A cost of maternal care in the dung beetle *Onthophagus taurus*?

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Abstract

Parental care theory assumes that investment in current offspring will trade against future investment. A number of field studies on birds have used clutch size manipulations to demonstrate a survival cost to chick rearing. However, such studies do not account for costs accrued during earlier stages of reproduction because not all aspects of reproductive effort are manipulated by varying the number of nestlings. In this study, we investigate the effect of reproductive effort on female survival in the dung beetle, *Onthophagus taurus*. By experimentally manipulating mating status and dung availability, we demonstrate that virgin females survive longer than mated females and that the survival of mated females was negatively associated with the number of brood masses produced. Using a novel manipulation of the mating system, we separated the effects of egg production and maternal care on female survival. Previously, we have shown that females provisioning with the assistance of a major male provide relatively less care than unassisted females. However, paternal assistance did not alter the number of brood masses produced and hence the amount of reproductive effort that was allocated to egg production. Therefore, our finding that female survival was increased when receiving paternal assistance provides, to our knowledge, the first definitive evidence that maternal care reduces female lifespan. These results are of major importance to theoretical models on the evolution of parental care.

Introduction

Theoretical studies of life-history evolution are based on the assumption that trade-offs exist between important life-history variables (Williams, 1966; Gadgil & Bossert, 1970; Charnov & Krebs, 1974; Schaffer, 1974; Pianka & Parker, 1975; Charlesworth, 1980). The most prominent of these trade-offs involves the cost of reproduction, where an increase in current reproductive effort has a negative effect on future reproduction (Williams, 1966). Consequently, natural selection is unable to simultaneously maximize reproductive effort over consecutive breeding attempts (Williams, 1966; Gadgil & Bossert,

1970; Schaffer, 1974; Charlesworth, 1980). Rather, parents are expected to optimize their reproductive success from current and future reproduction by balancing the fitness benefits of continued investment in current offspring against the resulting costs to future survival and/or fecundity (Williams, 1966). As fitness is a product of lifetime reproductive performance, the trade-off between current and future reproduction has important consequences for the evolution of major life-history traits (reviewed by Bell, 1980; Partridge & Harvey, 1985, 1988; Reznick, 1985; Bell & Koufopanoa, 1986). Although the notion of reproductive costs are a central assumption in the study of life-histories, its empirical basis has been the subject of much controversy (see Bell, 1980, 1986; Reznick *et al.*, 1986).

Much of the controversy arises because of the way reproductive costs have been measured empirically. Traditionally, reproductive costs have been estimated

using a combination of phenotypic and genetic correlations (reviews by Bell, 1980; Reznick, 1985; Bell & Koufopanoa, 1986; Stearns, 1989). Measures based purely on phenotypic correlations may often be confounded by a third variable acting simultaneously on early and late reproduction (Reznick, 1985; Partridge & Harvey, 1985, 1988; Bell & Koufopanoa, 1986). Given the difficulty in assigning causality, it is not surprising that empirical studies based exclusively on phenotypic correlations typically yield mixed results (Reznick, 1985; Partridge & Harvey, 1985, 1988) and have lead several authors to argue that only genetic correlations provide valid evidence of a reproductive cost (Rose & Charlesworth, 1981; Lande, 1982; Reznick, 1985). Therefore, when investigating reproductive costs using phenotypic correlations, it is crucial to perform manipulative experiments in which all other confounding variables are kept constant (Partridge & Harvey, 1985, 1988). A particularly useful approach has been to experimentally manipulate an individuals reproductive effort when it has been randomly assigned to groups across comparable environments (Partridge & Harvey, 1988). Studies adopting this procedure generally reveal costs to subsequent survival and fecundity (Partridge & Harvey, 1985, 1988; Reznick, 1985; Bell & Koufopanoa, 1986; Stearns, 1989), although the extent and magnitude of these costs often critically depend upon the environmental conditions in which they were measured (Bell & Koufopanoa, 1986).

