Title: Ultra-local adaptation due to genetic accommodation

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Keywords: interspecific competition, phenotypic plasticity, plasticity-led evolution, burying beetles, *Nicrophorus vespilloides,* niche expansion, local adaptation.

Statement of authorship: R.M.K. conceived the idea. S.-J.S., A.M.C. S.P and R.M.K designed the study. S.-J.S., A.M.C. and B.J.M.J. acquired the phenotypic data, which was analysed by S.-J.S. and A.M.C. M.J.S. and S.P designed the genetic's component, which was collected by S.P and analysed by M.J.S and S.E.M. All authors contributed to discussion of the results and writing the manuscript.

Abstract

Models of 'plasticity-first' evolution suggest that phenotypic plasticity precedes and facilitates the rapid evolution of new adaptations. Previous work has emphasised how new adaptations arise through the loss of plasticity, when populations split to occupy new environments and become reproductively isolated from the ancestral population. Here we analyse the role of 'plasticity-first' evolution in generating divergent new adaptations when populations are still connected by gene flow. We report that neighbouring populations of burying beetles *Nicrophorus vespilloides*, in different Cambridgeshire woodlands, have divergently adapted to breed on different-sized carrion despite ongoing gene flow. We show experimentally that this ultra-local adaptation is due to evolution of the reaction norm linking clutch size to carrion size, a process known as genetic accommodation. Plasticity is not lost in the derived population, but instead is fine-tuned by evolution to match the greater range in carrion available naturally. Accordingly, we find that loci associated with oogenesis have become differentiated in beetles from the different woodlands. We suggest that genetic accommodation can enable organisms to rapidly and specifically adapt to very fine-scale differences in their local environment, even while gene flow persists.

Introduction

A key challenge in evolutionary biology is to explain how natural populations evolve new adaptations. An old idea [1–3], which has recently been revitalized [4,5], is that pre-existing phenotypic plasticity can facilitate adaptive evolution in new environments ('plasticity-first evolution'). The extent of phenotypic plasticity in any trait is described by the reaction norm which relates environmental variation to the phenotype it induces. Plasticity-first evolution is possible because the shape, slope and elevation of a reaction norm each have genetic components, upon which selection can act [6]. Focusing mainly on traits that promote survival, such as melanic colouration or morphology, recent experimental work on natural populations has shown that novel adaptive phenotypes can indeed evolve as a consequence of plasticity-first evolution [7, 8]. In populations of toads and lizards, for example, selection in a new environment has greatly reduced the slope of the reaction norm, thus eliminating phenotypic plasticity and causing the constitutive expression of novel, canalised traits [7,8]. New adaptations can therefore evolve through 'genetic assimilation' (sensu [9]) of traits that were once induced environmentally.

However, genetic assimilation is predicted to occur only when there is little or no gene flow between populations that inhabit different local environments [10–12]. Consistent with

this prediction, the populations of toads and lizards that have evolved new traits through genetic assimilation are apparently reproductively isolated [7,8]. While gene flow still persists, theory predicts that selection will instead act to maintain plasticity in adaptive traits [11,12]. Nevertheless, plasticity-first evolution can still facilitate local adaptation because selection can fine-tune the slope or elevation of the reaction norm within each population to match local conditions. This is the process of genetic accommodation [13]. In principle, genetic accommodation of the reaction norm provides a mechanism for the ultra-local adaptation seen in populations that still experience gene flow [14]. However, whether this mechanism is at work in natural animal populations is unclear (but see [15] for evidence from a plant species).

We tested for evidence of plasticity-first adaptation through the genetic accommodation of a reaction norm, in wild populations of burying beetles *Nicrophorus vespilloides.* We focused on populations occupying neighbouring woodlands in Cambridgeshire, UK, which are c. 2.5 km apart: Gamlingay Wood (Latitude: 52.15555°; Longitude: −0.19286°), and Waresley Wood (Latitude: 52.17487°; Longitude: −0.17354°). In previous work, we showed that there is gene flow between the *N. vespilloides* populations inhabiting each woodland because they cannot be differentiated at neutral genetic markers [16]. Here we show that the burying beetle guilds differ between the two woodlands. We then test whether genetic accommodation of traits related to fecundity has enabled each population of *N. vespilloides* to become ultra-locally adapted to the contrasting levels of competition from other *Nicrophorus* species.

Burying beetles (*Nicrophorus* spp.) breed on the dead body of small vertebrates, such as rodents, and typically match their clutch size to the size of the carcass they opportunistically secure for reproduction [17]. The adaptive clutch size therefore depends on the size of the carrion that is routinely available for reproduction. This is the population's carrion niche. It is common for several species of *Nicrophorus* beetle to exist in sympatry within the same woodland and then the carrion niche is divided between them, according to their relative size [18]. Burying beetles compete within and between species to obtain exclusive ownership of a carcass to breeding upon, and larger beetles are more likely to win these fights [19,20]. Competition for carrion divides the carrion niche so that larger burying beetle species tend to breed on larger carrion, while the smallest species (*N. vespilloides* in the UK) usually breeds on the smaller carrion [18,21]. Larger carrion potentially yields more larvae than small carcasses, and so is more intensely fought over [22].

In this study, we collected ecological, genomic and experimental data to test three of the criteria for plasticity-first evolution, set out by Levis & Pfennig (2016). With ecological and

genomic data, we show that *N. vespilloides* from Gamlingay Wood is an ancestral-proxy population while the *N. vespilloides* population from Waresley Wood represents a derived condition. Through experimentation and analysis of ecological data, we find that *N. vespilloides* from each woodland now occupies a different carrion niche. Gamlingay *N. vespilloides* faces competition for carrion from three larger *Nicrophorus* species and has a narrow carrion niche in which reproduction is specialised on small carcasses. By contrast, Waresley *N. vespilloides* competes with only one rival *Nicrophorus* species, and has expanded its carrion niche to breed successfully on carcasses that range more widely in size. To test whether this niche expansion has evolved under genetic accommodation we experimentally: (1) expose Gamlingay *N. vespilloides* to larger carrion and test whether this induces an increase in clutch size; (2) characterise the equivalent reaction norm in Waresley *N. vespilloides* to test whether the slope or elevation has evolved to be different; (3) test whether any population divergence in the reaction norm is adaptive by quantifying how effectively *N. vespilloides* from each population converts small and large carrion into offspring. Finally, we use genomic analyses to test for evidence of divergence between the populations at loci associated with oogenesis.

Results and Discussion:

Gamlingay and Waresley Woods are 'woodland islands' that have existed for 1000-4000 years

Our study focuses on the *Nicrophorus* populations that inhabit Gamlingay Wood and Waresley Wood, in Cambridgeshire, England, UK. Five thousand years ago, England was almost uniformly covered in ancient forest: the so-called 'Wild Wood' [23]. During the Bronze Age, and especially from the Iron Age onwards (i.e. from 3000-4000 years ago), the Wild Wood underwent extensive destruction [23]. We know from the Domesday Book (a land survey commissioned almost 1000 years ago by William the Conqueror) that Gamlingay Wood and Waresley Wood have existed as distinct woodlands since at least 1086, meaning that they separated c.1000-4000 years ago. In common with other ancient woodlands created at this time, these woods have stayed approximately the same size since then [24]. Gamlingay Wood was acquired and managed by Merton College, Oxford for c. 800 years [25]. Its ecology was described in detail in 1912 [25]. The modern history of Waresley Wood is less well-known [24,26]. Both sites are now designated as 'ancient woodland' and are managed by the Bedfordshire, Cambridgeshire and Northamptonshire Wildlife Trusts. They are woodland islands of approximately the same size (c 50ha) in a landscape dominated by arable farming (S1A Fig).

131

132 ***The Nicrophorus guild differs between Gamlingay and Waresley Woods***

133 Each year from 2014-2017 we set five beetle traps per woodland, at exactly the same five
134 locations within each wood, and checked the contents every 2-3 weeks from June until
135 October (see Materials and Methods; S1A Fig). In general, we found that the two woodlands
136 harboured a similar number of *Nicrophorus* beetles: we caught a total of 1873 *Nicrophorus*
137 individuals in Gamlingay Wood over the four-year sampling period compared with 1806
138 *Nicrophorus* individuals in Waresley Wood. However, we discovered that whereas
139 Gamlingay Wood routinely supports four *Nicrophorus* species, Waresley Wood is routinely
140 inhabited by only two species (Fig 1A). The average abundance per trap of each *Nicrophorus*
141 species differed between the two woods (species x woodland interaction: $\chi^2 = 142.68$, d.f. =
142 4, $p < 0.001$). The smallest species (Fig 1B; S1 and S2 Tables), *N. vespilloides*, was by far the
143 most abundant in each woodland; 80.6% (1510 individuals) of all *Nicrophorus* beetles
144 trapped in Gamlingay Wood and 93.9% (1695 individuals) of those trapped in Waresley
145 Wood were *N. vespilloides*. Both sites also contained stable populations of the largest species,
146 *N. humator* (Fig 1B; S1 and S2 Tables) Only Gamlingay Wood contained populations of
147 intermediate-sized *N. interruptus* and *N. investigator* in all four years of the study (Fig 1B; S1
148 and S2 Tables). Overall, each of these two species was significantly more abundant in
149 Gamlingay than in Waresley Woods (Tukey HSD, $z = 7.90$, $p < 0.001$ and $z = 5.80$, $p <$
150 0.001, respectively). The *Nicrophorus* guild is therefore significantly different between the
151 two woodlands (PERMANOVA (permutational multivariate analysis of variance) test $F =$
152 0.024, $p < 0.001$). A clear separation of the guild structure between the woods can be seen for
153 each sampling time across the four years in NMDS (nonmetric multidimensional scaling)
154 ordination (S1B Fig).

