Evidence that sex-specific signals may support mate finding and limit aggregation in the dung beetle Aphodius fossor

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Abstract:

1. In temperate climates dung is often colonised by several species of endocoprid (dwelling) dung beetles which use pats for feeding, mating, and reproduction.
2. Endocoprid beetles aggregate even when offered patches (dung pats) of consistent age, size, and origin, suggesting that beetles themselves might influence the attractiveness of the patches they colonise to members of their own species. Both pheromones and physical changes to the structure of dung pats caused by colonising beetles have been suggested as mechanisms facilitating intraspecific aggregation, but neither of these hypotheses has been empirically tested.
3. Using a common European dung beetle (*Aphodius fossor*), we conducted a simple choice experiment designed to test whether 1) earlier colonisation by conspecifics could alter dung attractiveness and 2) whether attraction was influenced by sex-specific signals.
4. We show that female beetles are repelled by dung colonised by conspecific females and are attracted to dung colonised by conspecific males. Male beetles show no evidence of attraction or repellence for dung colonised by either sex. Neither in females nor in males was uncolonised dung found to be significantly more or less attractive than predicted by non-preference.
5. Our results suggest that male-produced signals may support mate finding in patchy environments, and that female-produced signals may serve to discourage subsequent colonisation by additional females.

Introduction

Dung beetles (Coleoptera: Scarabaeidae) are a popular model taxon for testing ecological theory, including biodiversity-ecosystem functioning relationships (Beynon *et al.*, 2012), metapopulation dynamics (Roslin, 2000; Roslin & Koivunen, 2001), and species-area relationships (Lobo & Martín-Piera, 1999). The spatial ecology of these beetles has been studied extensively, as habitat patches (dung pats) are easily delimited and species distributions can be studied readily over a wide range of scales (e.g. Finn *et al.*, 1998).

In contrast to the intense competition experienced in tropical dung beetle communities (Hanski & Cambefort, 1991), in temperate ecosystems competition is relatively weak and dung monopolisation rarely occurs (for review see Finn & Gittings, 2003). Instead, dung beetle communities tend to be dominated by dwelling beetles, with density and diversity showing an aggregated distribution across habitat patches (Palestrini *et al.*, 1998).

An important element that influences dung beetles aggregation within a localised patch is dung attractiveness. This can be influenced by a variety of factors including time of day at which the dung was produced (Holter, 1979), exposure of the dung pat (Landin, 1961), weather during dispersal events (Finn *et al.*, 1998), age of the dung (Landin, 1961), and presence of veterinary pharmaceutical residues (Floate, 2007). However, highly variable beetle aggregation is observed even when using dung from a homogenous source with patches having both identical size and age (Hanski & Cambefort, 1991; Palestrini *et al.*, 1998). This suggests additional unidentified mechanisms may be promoting aggregation within dung pats.

A study exploring spatial distribution of temperate dung beetles (Palestrini *et al.* 1998) found both inter- and intraspecific aggregation. They proposed that activity of early colonising beetles might increase attractiveness and habitability of dung for potential future colonisers.

A second hypothesis offered in discussion within the same paper, was that pheromones might be contributing to intraspecific aggregation.

We conducted a preliminary investigation of these hypotheses with a choice experiment using the widely-distributed temperate dung beetle *Aphodius fossor* L. We offered male and female beetles choice of three dung pats which were either: uncolonised or manipulated to contain either four conspecific males or four conspecific females. By comparing observed frequencies with expectations of non-preference, we were able to test whether colonisation patterns supported aggregations promoted by (1) enhanced attractiveness due to activity of colonised beetles, or (2) sex-specific responses.

Methods

Collection of dung beetles

Adult *A. fossor* were hand-collected from cattle dung at Dr Beynon's Bug Farm, St Davids, Pembrokeshire, United Kingdom (51°53′20″, 5°14′09) on May 30th 2015. Adults were separated by sex into well-ventilated 4 L plastic tubs containing damp builder's sand and 500 mL of fresh cattle dung. All beetles were stored in a cool, dark shed before beginning the experiment.