One important life-history trait that is subject to reproductive trade-offs is parental investment (Trivers, 1972; Clutton-Brock, 1991). Broadly defined, parental investment represents any behaviour that increases the fitness of offspring at the expense of investment to future offspring (Trivers, 1972). It is this fundamental trade-off that should determine how parents allocate resources to parental duties in current and future breeding events (Trivers, 1972; Clutton-Brock, 1991). Numerous field studies on birds have used clutch size manipulation to demonstrate that parents often experience a cost as a result of chick rearing (reviewed by Lindén & Møller, 1989). However, such studies have only gone part way in demonstrating a cost of parental care because not all aspects of reproductive effort are manipulated by varying the number of nestlings (Partridge & Harvey, 1988). For example, a number of empirical studies have demonstrated that both mating (Fowler & Partridge, 1989; Chapman, 1992; Chapman *et al.*, 1995; Stutt & Siva-Jothy, 2001) and the production of eggs can be costly to females (Maynard Smith, 1958; Dean, 1981; Winkler, 1985; Partridge *et al.*, 1987; Monaghan *et al.*, 1995; Visser & Lessells, 2001). Therefore, to provide direct evidence for a cost to parental care, manipulative experiments are required that control for costs that may be accrued during earlier reproductive stages (e.g. Visser & Lessells, 2001).

Parental care of eggs or young is typically uncommon among invertebrates (Clutton-Brock, 1991). However, in dung beetles belonging to the genus *Onthophagus*, par-

ental care appears common (Lee & Peng, 1982; Cook, 1988; Sowig, 1996; Hunt & Simmons, 1998a, 2000). During reproduction, members of this genus remove portions of dung from the pad and pack it into the blind end of tunnels constructed beneath the dung pad (Halffter & Edmonds, 1982). A single egg is then deposited into an egg chamber and sealed: one egg and its associated dung provision constitutes a brood mass (Halffter & Edmonds, 1982). Many onthophagine dung beetles are characterized by morphological dimorphisms in which large 'major' males develop enlarged head and/or pronotal horns, whereas small 'minor' males remain hornless (Cook, 1987; Emlen, 1996; Hunt & Simmons, 1997, 1998b). In *O. taurus*, major males provide females with assistance during brood mass construction that results in the production of significantly heavier brood masses (Hunt & Simmons, 1998a, 2000). As the adult body size is largely determined by the quantity of dung provided in the brood mass (Hunt & Simmons, 1997, 2000), parental care has a substantial effect on the phenotype (Hunt & Simmons, 1997) and future adult fitness of offspring (Hunt & Simmons, 2001a).

In this study, we conduct two experiments to directly quantify the survival costs experienced by females providing care. In the first experiment, we simultaneously manipulate mating status (unmated vs. mated) and dung availability to vary a females reproductive effort. We predict that females will have higher survival when dung supply, and thus reproductive opportunity, is limited. However, this experimental design does not allow us to determine whether differences in survival are the result of egg production or maternal care, because a single egg is deposited in each brood mass produced. In the second experiment, we simultaneously manipulate dung availability and the mating system (paternal assisted vs. unassisted) of *O. taurus* to separate the effects of egg production and maternal care on female survival. Females receiving male assistance provide relatively less care than unassisted females (J. Hunt & L.W. Simmons, 2001b), while still producing the same number of brood masses (Hunt & Simmons, 1998b, 2000). Therefore, we predict that male assistance will increase female survival at each dung level and that the magnitude of this difference in survival will be attributable to differences in maternal care.

