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# Intraspecific female brood parasitism in the dung beetle *Onthophagus taurus*

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**Abstract.** 1. Brood parasitism occurs when individuals parasitise each others' investment into parental care, and has been documented primarily as an interspecific interaction. Intraspecific brood parasitism, in contrast, is often difficult to detect and quantify, and evidence for it is comparatively scarce. The present study documents the occurrence of intraspecific brood parasitism by females of the tunnelling dung beetle *Onthophagus taurus*, and investigates the contributions of two variables to the propensity of female brood parasitism: female body size and dung desiccation rate.

2. Female *O. taurus* were found to routinely utilise brood balls made by conspecific females as food provisions for their own offspring.

3. Contrary to expectations, large and small females did not differ in the likelihood of engaging in brood-parasitic behaviour.

4. Dung desiccation rate appeared to influence likelihood of brood parasitism. Females that were given access to rapidly drying dung were significantly more likely to detect and utilise brood balls produced by conspecific females.

5. While interspecific brood parasitism has been documented in dung beetles before, the present study is among the first to present evidence for intraspecific brood parasitism as an alternative reproductive tactic of female dung beetles. Results are discussed in the context of the evolutionary ecology of onthophagine beetles.

**Key words.** Alternative reproductive tactics, brood parasitism, horned beetle, *Onthophagus*, phenotypic plasticity, resource competition.



## Introduction

Parental care is generally defined as the allocation of resources to offspring to enhance offspring survival and fecundity (Krebs & Davis, 1993). Parental care is phylogenetically widespread and often includes the preparation and maintenance of a nesting site, offspring guarding and defence, and food provisioning (Clutton-Brock, 1991; Choe & Crespi, 1997). Such resources can sometimes be open to exploitation by other, unrelated individuals (Vollrath, 1984; Gonzalez-Megias & Sanchez-Pinero, 2003). Brood parasitism, or kleptoparasitism, is an interaction in which an individual usurps some or all of the resources allocated to parental care by another individual (Vollrath, 1984; Smith *et al.*, 2000). This kind of parasitism has been described mostly as an interspecific interaction, in particular among birds (Rothstein & Robinson, 1998; Smith *et al.*, 2000) and to a lesser extent certain groups of insects (Rougon & Rougon, 1980; Gill, 1991; Rasa, 1996). Intraspecific brood parasitism, in contrast, occurs when individuals of the same species parasitise each others' parental care efforts (Andersson, 1994). Intraspecific brood parasitism is often difficult to detect and quantify, and evidence for it is relatively scarce.

The present study investigates intraspecific brood parasitism in female *Onthophagus taurus* dung beetles. Female *O. taurus* reproduce by provisioning cow or horse dung for their offspring in subterranean tunnels in the form of so-called brood balls (Halffter & Edmonds, 1982). Each brood ball contains a hollow chamber at one end, which in turn contains one egg. Females only oviposit one egg per brood ball, which constitutes the sole amount of food available for larvae to complete larval development (Moczek, 1998). The present study documents that females routinely access brood balls made by other females and replace existing eggs with their own, and investigates the contributions of two variables to the propensity of female brood parasitism: female body size and dung desiccation rate.

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*(i) Brood parasitism and female body size*

Brood-ball size determines offspring body size and fecundity, and larger females produce on average larger brood balls compared with smaller females (Hunt & Simmons, 2002a). Small females may thus have relatively more to gain from parasitising brood balls compared with large females, and may therefore be more likely to engage in brood parasitism. Here the influence of female body size on parasitism rate is investigated.

*(ii) Brood parasitism and dung availability*

Dung pads are ephemeral resource patches available for brood-ball production only as long as dung remains moist enough for the production of brood balls. The duration over which a given dung pad remains useable by *Onthophagus* beetles is heavily dependent upon climatic conditions, in particular temperature and humidity, and can range from a few hours to several days (Moczek *et al.*, 2002). Time expended on tunnelling and brood-ball production in *O. taurus* have been quantified by several studies and require at least several hours (Moczek, 1998; Hunt & Simmons, 2002b, 2004), and adverse climatic conditions such as increased temperatures or evaporation severely reduce dung beetles' ability to convert dung into brood balls. Brood parasitism may thus be a behaviour that females engage in when their own chances of constructing tunnels and brood balls in a timely manner are reduced due to adverse climatic conditions. Here, experimental manipulation of dung desiccation rate is used to investigate its effects on female propensity to parasitise conspecific brood balls.

