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## Traits reveal ecological strategies driving carrion insect community assembly

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**Abstract**

1. The succession of carrion-associated (necrophilous) insects on decomposing carrion is well documented. To exploit the changing nutritious and dynamic resources available throughout the carrion decomposition process, different species colonise and consume carrion in a predictable temporal sequence. The traits of these necrophilous insects should reflect their ecological strategies. Morphological traits of these insects such as body size and wing size, however, have not previously been examined during active and advanced decomposition.
2. We used fourth-corner multivariate generalised linear models to identify insect community morphological trait patterns, and to quantify their change through time at decomposing rabbit carcasses in grassland and woodland environments.
3. We found that larger-bodied species of flies and carrion-specialist beetles were associated with early stages of decomposition. The morphological traits of ants, in contrast, showed no changes at carcasses through time, and instead showed body size differences between grassland and woodland environments.
4. Our findings indicate that specialist flies and beetles that arrive early in the decomposition process possess traits that enable rapid discovery of carrion at a large scale. Generalist beetles and ants do not share this same trait and are instead adapted to locate and consume a wider variety of resources in their preferred habitat type at their local scale.
5. Our results provide insights into the morphological adaptations linked to the ecological strategies of distinct components of carrion insect communities. Further,

our results offer insights into the community assembly dynamics that structure necrophilous insect species communities.

## Introduction

Identifying general and predictable responses of species to common environmental gradients is a major goal in community ecology. This has led to a trait-based approach where the attributes or ‘traits’ of species are the subject of analyses, rather than species identities (e.g. Evans *et al.* 2016; Ng *et al.* 2018; Evans *et al.* 2019). Traits of species include their morphology (Grimbacher & Stork 2007; Evans *et al.* 2018; Yama *et al.* 2019), diet (Woodcock *et al.* 2009; Evans *et al.* 2016; Evans *et al.* 2019), mode of dispersal (Zalewski *et al.* 2012), or reproductive strategy (Tonelli, Verdú & Zunino 2019). Examining such traits offers a way of understanding species-environment interactions - it reveals species trait patterns that indicate their ecological strategies for exploiting resources or for surviving in certain habitats (Li *et al.* 2017; Comte & Olden 2018). Linking species traits to their environmental context can therefore reveal key ecological processes shaping the structure of biological communities (Ribera *et al.* 2001; Grimbacher & Stork 2007; Woodcock *et al.* 2009; Evans *et al.* 2019).

An important ecological process fundamental to all ecosystems is the decomposition of dead animal biomass, or carrion (Barton *et al.* 2019; Benbow *et al.* 2019a). The decomposition of carrion in terrestrial ecosystems is enhanced by the activity of carrion-associated (i.e. necrophilous) insects that colonise and consume decomposing carcasses (Barton & Evans 2017; Benbow *et al.* 2019b). The succession of necrophilous insects on

decomposing carrion is well documented – different species colonise and consume carrion in a predictable sequence (Payne 1965; Peschke, Krapf & Fuldner 1987; Schoenly & Reid 1987; Anderson & VanLaerhoven 1996; Sharanowski, Walker & Anderson 2008; Tomberlin *et al.* 2011b; Barton & Evans 2017; Barton *et al.* 2017; Cruise, Watson & Schal 2018). Although there are some widespread necrophilous insects (e.g. house fly *Musca domestica* (Muscidae); hairy maggot blowfly *Chrysomya rufifacies* (Calliphoridae); hide beetle *Dermestes maculatus* (Dermestidae)), the majority of necrophilous species (e.g. flies, beetles, ants) have a more limited distribution (Cruise, Watson & Schal 2018; Anderson *et al.* 2019) – individual species are usually restricted to particular geographic regions (Michaud *et al.* 2010; Barton & Evans 2017; Anderson *et al.* 2019; Langer *et al.* 2019) or environments (Kavazos & Wallman 2012; Farwig *et al.* 2014; Babcock, Pechal & Benbow 2019).

The unpredictable temporal and spatial availability of carrion means that specialist insects have traits that enable the rapid discovery, colonisation, and exploitation of carcasses (Tomberlin *et al.* 2011a; Barton *et al.* 2013). Well known early colonisers of carrion include specialists such as blowflies (Calliphoridae) or carrion beetles (Silphidae). These species can fly long distances and are highly sensitive to carrion odour cues (Tomberlin *et al.* 2011b; Barton *et al.* 2013). Other necrophilous species include trogid and dermestid beetles that specialise on the hair, skin and other tough or dehydrated tissues that persist after much of the soft tissues are consumed (Anderson *et al.* 2019). A further suite of predatory species (e.g. staphylinid beetles, ants) also visit carcasses to feed on the abundant maggots and larvae of other insects (Braack 1987). Necrophilous insect communities therefore comprise distinct taxa with contrasting ecological requirements and strategies for exploiting carrion resources.