Materials and methods

General procedure

Onthophagus taurus were collected from Margaret River in the south-west of Western Australia using baited pitfall traps (see Hunt *et al.*, 1999). Beetles were maintained in the laboratory in a mixed sex population for 2 weeks with constant access to fresh cow dung to ensure that females were reproductively mature and had been mated. A total of 500 females were placed in independent

breeding chambers (PVC piping 25 cm in length and 6 cm in diameter), three-quarters filled with moist sand and 250 mL of cow dung, and maintained for 1 week. Chambers were then sieved and brood masses collected. The brood masses were buried in moist sand in individual containers (5 × 5 × 4 cm) and maintained at 25 °C until beetles emerged as adults. On emergence, the pronotum widths of males and females were measured using digital calipers and the horn length of males were measured using an eyepiece graticule in a binocular microscope. Beetles were maintained for 2 weeks in individual containers to ensure they were reproductively mature prior to use in experiments. Containers were three-quarters filled with fresh moist sand and supplied with 50 mL of fresh cow dung. Fresh sand and dung were replenished at the end of the first week.

Experiment 1: Measuring the survival cost of reproduction

To examine the survival costs of reproduction, we simultaneously manipulated the mating status and the quantity of dung available to females provisioning offspring. We randomly assigned 135 unmated females to one of the following three experimental treatments: (i) females were mated with a minor male but provisioned alone, (ii) females were mated with a major male but provisioned alone and (iii) females were maintained as unmated females. In the first two treatments, each female was randomly paired with a male of the appropriate morphology and placed in an independent container (5 × 5 × 4 cm), three quarters filled with moist sand and provided with 50 mL of cow dung. The pair were left for 1 day. Unmated females were maintained in the same way, except a male was not added during this period.

To manipulate the quantity of dung available to females, we randomly allocated the females in each mating status treatment to three dung level treatments. Females were provided with either 75 mL (low), 150 mL (medium), or 300 mL (high) of dung. Each female was established in an independent breeding chamber (PVC piping 25 cm in length and 6 cm in diameter), three quarters filled with moist sand and the appropriate quantity of dung. Chambers were maintained for 10 days at 25 °C and then sieved and brood masses removed. Surviving females were then established for a further 10 days under identical conditions. We continued this process for the entire lifespan of the female. It is important to note that dead females were located in each sampling period and thus all females were of known fate. For each 10-day sampling period, excess sand was removed from brood masses using a dissecting probe and the brood masses were dried to a constant weight at 60 °C. After drying, any remaining sand was removed and all brood masses were counted and weighed to the nearest 0.01 g.

Experiment 2: Measuring the survival cost of maternal care

To examine the survival costs of maternal care, we simultaneously manipulated the mating system and the quantity of dung available to females provisioning offspring. We randomly allocated 90 unmated females to the following two mating system treatments: (i) females were mated with a minor male who remained with the female during brood mass provisioning and (ii) females were mated with a major male who also remained with the female during brood mass provisioning. Only major males assist the female during brood mass provisioning (Hunt & Simmons, 1998a, 2000). Each female was randomly paired with a male of the appropriate morphology, placed in an independent container for a day and maintained as above. Pairs in each treatment were then randomly assigned to the same three dung level treatments and the weight and number of brood masses produced every 10 days over the females lifespan measured as above. Each female was provided with a fresh male every 10-day sampling period to reduce the likelihood that males died whilst paired with females.

Statistical analysis

All data were analysed using two-way ANCOVA's, with mating status (or mating system) and dung level as the main effects and female body size as the covariate. All data was log transformed to meet the underlying assumptions of normality and homogeneity of slopes across treatments (Zar, 1984) but were graphically presented using raw data for ease of interpretation. We used Tukey tests (Zar, 1984) to examine differences between treatment means.

Results

Experiment 1: Cost of reproduction

Both mating status and dung availability significantly influenced the survival of females (two-way ANCOVA: mating status, $F_{2,125} = 73.096$, $P < 0.001$; dung level, $F_{2,125} = 4.62$, $P = 0.01$; mating status × dung level, $F_{4,125} = 1.42$, n.s.; size, $F_{1,125} = 6.88$, $P < 0.01$). Unmated females survived longer than mated females at each dung level and the survival of mated females decreased as dung availability increased (Fig. 1a). Interestingly, the survival cost of reproduction was size dependent, with larger females surviving longer than smaller females. Qualitatively similar results were attained if we analysed the survival data using a Cox regression (mating status, $\chi^2_2 = 54.58$, $P < 0.001$; dung level, $\chi^2_2 = 7.08$, $P < 0.05$; mating status × dung level, $\chi^2_4 = 1.16$, n.s).

