Population density, body size, and phenotypic plasticity of brood size in a burying beetle

J. Curtis Creighton
Department of Biological Sciences, Purdue University Calumet, Hammond, IN 46323, USA

Theory predicts that organisms living in heterogeneous environments will exhibit phenotypic plasticity. One trait that may be particularly important in this context is the clutch or brood size because it is simultaneously a maternal and offspring characteristic. In this paper, I test the hypothesis that the burying beetle, *Nicrophorus orbicollis*, adjusts brood size, in part, in anticipation of the reproductive environment of its adult offspring. *N. orbicollis* use a small vertebrate carcass as a food resource for their young. Both parents provide parental care and actively regulate brood size through filial cannibalism. The result is a positive correlation between brood size and carcass size. Adult body size is an important determinant of reproductive success for both sexes, but only at higher population densities. I test three predictions generated by the hypothesis that beetles adjust brood size in response to population density. First, average adult body size should vary positively with population density; second, brood size on a given-sized carcass should be larger (producing more but smaller young) in low-density populations than in high-density populations. Third, females should respond adaptively to changes in local population density by producing larger broods when population density is low and small broods when population density is high. All three predictions were supported using a combination of field and laboratory experiments. These results (1) show that brood size is a phenotypically plastic trait and (2) support the idea that brood size decisions are an intergenerational phenomenon that varies with the anticipated competitive environment of the offspring. Key words: brood size, burying beetle, clutch size, maternal effects, *Nicrophorus*, phenotypic plasticity, transgenerational phenotypic plasticity. [Behav Ecol 16:1031–1036 (2005)]

Many female insects lay their eggs on discrete resource patches. Examples include butterflies ovipositing on host plants, beetles and flies on dung pats, parasitoid wasps on insect hosts, and many insects on seeds or fruit. For these females, the decision of how many young to produce can profoundly affect the future fitness of their young due to density-dependent competition for food (Mock and Parker, 1997). Consequences of larger broods include decreased survivorship and/or smaller adult body size of offspring (Hardy et al., 1992; Taylor, 1988; Vet et al., 1994; Wilson, 1994). Body size, in turn, affects components of fitness such as fecundity, competitive ability, and life span (e.g., Ellers et al., 1998; Fincke, 1992; Hardy et al., 1992; Ottonen, 1988; Partridge et al., 1987; Sigurjónsdóttir and Parker, 1981; Vet et al., 1994; Visser, 1994; West et al., 1996). Theoretical models that have addressed optimal offspring size predict that larger offspring should be produced if environmental conditions are poor whether due to competition or limited resources (Parker and Begon, 1986; Sibley and Calow, 1983).

When environmental conditions vary across time, selection can result in the evolution of adaptive phenotypic plasticity: a condition where genotypes produce different phenotypes depending on environmental conditions (Stearns, 1992). Because optimal clutch size is a function of female condition and effort and of offspring fitness (Godfray, 1987; Wilson and Lessells, 1994), environmental conditions experienced by the mother as well as the future environmental conditions experienced by her offspring have an effect on clutch size (Mangel et al., 1994). As a consequence, clutch size is expected to be a trait where phenotypic plasticity or maternal effects would be especially prevalent. Maternal effects that change offspring phenotype and are based on environmental cues that predict offspring environment are known as transgenerational phenotypic plasticity (Mousseau and Dingle, 1991).

One factor that affects clutch size is population density (which affects the intensity of intraspecific competition; Parker and Begon, 1986). Intraspecific competition for reproductive resources can influence clutch size decisions with females predicted to produce smaller clutches when the risk of superparasitism is high (Ives, 1989; Strand and Godfray, 1989). The parasitoid wasp, *Aphaereta minuta*, produces smaller clutches when introduced singly to a patch containing unparasitized hosts if first kept in simulated crowded conditions versus kept alone (Visser, 1996). Similarly, the cowpea weevil, *Callosobruchus maculatus*, produces larger eggs when kept under crowded conditions (Kawecki, 1995). Both are examples of transgenerational phenotypic plasticity based on the predicted larval conditions of the offspring. In this paper, I test the hypothesis that the burying beetle, *Nicrophorus orbicollis*, adjusts its brood size, in part, in anticipation of the reproductive environment of its adult offspring.

