and

98    beetles while they breed on the same carrion are highly variable, ranging from

99    parasitic (De Gasperin and Kilner 2015) to commensal to mutualistic (Sun et al. 2019;

100   S.-J. Sun & R. M. Kilner, unpublished manuscript). This means it is likely that the

101   extent of local reciprocal adaptation between burying beetles and their phoretic mites

102   will vary accordingly, from population to population.

103        Although Gamlingay and Waresley Woods are no more than 2.5 km apart, we

104   have discovered differences in the *Nicrophorus* guild inhabiting each woodland (S.-J.

105   Sun et al. unpublished manuscript). Both woods contained the smallest burying

106   beetles *N. vespilloides* and largest burying beetle *N. humator*. However, only

107   Gamlingay Wood is additionally routinely inhabited by intermediate-sized *N.*

108   *interruptus* and *N. investigator*. As a result, *N. vespilloides* in Waresley Wood has

109   adapted to breed on larger carrion, whereas *N. vespilloides* in Gamlingay Wood is

110   confined to breeding on smaller carrion (S.-J. Sun et al. unpublished manuscript).

111        We tested whether these ecological differences between the two woodlands, and

112   the adaptations that have ensued, could potentially affect the extent of reciprocal local

113   adaptation between burying beetles and their mites. We tested for local co-adaptation

114   by exposing beetles from each woodland to mites from the alternative woodland. In

115 addition, we tested whether carcass size plays an important role in modulating the

116 extent of local adaptation. Specifically, we predicted (1) that Gamlingay *N.*

117 *vespilloides* and mites should be less adapted to each other on a larger carcass,

118 because they are seldom exposed to this breeding resource (S.-J. Sun et al.

119 unpublished manuscript). Second, we investigated the composition of *P. carabi* mite

120 community carried by *N. vespilloides* from each woodland. *P. carabi* exists as a

121 species complex, comprising distinct races of mites that are each specialised to breed

122 on different species of burying beetle. The mite's lifecycle is timed to match the

123 duration of parental care in the host burying beetle species, so that the new generation

124 of mites can disperse on the burying beetle parents when they have finished looking

125 after their young. However, each burying beetle species differs slightly in the time it

126 spends tending its larvae. This has favoured local adaptation in the mite populations

127 associated with each species of burying beetle, which in turn has generated distinct

128 mite races (Wilson 1982; Müller and Schwarz 1990, Brown & Wilson 1992).

129 Nevertheless, mite races can still interbreed and cannot be told apart phenotypically

130 except behaviourally, through their relative preference for different burying beetle

131 species (Wilson 1982; Müller and Schwarz 1990, Brown & Wilson 1992). Previous

132 work suggests that when sympatric *Nicrophorus* species do not differ much in their

133 duration of care, then each species of burying beetles carries a mixture of the different

134 mite races associated with each of the sympatric beetles and the mites hybridise

135 across races (Brown and Wilson 1992). Mixing happens when *Nicrophorus* species

136 gather to feed, for example on larger carrion (Brown and Wilson 1992). However, in

137 populations where *Nicrophorus* species differ considerably in their duration of care,

138 each species is more likely to carry its own specialist mite race and mites are more

139 likely to be reproductively isolated (Brown and Wilson 1992). Accordingly, we

140 predicted: (2) that *N. vespilloides* from Gamlingay Wood should be more likely to

141 carry a mixture of mite races, whereas *N. vespilloides* from Waresley Wood should be

142 more likely to carry the *N. vespilloides* race of mites. We further predicted (3) that

143 this should affect the extent of local adaptation. Specifically, *P. carabi* mites and *N.*

144 *vespilloides* from Gamlingay Wood should show greater levels of reciprocal local

145 adaptation than those from Waresley Wood.

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## 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 $21 \pm 2°C$ and on a 16:8 light to dark cycle.