As female survival differed across treatments, we included this variable as an additional covariate in our analysis of brood mass weight and number. We limited
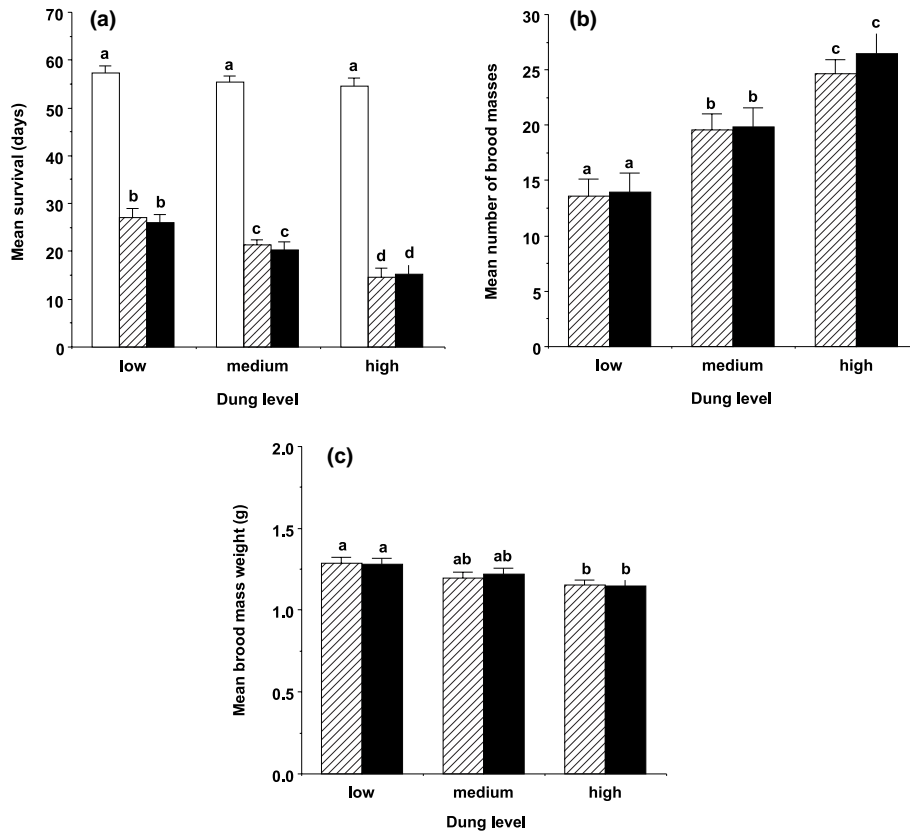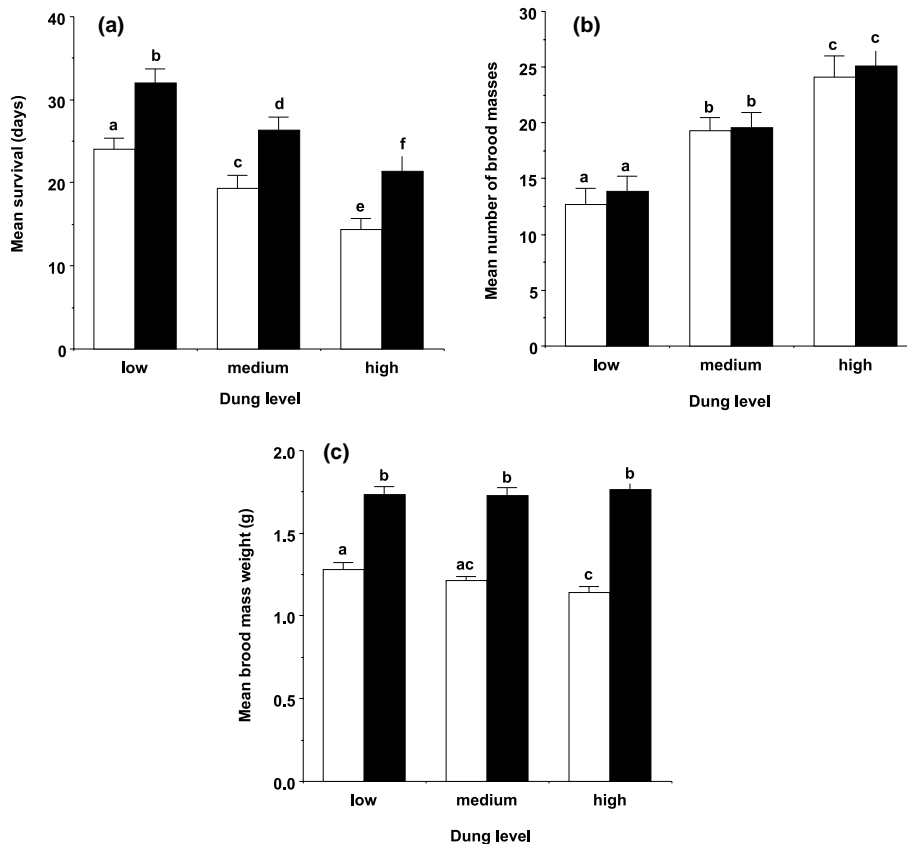
Fig. 1 The mean (±SE) (a) survival, (b) lifetime number of brood masses produced and (c) lifetime weight of brood masses produced by females at each dung level. In each instance, unmated females are represented by clear bars, females mated to minors by hatched bars and females mated to majors by dark bars. Treatments with different letters differ significantly at $P = 0.05$.