## Materials and methods

*Source population*

All animals used in this study were derived from a laboratory population of *O. taurus* kept at an insectary at the University of Arizona at 25 °C and a LD 16:8 h cycle. This colony was originally founded by approximately 1500 individuals collected in Durham and Orange Counties, North Carolina. Each individual was only used once.

*Markers*

A marker method was used to mark brood ball and egg identity independent of each other. Rhodamine B (Sigma) was used at a concentration of 20 mg $kg^{-1}$ dung to distinguish eggs produced by females that had access to Rhodamine-marked or unmarked dung. Rhodamine B is a very powerful industrial dye (also known as Basic Violet 10; $C_{28}H_{31}N_2O_3Cl$) and is highly soluble in aqueous solutions and moderately soluble in lipids. Females incorporated Rhodamine B into eggs during brood-ball production, which in turn could be visualised using fluorescence microscopy (Fig. 1a). Vermiculite (Sta-Green) was used at a concentration of approximately 25 g $kg^{-1}$ dung to distinguish brood balls made by females that had access to vermiculite-marked or unmarked dung (Fig. 1b). Vermiculite is an inert mineral commonly used in horticulture. When mixed into dung, females readily incorporate it into brood balls, which is apparent macroscopically. Using these two markers all experiments were then executed in two steps, as described below.

*Experimental design*

*General.* The experimental design uses two groups of females, referred to as cohort 1 and cohort 2 (Fig. 2). For each experiment, 10 cohort 1 females selected at random from the laboratory colony were given access to unmarked dung *ad libitum* for 3 days and allowed to produce brood balls in standard breeding containers (for details on breeding procedures see Moczek, 1998). After 3 days all cohort 1 females were removed, all cohort 1 brood balls collected (28 ± 1.5 averaged over all trials), and divided at random into two groups that were re-buried in two new breeding containers. Ten cohort 2 females were then added to each of these breeding containers and allowed to breed for 2 days. Cohort 2 females were given *ad libitum* access to dung marked with both vermiculite and Rhodamine B. After 2 days all brood balls were collected and assayed immediately for the presence of vermiculite, allowing an unambiguous distinction between brood balls produced by the two different female cohorts. Eggs were stored in 100% glycerol and subsequently examined for the presence of Rhodamine B. If brood parasitism does not occur, all cohort 1 brood balls (vermiculite-free) are predicted to contain eggs free of Rhodamine B, whereas all cohort 2 brood balls (with vermiculite) are predicted to contain Rhodamine B-marked eggs. However, brood parasitism of cohort 1 brood balls by cohort 2 females should be manifest in a mismatch, that is the presence of a Rhodamine B-marked egg inside a vermiculite-free brood ball (Fig. 2).

*Possible limitations.* Pilot breeding experiments were used to optimise the concentration of both vermiculite and Rhodamine B in the dung. Experimenters' ability to identify the origin of brood balls and eggs was then quantified in a blind experiment using a series of anonymised brood balls and eggs of unknown treatment. The concentrations used here permitted unambiguous identification of brood-ball identity in 100% of all brood balls ($n$ = 20 including 10 vermiculite-positive brood balls) as well as eggs ($n$ = 20 including 10 Rhodamine-positive eggs) assayed during these tests. This present method can therefore be considered very sensitive for the detection of certain parasitism events. Once eggs were transferred to 100% glycerol, Rhodamine B staining did not fade and instead remained visible for weeks after the experiment. At the same time, neither marker had any obvious effects on female brood-ball production. Rhodamine B containing eggs hatched and completed larval and pupal development similar to untreated larvae (J. Cochrane & A. P. Moczek, unpublished). However, the present method does not allow for a quantification of brood parasitism *within* cohorts 1 and 2 respectively. Estimates of parasitism frequency derived from this method therefore need to be considered conservative, and real frequencies may be higher.