Beetles 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     <u>Mites</u> 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

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

219 To investigate population differences in the number of mite races present, we used

220 consecutive choice experiments. *P. carabi* from either Gamlingay or Waresley Woods

221 were allowed to choose between one of the following field-collected burying beetle

222 species: *N. vespilloides, N. humator, N. interruptus, and N. investigator*. Each species

223 was represented by one individual, drawn at random from a pool of field beetles (184

224 *N. vespilloides*, 98 *N. humator*, 129 *N. interruptus* and 100 *N. investigator*). Burying

225 beetles in this pool were haphazardly chosen from four field populations (Gamlingay

226 Wood, Waresley Wood, Madingley Wood (Latitude: 52.22658°; Longitude:

227 0.04303°), and Thetford Forest (Latitude: 52.41286°; Longitude: 0.75167°). This

228 allowed us to remove any population specific effect on mite preferences, and to focus

229 entirely on the species effect.

230 We used mites that had been bred for one generation in the lab without burying

231 beetles and field-caught beetles. The four burying beetles (of the same sex, one from

232 each species) were introduced into a plastic container (17 x 12 x 6 cm), around which

233 they could move freely. At the same time 50 mites were also introduced – either from

234 the Gamlingay population or from the Waresley population. The container held moist

235 soil to a depth of 2 cm and minced beef in *ad libitum* quantities to prevent intraguild

236 predation. The number of mites carried by each beetle species was recorded 24 h

237 later, and used to assess the mixing of different mite races in each population. Mites

238 that chose *N. vespilloides, N. humator, N. interruptus, and N. investigator* were

239 defined as P-ves, P-hum, P-int, and P-inv, respectively. We then bred these mites

240 separately on a fresh mouse carcass, with one mouse carcass for each race of mite

241 identified in the first experiment. The offspring of these breedings were then tested

242 again for their burying beetle preferences, as a further test of extent to which the mite

243 races were mixed in each woodland. Just as before, we introduced 10 mites and one

244 beetle from each of the four species in a plastic container, and counted the number of

245 mites on each beetle after 24 h.

246

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

248 These experiments were focused on *N. vespilloides* and *P. carabi* mites drawn

249 specifically from Gamlingay Wood. We experimentally manipulated the composition

250 of the mite community associated with each burying beetle ($n = 10$ deutonymphs),

251 generating three treatments in all: a) pure *N. vespilloides* race of *P. carabi*; b) a

252 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.

287   *Local adaptation of mites to beetles* We analysed the number of dispersing mite

288   deutonymphs present at the end of each reproductive bout using a negative binomial

289   GLMM. Beetle treatment (control/local/foreign), mite treatment

290   (Gamlingay/Waresley), carcass size (large/small) and their interaction were included

291   as explanatory variables. Block was included as a random factor.

292

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

294   We analysed variation in the number of mites carried by each beetle species using a

295   Poisson model, with an offset of the log total number of mites allowed to make a

296   choice in each trial. We included mite population (Gamlingay/Waresley), beetle

297   species (*N. vespilloides*, *N. humator*, *N. interruptus*, *and N. investigator*), and their

298   interaction as explanatory variables. Sex and body size of beetles were included as

299   covariates. We also included as random factors the sampling year (2017 or 2018), and

300   trial ID. We analysed the data in a similar manner for the second choice experiment,

301   testing for consistency of beetle species preference among mites in the next

302   generation. However, this time we analysed only those the mites that made the same

303   choice as their parents.

304

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

306   We used generalized linear models (GLM) to analyse two measures of beetle

307   reproductive success when exposed to different mite populations: brood size (using a

308   Poisson distribution) and average larval mass (using a Gaussian distribution). We

309   included mite treatments and carcass mass as explanatory variables in each model.

310   When analysing variation in average larval mass, we additionally included larval

311   density as a covariate. We also analysed variation in mite reproductive success using

312   a negative binomial GLM with mite treatments and carcass mass as explanatory

313   variables.

314

315   **Results**

316   *Field observation*

317   In total, 1464 *Nicrophorus* individuals were caught over the two sampling years (779

318   and 685 for Gamlingay and Waresley Woods, respectively), carrying a total of 17,171

319   *P. carabi* mite deutonymphs on four beetle species (fig. A1). We found that the mite

320   load on each *Nicrophorus* species varied differently between populations (beetle

321   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.