155

156 ***No evidence for divergence in the small mammal population between Gamlingay and***
157 ***Waresley Woods***

158 In 2016, we sampled the small mammal population in Gamlingay and Waresley Woods to
159 estimate the abundance and type of carrion that might be available to the burying beetles to
160 breed upon (see Materials and Methods). In Gamlingay, 32 animals were caught across 50
161 trap sessions (23 new catches and 9 recaptures); in Waresley 41 animals were caught across
162 50 trap sessions (30 new catches and 11 recaptures). Across both woods, bank voles (*Myodes*
163 *glareolus*; range: 15-40 g) and wood mice (*Apodemus sylvaticus*; range: 13-27 g) were the
164 dominant species, constituting 53% and 43% of all trapped mammals respectively. One
165 yellow-necked mouse (*Apodemus flavicollis*; range: 14-45 g) and one common shrew (*Sorex*

araneus; range: 5-14 g) were caught in Waresley. There was no difference in the mean body mass of small mammals sampled between the two sites ($\chi^2 = 0.19$, d.f. = 1, $p = 0.661$; S2 Fig). We have no reason to think that the mortality of these rodent species should differ between woodlands that are in such close proximity and that are subjected to similar levels of ecological management.

Greater competition among Nicrophorus for carrion in Gamlingay versus Waresley Woods

We conclude from these data that there are approximately similar numbers of *Nicrophorus* beetles within each woodland competing for an approximately similar size, abundance and type of rodent carrion to breed upon. The key difference lies in the number of burying beetle species in each wood. Evidence from other populations suggests that our measurements reflect long-term differences because abundance measures of *Nicrophorus* are robust over time, even when there are marked change in woodland management [25].

Tests of plasticity-led evolution critically depend on the ability to distinguish ancestral populations from more recently evolved populations [6]. We infer from the ecological data that the more speciose burying beetle guild in Gamlingay Wood more closely approximates the ancestral burying beetle guild that was present in the 'Wild Wood', whereas the guild in Waresley Wood represents a more recent, derived condition. This is because burying beetle guilds in pristine ancient forests in North America are more speciose than those present in degraded woodlands [27], in keeping with the general observation that there is a positive relationship between habitat size and species richness [28]. Furthermore, smaller-bodied generalist carrion beetles are more likely to survive in fragmented forests [27].

Niche expansion by N. vespilloides beetles in Waresley Wood

The ecological data additionally suggest that in Gamlingay Wood, *N. vespilloides* is more likely to be confined to breeding only on smaller carrion, whereas in Waresley Wood this species' carrion niche is likely to extend to include larger carrion because it very rarely faces competition from *N. interruptus* and *N. investigator* for larger carcasses (see [18]). Although the proportion of intermediate-sized *Nicrophorus* species is relatively small (Fig 1C), *N. vespilloides* in Gamlingay Wood is more likely to face competition from congenerics for rarer, larger carrion than *N. vespilloides* in Waresley Wood [18,22]. This inference is further supported by previous experimental work on *Nicrophorus*. In continental forests, which are presumably also rich in *Nicrophorus* species, Urbański & Baraniak (2015) found that *N. vespilloides* was more than twice as likely to be found on small carcasses than on large

carcasses. In addition, larger species appear to be under selection to breed on larger carcasses [29,30].

To test these inferences about the division of the carrion niche among *Nicrophorus* species within each woodland, we measured the reproductive performance of *N. interruptus* (from Gamlingay Wood), *N. investigator* (from Gamlingay Wood) and *N. vespilloides* beetles (from both Gamlingay and Waresley Woods) in the laboratory (see Materials and Methods). For each species, we experimentally varied the size of carrion available for reproduction, presenting pairs with either a small mouse carcass (range: 12-20 g; mean ± S.E.M: 16.80 ± 0.55 g) or a large mouse carcass (range: 25-31 g; mean ± S.E.M: 28.27 ± 0.53 g; natural carcass range = 8.5-41 g). To quantify reproductive performance on each carcass size, we measured 'carcass use efficiency': this is the extent to which each species converted the carcass resource into larvae, and was calculated by dividing the total brood mass at the end of larval development (measured when larvae had dispersed from the carcass) by the size of the carcass the brood was reared on. The rationale was that beetles should exhibit greatest efficiency when breeding on the carcass size to which they are locally adapted in nature. Therefore, if there is local adaptation, *N. interruptus* and *N. investigator* should each exhibit greatest efficiency when breeding on a large carcass, while *N. vespilloides* from Gamlingay Wood should exhibit greatest efficiency when breeding on a small carcass. *N. vespilloides* from Waresley Wood should show similar levels of efficiency whether breeding on a small or large carcass.

We found that the efficiency of converting the carcass into larvae varied with carcass size, but in a different way for each *Nicrophorus* species (carcass size x *Nicrophorus* species interaction term: $\chi^2 = 28.85$, d.f. = 3, $p < 0.001$; Fig 2 and S3 Table). Specifically, *N. interruptus* had an equal efficiency when breeding on small and large carcasses ($t = 0.99$, $p = 0.325$), whereas the larger *N. investigator* exhibited a greater efficiency when breeding on large carcasses than on small carcasses ($t = 3.51$, $p < 0.001$). *N. vespilloides* from Gamlingay Wood exhibited greatest efficiency on small carcasses ($t = -4.16$ $p < 0.001$), whereas *N. vespilloides* from Waresley had similar levels of efficiency on small and large carcasses ($t = -0.27$, $p = 0.791$). Confining our analyses to reproductive efficiency on larger carcasses, we found that Gamlingay *N. vespilloides* was less efficient than both *N. interruptus* ($t = -3.92$, $p < 0.001$) and *N. investigator* ($t = -2.69$, $p = 0.038$), as well as *N. vespilloides* from Waresley ($t = -3.11$, $p = 0.011$).

Therefore, we conclude that the population of *N. vespilloides* in Waresley Wood has adaptively expanded its carrion niche to breed on larger carcasses, in response to the reduction in competition from *N. interruptus* and *N. investigator* for larger carrion.

Niche expansion by Waresley N. vespilloides is due genetic accommodation

To test whether niche expansion was facilitated by genetic accommodation, we first generated reaction norms relating carcass size to brood size for *N. vespilloides* from the two woodland populations (see Materials and Methods). We predicted that the reaction norm for *N. vespilloides* from Waresley Wood should be significantly steeper than the reaction norm for *N. vespilloides* from Gamlingay Wood, and this was indeed the case (woodland x carcass size interaction: $\chi^2 = 4.80$, d.f. = 1, $p = 0.029$; Fig 3; S4 Table). When given a larger carcass for reproduction, *N. vespilloides* from Gamlingay produced only a marginally larger brood than when breeding on a small carcass ($z = 1.82$, $p = 0.068$). By contrast, Waresley *N. vespilloides* produced significantly larger broods on larger carcasses than on smaller carcasses ($z = 5.53$, $p < 0.001$). Thus, on a large carcass, Waresley *N. vespilloides* produced more larvae than Gamlingay *N. vespilloides* ($z = -1.97$, $p = 0.049$), whereas on a small carcass the number of larvae produced did not differ between the two populations ($z = 0.76$, $p = 0.447$). Whether they bred on small or large carcasses, the variance was similar for Gamlingay and Waresley *N. vespilloides* in brood size (Bartlett's test, $p = 0.615$) and carcass use efficiency (Bartlett's test, $p = 0.943$). Females produced heavier larvae at dispersal when breeding on a large carcass than on a small carcass ($\chi^2 = 139.05$, d.f. = 1, $p < 0.001$; S4 Table), irrespective of their woodland of origin ($\chi^2 = 1.06$, d.f. = 1, $p = 0.304$; S4 Table). We could find no difference in the size of adult *N. vespilloides* trapped in Gamlingay and Waresley Woods, ($\chi^2 = 2.02$, d.f. = 1, $p = 0.156$; S3A Fig), even after controlling for sex ($\chi^2 = 1.41$, d.f. = 1, $p = 0.235$). Nor could we detect any differences in body size distribution between Gamlingay and Waresley *N. vespilloides* ($D = 0.044$, $p = 0.410$; S3B Fig). Thus, given an abundance of resources for reproduction, *N. vespilloides* allocates them to producing more offspring rather than larger offspring.