Members of the genus *Nicrophorus* (Coleoptera: Silphidae) use small vertebrate carcasses as a food resource for their young. Competition among adults for carcasses can be intense (Müller et al., 1990a; Trumbo, 1990b; Wilson and Fudge, 1984), and in *N. orbicollis*, a carcass is usually monopolized by a single pair of beetles (Creighton, 1995; Trumbo, 1992). Larger *N. orbicollis* usually are victorious over smaller individuals in fights for possession of unburied carcasses (Creighton, 1995). Similar trends have been observed in other *Nicrophorus* species (Bartlett and Ashworth, 1988; Müller et al., 1990a; Ottonen, 1988). After a carcass is buried, intruders are more likely to take over a carcass when they are larger than the resident (Robertson, 1993; Scott and Gladstein, 1993).

While the advantage of large size in individual interactions is clear, at the population level, the advantage of large size is dependent on population density (Eggert and Müller, 1997), which indirectly reflects the intensity of competition for resources. In two studies done with low-density populations of...
N. orbicollis, either no differences in body size between breeding and nonbreeding individuals (Trumbo, 1990c) or differences only for males (Scott and Tranieri, 1990) were found. In contrast, in one study done with a relatively high-density population of N. orbicollis, the mean body size of reproducing beetles was significantly larger than the mean body size in the adult population as a whole (Creighton, 1995). Similarly, in dense populations, Nicrophorus vespilloides reproducing on carcasses were larger than individuals captured in pitfall traps (Otronen, 1988). Finally, the number of N. orbicollis captured in pitfall traps missing tarsal segments or antennae (most likely lost as a result of contests for carcasses) was much higher in dense populations (Trumbo, 1990c).

Both sexes of N. orbicollis provide parental care. Eggs are laid in the soil surrounding the carcass, and the young usually arrive on the carcass 5–7 days after carcass burial. Males usually disperse from the carcass after the young reach the third and final instar stage, approximately 10 days after carcass burial. Females stay until the young disperse into the soil to pupate, which can be 17 days after carcass burial (Scott and Tranieri, 1990; Trumbo, 1991). Care is extensive and includes burial and preservation of the carcass, defense of the young from predators, and regurgitation of food to the young (Fetherston et al., 1990; Scott; 1990; Trumbo, 1990b). Both sexes are fully capable of all parental behaviors in the absence of the other member of a pair (Fetherston et al., 1990).

In all burying beetles studied to date, including N. orbicollis, there is a negative relationship between offspring body size and brood size on a given-sized carcass and a positive relationship between brood size and carcass size (Bartlett, 1987; Creighton; 1995; Scott and Tranieri, 1990; Trumbo, 1992; Wilson and Fudge, 1984). The number of offspring is controlled directly at two points. First, females reduce the number of eggs laid on very small carcasses (Müller et al., 1990b). Second, both parents regulate brood size through filial cannibalism (Bartlett, 1987; Trumbo, 1990a; Wilson and Fudge, 1984; Creighton JC, personal observation). Thus, parents have direct control over the number and size of the offspring they produce. In this paper, the focus is on the number of offspring raised. For this reason, both mechanisms of regulation could be involved, and I will refer to the term brood size when discussing the number of offspring raised.

Population densities of burying beetles vary considerably over space and time (Scott, 1990; Scott and Gladstein, 1993; Smith et al., 2000; Creighton JC, personal observation). As the importance of large body size in interspecific competition changes with population density, females would be expected to alter brood sizes as well. Specifically, if females respond adaptively to changes in population density, they should produce large broods of small young at low density and small broods of large young at high density. In contrast, if females do not respond adaptively, brood size should not vary with population density. I evaluate the hypothesis of adaptive plasticity by testing the following three predictions: (1) across populations, average body size of adult beetles should be positively correlated with population density; (2) average brood size should be larger in low-density populations than in high-density populations, given the same-sized carcass; and (3) within a population, females should produce larger broods of small young when population density is low and smaller broods of larger young when population density is high.

**METHODS**

**Source of animals**

Burying beetles were captured using pitfall traps baited with rotted chicken as outlined below. Laboratory mouse (Mus musculus) carcasses used in this study were obtained from the Animal Care Facility at the University of Oklahoma. Mice were killed using CO2 and kept frozen until used in experiments.