391       In Gamlingay Wood, we found that mites produced more offspring on larger

392    carcasses, but that this effect was modulated by the presence of beetles on the carcass

393    and their woodland of origin (beetle origin x carcass size interaction, $\chi^2 = 8.80$, d.f. =

394    2, $p = 0.012$; fig. 2*A*). In general, Gamlingay mites had greater reproductive success

395    when they bred alongside beetles from their own woodland than when there were no

396    beetles present at all, whether they bred on a small (*post-hoc* comparison, no beetles

397    v. local beetles: $z = -8.82$, $p < 0.001$) or large carcass (*post-hoc* comparison, no

398    beetles v. local beetles: $z = -12.45$, $p < 0.001$). On smaller carcasses, Gamlingay mite

399    reproductive success was enhanced to a similar extent, whether beetles were from

400    Gamlingay or Waresley Woods (*post-hoc* comparison, local beetles v. foreign beetles:

401    $z = -2.02$, $p = 0.106$). On larger carcasses, mites had even greater reproductive success

402    when breeding alongside Gamlingay beetles rather than Waresley beetles (*post-hoc*

403    comparison, local beetles v. foreign beetles: $z = 2.42$, $p = 0.041$).

404       Similarly, we found that Waresley mites produced more offspring on larger

405    carcasses, but that the magnitude of this effect depended on the presence of beetles on

406    the carcass and their woodland of origin (carcass size x beetle origin interaction, $\chi^2 =$

407    6.93, d.f. = 2, $p = 0.031$; fig. 2*B*). This time, we found that mites produced a similar

408    number of offspring when breeding on a small carcass regardless of whether beetles

409    were present or not (*post-hoc* comparison, no beetles v. local beetles: $z = -1.69$, $p =$

410    0.208) and regardless of the beetles' woodland of origin (*post-hoc* comparison, local

411    beetles v. foreign beetles: $z = -1.07$, $p = 0.531$). However, on larger carcasses, mites

412    tended to produce more offspring when breeding alongside Waresley beetles than

413    when breeding alongside Gamlingay beetles (*post-hoc* comparison, local beetles v.

414    foreign beetles: $z = 2.22$, $p = 0.067$). They produced a similar number of offspring

415    with Waresley beetles as when they did breeding with no beetles at all (*post-hoc*

416    comparison, no beetles v. local beetles: $z = -1.69$, $p = 0.209$).

417

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

419    The first lab-bred generation of *P. carabi* differed significantly from each other in

420    their relative preference for the different *Nicrophorus* species, depending on whether

421    they were derived originally from Gamlingay or Waresley Woods (mite population x

422    beetle species interaction, $\chi^2 = 151.61$, d.f. = 3, $p < 0.001$; fig. 3*A*; table A1). *P. carabi*

423    derived from each woodland showed clear preferences for the different *Nicrophorus*

424    species (Gamlingay: $\chi^2 = 293.06$, d.f. = 3, $p < 0.001$; Waresley: $\chi^2 = 459.69$, d.f. = 3, $p$