The relationship between carcass size and brood size at dispersal depends partly on the number of eggs laid, and partly on any mortality that occurs between egg-laying and the dispersal of larvae away from the carcass at the end of their development to pupate. In further analyses, we compared the reaction norms relating carcass size to clutch size for each population. Again, we found that they differed. This time the difference between reaction norms was in their intercept, rather than in their slope (Fig 4A). Female *N. vespilloides* from both woodlands laid more eggs when given a larger carcass to breed upon (carcass size effect: $\chi^2 = 10.97$, d.f. = 1, $p < 0.001$; S4 Table), but Waresley *N. vespilloides* consistently laid more eggs than Gamlingay *N. vespilloides* (woodland effect: $\chi^2 = 21.07$, d.f. = 1, $p < 0.001$; S4 Table), irrespective of carcass size (carcass size x woodland interaction: $\chi^2 = 1.44$, d.f. = 1, p

= 0.231) or female size (woodland x female size interaction: $\chi^2 = 1.30$, d.f. = 1, $p = 0.254$). Likewise, female *N. vespilloides* from both woodlands laid smaller eggs when given a larger carcass to breed upon (carcass size effect: $\chi^2 = 5.90$, d.f. = 1, $p = 0.015$; Fig 4B), but Waresley *N. vespilloides* consistently laid smaller eggs than Gamlingay *N. vespilloides* (woodland effect: $\chi^2 = 4.59$, d.f. = 1, $p = 0.032$), irrespective of carcass size (carcass size x woodland interaction: $\chi^2 = 0.33$, d.f. = 1, $p = 0.565$). Mean egg volume per clutch did not predict mean larval mass per brood at dispersal ($\chi^2 = 0.28$, d.f. = 1, $p = 0.595$), regardless of the population of origin. This suggests that any under-provisioning of eggs is compensated by the over-abundance of resources available on the carcass after hatching [31,32]. The total volume of all eggs in a clutch did not differ between populations ($\chi^2 = 2.16$, d.f. = 1, $p = 0.141$; S4 Table) or carcass size treatments ($\chi^2 = 2.38$, d.f. = 1, $p = 0.123$; S4 Table).

We conclude that the reaction norms relating carcass size to brood size differ in slope between the two woodlands (Fig 3), partly because Waresley females routinely lay more eggs when they breed (Fig 4A), and partly because Waresley broods on smaller carcasses exhibit greater mortality between egg-laying and larvae dispersal. Niche expansion by Waresley *N. vespilloides* is due to genetic accommodation of the reaction norm linking carrion size and clutch size.

Divergence at loci associated with oogenesis in N. vespilloides from Gamlingay v. Waresley Woods

To test whether these differences between woodlands really are due to evolutionary change, and hence genetic change, we generated low-coverage whole genome sequences for 40 individuals collected from each wood ($n = 80$ chromosomes per population). Population genetic summary statistics were similar for both populations (mean of 2 kb windows – Gamlingay Pi = 0.0055 ± 1.02e-5, Tajima's D = -0.70 ± 0.002; Waresley Pi = 0.0056 ± 1.0e-5, Tajima's D = -0.67 ± 0.002). Consistent with previous microsatellite analyses [16], we found little to no genetic differentiation between populations from Gamlingay and Waresley Woods (unweighted *Fst* = 0.0069; weighted *Fst* = 0.013), strongly suggesting there is ongoing gene flow between the two populations. There was minimal divergence across the genome with no instances of extreme outliers (S4 Fig). That no loci were highly diverged between the two populations indicates that the divergent traits are likely controlled by many loci, as is typical for behavioural and life history traits. It is consistent with the predicted quantitative genetic basis of genetic accommodation [6]. The highest *Fst*-windows in the genome showed only modest absolute values of divergence. Nevertheless, they were extreme outliers due to the otherwise consistently low pattern of *Fst* between populations. For

example, the top window of divergence between the two populations fell in *transmembrane protein 214* (*Fst* = 0.11, *zFst* = 19.2, *p* = 7.2e-82). This gene is highly expressed in the ovaries in *Drosophila melanogaster*, suggesting a potential candidate gene that may influence differences in *N. vespilloides* egg laying behaviour between Gamlingay and Waresley Woods.

Next, we investigated whether the divergent *Fst* scores could be attributed to shifts in allele frequency in *N. vespilloides* from Waresley Wood, Gamlingay Wood, or both woodland populations. Greater differentiation attributable to Waresley compared to Gamlingay is the pattern we would expect if the Waresley *N. vespilloides* is the derived population. By comparing differentiation between both woodlands and a third population, we can polarise the divergence between populations. For these analyses, we generated low-coverage whole genome sequences from 22 individuals trapped in woods near Swansea, Wales, UK, approximately 300 km away from Gamlingay and Waresley Woods. We calculated all pairwise *Fst* and assigned the relative divergence of each population using population branch statistics (PBS) to understand which population was driving divergence across the genome [33,34]. Not surprisingly, the distant Welsh population showed the highest genome-wide PBS. Interestingly, the population from Waresley Wood showed higher differentiation compared to the population from Gamlingay Wood (mean PBS: Waresley = 0.0074, Gamlingay = 0.0056, Wales = 0.0109). These analyses therefore support the ecological data in indicating that the Gamlingay *N. vespilloides* represents the ancestral condition, whereas the Waresley *N. vespilloides* are the derived population (S5 Fig).

To visualise the relative divergence between populations across the genome, we generated a scatterplot of the PBS values for 2kb non-overlapping windows for each population (Fig 5A). The analysis highlighted multiple potential candidate genes associated with the differences in egg-laying behaviour, again consistent with this trait being controlled by many loci of small effect. These genes showed elevated PBS in *N. vespilloides* from Waresley Wood (PBS > 0.05), but not Gamlingay Wood, and they are linked to arthropod oogenesis. For example, homologs of three of the highly differentiated genes in the Waresley Wood population – *obg-like ATPase*, *transmembrane protein 214*, and *liprin-alpha* – show elevated expression in the ovaries of fruit flies [35]. FlyAtlas: survey of adult and larval expression. http://www.flyatlas.org/) and other arthropods [36], suggesting a plausible role in regulating egg production. Another gene, *kekkon1*, is a transmembrane protein known to regulate the activity of the epidermal growth factor receptor (EGFR) during oogenesis in *Drosophila* [37,38]. A different portion of *kek1* is moderately differentiated in *N. vespilloides* from Gamlingay Wood (Fig 5A), highlighting that differentiation related to oogenesis is not

limited to *N. vespilloides* from Waresley Wood. One of the stronger signals of differentiation in Waresley Wood that may contribute to the phenotypic differences observed between populations is found in a serotonin receptor (Fig 5B). Serotonin has been linked to reproduction via effects on the production of ecdysteroids such as juvenile hormone in multiple insects [39–41]. Serotonin has also been related to the intensity of aggressive behaviour in contests across diverse insect groups [42–45].

Finally, we asked whether genes involved in oogenesis generally showed elevated levels of differentiation in each population, in comparison with the rest of the genome. For each population, we ranked genes by the highest PBS score in 500bp windows overlapping with the gene-body and conducted a gene set enrichment analysis for each population. N. *vespilloides* from both Waresley and Gamlingay Woods showed enrichment in multiple GO terms associated with ovaries and oogenesis (Table 1). Consistent with the outlier analyses, oogenesis-related GO-terms tended to have higher enrichment scores in *N. vespilloides* from Waresley Wood compared to Gamlingay Wood. The gene enrichment analysis also revealed local divergence between beetles from Waresley and Gamlingay Woods at genes associated with other traits, including learning and memory and sensory systems (S1 Data).

Conclusions

Our analyses support three of the criteria required to show evidence for plasticity-first evolution [6]. We show that the ancestral reaction norm linking carrion size to clutch size has evolved to have a greater intercept in *N. vespilloides* from Waresley Wood. This change has enabled Waresley N. *vespilloides* beetles to expand their carrion niche in the absence of rival congenerics. It is adaptive because Waresley *N. vespilloides* are now more effective than Gamlingay *N. vespilloides* at converting carrion that ranges widely in size into offspring.

Previous analyses of plasticity-first evolution in natural populations have emphasised how new adaptations can evolve from the genetic assimilation of plastic traits, and are caused by the loss of plasticity [6–8,46]. In these studies, there is little evidence of ongoing gene flow between diverging populations [7,46]. The new adaptations presumably arise from a loss-of-function mutation(s), that makes trait expression independent of wider environmental conditions.

Fewer studies have explicitly shown how new adaptations can also evolve from the genetic accommodation of previously plastic traits. Here, adaptation involves re-tuning the reaction norm to change the extent of plasticity that is induced by an environmental cue (Bock et al. (2018) [15] and this study, but see also [47,48]). The candidate loci for evolutionary change are thus likely to function in gene regulation. Our data suggest that the

candidate loci that have re-tuned the fecundity reaction norm in *N. vespilloides* include genes associated with the regulation of oogenesis. In both Bock et al. (2018) and this study, plasticity-first evolution through genetic accommodation was possible despite gene flow between the diverging populations. Ultra-local adaptations, between populations that are separated by just a few kilometres and that exhibit ongoing gene flow, have now been reported from diverse contexts (e.g. [14]). We suggest that these types of adaptation are likely to have evolved by genetic accommodation as well.

The emerging empirical evidence therefore suggests that plasticity-first evolution could frequently be the source of diverse new adaptations in natural populations. Where gene flow is minimal, new adaptations have been shown to arise through a loss of plasticity. By contrast, while gene flow continues, our study suggests that new adaptations are more likely to arise through changes in the extent of plasticity, just as theory predicts. Through this mechanism, organisms can rapidly and specifically adapt to very fine-scale differences in their local environment.

Materials and Methods

Competition for carrion in Gamlingay and Waresley Woods

Burying beetle trapping

Surveys of burying beetle species in Gamlingay and Waresley Woods were conducted from June to October in 2014-2017. Five carrion-baited soil-filled traps were placed in each site to attract *Nicrophorus* beetles, with traps set at least 150 m apart from each other and at the same location each year (S1A Fig). Every 2-3 weeks, we collected all the *Nicrophorus* spp. within each trap, and rebaited the trap with fresh compost and a dead mouse. In the lab, we identified each burying beetle to species-level, and measured the pronotum width (to the nearest 0.01 mm) as an index of body size (see below).