our analysis to mated females because unmated females do not produce brood masses. Controlling for differences in female size and survival, the number of brood masses produced was significantly influenced by dung level but not by mating status (mating status, $F_{1,82} = 2.14$, n.s.; dung level, $F_{2,82} = 27.77$, $P < 0.001$; mating status × dung level, $F_{2,82} = 0.73$, n.s.; survival, $F_{1,82} = 1.77$, n.s.; size, $F_{1,82} = 0.38$, n.s). The mean number of brood masses produced by mated females increased with dung availability. We note that there was a trend for females mated to major males to produce more brood masses, and that the power to detect differences because of mating status was low in our experiment ($\beta - 1 = 0.31$) (Fig. 1b). Similarly, the mean weight of brood masses was significantly influenced by dung level but not by mating status (mating status, $F_{1,82} = 0.36$, n.s.; dung level, $F_{2,82} = 8.49$, $P < 0.001$; mating status × dung level, $F_{2,82} = 0.40$, n.s.; survival, $F_{1,82} = 1.61$, n.s.; size, $F_{1,82} = 25.94$, $P < 0.001$). While brood mass weight was positively related to female size, the mean weight of brood masses produced by mated females decreased with increasing dung availability (Fig. 1c).

Experiment 2: Cost of maternal care

Both mating system and dung level had a significant effect on the survival of females (two-way ANCOVA: mating system, $F_{1,83} = 50.06$, $P < 0.001$; dung level, $F_{2,82} = 31.95$, $P < 0.001$; mating system × dung level, $F_{2,83} = 1.13$, n.s.; size, $F_{1,83} = 177.11$, $P < 0.001$). The mean survival of both assisted and unassisted females decreased with dung availability but assisted females survived longer than unassisted females at each dung level (Fig. 2a). The survival of females was again size dependent, with larger females surviving longer than smaller females. Similar results were attained if we analysed the survival data using a Cox regression (mating system, $\chi^2_1 = 26.54$, $P < 0.001$; dung level, $\chi^2_2 = 17.91$, $P < 0.001$; mating system × dung level, $\chi^2_3 = 1.24$, n.s.).