**Population density and body size**

During the summers of 1991, 1993, 1994, and 1998, baited pitfall traps were used to capture N. orbicollis at five sites located in eastern and central Oklahoma and one site in central Arkansas. The number of plots placed at each site ranged from 5 to 60 and was surveyed as part of a project on the ecology of the endangered American burying beetle (Nicrophorus americanus). Plots were located in a diversity of habitats and were composed of eight pitfall traps spaced 20 m apart. Each trap was kept in the field for three nights and covered with an elevated plastic dome to prevent the loss of beetles from heat stress or drowning (see Lomolino and Creighton, 1996; Lomolino et al., 1995, for additional details). Traps were checked daily before 1000 h (Daylight Savings Time), and the number of N. orbicollis captured was recorded.

Population density of N. orbicollis at each of the six sites was calculated as the number of individuals captured per functional trapnight. Functional trapnights were calculated as total number of trapnights at each site minus the number of traps that were completely disturbed and minus 0.5 times the number of traps missing bait but otherwise intact when checked the next morning. Pronotum width of a subset of captured beetles (measured to the nearest 0.1 mm with digital calipers) was used as an estimate of beetle body size.

**Brood sizes in populations with low and high beetle density**

During June 1992 at the Cherokee Wildlife Management Area (a site previously determined to have a relatively high density of N. orbicollis) and June 1993 and 1994 at the Lexington Wildlife Management Area (a site previously determined to have a relatively low density), I captured beetles using baited pitfall traps. On the same day as their capture, I placed pairs of N. orbicollis in the field on 10-, 20-, or 30-g carcasses. The carcass was then covered with a 26 × 20 × 15-cm plastic tub. If no beetle activity was noted on a carcass within 12 h, I placed a second pair of beetles on that carcass.

After 2–7 days, I exhumed each carcass and, if present, recorded the number of young. I then placed the carcass back in the ground and covered it with leaves, soil, and a small rock. The rock could then be easily removed during subsequent observations. During these observations, I recorded the condition of the carcass, presence of parents, and number and instar stage of young. There is no relationship between the number of third instar larvae (which is the final instar stage) and probability of survival to eclosion (Creighton JC, in preparation), and filial cannibalism occurs during the first two instar stages. For these reasons, the number of third instar present prior to dispersal was the brood size used for data analysis. Statistical analyses were completed using the SYSTAT statistical package (SYSTAT, 1992). Because of the a priori prediction that brood size from the high-density population would be smaller than the low-density population, all comparisons were one tailed.

**Phenotypic plasticity of brood size**

In June 1996, I collected N. orbicollis at the Cherokee Wildlife Management Area using baited pitfall traps to test the prediction that burying beetles should adjust brood size according to perceived population density. Individual pairs were placed in 3.84 soil-filled glass jars with a 20-g carcass. I then placed the jars in an environmental chamber kept on a 14:10 h light:dark
cycle at 21°C. The young resulting from these pairings were then used for this experiment.

Immediately after eclosion, females were randomly assigned to one of the two treatments: to the “low-density” population treatment by being placed singly in a 3.84 glass jar or to the “high-density” population treatment by being placed in groups of five in 3.84 jars. I further isolated each replicate by wrapping a piece of paper around the outside of the jars. All jars were supplied with moist paper towels and ad libidum food (1- to 2-g pieces of chicken liver) and were placed in an environmental chamber kept on a 14:10 h light: dark cycle at 21°C. The males were kept in groups of five under the same conditions in a separate environmental chamber. Three weeks after eclosion (the approximate length of time necessary to reach sexual maturity), pairs were placed in 3.84 soil-filled glass jars with a 20-g carcass. After 2 days (before the eggs are laid but after mating takes place), all males were removed from the jars. After 10 days, I checked each carcass and counted the number of larvae present. I measured the pronotum width of each young immediately after eclosion. Statistical analyses were done using the SYSTAT statistical package (SYSTAT, 1992), and all comparisons were one tailed.

RESULTS

Average population body size ranged from 6.41- to 7.17-mm pronotum width (Table 1). Consistent with prediction one, body size was positively correlated with beetle density across the populations sampled (r = .78, p < .05, n = 6 populations; Figure 1).

Broods raised on 10-, 20-, and 30-g carcasses increased in size with increasing carcass size in both the low-density and high-density populations (low-density population: F1,39 = 82.35, p = .08, p < .001; high-density population: F1,35 = 46.71, p < .001). Broods raised on 10- and 30-g carcasses in the low-density population were significantly larger than broods raised on the same-sized carcasses in the high-density population (10-g carcasses: t = 1.71, df = 29, p < .05; 30-g carcasses: t = 4.25, df = 33, p < .001; Figure 2). Brood size on 20-g carcasses in the low-density population was larger than brood size in the high-density population but not significantly (t = 0.45, df = 30, p = .33; Figure 2).