We caught five species in total (in increasing order of size): *N. vespilloides*, *N. interruptus*, *N. vespillo, N. investigator* and *N. humator*. However, *N. vespillo* were only caught 5 and 3 times within the four years in Gamlingay and Waresley respectively, indicating that there is no stable population of *N. vespillo* in either wood.

After removing any phoretic mites, beetles were retained and kept individually in plastic boxes (12cm x 8cm x 2cm) filled with moist soil in a laboratory kept at 20°C and on a 16:8 light to dark cycle. Beetles were fed twice a week with minced beef. We kept all field-caught individuals for at least two weeks before breeding to ensure that they were sexually mature and to reduce any variation in nutritional status. We then maintained stock

409 populations of both Gamlingay and Waresley Woods by breeding pairs of unrelated
410 individuals on 8-16 g mice carcasses.
411 For the genomic analyses, we analysed *N. vespilloides* from three ancient woodlands
412 near Swansea in Wales, trapped by Dr Chris Cunningham in 2017. The three sites in Wales
413 (Park Wood (Latitude: 51.57258°; Longitude: −4.03096°); Clyne Valley Wood (Latitude:
414 51.61262°; Longitude: −4.02293°); and Caswell Bay Wood (Latitude: 51.57258°; Longitude:
415 −4.03096°)) are approximately 300 km away from our two study sites in Cambridgeshire.
416
417 ***Size distributions of the Nicrophorus spp.***
418 Body size was measured for *Nicrophorus* spp. collected from 2014 to 2017 (except in 2016).
419 In total, 838 *N. vespilloides*, 41 *N. humator*, 127 *N. interruptus*, 54 *N. investigator*, and 5 *N.*
420 *vespillo* were measured for Gamlingay Wood, whereas 824 *N. vespilloides*, 51 *N. humator*,
421 25 *N. interruptus*, 4 *N.* investigator, and 6 *N. vespillo* were measured for Waresley Wood.
422 Mean body size of *Nicrophorus* spp. significantly varied among species (GLMM: χ^2 =
423 2069.44, d.f. = 4, $p < 0.001$; S2 Table). Post-hoc comparisons revealed that *N. vespilloides*
424 was the smallest species compared to other *Nicrophorus* spp. (S1 and S2 Tables).
425 Kolmogorov-Smirnov test comparing pairwise differences in *Nicrophorus* spp. body size
426 frequency distribution also revealed similar patterns found in differences of mean body size
427 (Fig 1 and S5 Table).
428
429 ***Mark-recapture experiment***
430 In 2014, we investigated the interconnectivity of populations of burying beetles between
431 Gamlingay and Waresley Woods using a mark-recapture survey. In total, 98 *N. vespilloides*,
432 9 *N. humator*, 17 *N. interruptus*, 9 *N. investigator,* and 2 *N. vespillo* were marked for
433 Gamlingay Wood, whereas 113 *N. vespilloides*, 5 *N. humator*, 1 *N. interruptus*, 1 *N.*
434 *investigator* were marked for Waresley Wood. Beetles were marked with a numbered plastic
435 bee tag on either the right or left elytra for those found in Gamlingay or Waresley Woods
436 respectively. To identify any previously caught beetles that lost their tags, we permanently
437 marked them by cutting a small portion of the elytra (less than 2%). All marked beetles from
438 each wood were released from a designated location at the geographic midpoint with the
439 minimum total distance to all trapping sites for Gamlingay (Latitude: 52.16294°; Longitude:
440 −0.18984°) and Waresley Woods (Latitude: 52.176508°; Longitude: −0.156776°). We found
441 no evidence of migration between the two sites. We recaught 8 of 98 marked *N. vespilloides*
442 from Gamlingay Wood in Gamlingay Wood and 8 out of 113 marked *N. vespilloides* from

Waresley Wood in Waresley Wood. None of the other marked *Nicrophorus* spp. was recaptured in either wood.

Small mammal trapping

To assess the rodent carrion available for *Nicrophorus* spp. reproduction, we sampled the small mammal communities in the two woodlands. In general, rodent populations peak in the autumn, because breeding for the year has just ceased and there has yet to be any winter-induced mortality [49]. Sampling at this time is therefore ideal for detecting which species are present and for determining their relative abundance. We placed Longworth traps in both woodlands in November 2016. Traps were baited with oats and blowfly maggots (with hay provided as bedding) and set in pairs within 20 m of each original beetle trapping site (S1A Fig), with 10 traps set per wood. We continuously trapped rodents for three days, generating 50 trap sessions per woodland. Traps were checked daily at approximately 0830 and 1500 (generating a total of 30 trap sessions overnight and 20 trap sessions in daylight hours). Trapped mammals were identified, weighed, sexed, marked by a fur clip on either the right or left rear flank, and released *in situ*. Any recaptured mammal was recorded in subsequent censuses. All traps were reset and rebaited immediately after checking.

Division of the carrion niche by Nicrophorus beetles in Gamlingay and Waresley Woods

During the field season from 2017 to 2018, pairs of wild-caught *N. vespilloides*, *N. interruptus*, and *N. investigator* were bred on either small (12-20 g; 16.80 ± 0.55 g) or large carcasses (25-31 g; 28.27 ± 0.53 g) within a breeding box (17 x 12 x 6 cm) filled with 2 cm of moist soil. All field-caught beetles were kept for two weeks in the laboratory and fed twice a week prior to breeding. In total, we established eight treatments: large (n = 47) and small (n = 48) carcasses for Gamlingay *N. vespilloides*; large (n = 42) and small (n = 33) carcasses for Waresley *N. vespilloides*; large (n = 25) and small (n = 18) carcasses for *N. interruptus*; large (n = 9) and small (n = 13) carcasses for *N. investigator*. *N. interruptus* and *N. investigator* were both drawn from Gamlingay Wood as the populations of these species in Waresley were too small to be used experimentally.

Approximately eight days after parents were given a carcass to breed on, larvae switch from aggregating on the carcass to dispersing away into the soil to pupate. When one or more larvae from each brood switched their behaviour we scored the whole brood as having reached the dispersal stage. At this point, all larvae were counted and total brood mass was weighed to the nearest 0.001 g. We also calculated average larval mass for each brood by dividing total brood mass by number of larvae.

478

479 **Niche expansion in *N. vespilloides* by genetic accommodation**

480 ***The reaction norm relating carcass size to brood size in each woodland***

481 This experiment was conducted in the laboratory, over two blocks in 2017, using the second
482 and third descendant generations of field-caught beetles from Gamlingay and Waresley
483 Woods. By rearing beetles from both woodlands in the lab in a common garden environment
484 for at least one generation prior to testing, we minimized any residual environmental effects
485 when quantifying the reaction norm for each population. To pair beetles for reproduction, we
486 began by haphazardly casting broods into dyads when larvae had matured into adults. Within
487 each dyad, we haphazardly chose four males from one brood, and paired them with four
488 haphazardly chosen females from the second brood. Two of these pairs were then given a
489 small mouse to breed upon (12-17 g; 15.03 ± 0.67 g), while the remaining two pairs were
490 given a large mouse to breed upon (26-31 g; 28.86 ± 0.67 g). By using sibships to generate
491 pairs in this way, we were able to compare how very similar genotypes responded to the
492 opportunity to breed on either a small or large mouse. Each pair, and their mouse, was housed
493 in a clear plastic box (17 x 12 x 6 cm), with 2 cm depth of Miracle-Gro compost. The box
494 was placed in a dark cupboard for eight days after pairing the beetles, at which point larvae
495 started to disperse away from the carcass. We measured the number of larvae present at
496 dispersal and weighed the whole brood.

497

498 ***The reaction norm relating carcass size to clutch size in each woodland***

499 In the second block of the experiment, we also measured clutch size. Fifty-six hours after we
500 introduced the beetles to the carcass, we photographed the base of each transparent breeding
501 box. Using Digimizer ver. 5.1.0, we then counted the number of visible eggs, and also
502 measured the length and width of all eggs that were able to be measured accurately (i.e. those
503 that were fully visible and lying flat on the base of the box). All counting and measuring of
504 eggs was performed blind to the carcass size treatment and the population from whence the
505 breeding beetles came. Egg volume was then calculated using the formula V=1/6 *π*w2**L,
506 which assumes eggs to be a prolate spheroid (following [50]).