Experimental set-up

Freshly-excreted dung was collected before any invertebrates were able to colonise from a herd of Welsh Black cattle at the same farm. The dung was homogenised, and formed into nine 250 mL dung pats. Each dung pat was placed into a well-ventilated, 4 L rectangular tub (22 cm × 15 cm × 15 cm), on top of 8 cm of moist, washed builders sand. Either four female beetles (n=3) or four male beetles (n=3) were randomly assigned to tubs. The three remaining dung pats were left uncolonised to serve as controls. Tubs were stored for 48 hours in a cool, dark shed to allow beetles to acclimatise before beginning the experiment.

Three arenas were constructed using circular, 30 cm diameter, 14 L black plastic plant pots filled to 10 cm with moist, washed builders sand (Figure 1). Three circular holes measuring 2 cm in diameter were drilled at intervals of 120^{0}, with the bottom of the hole sitting flush with the sand surface. A 4 cm length of clear plastic tubing (2 cm diameter) was used to join the larger arena to the tubs containing the dung pats. One end of the tubing was placed flush against the side of the arena with the other end extended into the smaller tubs (Figure 1).

Dung choice tests

On June 1st at 19:00h, two hours before the experiment began, the tubs containing dung pats were attached to the larger arena. The first round of the experiment began at 21:00h when *A. fossor* becomes most active (P. Manning, pers. obs.). A total of 90 males and 90 females were run through the experiment for a total of 18 separate rounds (occurring in six concurrent periods). Sand was re-moistened with water between rounds, using a spray bottle. The experiment was conducted indoors, under fluorescent light. Arenas and tubs were rotated 120^{0} following each round to account for any differences caused by differences in ambient light. We ran male beetles through the experiment first, replacing the top c. 3 cm of sand within the arena before testing female beetles.

Three arenas were run simultaneously. As pilot trials found that beetles frequently took longer than twenty minutes to select a dung pat, beetles were run in groups of ten. In each round, the 10 beetles of a single sex were added to the middle of each arena and were initially confined to the innermost area of the arena using a circular, steel tea strainer (8 cm diameter). Beetles were kept in place for 120 seconds before the tea strainer was lifted and beetles were able to move towards a dung pat in one of the surrounding tubs. Each round of the experiment continued until either all beetles had reached a tub (each beetle being promptly captured and removed from the experiment before it could enter the dung) or after 25 minutes, when the round was terminated.

Analysis

Data for male and female beetles were analysed separately using a goodness-of-fit test for discrete multivariate data, comparing observations to null expectations of non-preference. When observed counts were found to be significantly different from null expectations ($P <$ 0.05), a post-hoc test was performed, testing each category of observation against its expected frequency (McDonald, 2009) using a Bonferroni corrected significance threshold ($\alpha =$ 0.0167). Analyses were carried out using the "EMT" package (Menzel, 2013) for R 3.1.1 (R Core Team, 2014).

Results and Discussion

Of the 90 females tested, 67 reached the dung within the allotted 25 minutes. The distribution of observed counts of females attempting to colonise dung differed significantly from expectation ($X^2 = 11.32$, df=2, P=0.002). Post-hoc testing suggested that female beetles avoided dung colonised by other females ($X^2 = 10.024$, df=1, $P = 0.001$) while preferentially selecting dung colonised by males ($X^2 = 6.52$, df=1, P=.013) (Figure 2a). Of the 90 males tested, 62 reached the dung within the allotted 25 minutes. The majority of male beetles were attracted to dung colonised by females (Figure 2b), but the observed values were not significantly different from expectations of indiscriminate choice ($X^2 = 4.004$, df = 2, P=0.146). In neither case did we find uncolonised dung was selected more or less frequently than predicted by non-preference, suggesting there was no significant influence of beetle colonisation on dung attractiveness.

The most striking result of our experiment was the avoidance of female-colonised dung by newly colonising female beetles. We suggest this might be attributable to deterrent pheromones produced by females, which serve to repel other females, many being classified as 'anti-aggregation' or 'oviposition deterring' pheromones. These signals discourage crowding and oviposition, thus reducing potential competition for offspring. Production of deterrent pheromones are known from many insects, e.g.: tephritid flies (Prokopy, 1975), weevils (Njihia *et al.*, 2014) and lacewings (Růžička, 2013).We suggest that upon receiving a signal indicating the presence of a female beetle, the dispersing female selects an alternative resource where her offspring might be less likely to experience density-dependent mortality. There is strong evidence suggesting that dung beetle larvae can be limited by space within a dung pat (Finn & Gittings, 2003). As *A. fossor* is one of the largest species of endocoprid dung beetle in Europe (Jessop, 1986), low-cost strategies (production and reception of chemical signals) could play an important role in reducing density-dependent mortality.