The different insects that exploit carrion resources at particular stages of decomposition should result in community trait patterns that change over time as decomposition progresses. There has been limited previous research on this topic (but see Barton *et al.* 2013), and no comparisons of traits of contrasting insect taxa have been made to identify possible convergent trait patterns or a common ecological strategy. Identifying trait patterns of necrophilous insects, therefore, offers a way to characterise the ecological strategies of species associated with patchy, dynamic, and ephemeral carrion resources. In this study, we examined the temporal change in morphological traits of flies, beetles, and ants found at small vertebrate carcasses (rabbits) over 12 days of decomposition spanning fresh to dry decay stages (*sensu* Payne 1965) in the Australian summer. Our overarching aim was to identify what traits are associated with contrasting ecological strategies of specialist and generalist necrophilous insects from three different insect taxa (flies, beetles, ants). We therefore asked:

1. *What are the main traits that characterise fly, beetle, and ant assemblages found at carrion?* We first wanted to identify what morphological traits varied most among insect species found at carrion, and how these differed among specialist and generalist fly, beetle and ant species. These taxa each occur at carrion in high abundances (Anderson & VanLaerhoven 1996; Sharanowski, Walker & Anderson 2008; Barton & Evans 2017; Barton *et al.* 2017; Cruise, Watson & Schal 2018), but also differ markedly in their morphology, and so allow for comparisons of traits associated with ecological strategies at the same resource.

2. *What traits of fly, beetle, and ant assemblages change over time during decomposition of carrion?* We hypothesise that specialists associated with early stages of decomposition will be dominated by species with traits that enable rapid colonisation, such as a large body size that enables species to disperse further and faster with larger energy reserves and increased competitiveness against smaller species (Peters 1983; Whitmee & Orme 2013). In contrast, Barton and Evans (2017) showed that generalist ant and beetle species at carrion were strongly associated with the habitat context of the carrion (tree or grassland sites). This suggests that their traits might differ from those of specialists and instead be more strongly associated with the context in which the carrion is decomposing (i.e. habitat context).

## **Materials and methods**

### *Study area and design*

We conducted our research at the Mulligans Flat – Gorooyarroo Woodland Experiment near Canberra in south-eastern Australia (35.165°S, 149.171°E) (Shorthouse *et al.* 2012). The area is part of a temperate eucalypt woodland reserve characterised by open grassland dominated by native perennials (*Themeda*, *Aristida*, *Rytidosperma*) and is interspersed with stands of yellow box (*Eucalyptus melliodora*), red gum (*Eucalyptus blakelyi*) and red stringy bark (*Eucalyptus macrorhyncha*) trees (McIntyre *et al.* 2010; Barton & Evans 2017).

We placed one rabbit carcass (*Oryctolagus cuniculus*) at each of 18 sites within the reserve – nine under trees and nine in open grassland. We arranged the sites into nine pairs of one tree and one open site. We placed the two sites within pairs a minimum of 100m apart

and site pairs at a minimum of 400m apart. We defined tree sites as wooded areas with many trees (*Eucalyptus* spp.), an obvious canopy and continuous leaf litter ground cover. We defined open grassland sites as having a continuous grass layer dominated by native perennial species (*Themeda*, *Aristida*, *Rytidosperma*), typically more than 80 m from the nearest tree. We acquired rabbits from pest-control shooting conducted by the Australian Capital Territory (ACT) government. We used only intact carcasses (average mass = 1.75 kg) which were bagged and frozen until needed. We defrosted carcasses in a refrigerator 48 hours before deployment. We placed each carcass in a random direction on its side and in direct contact with the ground. We surrounded each carcass in a small cage to prevent scavenging by ravens and foxes and covered the structure in a white plastic sheet to reduce the effect of direct sunlight that differed between the open and tree sites. We conducted the study in the Australian summer (January 2016). We used temperature data loggers placed on each carcass to monitor any differences in temperature between sites. We derived an accumulated-degree-day (ADD) value for each carcass by summing the hourly temperatures recorded over the period of the study (288 hours) and dividing that value by 24. The average temperature during the experiment was 25°C with a range of 9.2 to 42°C. Temperatures were slightly more extreme in the grassland sites (8.6-44.5°C) than the tree sites (9.9-40.9°C). This resulted in slightly higher ADD values for the open grassland sites.

### *Insect sampling*

We collected adult insects using a single pitfall trap (250 ml volume, 8 cm diameter) positioned next to the ventral surface of each rabbit carcass. We filled the pitfall traps with a glycol and ethanol solution. We collected and replaced the contents of each trap on days 1, 2,



3, 4, 6, 8, 10 and 12 of the decomposition process. We separated the contents of each trap into ants, flies and beetles, and then identified individuals to morphospecies (sensu Oliver & Beattie 1996, hereafter referred to as species), using a variety of taxon-specific keys (Mathews 1987; Andersen 1991; CSIRO 1991; Shattuck 2000; Wallman 2001; Kavazos *et al.* 2012). We established a reference collection of species/morphospecies which were verified with help from taxonomists at the Australian National Insect Collection and the Australian Museum. The final dataset comprised 15 species of flies (3,245 individuals), 31 species of beetles (6,469 individuals), and 34 species of ants (10,288 individuals); see Barton and Evans (2017) for more details.