507 In total, 2518 eggs were counted across 132 breeding boxes, of which 1633 could be
508 measured. No eggs could be seen in four of these boxes, and these were excluded from
509 further analysis (hence $n = 128$). Both the total number of eggs counted (observed clutch
510 size) and the number of eggs which could accurately be measured correlated positively with
511 brood size (number of eggs counted: $\chi^2 = 27.18$, d.f. = 1, $p < 0.001$; number of eggs

512 measured: $\chi^2 = 24.26$, d.f. = 1, $p < 0.001$), indicating these counts capture biologically
513 meaningful information.
514
515 **Divergence at loci associated with oogenesis in *N. vespilloides* from Gamlingay *v.***
516 **Waresley Woods**
517 We generated low-coverage whole genome sequences for three populations of *N. vespilloides*
518 from Waresely Wood, Gamlingay Wood and Swansea, Wales, UK. DNA was individually
519 extracted from beetle heads using the DNeasy Blood and Tissue kit (Qiagen) and
520 subsequently quantified and quality checked using NanoDrop and Qubit. DNA was then
521 shipped to Cornell University, where libraries were prepared using partial reactions of a
522 Nexterra kit by the Cornell Genomics Core. Libraries were subsequently sequenced by
523 Novogene (Davis, CA, USA) at an average depth of approximately 5x coverage. Paired-end
524 550 bp insert libraries were prepared for each sample with the Nextera library preparation kit.
525 Libraries were sequenced using the Illumina HiSeq (Novogene, Davis, CA) at an average
526 coverage of 3.4X. Trimmomatic (v0.36) was used to removed adaptors and poor-quality
527 sequence. Trimmed reads were mapped to the *N. vespilloides* reference genome using the
528 Burrows-Wheeler Aligner (v0.7.13) [51]. SNPs were identified using Picard (v2.8.2) and
529 GATK (v3.6) HaplotypeCaller following best practice recommendations [52]. After
530 alignment, SNPs were hard filtered using the parameters: QD < 2.0 || SOR > 3.0 || FS > 200.
531 We used VCFtools to calculate population genetic statistics for each population. To examine
532 population structure, we generated a thinned VCF file with one SNP per 5kb and used Tassel
533 (version 5) [53] to calculate generate an MDS plot. For calculation of *Fst* and PBS values,
534 aligned bam files were analysed in ANGSD (v 0.911) [54], which is specifically designed for
535 analysis of low-coverage genome sequencing data. Data for this project is available at the
536 NCBI Sequence Read Archive under Bioproject PRJNA530213.
537 Genes were assigned gene ontology (GO) terms using the BLAST2GO workflow
538 (v5.1.1). In brief, gene identity was determined based upon BLAST searches to the
539 Arthropod or Drosophila non-redundant protein databases and protein domains were
540 identified based on matches to Interpro database. GO terms were assigned to each gene
541 model based upon mapping results. GO terms were filtered with the "Filter Annotation by
542 GO Taxa" option to remove GO terms that are incompatible for Arthropods.
543
544 **Statistical analyses**

All statistical analyses were performed in R version 3.4.3 (R Development Core Team), with NMDS in the package *vegan*, GLM and GLMM in the package *lme4* [55], and Tukey's HSD post hoc comparisons in the package *lsmeans* [56].

Competition for carrion in Gamlingay and Waresley Woods: field data

To test for a difference in the *Nicrophorus* guild between Gamlingay and Waresley Woods, a frequency table of beetle communities was analysed using permutational multivariate analysis of variance (PERMANOVA) on two-dimensional nonmetric multidimensional scaling (NMDS) based on Bray-Curtis distances [57], with the 'adonis' function (vegan package). We tested the effect of study site on the composition of the beetle community, using sampling year as strata. The analysis was based on 10000 permutations of the data. We visualized the difference of beetle community between Gamlingay and Waresley population in two dimensions of a NMDS plot. NMDS two-dimensional stress values (a measure of goodness of fit) were below 0.1 (0.085), indicating the ordination provides a good fit to the data [58].

We used general linear mixed models (GLMM) with a Poisson error structure to test for differences across beetle species and sites on average abundance for each species per trap as an independent variable and year as a random factor. Beetle species and population (Gamlingay/Waresley) were included as fixed effects, whereas trap ID and sampling year were included as random factors.

We assessed the significant difference in body size frequency distribution between Gamlingay and Waresley *N. vespilloides* and among *Nicrophorus* spp. using the Kolmogorov-Smirnov (K-S) two-sample test, which compares if the cumulative distributions of two data sets are derived from the identical distribution. We also tested for significant differences in mean body size between *N. vespilloides* in Gamlingay and Waresley, using a GLMM that included population (Gamlingay/Waresley) and sex (male/female) as fixed effects, and sampling year as a random factor. Differences of mean body size among *Nicrophorus* spp. was assessed in a GLMM that included species and sex as fixed effects, and sampling year as a random factor.

We assessed the significant difference in mean body mass of rodents in Gamlingay and Waresley using a GLM by including rodent species and population (Gamlingay/Waresley) as fixed effects.

Division of the carrion niche by Nicrophorus beetles in Gamlingay and Waresley Woods

To test for significant differences in reproductive performance between species, we conducted a GLMM regression to analyse differences in efficiency (total brood mass divided by carcass mass), which was logit transformed prior to analysis. Beetle species, carcass size (small/large), and their interaction were included as explanatory variables. Sampling year was included as a random factor. In this analysis, we included beetle species as *N. interruptus*, *N. investigator*, Gamlingay *N. vespilloides* and Waresley *N. vespilloides* to fully compare differences not only between *Nicrophorus* beetle species, but also *N. vespilloides* between populations.

Niche expansion in N. vespilloides by genetic accommodation

For the reaction norm experiment, we used GLMMs to test the interacting effect of population and carcass size on brood size and average larval mass, with dyad identity nested within block and included as a random factor. In all models, brood size and average larval mass were analysed with a Poisson and Gaussian error distribution, respectively. A similar statistical approach was used for analyses of clutch traits to test for the significant differences on clutch size and average egg volume in GLMMs with a Poisson and Gaussian error distribution, respectively. The effect of population of origin, carcass size, and their interaction were included as fixed effects, whereas dyad identity was included as a random factor If a significant interaction was found, a Tukey's post-hoc test was performed to detect significant effects using multiple pairwise comparisons.

Acknowledgements

We thank P. Pilbeam for carrying out small mammal trapping in the field. We thank A. Attisano, C. Swannack, and R. Mashoodh for help with beetle trapping. S.-J.S. was supported by the Taiwan Cambridge Scholarship from the Cambridge Commonwealth, European & International Trust. A.M.C. was supported by a studentship from the National Environmental Research Council's Earth System Sciences Doctoral Training Partnership at Cambridge University and a Victoria Brahm Schild Internship Grant from Homerton College. R.M.K. was supported by a European Research Council Consolidator's grant 301785 BALDWINIAN_BEETLES and a Wolfson Merit Award from the Royal Society.

612

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764 **Figures**

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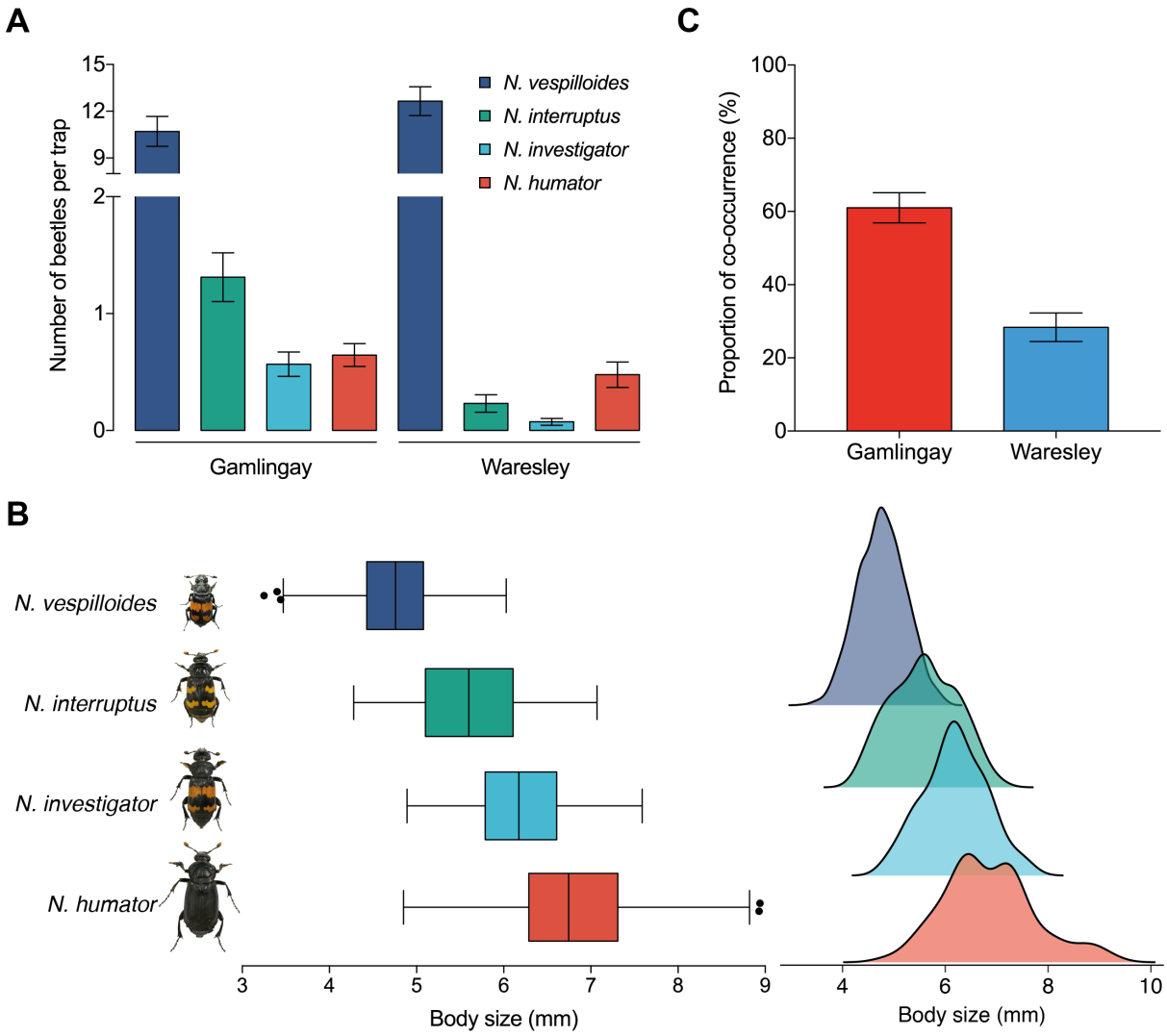

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769 **Figure 1.** (A) Number of *Nicrophorus* beetles caught per trap from 2014-2017. (B) Body size
770 diversity and frequency distribution of field-caught *Nicrophorus* spp. as illustrated in
771 box-and-whisker plots and kernel density estimation. Outliers are depicted as points. (C) The
772 proportion of traps set at which *N. vespilloides* was trapped with another *Nicrophorus*
773 species, in the two woodlands. Error bars indicate mean ± S.E.M.