Phenotypic plasticity of brood size

_N. orbicollis_ adjusts its brood size in response to population density: females kept in the simulated low-density population raised significantly more young on a 20-g carcass than did females kept in the simulated high-density population (t = 4.05, df = 41, p < .001; Figure 3A). As a consequence, the offspring resulting from broods in the low-density treatment were significantly smaller than offspring from the high-density population (t = -2.40, df = 41, p < .05; Figure 3B).

DISCUSSION

Burying beetles are relatively unique among insects in that they regulate brood size through filial cannibalism after the eggs hatch. The result is an increase in brood size as carcass size increases. This behavior also provides an opportunity to adjust brood size, depending on the predicted reproductive environment of their adult offspring. In this paper, I tested three predictions generated by this hypothesis. Consistent with the hypothesis’s predictions, average body size of _N. orbicollis_ populations were positively correlated with population density. Also, average brood size was smaller in a relatively high-density population compared to a relatively low-density population. These two results could come about through, at least two non-exclusive, selective scenarios. First, natural selection could select for brood sizes that reflect populations consistently at different densities. These changes would be genetically based. Second, changes in brood size and the resulting body size could come about through maternal effects. In strong support

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**Table 1**

Summary of population density and body size (mean ± SE) data

<table>
<thead>
<tr>
<th>Site</th>
<th>Number captured</th>
<th>Functional trapnights</th>
<th>Densitya</th>
<th>Body sizeb</th>
<th>ne</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cookson WMA⁴</td>
<td>89</td>
<td>85.5</td>
<td>1.04</td>
<td>6.86 ± 0.13</td>
<td>29</td>
</tr>
<tr>
<td>Lexington WMA</td>
<td>110</td>
<td>199.5</td>
<td>0.55</td>
<td>6.41 ± 0.07</td>
<td>82</td>
</tr>
<tr>
<td>Spavaw WMA</td>
<td>522</td>
<td>256.0</td>
<td>2.04</td>
<td>6.82 ± 0.08</td>
<td>78</td>
</tr>
<tr>
<td>Latimer County, OK</td>
<td>110</td>
<td>291.5</td>
<td>0.78</td>
<td>6.53 ± 0.06</td>
<td>107</td>
</tr>
<tr>
<td>Camp Gruber, OK</td>
<td>3364</td>
<td>1930.0</td>
<td>1.74</td>
<td>7.17 ± 0.13</td>
<td>26</td>
</tr>
<tr>
<td>Camp Robinson, AR</td>
<td>623</td>
<td>979.5</td>
<td>0.64</td>
<td>6.58 ± 0.08</td>
<td>58</td>
</tr>
</tbody>
</table>

⁴ WMA refers to Wildlife Management Area.

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*a* Density measured as number beetles/functional trapnight.

*b* Body size as pronotum width (mm).

*c* Represents the number of beetles measured to estimate mean population body size not the total number of beetles captured.
of this scenario, females kept under a simulated low-density population produced significantly larger broods of smaller offspring than did females from the same population that were kept under simulated high-density conditions.

Several studies have demonstrated plasticity of egg and/or clutch size. Visser (1996) demonstrated a similar pattern of smaller clutches in a simulated high-density population in the parasitoid wasp, A. minuta. After emergence, female wasps were mated and then kept for 4 days either in groups of four or alone. Wasps were then introduced singly into a patch containing 20 hosts. Visser (1996) found that females produced, on average, 0.74 fewer eggs (a 14% decrease) when first kept in groups. These results were interpreted as a response by females to an increased probability of superparasitism. Similarly, Kawecki (1995) found that cowpea weevil (C. maculatus) females produced larger eggs under crowded conditions.

However, Wilson (1994) found no effect of population density on clutch size in C. maculatus. Wilson (1994) kept females isolated, with one female, or with four other females for 24 h and then presented them with a seed on which to oviposit. There were no significant differences in the size of clutch laid by females among the three treatments. Fox et al. (1997) showed that the seed beetle, Stator limbatus, will produce larger eggs (and smaller clutches because of a size-number trade-off) when ovipositing on low-quality seeds.