#### *Insect categories*

We were interested in identifying traits of necrophilous specialists that differed from generalist species; therefore, we assigned each species to one of these categories (Tables S1-S3). We defined specialist species as those species that breed at carrion (commonly referred to as obligates; Olea, Mateo-Tomás & Sánchez-Zapata 2019), as well as species that are known to feed on carrion, or prey on other species that do. We included dung beetles in this category as they are well-known specialists on ephemeral and patchy dung resources, as well as carrion. They contrast with species considered generalist and opportunistic feeders such as carabid beetles and many species of ants. Examples of specialist species include both the flesh fly *Sarcophaga misera* (Sarcophagidae) that feeds on the flesh of carrion (as larvae) and the predatory beetle species *Creophilus erythrocephalus* (Staphylinidae) that feeds on fly larvae (as adults and larvae) (Kavazos *et al.* 2012; Barton & Evans 2017).

We defined generalist species as those that might utilise, but are not restricted to, carrion for part of their life cycle, and which typically have a broader diet (commonly referred to as facultative or opportunistic scavengers; Olea, Mateo-Tomás & Sánchez-Zapata 2019) and are therefore not restricted to nutrient-rich ephemeral patchy resources such as carrion. This included many species of predatory beetles (e.g. species in the families Carabidae and Staphylinidae), all ants (especially the abundant species of *Iridomyrmex* (Formicidae) observed scavenging directly on the carcasses or feeding on fly larvae), and some generalist saprophage beetles (e.g. species of Anthicidae, Leiodidae).

### *Morphological traits*

We measured several morphological traits from a representative of each species (Table 1) (Grimbacher & Stork 2007). We captured images of the individuals using a Nikon SMZ1000 stereo microscope and TrueChrome 2 digital camera. We then used these images to make accurate linear measurements of the traits using ImageJ image measurement software (Rasband 2016). Traits measured were different between the major taxa (Diptera, Coleoptera, Hymenoptera: Formicidae) owing to the broad morphological differences between the groups (e.g. flies have aristae, ants do not). We were primarily interested in insect traits that might be associated with species' ability to compete for resources at carrion. These included dispersal related traits that allow rapid colonisation of carrion such as larger body size (Peters 1983) and larger wing size (McCulloch, Wallis & Waters 2017). It also included traits that are potentially associated with the sensory detection of carrion resource, such as antennae and aristae in flies (Sukontason *et al.* 2004).

### *Statistical analysis*

To standardise sampling effort, we pooled the data from days 1 and 2, and days 3 and 4. We analysed the three major groups of insects separately, given their differences in types of morphological traits (i.e. they could not be added to the same analysis). Further, to compare the trait responses of specialist and generalist species, we split the flies and beetles into two groups each (specialists and generalists; note: all ants were classified as generalists).

To identify traits (Question 1), we used a Principal Component Analysis (PCA) ordination to characterise species in morphological trait space for specialist flies, specialist beetles, generalist flies, generalist beetles, and ants separately using the ‘FactoMineR’ package (Le, Josse & Husson 2008) in R. We transformed the trait values ( $\log^{10}[x+1]$ ) before this analysis to reflect the static allometry inherent in insect communities (Ribera *et al.* 1999). We gave flightless beetle species a wing length and width of zero. We then used the axes of the first two principle components of each analysis as representatives of the dominant morphology of each species grouping. We expected that body size would constitute the first component because the dominant morphological trait of animals is their body size (Peters 1983).

To test how species traits varied through the decomposition process (Question 2), we fitted fourth corner multivariate generalised linear models of the PCA-derived axes against time (days 2, 4, 6, 8, 10 and 12) (Brown *et al.* 2014). We were also interested in knowing how traits differed between habitat contexts (tree and open habitats). Fourth corner modelling provides a way to analyse the relationships between species abundances and occurrences (L), their traits (Q) and environmental variables (R) (Legendre, Galzin & HarmelinVivien 1997).

The fourth corner – the interaction between R and Q – is quantified with fourth corner model coefficients. We fitted models using the ‘traitglm’ function in the ‘mvabund’ package (Wang *et al.* 2018). Our species data were count data (abundances of species) so we assumed a negative binomial error distribution. We fitted the models with LASSO penalties; an approach that uses iteratively reweighted local linearisation to penalise estimates to zero using BIC scores should they not improve the fit of the model. This essentially means that non-zero estimates should be treated as significant. Our model formula was:

$$\sim \text{day}*\text{habitat}*\text{trait},$$

Where *day* was a categorical factor with six levels (2, 4, 6, 8, 10, 12), *habitat* was a factor with two levels (open, tree) and *trait* was one of two continuous principal components axes scores for each species group. In our analysis, we treated the day and habitat variables as environment variables (R).

We were also interested in whether our specialist categorisation (generalist/specialist) changed through time – i.e. did the relative contributions of generalists and specialists change through the decomposition process. To test this, we fitted similar fourth corner models as described above, instead modelling all flies in one analysis using a binomial dummy variable as a trait to signify whether the species was a specialist (1) or generalist (0). We did the same for the beetle community.

We used the FactoMineR’ (Le, Josse & Husson 2008), ‘ggplot2’ (Wickham 2016) and ‘mvabund’ (Wang *et al.* 2018) packages in R (R Core Team 2019) for all of our analyses and plotting.