The number of brood masses produced was significantly influenced by dung level but not mating system (mating system, $F_{1,82} = 2.09$, n.s.; dung level, $F_{2,82} = 168.52$, $P < 0.001$; mating system × dung level, $F_{2,82} = 1.37$, n.s.; survival, $F_{1,82} = 0.47$, n.s.; size, $F_{1,82} = 0.005$, n.s.). The mean number of brood masses produced by assisted and unassisted females increased with dung availability

Fig. 2 The mean (±SE) (a) survival (b) lifetime number of brood mass produced and (c) lifetime weight of brood masses produced by male-female pairs at each dung level. In each instance, females paired with a minor male are represented by clear bars and females assisted by a major male by dark bars. Treatments with different letters differ significantly at $P = 0.05$.

(Fig. 2b). The mean number of brood masses produced by females did not differ significantly with regard to mating system. Although the power to detect differences between mating system treatments was low ($\beta - 1 = 0.49$), previous studies have likewise failed to revealed any influence of male morph on the number of brood masses produced (Hunt & Simmons, 1998a, 2000). Mating system but not dung level significantly influenced the mean weight of brood masses produced and there was a significant interaction (mating system, $F_{1,82} = 435.31$, $P < 0.001$; dung level, $F_{2,82} = 0.31$, n.s.; mating system × dung level, $F_{2,82} = 3.71$, $P < 0.05$; survival, $F_{1,82} = 0.01$, n.s.; size, $F_{1,82} = 4.63$, $P < 0.05$). Cooperative pairs produced heavier brood masses than unassisted females at all dung levels (Fig. 2c). However, while mean brood mass weight decreased with dung availability in unassisted females, mean brood mass weight produced by cooperative pairs was unaffected by dung availability.

Discussion

The results of this study add to the already extensive list of empirical studies demonstrating a cost of reproduction

(reviewed by Reznick, 1985; Bell & Koufopanoa, 1986). By far the most widely used experimental technique to measure reproductive costs has been the manipulation of mating status by exposing one group of females to males and preventing the other group from mating (Bell & Koufopanoa, 1986). Amongst insects, the results of such experiments are generally clear; unmated females typically survive longer than mated females (reviewed by Reznick, 1985; Bell & Koufopanoa, 1986). Indeed, the results obtained for *O. taurus* are in general agreement with the notion that unmated females survive longer than mated females. However, assigning direct causality in manipulative experiments is often difficult because unmated and mated females not only differ in their mating status but also frequently differ in the reproductive effort they expend (Reznick, 1985; Bell & Koufopanoa, 1986). Therefore, the lower survival of mated females in our study may be caused either by the direct effects of mating on survival and/or because mating increases reproductive effort, which in turn directly reduces survival. A number of empirical studies have demonstrated that both reproductive effort (Maynard Smith, 1958; Callow & Woolhead, 1977; Hirshfield, 1980;

Dean, 1981; Winkler, 1985; Partridge *et al.*, 1987) and mating (Fowler & Partridge, 1989; Chapman, 1992; Chapman *et al.*, 1995; Stutt & Siva-Jothy, 2001) can have independent negative effects on female survival.

However, in this study we provide two lines of evidence suggesting that the reduced survival of mated females is the direct result of an increased reproductive effort rather than a cost of mating. Firstly, the survival of females with restricted access to males (Fig. 1a) and with constant access to minor males (Fig. 2b) did not differ markedly suggesting that continued courtship and mating does not significantly reduce female survival. Secondly, our experimental manipulation of dung availability influenced female reproductive effort, with the number of brood masses produced being directly related to dung availability. As predicted, the increased reproductive effort with dung availability was associated with reduced female survival. While it is possible that this observed trade-off is the result of dung availability independently affecting both reproductive effort and survival without direct causation, this is unlikely given that the survival of unmated females was not influenced by dung availability. Thus, our experiments provide strong evidence for a direct causal relationship between reproductive effort and survival. However, as a single egg is deposited in each brood mass, this experiment alone is unable to distinguish between a cost of egg production and a cost of maternal care via brood mass provisioning.