The preference of male-colonised dung by female beetles suggests that males may produce an attraction pheromone, a phenomenon documented in other male Scarabaeidae (e.g. Tribe, 1975; Edwards & Aschenborn, 1988; Larsson *et al.*, 2003). The timing of our study occurred approximately two weeks after teneral adults began emerging, which roughly corresponds to when *A. fossor* females reach reproductive maturity (Gittings & Giller, 1997). The release of attractant pheromones by males could support mate-finding by females in patchy environments (Larsson *et al.*, 2003). In the case of *A. fossor*, beetles are almost exclusively found in coupled pairs, although density may exceed six pairs within a single dung pat (P. Manning, pers. obs.). Individual males of the temperate species *Typhaeus typhoeus* L. have been observed tunnelling part-way into dung where they defecate on the dung surface (Brussaard, 1983). This behaviour is thought to represent a simultaneous pheromone release. During our experiment, we observed both males and females tunnelling part-way into the dung where they remained for several minutes with their abdomen partially exposed: while we did not observe defecation, this stance may have facilitated defecation and pheromone release.

Alternatively, sex-specific chemical signals influencing dung beetle aggregation may not be pheromone based. The activity of endocoprid dung beetles alters the composition of gases fluxing from cow dung (Penttilä *et al.*, 2013), which might act to modify its attractiveness to colonising beetles. If male or female beetles behave differently within the dung pat (eg: higher activity), this could potentially induce different gas fluxes. As we have no mechanistic evidence for the sex-specific responses, non-pheromone based chemical cues should not be discounted.

Furthermore, sex-specific signals in dung beetles may not be chemical: acoustic signals have been shown to play a role in aggregation in other *Aphodius* species (Hirschberger, 2001; Kasper & Hirschberger, 2005). However, acoustic signals are unlikely to have contributed to dung choice in this experiment as previous evidence suggests they are effective only at short range, being received mechanically from within the dung pat (Hirschberger, 2001).

As this experiment was conducted only 48 hours after dung was first produced, these data are not fully representative of the entirety that beetles remain resident within a dung pat. Adult *A. fossor* often inhabit older dung and it is likely that beetle activity could play a more influential role in altering attractiveness as dung ages and desiccates.

While our experimental design necessitated running beetles in groups of 10 as a consequence of logistical constraints, we recognise that individuals may have been influenced by odours released, and/or signals displayed by other beetles in the group. However, we observed that beetles frequently stopped when reaching the tunnel end, with their head suspended into the airspace above the dung. After a brief (15 – 30s) period of antennal movement, beetles would either turn, walking back into the arena, or alternatively walk forward – dropping into the tub. This observation suggested that beetles were responding to cues emanating from the dung pat itself, rather than solely following chemical trails laid by other beetles in the group.

Although our data show female patch choice is influenced by the sex of earlier colonisers, it is less clear how this might lead to dung beetle aggregations observed in nature. Female preference for male-colonised patches can explain observations of mate pairing, but female-female avoidance would ostensibly counteract formations of larger aggregations. One explanation could be that when both males and females occupy the same pat, female attraction to males outweighs female-female repellence. A second explanation could be that the female-female repellence observed in our experiment was induced by high densities (four beetles per 250mL dung pat), and the effect we observed may not persist when beetles occupy larger patches.

Our results demonstrate that sex-specific attraction and repellence could have a strong influence in explaining intraspecific dung beetle aggregations. We also suggest that alongside more refined methodologies, simple olfactometer studies could play an important role in achieving a better understanding of the underlying mechanisms that influence dung beetle aggregations.