## Results

### *Trait characteristics of fly, beetle and ant assemblages*

We found that most of the variation in morphology in all taxonomic groups was explained by size (Figs. 1 & 2, Table S4). The first axis of the principal components analyses for all three taxa showed a strong correlation with body size (correlations vary from 1 for ants to 0.87 for generalist flies) and with most other measured traits (Tables S5-S9). Interpretation of the second axes differed among the taxonomic groups. For flies, relative antenna length and relative arista length explained most of the remaining morphological variation for specialist and generalist species, respectively (Tables S4, S5). For both specialist and generalist beetles, relative wing size explained most of the remaining morphological variation (Tables S4, S7, S8). For ants, relative petiole length explained most of the remaining morphological variation (Tables S4, S9).

### *Temporal change in insect species traits*

We found that insect body size changed over the decomposition process in a similar way for specialist taxonomic groups (Fig. 3). For both specialist flies and beetles, and in both open and tree sites, body size was larger than the mean in the early stages of decay and smaller than the mean in days 8 and 10. Body size then increased again to be larger in open habitats and similar to the mean in tree habitats at day 12.

The insect body size pattern of generalist species during decomposition differed from specialist species, as well as between taxonomic groups (Fig. 3). Body size for generalist flies showed a similar pattern to that of the specialist groups and was similar in open and tree sites. Flies were larger in the earlier stages of decomposition but smaller towards the later stages. In

contrast to the specialist groups, however, instead of an increase in body size in day 12, generalist flies were much smaller than in the preceding days. Body size of generalist beetles demonstrated different patterns to the other taxonomic groups. In both open and tree sites body size was smaller than, or the same, as the mean body size in day 2, but increased in day 6. It then decreased again and was smaller by day 12. Ant body size showed differing patterns in tree and open sites but no strong pattern over the period of decomposition. The patterns for the second principal component axes for all groups were idiosyncratic, with the exception of specialist beetles that showed a pattern of decreasing relative wing size over the time of decomposition, most notably in open sites (Fig. 4).

There were more specialist fly species in days 2 and 4 in both open and tree environments than in day 6 and onwards (Fig. 5). In the tree environment, there were more generalist species in day 12 than specialists. Beetles showed more specialists in days 4, 6 and 12 in the tree sites and only in day 4 in the open sites (Fig. 5). They were dominated by generalists in the tree sites on day 10 and in the open sites in days 2, 8 and 10 (Fig. 5).

## Discussion

Our objective was to identify traits that were associated with the contrasting ecological strategies of specialist and generalist necrophilous insects. Our results clearly demonstrate a strong body size trait pattern of insect succession at decomposing carrion. Specialist flies and beetles displayed a similar trend of decreasing body size over time, whereas generalist flies and beetles displayed contrasting body size trends. Ant species did not show any obvious trend in body size over time. What follows is a discussion of the likely mechanisms behind these patterns, paying close attention to the species involved and why

certain traits are selected for in certain contexts. We then discuss the wider implication of our discovery and the insights our approach gives us on the assembly processes of necrophilous communities.

### *Adaptations for rapid discovery*

We found that communities of specialist necrophilous flies and beetles had similar body size trait patterns during the decomposition process. Species within these communities were larger in the early stages of the decay process, but smaller during later decay. Being larger is a clear advantage for those species that rely on the resources available in the early stages of carrion decomposition. In the early stages of decay, carrion comprises soft tissues and fluids and is at its most nutrient rich and easily consumed (Barton & Bump 2019). As a result, it is highly sought after and consumed very quickly by necrophilous species (Benbow *et al.* 2019a). The most obvious competitive advantage, therefore, is to have the ability to locate this resource as quickly as possible. The species associated with these early stages that utilise the soft tissue and flesh, such as the blowflies (Calliphoridae) and carrion beetles (Silphidae) are, therefore, necessarily larger – allowing them to disperse comparatively further and faster than smaller species and possibly outcompeting them for space and oviposition sites. This is also true for some species that prey upon blowfly larvae, a nutrient rich resource that appears relatively early in the decomposition process. These species include the large rove beetle *C. erythrocephalus* (Staphylinidae) and carrion beetle *Ptomaphila lacrymosa* (Silphidae). The similarity of the trait patterns shown by specialist flies and beetles – two distinct insect clades – suggests convergent adaptation of similar traits required for similarly carrion-dependant life cycles.

Our finding that body size of flies reduced in the later stages of decomposition is confirmation that smaller species are often associated with the later stages of advanced and dry decay (Anderson & VanLaerhoven 1996; Martinez, Duque & Wolff 2007). *Leptocera* species (Sphaeroceridae), for example, are used as indicators of the later stages of decay in forensic studies (Martinez, Duque & Wolff 2007). Further, species of the Phoridae family (e.g. *Megaselia* spp.), which breed and feed on carrion, have been reported to arrive at exposed pig carcasses after 5-12 days (Grassberger & Frank 2004). The reasons for this could be driven by a number of factors. The diet of sphaerocerids and phorids includes drier tissues and associated microbial biofilms (Kavazos *et al.* 2012; Merritt & De Jong 2015), which tend to be available for a wider time window than fresh soft tissues. This means that rapid arrival is less important, and their smaller size and limited ability to move rapidly and over larger distances does not place them at a disadvantage. Further, the larvae of larger fly species, such as *C. rufifacies* and *Chrysomya varipes* (Calliphoridae), reach large numbers and may out-compete the smaller species in the early stages of decomposition. Species of Phoridae, for example, can gain extremely high abundances in the absence of larger species such as *C. rufifacies* and *C. varipes* (Campobasso *et al.* 2004).