*Documenting brood parasitism.* To document brood parasitism among *O. taurus* females, 41 (replicate 1) and 26 (replicate

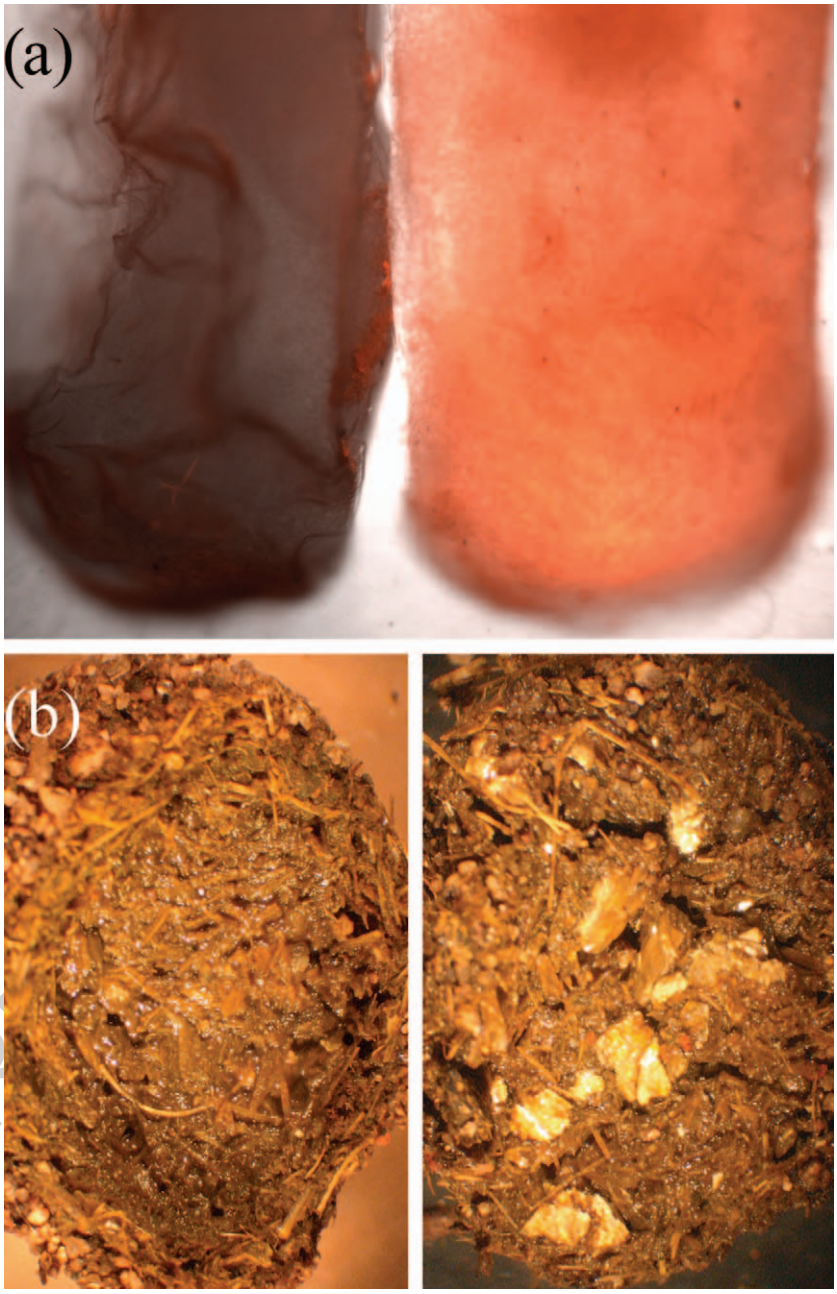


**Fig. 1.** Overview of marker methods used in the present study. (a) Close-up of eggs obtained from a female breeding with untreated dung (left) and Rhodamine B-treated dung (right) viewed through a fluorescence-compound scope. Control eggs show no signs of red fluorescence while Rhodamine B-containing eggs fluoresce bright red. (b) Cross-section through a brood ball made from untreated dung (left) and dung containing vermiculite (right) visible as bright shiny flakes inside the brood ball.