425    $< 0.001$). 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**

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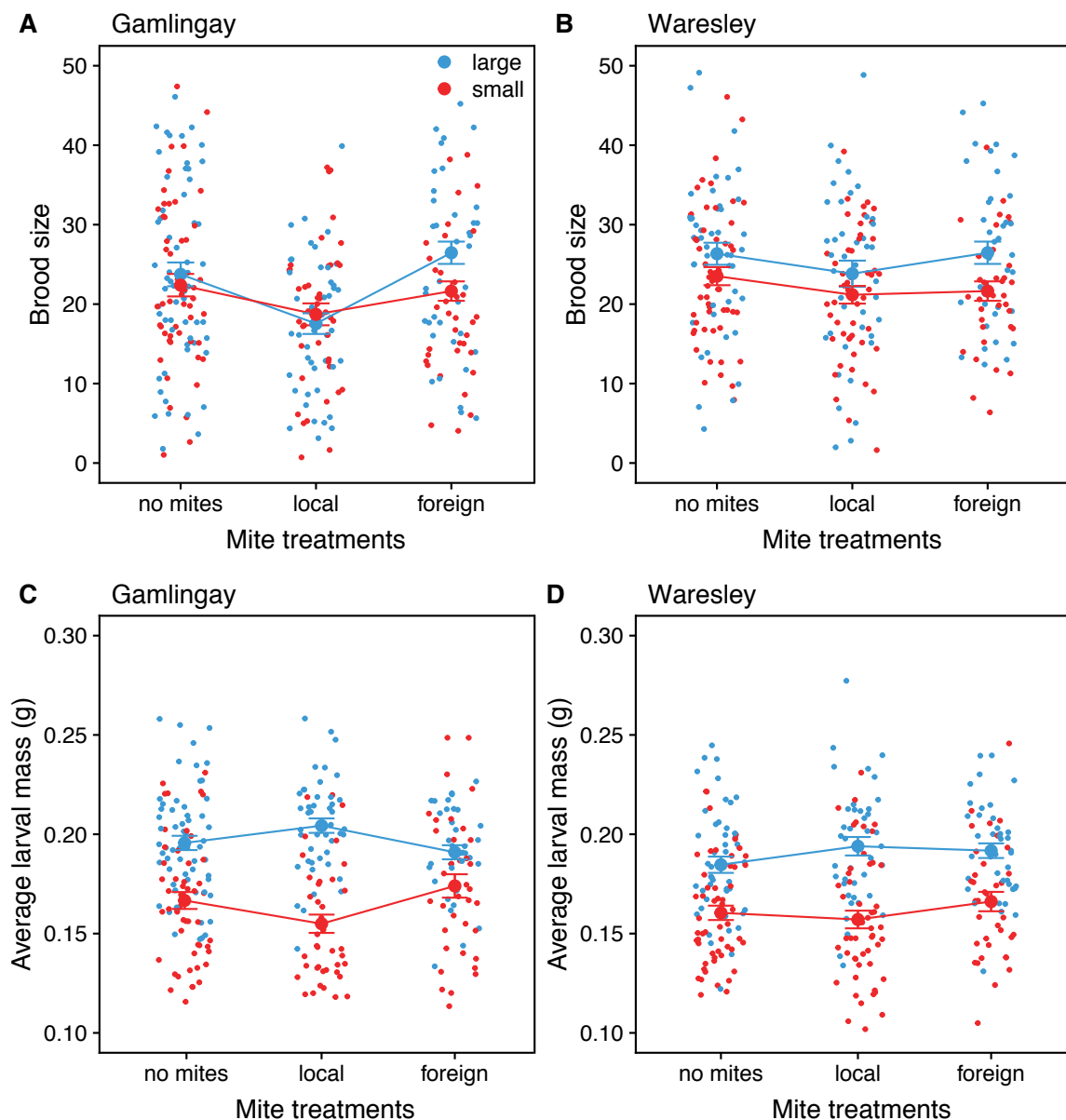640  Wilson, D. S. 1982. Genetic polymorphism for carrier preference in a phoretic mite.

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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.

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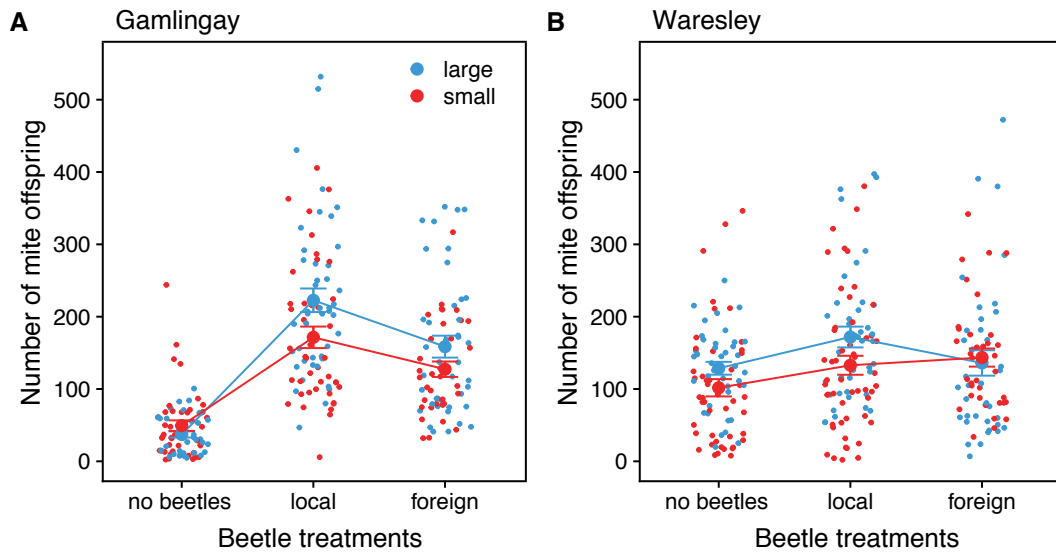
**Figure 2:** Mite reproductive success (mean ± SEM) in relation to woodland of origin, carcass size and the three beetle treatments. The number of mite offspring (deutonymphs) dispersing with adult beetles at the end of reproduction are shown for (A) Gamlingay mites and (B) Waresley mites. In the beetle 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.