Fox et al. (1997), Kawecki (1995), and Visser (1996) demonstrate that clutch size and/or egg size is a phenotypic plastic trait. In the later two studies, plasticity was an adaptive response based on the potential number of females ovipositing on a finite resource and the resulting expected environment of developing offspring. The larval environment does not appear to be the selective arena related to brood size plasticity in N. orbicollis. Because burying beetles actively regulate brood size through filial cannibalism after eggs hatch, observed brood sizes do not necessarily reflect the number of eggs laid, even if multiple females lay eggs on a carcass. For example, single female N. orbicollis breeding on a 20-g carcass produce an average of 22.7 eggs and, through filial cannibalism, reduce brood size (Creighton JC, unpublished data). In the study reported here, observed brood sizes on 20-g carcasses were 11.1 and 10.5 larva for low- and high-density populations, respectively. While overproduction of eggs is the norm on small carcasses, the question as to why female burying beetles typically produce more eggs than they anticipate raising remains unanswered (see Mock and Parker, 1997, for a recent review of hypotheses addressing the general issue of offspring overproduction). Thus, phenotypic plasticity in brood size demonstrated by female N. orbicollis appears to be in anticipation of the expected competitive conditions experienced by their adult offspring.

Pienaar and Greeff (2003), working with nonpollinating fig wasps, tested the hypothesis that adult females were sensitive to the potential breeding environment of their offspring. They developed a game theoretic model that allowed females ovipositing on discrete food patches to choose the sex ratio and male mating tactics (whether nondispersing fighter that mates in its natal patch or flightless disperser that disperses to the leaves of its tree before mating) of their offspring, based on the number of ovipositing females present. The predictions generated by their model were then tested using data from three species of fig wasp from the genus Otitesella. The results were consistent with the hypothesis that females were determining the sex ratio and the type of male morph based on population densities at individual figs (Pienaar and Greeff, 2003). These results suggest that females are making egg-laying decisions based on the reproductive environment of their adult offspring.

A number of studies have shown that avian clutch size exhibits strong maternal effects. For example, clutch size is dependent on multiple environmental factors including quality or condition of the female (Drent and Daan, 1980; Perrins and Moss, 1975), quality of the breeding habitat (Hugstedt, 1980), and rate of nest predation (Julliard et al., 1997). Quality of habitat, as measured by carcass size in the present study, is an important factor determining brood size in N. orbicollis.

The brood size decisions made by female N. orbicollis are, in part, dependent on the predicted competitive environment of their adult offspring. As a result, game theory can be used to describe the dynamics of the system. Recently, Mesterton-Gibbons and Hardy (2004) developed a game theoretic model patterned after a parasitoid wasp (Goniozus nephantidis) with a similar biology to N. orbicollis. In both systems, females guard a finite reproductive resource, and body size is an important determinant of the outcome of contests. In the model,

![Figure 2](http://beheco.oxfordjournals.org/) Mean (+SE) brood size on 10, 20, and 30-g carcasses of beetles from low-density (open bars) and high-density (filled bars) populations. Numbers above error bars represent sample sizes.

![Figure 3](http://beheco.oxfordjournals.org/) Mean (+SE) brood size (A) and offspring body size (B) for young raised on 20-g carcasses in simulated low-density (open bars) and high-density (filled bars) populations. n<sub>low</sub> = 21, n<sub>high</sub> = 22.
Mesterton-Gibbons and Hardy (2004) evaluated the effect of body size–dependent contests on clutch size decisions. They assumed that larger individuals develop from smaller clutches and larger individuals are successful over smaller individuals in contests for a limited resource. My results strongly support the model prediction that clutch size should be polymorphic with clutch size decreasing when contests occur more frequently.

Clutch size is simultaneously a mother and offspring characteristic (Sinervo, 1991). As a result, in variable environments where conditions experienced by parents and their offspring can be very different, organisms are predicted to exhibit transgenerational phenotypic plasticity (Mangel et al., 1994; Mousseau and Dingle, 1991). Maternal manipulation of offspring might be expected when environmental cues experienced by the mother are good predictors of conditions that will be experienced by her offspring. One potential variable condition is population density and its effect on intraspecific competition. Theoretical models have demonstrated the important role population density can play on female clutch and egg size decisions (Ives, 1989; Parker and Begon, 1986; Strand and Godfray, 1989). There is now growing experimental support that a range of female insects are sensitive to how density can influence the expected environment of their larval (Fox et al., 1997; Kawecki, 1995; Visser, 1996) and adult offspring (this study).

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