Another notable adaptation for rapid colonisation and exploitation of carrion is the live-bearing (viviparous) biology of *Calliphora augur* (Calliphoridae) and several species of Sarcophagidae. This adaptation is thought to enable quicker colonisation of carrion, and might also give a competitive advantage to viviparous species over oviparous species (Meier, Kotrba & Ferrar 1999). The ecological benefits of this reproductive trait have not been



investigated in detail, and might be worth examining in future studies of early colonisers of carrion.

Generalist flies showed a similar pattern of larger body sizes in the earlier stages of decomposition compared with specialist species. This was mostly driven by *M. domestica*. We classified this species as generalist because it uses other high-quality ephemeral patchy resources such as food waste and animal dung. These resources would likely also select for species that are able to disperse further and more quickly than others because of the similarity of these resources to carrion.

For generalist beetles, both body size and relative wing size demonstrated increases after day 4 and a decrease towards day 10. This may have been driven by *Acrossidius tasmaniae* (Scarabaeidae), a large generalist detritivore associated with grasslands. The larvae of this species feed on above ground foliage and dung, but the adults feed on detritus including carrion (Barton *et al.* 2017).

The body size patterns for ants did not show a successional pattern through the time of decomposition. This is likely a reflection of the ability of these generalist species to consume a wide variety of resources available at carrion. Ants were very quick to colonise the carcasses (Barton *et al.* 2017), reflecting their ubiquity and dominance in Australian systems (Wilson & Kinne 1990; Shattuck 2000). Ants have relatively poorer dispersal ability than flies or beetles yet can still reach resources quickly if locally abundant in the landscape. Their recruitment strategy also allows quick location of resources within the colony's territory (Wilson 1971). This means that the species of ants that are associated with carrion

are strongly associated with the context in which the carrion is located (Barton & Evans 2017). The most striking trait pattern shown for ants was that they differed in size according to the context of the carrion. Species were smaller in the open grassland habitat than in the woodland (tree) habitat. The structure and cover provided within the woodland environment results in a less variable microclimate, which might have a role in habitat partitioning among differently sized species. Larger bodies reach higher temperatures and lose heat more slowly than smaller bodies (Stevenson 1985) and ants in colder environments tend to be larger as a result (Bishop *et al.* 2016). It follows, therefore, that ants in the tree environment might gain an advantage by being larger, whereas ants in the open, where maximum temperatures are slightly higher, might benefit from being smaller, allowing their body temperature to remain cooler and release heat more quickly. Open sites, however, are also likely to be drier than tree sites given their increased mean temperatures and comparative lack of vegetative cover. This might increase the risk of desiccation for ants, particularly for smaller species that have larger surface to volume ratios than larger species. A more likely explanation for the size differences of ants between tree and open sites, therefore, is that the tree environments are more likely to contain a larger resource pool for ants in the form of increased nectar, sap, and invertebrates, than the open environments. Tree sites may provide enough resources to sustain a larger biomass of ants, including larger colonies of large species. The pattern we found is most likely driven by the dominant species in both environments: the smaller *Iridomyrmex rufoniger* in open environments and the larger *Iridomyrmex purpureus* in tree habitats (Barton *et al.* 2017). The trait pattern of ants at carrion reveals that their generalist

diet, habitat preference, and localised abundance results in no clear successional dynamics at decomposing carcasses.

Our results also demonstrate that some specialists do not necessarily appear in the early stages. For example, there were more specialist beetles on day 12 than at previous times in the decomposition process. This result is probably indicative of *Omorgus candidus* and *Dermestes maculatus*, species that feed on skin and fur; a resource that is more easily accessible once most of the high-quality resources such as flesh have been exhausted. Further, the increase in specialist beetles on days 4 and 6 is likely driven by specialist predators such as *Saprinus cupreus*, *Saprinus cyaneus* and *Creophilus erythrocephalus* that prey on the maggots of the obligate carrion feeders that appear in the first few days of decomposition.

#### *Ecological strategies driving necrophilous community assembly*

Our study gives insights into the strategies driving the community assembly of necrophilous communities. Our results confirm that the early stages of these communities are dominated by larger species whose competitive advantage enables them to rapidly locate discrete patches of high quality resources (Finn 2001) and possibly outcompete smaller species in the early stages of decomposition. This was particularly true for the specialist species (e.g. *C. rufifacies*, *C. varipes*), many of which are obligate carrion feeders (i.e. rely on carrion). This trait pattern is less strong for those species that feed - but are not entirely reliant - on carrion (i.e. omnivorous species; e.g. *I. rufoniger*, *I. purpureus*). These generalist species are more likely to possess traits linked to their habitat specialisation and broader dietary ecology. Our results, therefore, reveal that the necrophilous community is likely to consist of

a mix of generalists, with a strategy of utilising a wide range of resources at a local scale, and specialists with adaptations to locate and consume carrion as their primary resource at larger spatial scales. This pattern is likely to be widespread for invertebrate necrophilous communities in a variety of habitats worldwide. Indeed, the effect of macrohabitat in carrion insect community partitioning has also been identified in Germany (Peschke, Krapf & Fuldner 1987). We recognise that our findings are dependent, in part, on the categories we assigned to each species. Although we are confident that our classification of species as specialists reflects their ecology, we understand that other groupings might reveal additional insights into the ecological strategies of species at carrion. Nevertheless, our findings help to explain the globally general phenomenon of carrion insect succession, where early colonisers (e.g. blowflies and carrion beetles) have narrow dietary requirements and therefore a narrow time window for colonisation, and later colonisers have either broader dietary requirements or dietary requirements only available at later stages of decomposition (e.g. trogid beetles).