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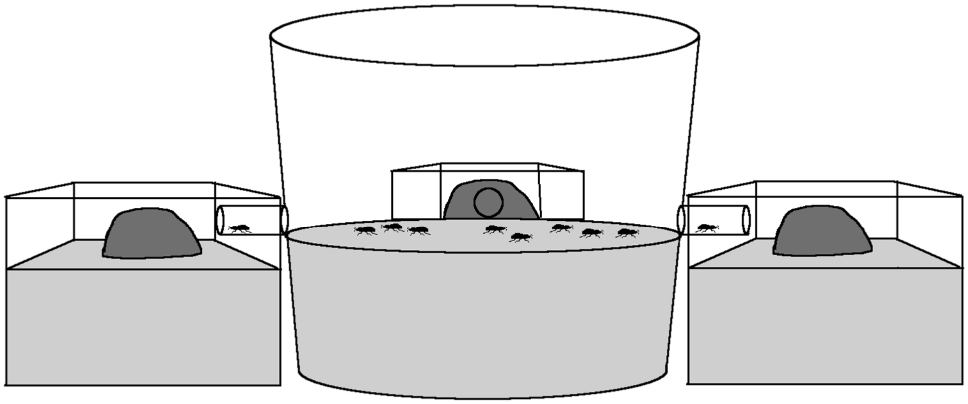

Figure 1. Dung choice experiment apparatus. Opaque tubs and arena are shown as transparent for clarity.
552x267mm (72 x 72 DPI)

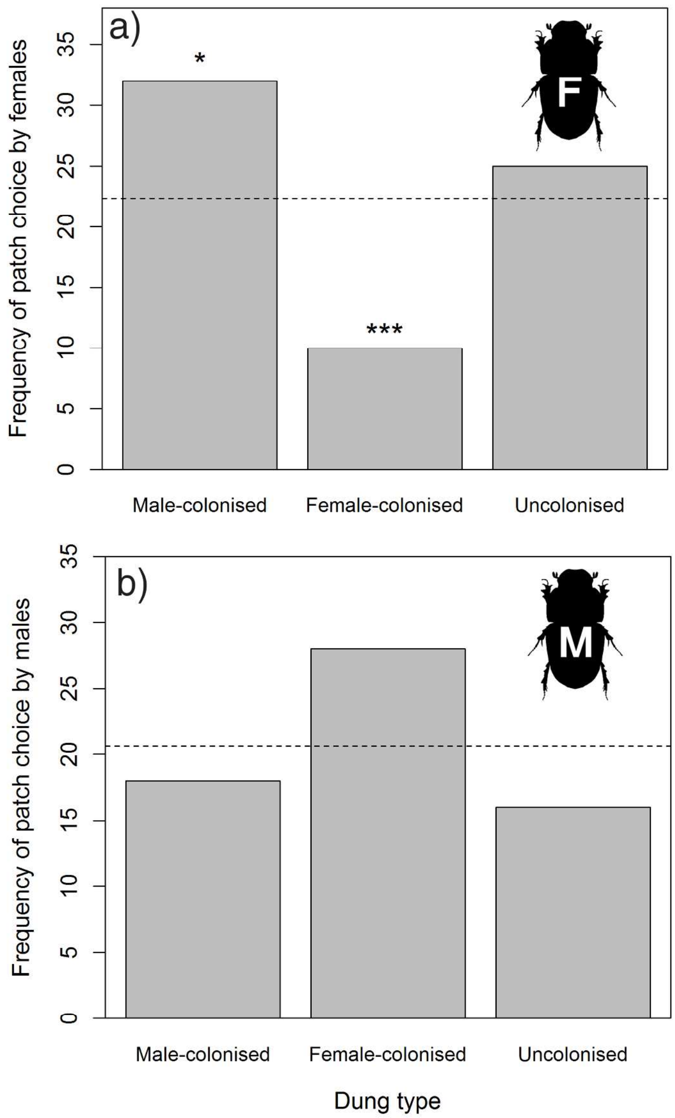

Figure 2. Preference of (a) female and (b) male Aphodius fossor in selecting dung which is uncolonised, or colonised by either male or female conspecifics. The dashed line marks the expected number of individual selections based on non-preference. Stars indicate frequencies which are significantly different from expectations of non-preference, values (*P<0.05, ***P<0.001).

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