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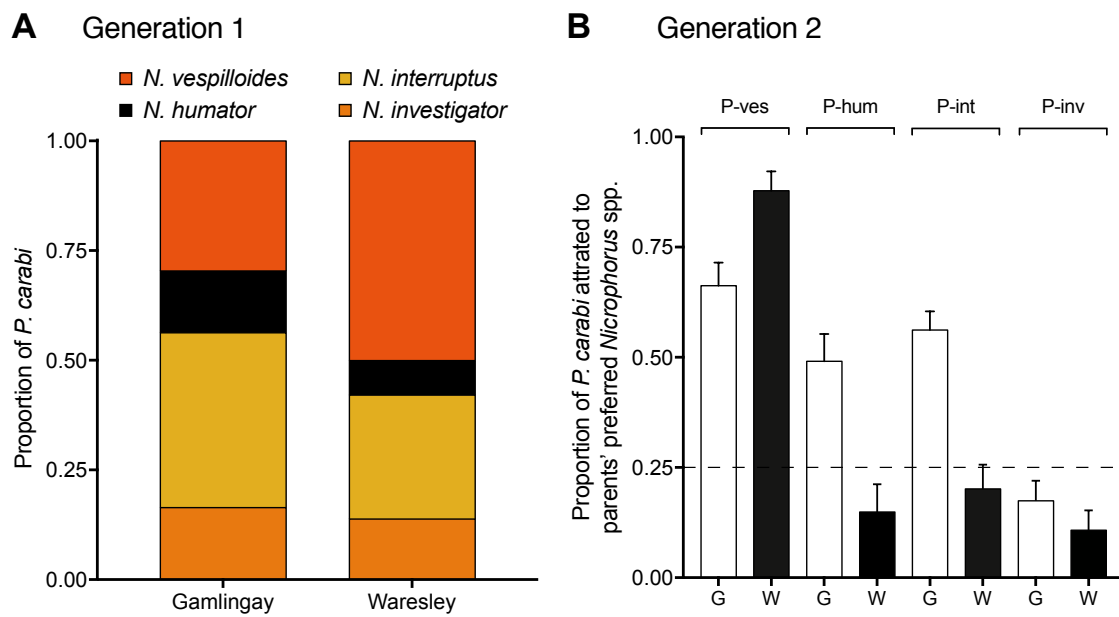
**Figure 3:** Population differences of mite preferences between Gamlingay (G) and Waresley (W) Woods. (A) Proportion of mites that were attracted to each *Nicrophorus* spp. in the first generation and (B) proportion of mites that were attracted to their parents' preferred *Nicrophorus* spp. P-ves, P-hum,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