Our work contributes to a long history of theoretical work on necrophilous ecological succession (Benbow *et al.* 2019a). We demonstrate a new perspective on the often-reported phenomenon of species succession of necrophilous animals and help to explain the patterns reported in forensic and other succession studies in a new light (e.g. Grassberger & Frank 2004; Matuszewski *et al.* 2010; Barton *et al.* 2017; Keshavarzi *et al.* 2019). Our interpretation of necrophilous community assembly via body size differences through time advances the fields of carrion ecology and entomology by highlighting the importance of trait patterns associated with the ecological strategies of specialist and generalist species.

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**Author Contributions:**

ME and PB conceived the ideas and designed the methodology. All authors collected the data. ME analysed the data. ME led the writing with contributions from PB and JW.

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## Tables and Figures

Table 1. Morphological traits measured for each major taxonomic group.

Trait category	Flies (Diptera)	Ants (Hymenoptera: Formicidae)	Beetles (Coleoptera)
Size	Total body length (head to opposite end of last abdominal segment) Head height	Total body length (sum of gaster, petiole, thorax and head lengths) Gaster length Petiole length Thorax length Head length Head width	Total body length (head to opposite end of elytra or abdomen)
Dispersal	Wing length	Rear femur length	Rear femur length Rear tibia length Wing area Wing length
Sensory	Antenna length Arista length Eye height		Inter-ocular width





### Figure Legends

Fig. 1. Fly and beetle generalist and specialist species in trait space (first two principal components). Point size represents the size of the species. The text on the axes are the interpretation of the morphological variation represented by each axis. Square points in the bottom-right plot represent flightless beetle species.

Fig. 2. Ant species in trait space (first two principal components). Note that all ant species were classified as generalists. Point size represents the size of the species. The text on the axes are the interpretation of the morphological variation represented by each axis.

Fig. 3. Fourth corner model estimates of mean size (PCA axis 1 scores) for each taxa community in each habitat context on days 2, 4, 6, 8, 10 and 12. Effect sizes can be interpreted as the effect on each trait compared to the mean trait value of all individuals in the community (zero-effect line). An estimate of zero indicates that the effect has been penalised by LASSO penalisation and can be interpreted as a non-significant effect.

Fig. 4. Fourth corner model estimates of PCA axis 2 scores for each community in each habitat context on days 2, 4, 6, 8, 10 and 12. The interpretation of traits represented by these scores is in italic text within each plot. Effect sizes can be interpreted as the effect on each trait compared to the mean trait of all individuals in the community (zero-effect line). An estimate of zero indicates that the effect has been penalised by LASSO penalisation and can be interpreted as a non-significant effect.

Fig. 5. Fourth corner model estimates of the generalist/specialist trait groupings in each habitat context on days 2, 4, 6, 8, 10 and 12. Positive values represent a community dominated by specialists, and negative values represent a community dominated by

generalists. An estimate of zero indicates that the effect has been penalised by LASSO penalisation and can be interpreted as a non-significant effect.

**List of legends for the supplementary tables:**

Table S1. Fly morphospecies classification and times caught in experiment (n).

Table S2. Beetle morphospecies classification and times caught in experiment (n).

Table S3. Ant morphospecies and times caught in experiment (n).

Table S4. Percentage of variation explained by the first 3 to 5 axes of the PCA ordination of taxonomic groups.

Table S5. Contribution of specialist fly traits to PCA axes.

Table S6. Contribution of generalist fly traits to PCA axes.

Table S7. Contribution of specialist beetle traits to PCA axes.

Table S8. Contribution of generalist beetle traits to PCA axes.

Table S9. Contribution of ant traits to PCA axes.