A number of empirical studies have attempted to measure the reproductive costs associated with providing parental care. By far, the majority of studies have focused on the experimental manipulation of clutch sizes in birds and have yielded mixed results (reviewed by Lindén & Møller, 1989). In some species, parents provisioning enlarged broods experience reduced survival (Askenmo, 1979; Reid, 1987), while in others no difference in parental survival was detected (DeSteven, 1980; Nur, 1984a,b; Røskaft, 1985; Hegner & Wingfield, 1987; Gustaffson & Sutherland, 1988; Orell & Koivulla, 1988; Pettifor *et al.*, 1988). In experiments involving the experimental manipulation of clutch size, it is frequently assumed that differences in the survival of parents between treatments largely reflects differences in parental care after offspring have hatched. Therefore, the findings of many studies may be potentially confounded by reproductive effort that is expended during incubation or the production of eggs. For example, in a recent study, Visser & Lessells (2001) experimentally manipulated the costs of egg production and incubation in a brood size enlargement experiment, demonstrating that both reproductive stages reduce female survival and are likely to play important roles in reproductive decisions.

In this study, we provide a novel manipulation of the mating system in *O. taurus* to demonstrate that increased maternal care reduces female survival, independent of egg production. Females provisioning offspring with male assistance provide relatively lower levels of care than unassisted females (J. Hunt & L.W. Simmons, in review). Therefore, although male assistance leads to the production of significantly heavier brood masses, a females independent contribution to parental care decreases when assisted. However, paternal assistance does not effect the number of brood masses produced and hence all females invest the same into egg production (this study; Hunt & Simmons, 1998a, 2000). Therefore, the finding that female survival is increased when receiving paternal assistance provides one of the first studies to demonstrate that maternal care reduces lifespan and is thus of major importance to theoretical models on the evolution of parental care (Clutton-Brock, 1991).

The results of this study also demonstrate that the observed relationship between reproductive effort and survival is dependent on female size. Larger females, whether provisioning with or without male assistance, had consistently higher survival than smaller females. Furthermore, this relationship was independent of egg production because the number of brood masses produced by a female was unrelated to body size in each instance. Therefore, although brood mass weight increases with female size (this study, Hunt & Simmons, 2000), larger females do not experience the same reductions in survival per unit of reproductive effort. This result may be expected if larger females are more efficient at constructing a brood mass. Our recent behavioural work suggests that this may indeed be the case with larger females provisioning more dung per unit time than smaller females (J. Hunt & L.W. Simmons, in review). However, the exact cause of this relationship warrants further investigation. Nevertheless, our results provide a further fitness advantage in producing large offspring in this species (see also Hunt & Simmons, 2001a).

Of interest is the finding that despite the decline in female survival with dung availability, females consistently produced more brood masses. Thus, females in this species appear to maximize current reproductive effort with little regard for future survival and by doing so maximize their reproductive fitness. Semelparity may be expected if the prospects for future survival and/or reproduction are limited (Bell, 1980; Sibley & Calow, 1983; Partridge & Harvey, 1988), as is likely to be the case for dung beetles that utilize an ephemeral dung resource for reproduction (Hanski & Cambefort, 1991). Similar results were attained in Reid's (1987) study on glaucous gulls (*Larus hyperboreus*) where female survival declined with an experimentally increased brood size but this higher mortality was associated with an increased number of chicks being fledged. Such findings lead to the obvious question, does the reduced survival of females represent a cost of reproduction? Whether a reduced survival represents a significant reproductive cost in *O. taurus* will depend on the relative strengths of the covariances between reproductive effort, survival and reproductive fitness. The results of our phenotypic manipulation suggests that the benefits of increasing

reproductive effort exceed the costs to survival. While phenotypic correlations determine the pattern of covariation presented to natural selection, unless the trade-off has a genetic basis it will not represent a significant evolutionary cost (Stearns, 1989). Clearly, future studies examining the genetic basis of this phenotypic trade-off are required to determine whether reduced survival represents an evolutionary cost in *O. taurus*.

Acknowledgments

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