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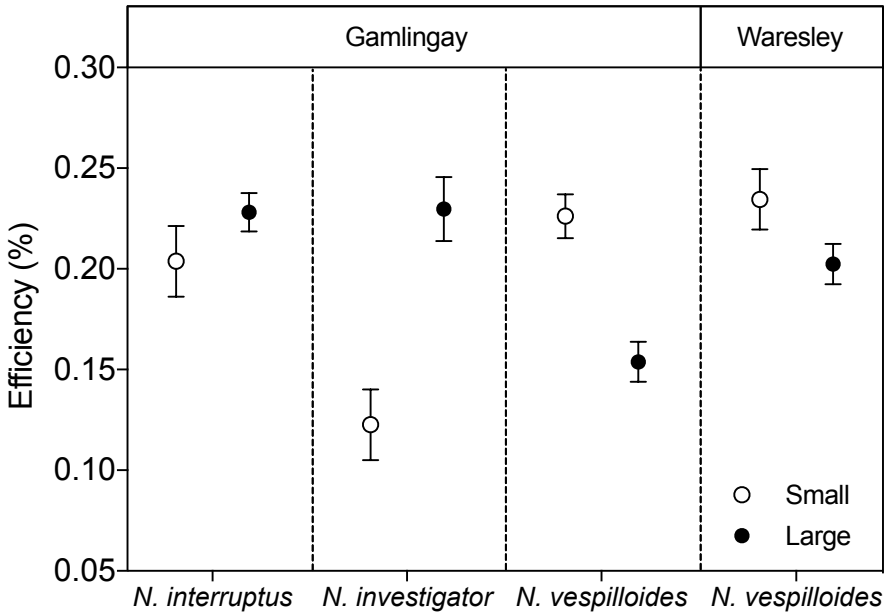

Figure 2. Efficiency (%) of carcass use (total brood mass divided by carcass mass) of *N. interruptus*, *N. investigator*, and *N. vespilloides* from Gamlingay Wood and *N. vespilloides* from Waresley Wood.

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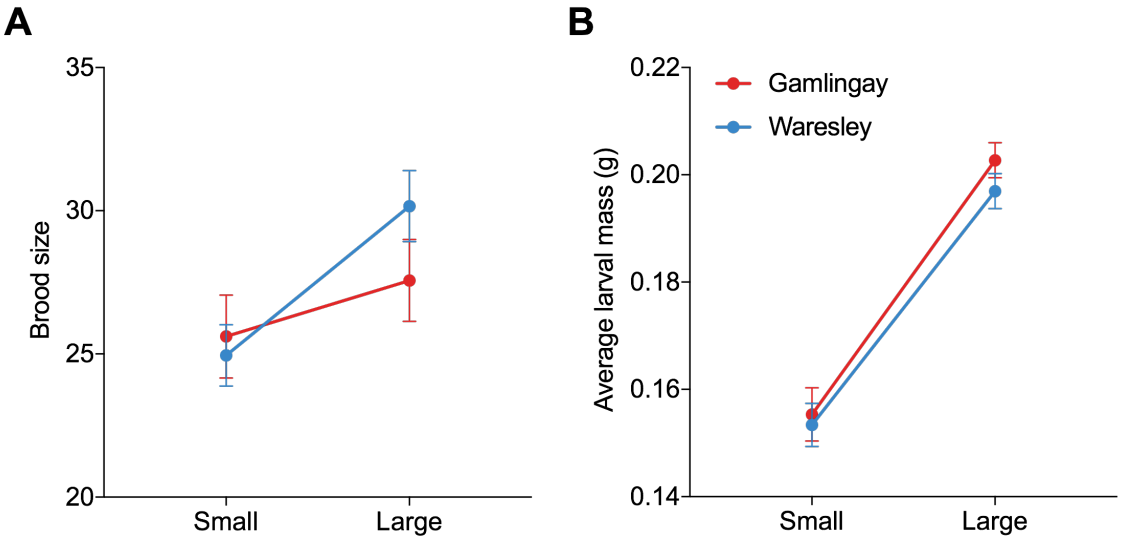

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785 **Figure 3.** The effect of carcass size on the brood size (A) and average larval mass (B) of *N.*
786 *vespilloides* in the reaction norm experiment (n = 46 and 46 for small and large carcasses in
787 Gamlingay Wood, and n = 62 and 62 for small and large carcasses in Waresley Wood).
788 Values represent the mean ± S.E.M.
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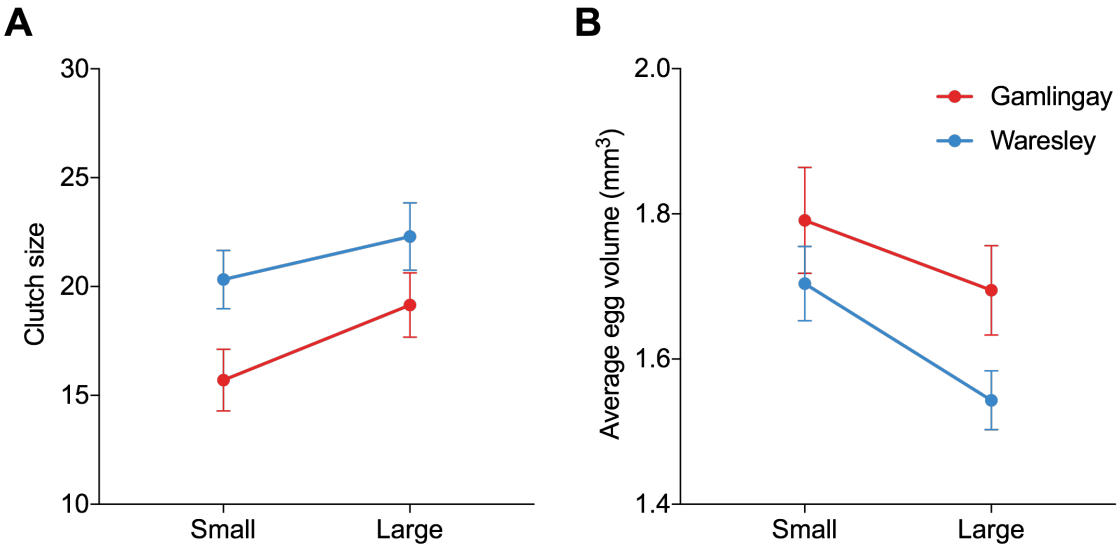

Figure 4. The effect of carcass size on the (A) clutch size and (B) average egg volume of *N. vespilloides*. $n = 27$ for small and large carcasses in Gamlingay Wood, and $n = 37$ for small and large carcasses in Waresley Wood. Values represent the mean ± S.E.M.