2)cohort 1 brood balls were exposed to possible brood parasitism by 20 cohort 2 females. Cohort 1 brood balls and cohort 2 females were separated randomly over two identical breeding containers. At the end of the experiment all brood balls were recovered and assayed as described above. Three cohort 1 eggs were removed prior to the second stage of the experiment and immediately stored in glycerol to serve as concurrent autofluorescence controls. Identical conditions were used in the low-desiccation control treatment (three replicates total; see below). To increase statistical power in obtaining a baseline value for female brood parasitism, all five replicates were combined in the subsequent statistical analysis.

*Effect of female size*

To explore the effect of female size on propensity to parasitise conspecific brood balls, the same experiment was executed using cohort 2 females that belonged to two different size categories: *large* and *small*. The first and fourth body mass quartile calculated from 56 body mass measurements of randomly selected females was used to define *large* and *small* females respectively. Twenty-five cohort 1 brood balls were allocated to two separate breeding containers, 10 *large* cohort 2 females were then added to one container and 10 *small* cohort 2 females to the other. Brood balls were collected and assayed as described

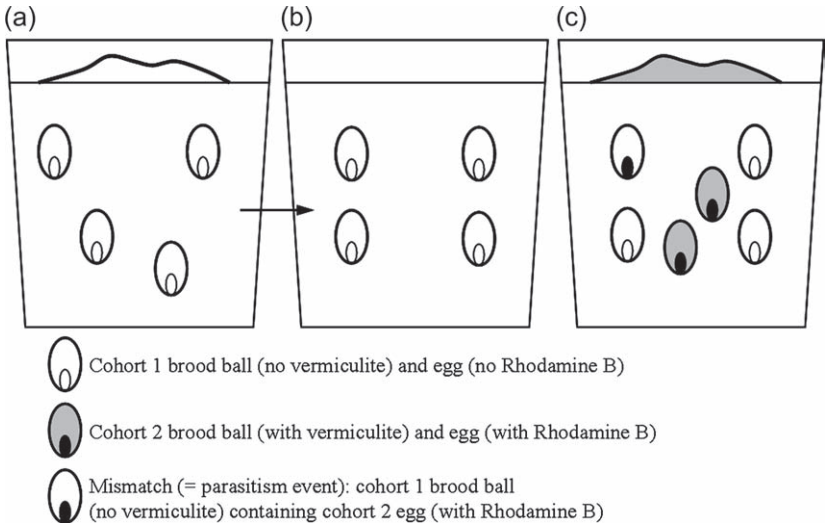


**Fig. 2.** Overview of general experimental design used in the present study. (a) Cohort 1 females are allowed to breed using dung free of vermiculite and Rhodamine B. (b) Brood balls are recovered after 3 days and re-buried in new breeding containers. (c) Cohort 2 females are added and allowed to breed on dung containing both vermiculite and Rhodamine B. All brood balls are collected after 2 days and assayed for presence (absence) of vermiculite in the brood ball and Rhodamine B in the egg. Parasitism events have occurred when Rhodamine B-containing eggs (cohort 2) are found inside a vermiculite-free brood ball (cohort 1).

above and three cohort 1 eggs were removed prior to the second stage of the experiment and immediately stored in glycerol to serve as concurrent autofluorescence controls. This experiment was replicated three times.

*Effect of desiccation rate*

To test the effect of dung desiccation rate on brood parasitism 25–30 cohort 1 brood balls were generated as described above and allocated randomly to two desiccation treatments. The control treatment was identical to standard breeding container set-up (see above), i.e. included a plastic cover to minimise evaporation at an ambient temperature of 25 °C and minimal air movement. The experimental treatment differed in three aspects: absence of protective plastic cover, increased ambient temperature to 30 °C, and increased air movement. Combined, these modifications more than doubled dung desiccation rates (measured as decrease in wet weight over time; J. Cochrane & A. P. Moczek, unpublished) such that most dung provided to cohort 2 females was dry by the end of the experiment. *Onthophagus taurus* routinely encounters similar desiccation regimes in parts of its natural range (A. P. Moczek, pers. observation). Brood balls were collected and assayed as described above. This experiment was replicated three times.