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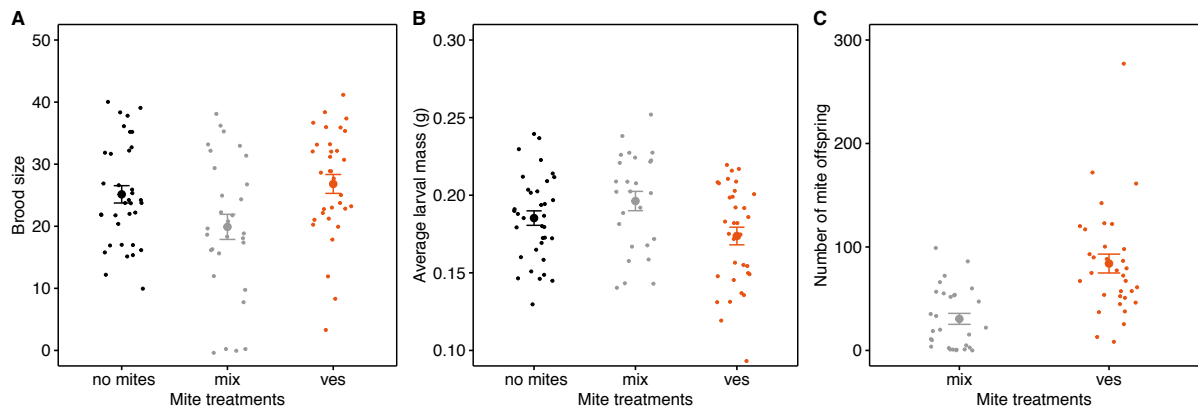


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676 **Figure 4:** Reproductive success of beetles and mites from Gamlingay Wood, following

677 experimental manipulations of mites on each carcass. Reproductive success of beetles

678 were measured as (A) brood size, (B) average larval mass, whereas mite reproductive

679 success was measured as (C) the number of deutonymphs dispersing with adult beetles.

680 In the mite treatments, 'mix' means that beetles bred alongside 10 mites as a mixture

681 of all four 'races' and 'ves' means that beetles bred alongside 10 mites from pure *N.*

682 *vespilloides* race. Each point represents one brood. Means and standard error bars are

683 shown.

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**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 |

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**Table 2:** Results from the final models analysing the fitness components of mite local adaptation
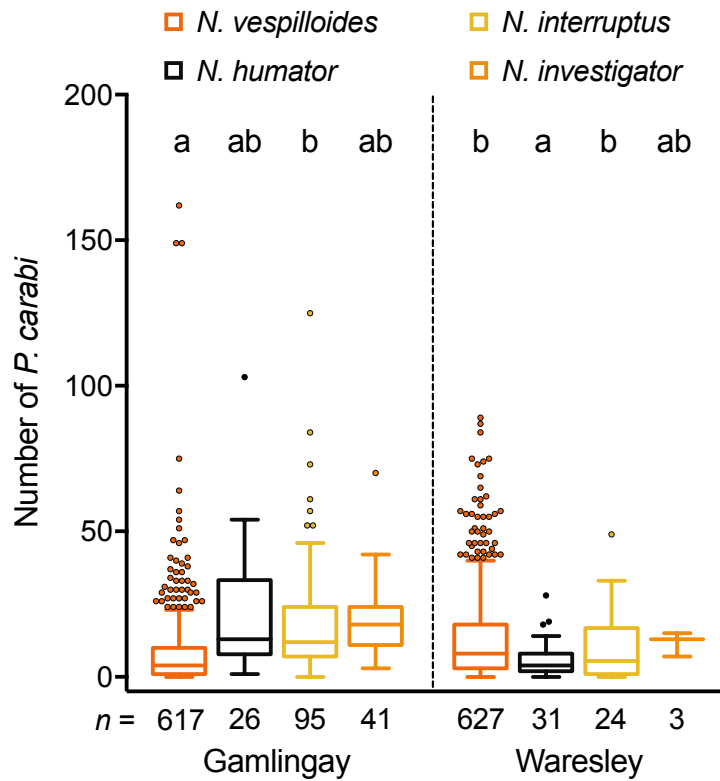
| Dependent variable | Explanatory variables | $X^2$ | d.f. | *p* value |
|---|---|---|---|---|
| Number of mite offspring | beetle origin | 152.61 | 2 | <0.001 |
| | mite population | 66.31 | 1 | <0.001 |
| | carcass size | 2.15 | 1 | 0.143 |
| | beetle origin*mite population | 65.29 | 2 | <0.001 |
| | 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 |

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**Appendix:**



**Figure A1:** Number of *P. carabi* carried by each *Nicrophorus* spp. in Gamlingay and Waresley Woods. The box plot show median values, the $25^{th}$ and $75^{th}$ 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 |

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