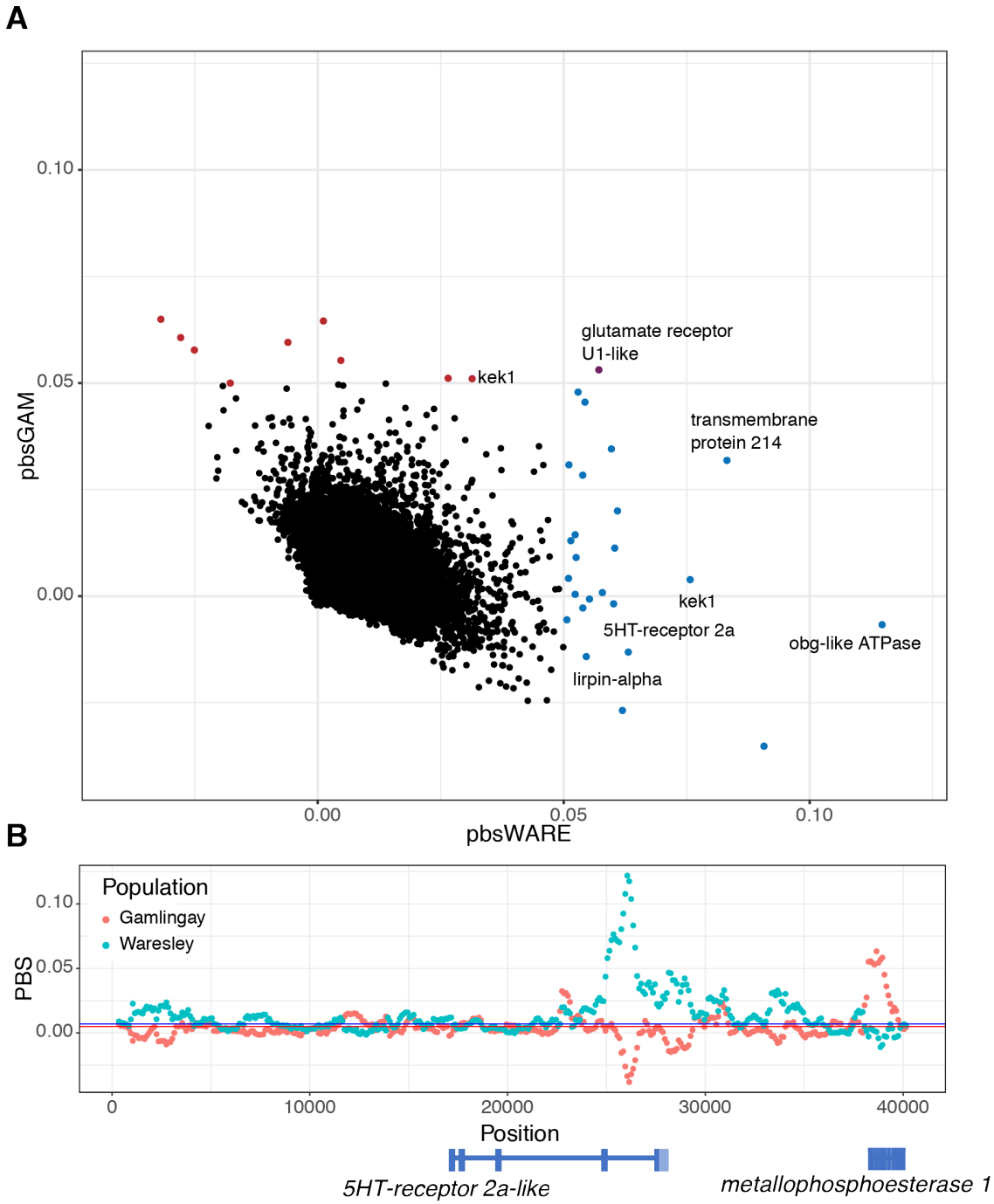

Figure 5. Differentiation at putative oogenesis genes. (A) A scatterplot of PBS values for Waresely and Gamlingay in 2kb windows genome-wide. Loci in the lower right hand of the graph show high differentiation in Waresely but Gamlingay. Loci with PBS scores greater than 0.05 are highlight – Waresley = blue, Gamlingay = red, Both = purple. Notable genes are highlighted. (B) Sliding window analysis (window = 500bp, slide = 100bp) of PBS values at the *5HT receptor 2a-like* receptor. The peak PBS in Waresley (blue) falls in the last intron of the gene.

Table 1. Population differences in enrichment scores in multiple GO terms associated with ovaries and oogenesis.

			Waresley		Gamling		Wales	
GO ID	**GO Name**	**Size**	**NES**	**FDR q-val**	**NES**	**FDR q-val**	**NES**	**FDR q-val**
GO:0030707	ovarian follicle cell development	345	3.36	0.00E+00	3.27	1.2E-05	2.52	1.35E-03
GO:0007297	ovarian follicle cell migration	129	3.32	0.00E+00	2.86	1.0E-04	3.09	4.10E-05
GO:1905879	regulation of oogenesis	66	2.22	7.31E-03	2.07	1.4E-02		
GO:0048599	oocyte development	165	2.16	9.70E-03	1.83	4.1E-02		
GO:0007304	chorion-containing eggshell formation	78	2.12	1.20E-02	2.41	2.0E-03		
GO:0007308	oocyte construction	157	2.07	1.55E-02	1.83	4.2E-02		
GO:0030703	eggshell formation	79	2.04	1.80E-02	2.50	1.3E-03		
GO:1905881	positive regulation of oogenesis	37	2.04	1.83E-02				
GO:0009994	oocyte differentiation	194	2.01	2.05E-02	2.25	5.1E-03		
GO:0007309	oocyte axis specification	145	1.90	3.44E-02	1.85	3.9E-02		
GO:0030728	ovulation	11	1.83	4.58E-02				
GO:0007306	eggshell chorion assembly	66			2.04	1.6E-02		
GO:0060281	regulation of oocyte development	29			1.79	4.9E-02		

812 **Supporting information**

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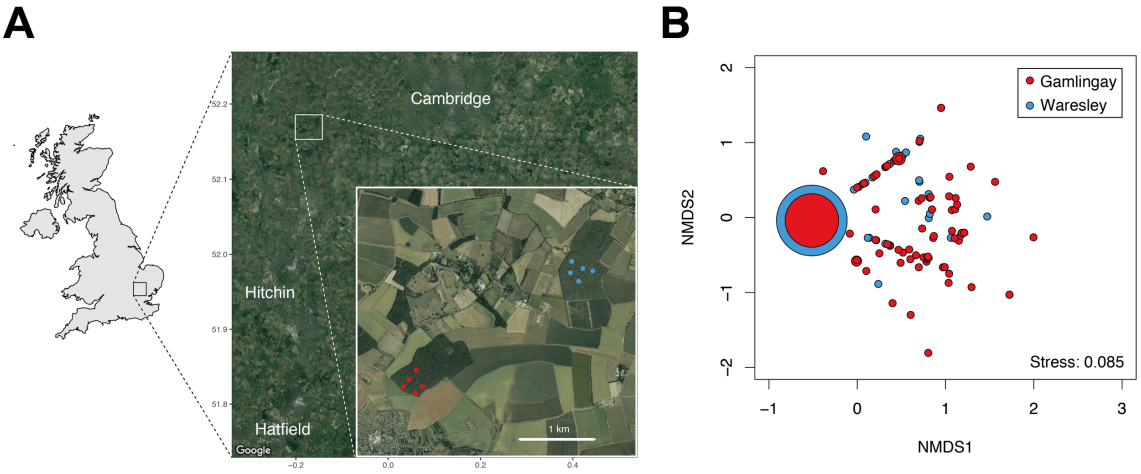

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815 **S1 Fig. Study sites and population differences in community structure.** (A) Location of
816 traps in Gamlingay Wood (red points) and Waresley Wood (blue points). (B) NMDS
817 ordination of Gamlingay (n = 141) and Waresley (n = 134) community structure. Each point
818 represents the sum of the beetle community collected in each trap per sampling time.

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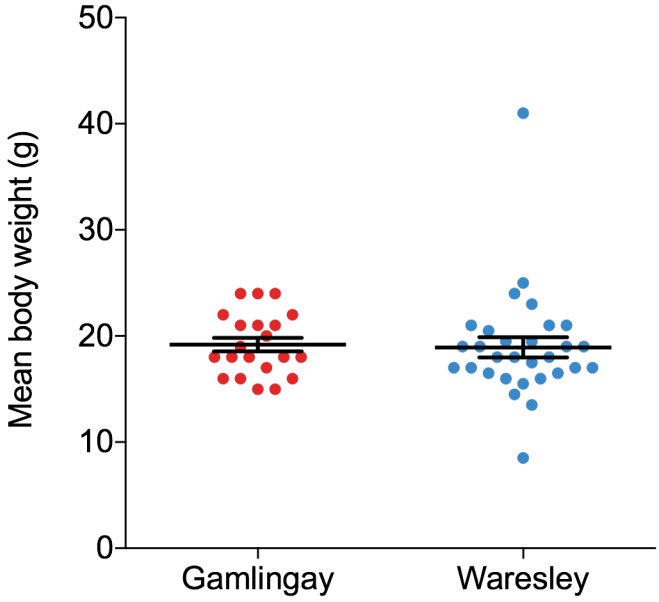

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822 **S2 Fig. Mean body weight of small mammals.** Gamlingay (*n* = 21, red) and Waresley
823 Woods (*n* = 30, blue). One bank vole and one wood mouse, each caught in in Gamlingay
824 Wood, escaped before they could be measured.

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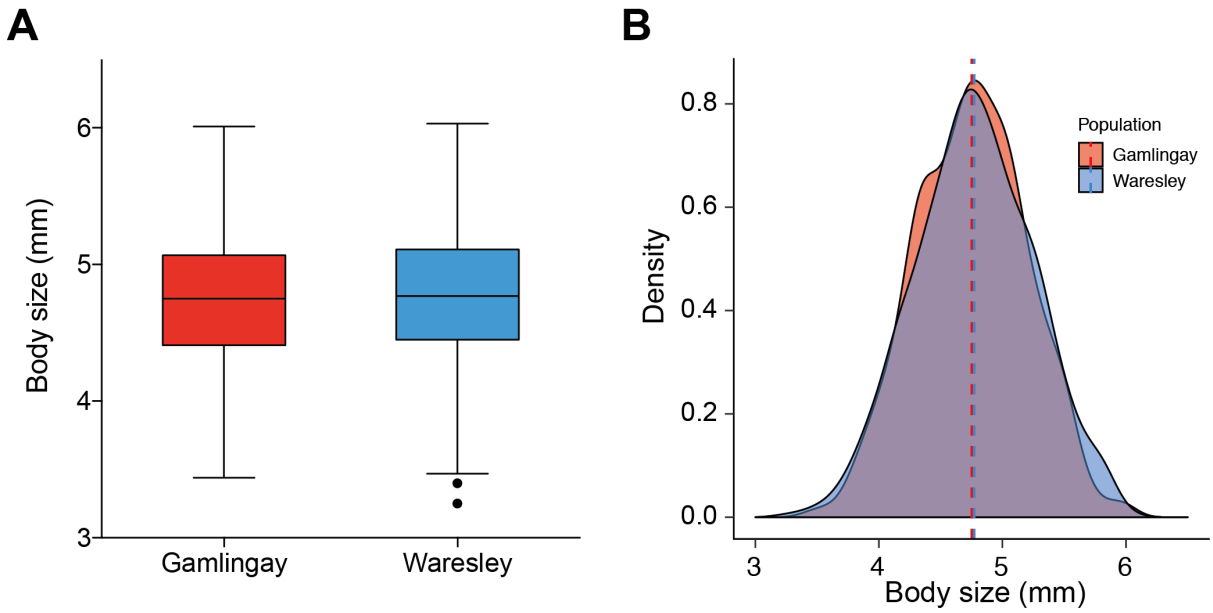

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830 **S3 Fig. Differences in body size (A) and frequency distribution (B) of field-caught *N.***
831 ***vespilloides* from Gamlingay and Waresley Woods.** Median values, inter-quartile range,
832 maximum, and minimum are as illustrated in box-and-whisker plots. Outliers are depicted as
833 points. Pronotum width was measured as an indicator of beetle body size of Gamlingay (n =
834 839) and Waresley Woods (n = 824).