*Categorisation and analysis of parasitism events*

Female *O. taurus* parasitised the breeding efforts of conspecific females through a variety of behaviours. Most strikingly, females replaced conspecific eggs inside brood balls produced by another females with their own egg. In addition, females sometimes added dung to an existing brood ball and then added their own breeding chamber and egg. These behaviours were scored as parasitism with oviposition. In addition, several parasitism events were scored that did not involve oviposition. These included cohort 1 brood balls that were enlarged by cohort 2 females (evidenced by a vermiculite-containing fraction added to an otherwise vermiculite-free brood ball) but were still lacking a new egg chamber and egg by the time the experiment was terminated. Cohort 2 females were also found to regularly burrow into egg chambers of pre-existing brood balls and to destroy the egg inside without replacing the removed egg with their own. Lastly, on occasion, cohort 2 females were found to not only destroy egg chambers and their content, but to also tear apart pre-existing brood balls, again without ovipositing. The last two types of behaviour are referred to as brood-ball raids. Parasitism events with and without oviposition were compared separately and combined across treatments using multiple $\chi^2$-tests. Unless otherwise noted, results are reported as mean percentages (± SE) of brood balls parasitised by cohort 2 females.

## Results

Females parasitised 14 out of a total of 122 brood balls under regular breeding conditions, which amounted to an average parasitism rate of 12.6% (± 2.46; $n = 5$) averaged over the five independent replicates (Fig. 3). The majority of parasitism events detected under these conditions included oviposition of a new egg into a pre-existing brood ball (6.8 ± 2.98%) or the incorporation of an existing brood ball into a new, larger brood ball (5.5 ± 2.34%). Parasitism events without oviposition included four incidences of brood-ball raiding.

Large females were as likely to parasitise conspecific brood balls as were small females [11.1 (± 2%) vs 8.4 (± 4%) for parasitism events with and without oviposition combined]. This time all parasitism events involved re-utilisation of a pre-existing brood ball, and no brood-ball raids were recorded during the experiment. These results reject the hypothesis that female size influences probability of engaging in brood-parasitic behaviour (Fig. 3).

Climatic conditions, however, appeared to influence female behaviour. Individual replicates failed to show a significant effects of desiccation rate on brood parasitic behaviour. However, lumping replicates into a single data set to increase number of

observations revealed a significant difference in the frequency of brood parasitic behaviours across treatments. High desiccation conditions resulted in a significantly higher rate of parasitism events without oviposition. Out of a total of 61 brood balls used over three replicates, 17 became subject to parasitism without oviposition under high desiccation conditions, including 14 raids. This was in contrast to four brood balls out of 57 under control conditions ($\chi^2 = 6.19$; $P < 0.025$). Interestingly, there were no significant differences in parasitism events with oviposition as a function of dung desiccation rate (Fig. 3). These results suggest that female *O. taurus* increasingly locate and utilise conspecific brood balls under adverse climatic conditions.

## Discussion

Female *O. taurus* routinely parasitised a subset of conspecific brood balls under regular breeding conditions. Brood-ball construction requires the excavation of tunnels, transport of dung into tunnels, and the assembly of a brood ball (Emlen, 1994). Time investment into brood-ball construction is typically on the order of several hours per brood ball (Moczek, 1999; Hunt & Simmons, 2002b). Parasitising conspecific brood balls thus allows parasitising females to divert the time and energy invested by other females into the construction of brood balls towards their own reproductive efforts. While interspecific brood parasitism has been documented in dung beetles before (Klemperer, 1980; Gill, 1991; Martin-Piera & Lobo, 1993; Gonzalez-Megias & Sanchez-Pinero, 2003), the current study presents the first evidence that suggests that conspecific females regularly parasitise each others' breeding efforts.