# Traits reveal ecological strategies driving carrion insect community assembly

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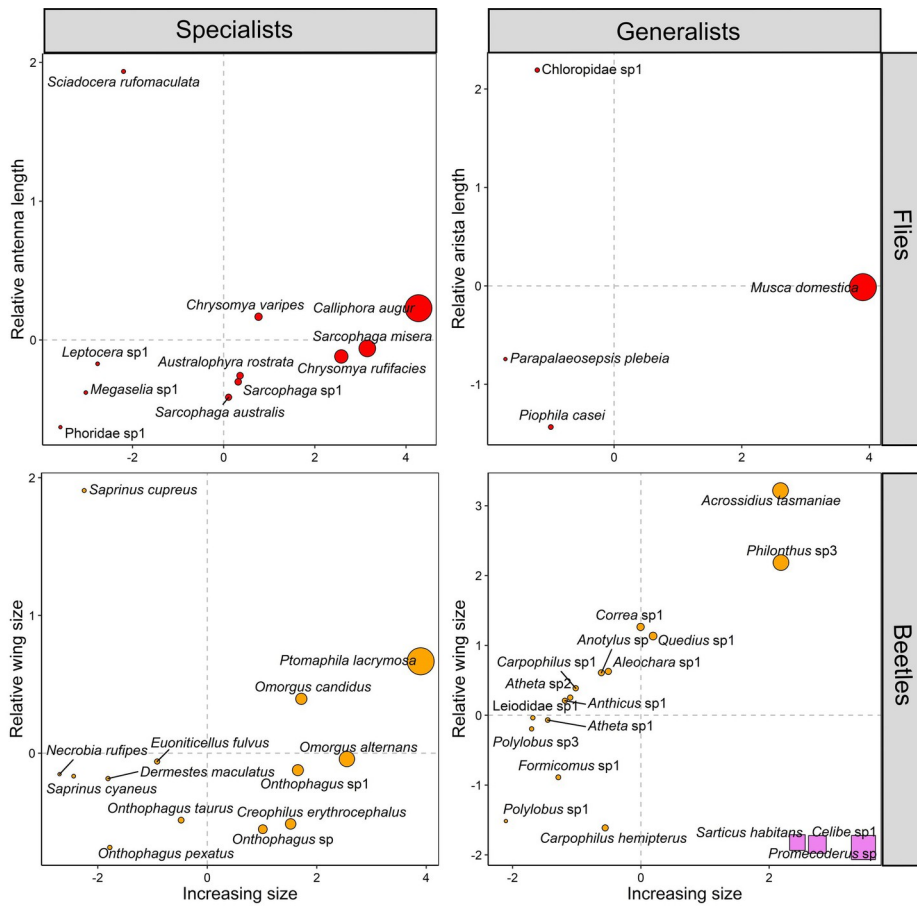
<sup>1</sup>Fenner School of Environment and Society, Australian National University

<sup>2</sup>Graduate School of Life and Agricultural Sciences, University of Tokyo

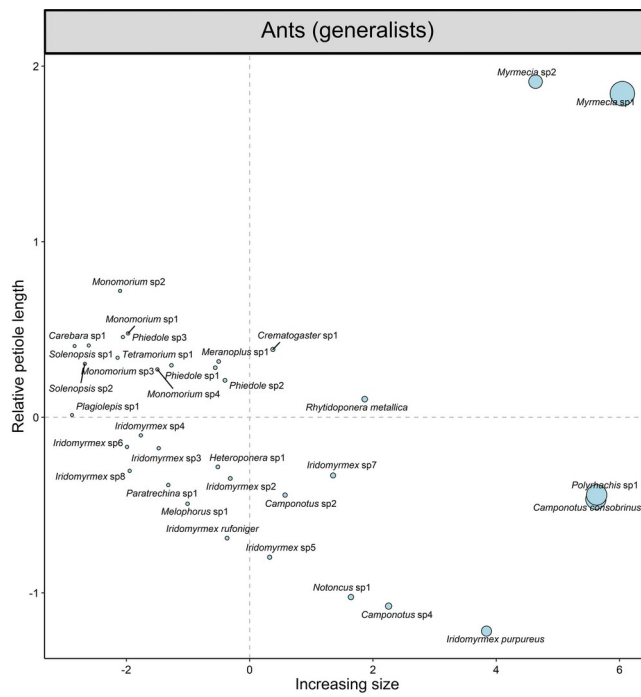
<sup>3</sup>School of Life Sciences, University of Technology Sydney

## Highlights

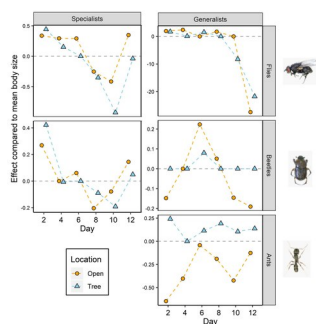
- Specialist flies and beetles that arrive early in the decomposition process possess traits that enable rapid discovery of carrion at a large scale.
- In contrast, generalist beetles and ants do not share this same trait and are instead adapted to locate and consume a wider variety of resources in their preferred habitat type at their local scale.
- These results offer insights into the community assembly dynamics that structure necrophilous insect species communities.