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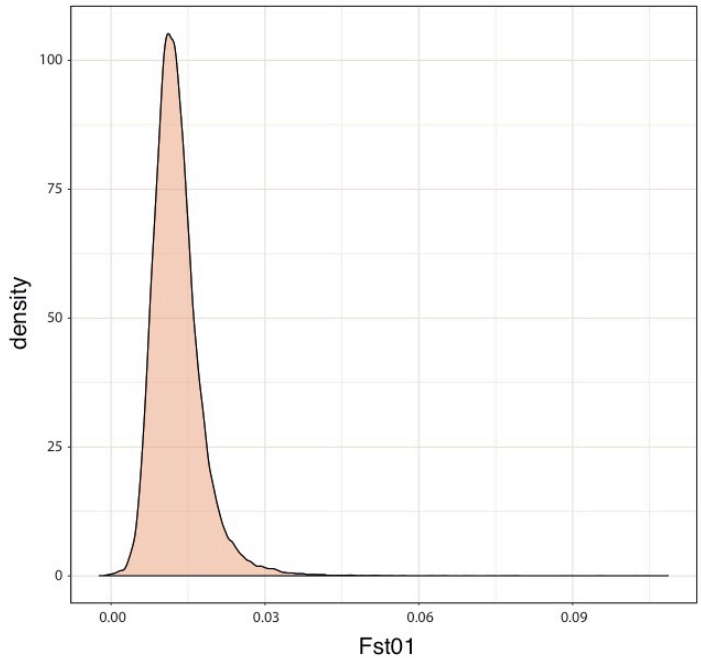
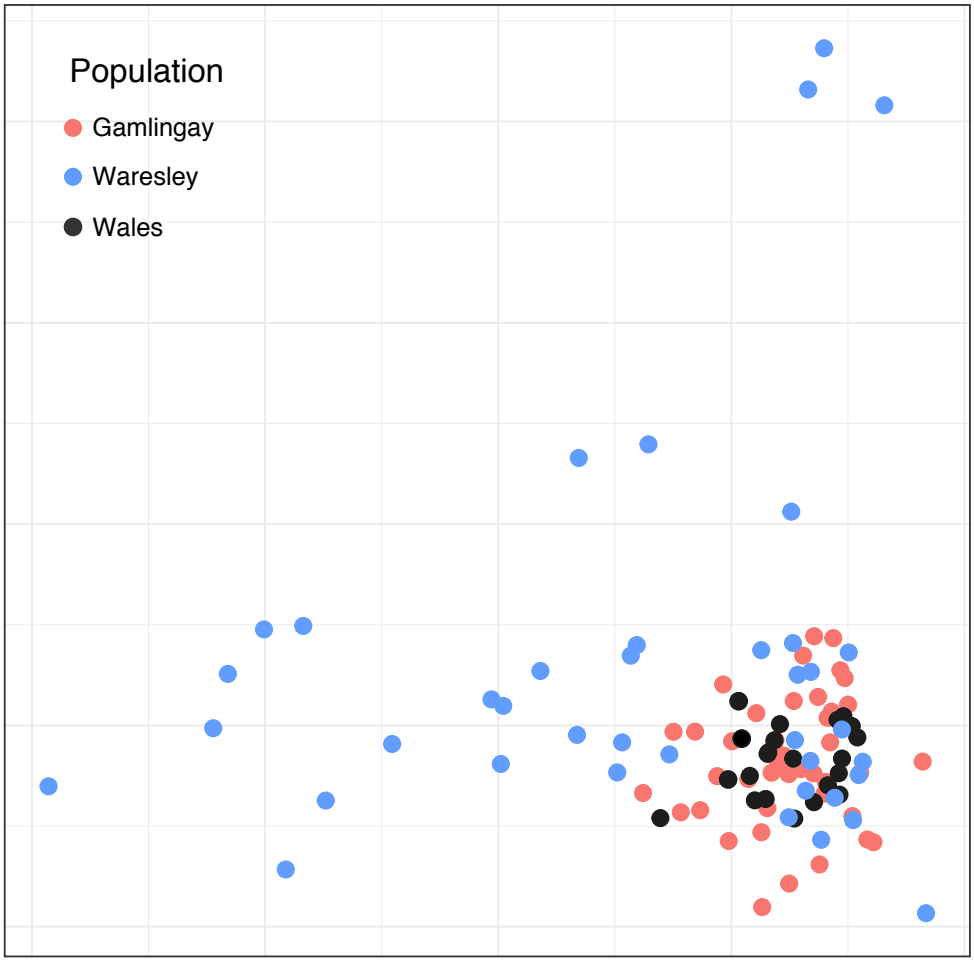


838

839 **S4 Fig. Density plot of *Fst* value between Gamlingay and Waresley Woods.** The plot
840 shows the narrow distribution of low *Fst* values between the two woodland populations.
841 Though there is a long tail to the right, the extreme values are modest in absolute terms.

842



S5 Fig. MDS plot of three burying beetle populations. The plot shows the first two dimensions of a multidimensional scaling analysis of genetic diversity among burying beetles in three populations. The populations do not separate out, indicating little to no genetic structure.

S1 Table. Quantifying *Nicrophorus* spp. body size (mm).

Species	*N*	Mean	SD	Minimum	Q1	Median	Q3	Maximum
N. vespilloides	1662	4.76	0.46	3.25	4.43	4.76	5.08	6.03
N. interruptus	152	5.59	0.64	4.28	5.10	5.60	6.10	7.07
N. investigator	58	6.19	0.61	4.89	5.80	6.18	6.58	7.59
N. humator	92	6.86	0.89	4.85	6.30	6.75	7.31	9.25
N. vespillo	11	5.74	0.59	4.86	5.44	5.76	5.98	6.93

Number of *Nicrophorus* spp. (*N*); Standard Deviation (SD); 25% Quantile (Q1); 75% Quantile (Q3).

S2 Table. Post-hoc Tukey HSD comparisons for differences in mean body size between *Nicrophorus* spp.

Species	***N. vespilloides***		***N. interruptus***		***N. investigator***		***N. humator***	
	t	*p*	*t*	*p*	*t*	*p*	*t*	*p*
N. interruptus	18.69	**<0.001**	-	-	-	-	-	-
N. investigator	20.62	**<0.001**	-7.61	**<0.001**	-	-	-	-
N. humator	38.15	**<0.001**	18.54	**<0.001**	7.69	**<0.001**	-	-
N. vespillo	6.31	**<0.001**	-0.93	0.268	2.68	0.058	6.85	**<0.001**

p value in bold indicate comparisons with significant difference, and dashes indicate comparisons that were not made.

866

867 **S3 Table. Results of the ANOVAs for division of carrion niche by *Nicrophorus* spp.**

Dependent variable	Explanatory variables	χ^2	d.f.	*p* value
Efficiency (%)	Carcass size	17.26	1	**<0.001**
	Beetle species	20.42	3	**<0.001**
	Carcass size x Beetle species	28.85	3	**<0.001**

868 *p* value in bold indicate significant differences in GLMMs.

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871 **S4 Table. Results of the ANOVAs for reaction norm experiment.**

Dependent variable	Explanatory variables	χ^2	d.f.	*p* value
Brood size	Carcass size	3.33	1	0.068
	Population	3.89	1	**0.048**
	Carcass size x Population	4.80	1	**0.029**
Average larval mass	Carcass size	139.05	1	**<0.001**
	Population	1.06	1	0.304
Clutch size	Carcass size	11.33	1	**<0.001**
	Population	21.07	1	**<0.001**
	Female size	4.26	1	**0.039**
Average egg volume	Carcass size	5.90	1	**0.015**
	Population	4.59	1	**0.032**
Total egg volume	Carcass size	2.38	1	0.123
	Population	2.16	1	0.141

872 *p* value in bold indicate significant differences in GLMMs.

873

S5 Table. Differences in body size frequency distribution between *Nicrophorus* spp.

Species	***N. vespilloides***		***N. interruptus***		***N. investigator***		***N. humator***	
	D	*p*	*D*	*p*	*D*	*p*	*D*	*p*
N. interruptus	0.54	**<0.001**	-	-	-	-	-	-
N. investigator	0.81	**<0.001**	0.41	**<0.001**	-	-	-	-
N. humator	0.91	**<0.001**	0.62	**<0.001**	0.38	**<0.001**	-	-
N. vespillo	0.70	**<0.001**	0.31	0.268	0.43	0.061	0.69	**<0.001**

D statistic and corresponding values of significant difference from Kolmogorov-Smirnov tests. *p* value in bold indicate comparisons with significant difference, and dashes indicate comparisons that were not made.

881

882 **S1 Data. Spreadsheet file of gene set enrichment analysis results of the multiple GO**

883 **terms for Gamlingay and Waresley *N. vespilloides*.**

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