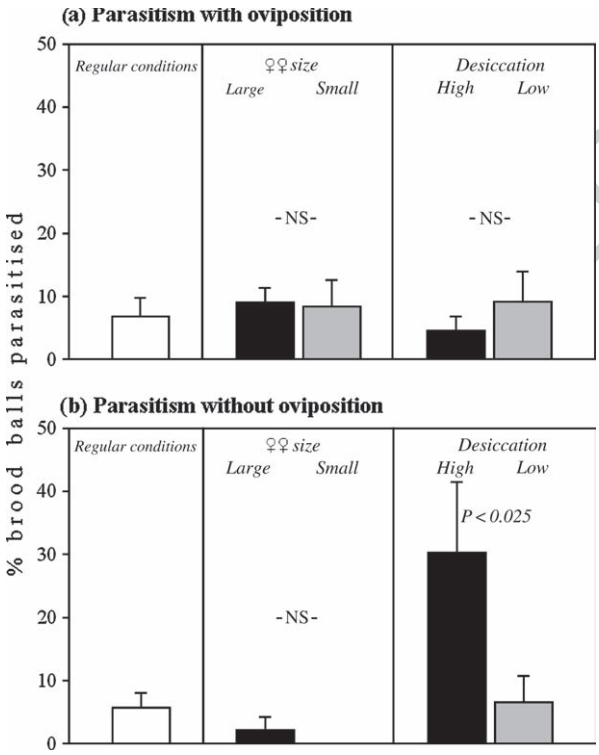


**Fig. 3.** Frequency of parasitism (a) with oviposition and (b) without oviposition of conspecific brood balls by female *Onthophagus taurus*. Data are shown as means + SE. Left: brood parasitism under regular breeding conditions. Centre: effect of female body size on brood parasitic behaviour. Right: effect of dung desiccation on brood parasitic behaviour. Females exposed to high desiccation conditions are significantly more likely to engage in parasitic behaviour without oviposition, including brood-ball raids. See text for further discussion.

Female body size exerted no effect on the propensity to engage in brood parasitic behaviour, and large females engaged in parasitic behaviour as frequently as did small females. Climatic conditions, on the other hand, measurably and significantly affected the frequency of at least some parasitic behaviours. While females oviposited into conspecific brood ball at the same rate independent of desiccation treatment, parasitism without oviposition occurred at a significantly higher rate under high desiccation conditions. The vast majority of these events were brood-ball raids, that is, destruction of the egg chamber or entire brood ball by cohort 2 females, but without a subsequent attempt to oviposit. This behaviour was unexpected, and suggests that females may utilise conspecific brood balls as a food source rather than oviposition opportunity during adverse climatic conditions. Conspecific eggs, in particular, may constitute a particularly valuable food source for *Onthophagus* females. Adult *Onthophagus* possess highly modified, membranous mouthparts with which they filter-feed through the liquid fraction of dung (Halffter & Edmonds, 1982). Adults cannot chew plant matter and rely entirely on fluid food. *Onthophagus* eggs are unusually large for an insect, packed with large quantities of yolk, and easily pierced. The results presented here raise the possibility that females raid brood balls to feed on dung and eggs, and that this behaviour increases in frequency as above-ground dung availability is diminished due to adverse climatic conditions.

Intraspecific brood parasitism by female *O. taurus* represents an alternative reproductive behaviour which, even though undocumented until now, may help explain at least two other aspects of *Onthophagus* behaviour that were noted in previous studies (Cook, 1990; Moczek, 1996, 1999). The first behaviour has been observed in females, which, after underground brood-ball construction has been completed, invariably spend up to several hours re-filling tunnels with previously excavated soil or sand (Moczek, 1996, 1999). Because un-filled tunnels would easily lead conspecific females to already produced brood balls, tunnel re-filling may help to limit parasitism by conspecific females by making it more difficult to locate brood balls underground. The second behaviour occurs in larval *Onthophagus*, which are typical grub-like and largely immobile immature stages adapted to maximise feeding efficiency inside brood balls. Despite their non-threatening appearance, larval *Onthophagus* are extremely aggressive and invariably attack forceps or other larvae placed in their vicinity. If two larvae are placed inside the same brood ball a highly aggressive fight ensues, typically within seconds after initial contact, and invariably results in the quick death of one of the larvae. Such high levels of aggression seem to contradict the notion that the brood ball constitutes a protective shelter isolating its owner from possible aggressive encounters (Halffter & Edmonds, 1982).

The results of the present study suggest that in nature brood balls may in fact exhibit a significant probability of being accessed by conspecific females, and high levels of larval aggression may thus help in deterring brood parasitic behaviour.

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