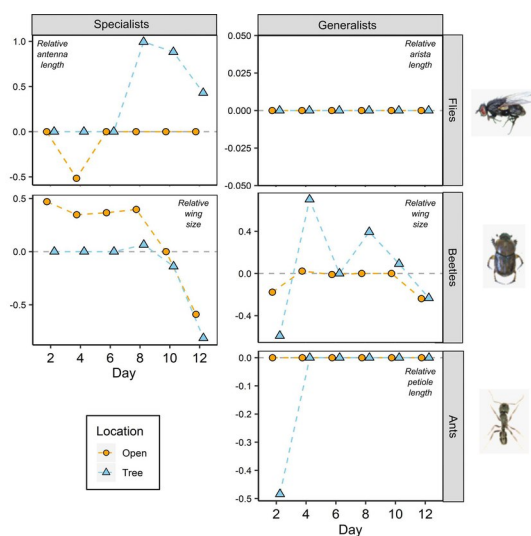
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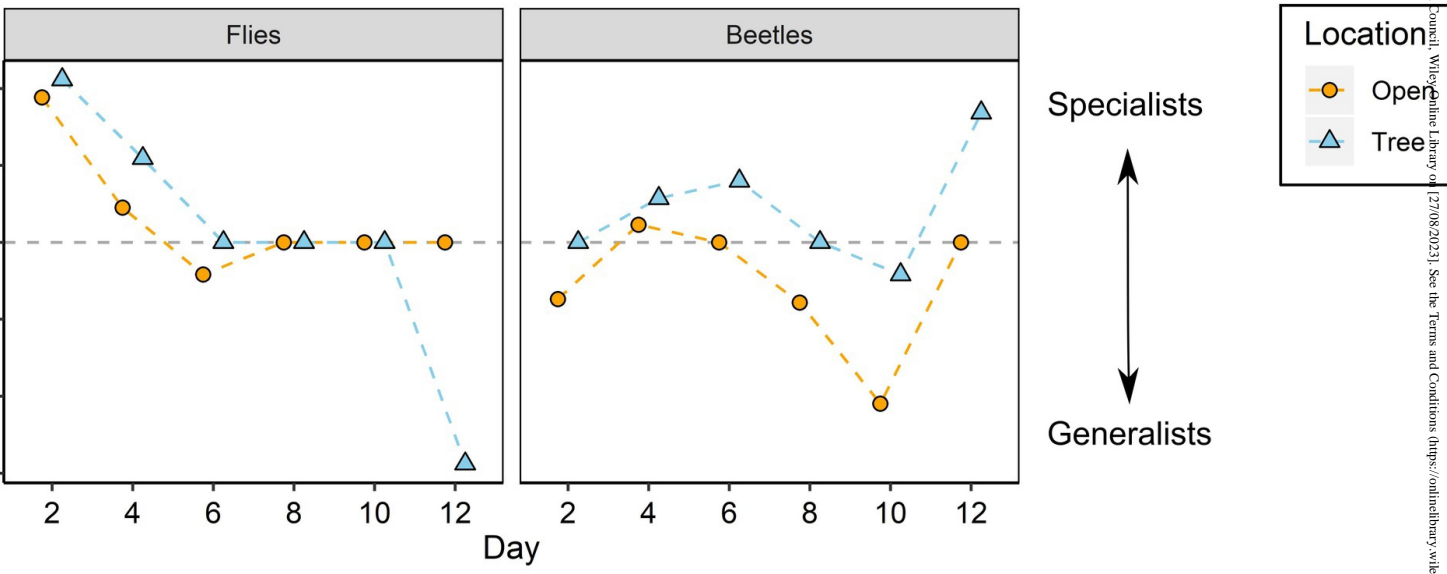
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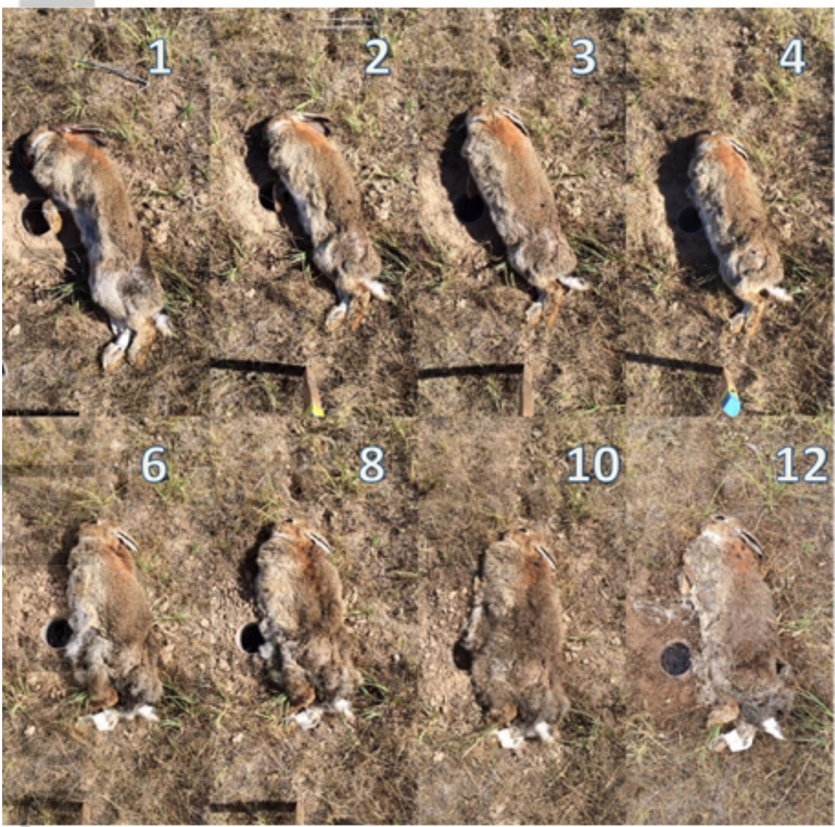


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