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ORIGINAL ARTICLE

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Insights into the costs of complex maternal care behavior in the burrower bug (*Sehirus cinctus*)

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Abstract The evolution of life history characters, including parental care behaviors, depends on costs and benefits. When offspring can influence parental behaviors, parent-offspring conflict over parental care can occur, but only if these parental behaviors are costly. Mother burrower bugs (*Sehirus cinctus*) exhibit extended and complex care of offspring. Mothers guard eggs until hatching and then attend and provision offspring for approximately 2 weeks after hatching. Using four experimental treatments, we attempted to identify the costs associated with specific components of these behaviors. Under laboratory conditions, egg guarding increases interclutch interval, but provisioning does not appear to be very costly. We discuss additional ecological factors that may be important in mediating provisioning costs under natural conditions. Through analysis of individual maternal performance, we find no evidence for trade-offs between successive clutches. These data suggest that variation in overall condition may obscure variation in allocation strategies.

Keywords Allocation strategies · Burrower bug · Egg guarding · Maternal care · *Sehirus cinctus*

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Introduction

In some species, maternal care behaviors are an important aspect of life history (Clutton-Brock 1991; Stearns 1991). Although maternal care can increase offspring growth and/or survival, such behaviors may be expensive to the parent with respect to other aspects of life history. For example, in birds, rearing of offspring has been found to have costs such as reduced future clutch size (Gustafsson and Sutherland 1988) and increased parental mortality (Ried 1987). The evolution of any reproductive trait depends on the balance of costs and benefits; natural selection favors those trait values that maximize an individual's lifetime reproductive success (Williams 1966). Indeed, a comparative analysis suggests that both costs and benefits are important in understanding the diversity of parental care among bird taxa (Owens and Bennett 1997). In the case of parenting behavior, the benefits to one generation must balance the cost paid by the other. It is these costs to parents that are expected to limit the evolution of extended parental investment and care behavior (Trivers 1974). A more sophisticated understanding of parental care involves recognizing that both costs and benefits can be state-dependent so that the optimal behavior depends on an individual's state (McNamara and Houston 1996). Nonetheless, a reasonable first-step to understanding parental care focuses on measuring the *average* costs and benefits of care.

Maternal care may consist of multiple components or phases. In oviparous organisms, such as birds and most arthropods, maternal care may consist of an egg tending phase as well as a post-hatching care phase (Clutton-Brock 1991). Different care components can differ in both the magnitude of cost as well as the form of cost (i.e., different maternal care behaviors can trade-off with different life history traits). For example, in the subsocial spider *Coelotes terrestris,* egg guarding increases the time between clutches whereas provisioning offspring drastically reduces maternal weight and the probability of producing a subsequent clutch (Gundermann et al. 1997).

When considering the evolution of multiple components of maternal care it is important to recognize the relationship among different care behaviors for two reasons. First, some elements of care may occur at the expense of others. For example, in several species of birds increased investment in egg incubation has been inferred to reduce provisioning behavior (Heaney and Monaghan 1996; Cichon 2000; Ilmonen et al. 2002). Second, some care behaviors may only occur if other behaviors already exist. For example, in oviparous organisms the latter phase of care is probably contingent on the former. That is, egg tending can occur without post-hatching care, but post-hatching care without egg tending does not seem to occur. This relationship determines which costs are important in the evolution of a particular care behavior. If egg tending is already established, then the evolution of provisioning is hindered only by the cost of provisioning. If egg tending is not established, then the evolution of provisioning might be prevented by the cost of egg tending, even if provisioning itself is cheap and highly beneficial.

Although maternal care is considered generally rare among insects, striking examples exist in many taxa (Wilson 1971; Tallamy and Schaefer 1997). In contrast, parental care and other forms of embryo protection (e.g., ovoviviparity, viviparity) are common among other terrestrial arthropods leading Zeh et al. (1989) to suggest that such characteristics probably arose early in the evolution of these taxa. These authors argue that key evolutionary innovations of the insect egg (e.g., desiccation resistance) removed the requirement of parental care and, in doing so, may have contributed substantially to the success of this lineage. According to Tallamy and Schaefer (1997), maternal care has been documented in 74 genera of 19 families within the insect order Hemiptera. They argue that these examples do not represent a derived state, but rather are evolutionary relics resulting from a failure to have evolved a better method to protect eggs (e.g., piercing ovipositors that allow injection of eggs into plant tissue). Such arguments assume that care, specifically egg guarding which is the most basic and common form of care, is very costly. This assumption has been examined in only two hemipteran species thus far (Tallamy and Denno 1982; Kaitala and Mappes 1997); egg-guarding behavior was shown to be costly in both cases.

Though examples of egg or nymph guarding are numerous in the Hemiptera, more extensive care behaviors are very rare (Eickwort 1981). In the family Cydnidae, progressive provisioning has been confirmed in only three species (*Sehirus cinctus*, *Parastrachia japonensis*, and *Adomerus triguttulus*) according to Filippi et al. (2001). Costs of care have not been rigorously examined in any of these species that show both egg guarding and progressive provisioning.

In the Midwestern United States, burrower bug (*Sehirus cinctus*) mothers lay eggs as the mint *Lamium purpureum* begins to produce nutlets. Egg clutches are typically laid on the soil surface beneath leaf litter, sometimes in shallow depressions or crevices. Mothers tend and guard eggs until they hatch (ca. 10 days), in most cases never leaving the eggs during this time (Kight 1997; A.F. Agrawal, personal observation). Although egg-tending mothers show defensive behaviors against artificial attacks in the laboratory it is unclear how successful these defenses are against natural enemies (S. Kight, personal communication). After the eggs hatch, the mothers drag mint nutlets with their stylets to their offspring (Sites and McPherson 1982). In the laboratory, mothers typically provision offspring up to the end of the second instar (ca. 10–13 days). The typical sizes of mothers, first- and second-instar nymphs are 5 mm, 0.9 mm, and 1.2 mm long, respectively (Sites and McPherson 1982); *L. purpureum* nutlets are ~1.5 mm in length. We have found evidence that the extent of provisioning exhibited by mothers depends on offspring number, condition, and genotype (Agrawal et al. 2001). Consequently, maternal provisioning could be at the center of a parent-offspring conflict (Trivers 1974) if this behavior is costly.

Here, we attempt to identify costs of the two phases of maternal care in this system: egg guarding and provisioning. We performed a laboratory experiment in which 200 mothers were assigned to different treatments determining which components of care they could perform (i.e., no care, guard eggs only, guard eggs and provision hatched offspring). If a care behavior was costly then individuals that did not perform that behavior would be expected to have higher lifetime fecundity than those that did. We predicted mothers that guarded eggs would have lower lifetime fecundity than mothers that did no care. Likewise, we predicted that mothers that both guarded eggs and provisioned offspring would have lower lifetime fecundity than mothers that guarded but did not provision. One constraint of laboratory experiments of this sort is that costs and benefits can be environmentally-dependent (Lindén and Møller 1989; Stearns 1991). For example, maternal provisioning may be inexpensive when food is abundant or predators are rare.

Methods

Two hundred female burrower bugs were collected in late April 2002, before any clutches had been laid, from an agricultural field in Bloomington, Indiana. Females were randomly assigned to one of four treatments (see below) and housed under laboratory conditions. Each treatment contained 50 females. All the females laid their first clutch within a 6-day period. Each mother remained in her assigned treatment for her entire life. Because the same treatment was applied to each clutch that a mother produced, our power to detect trade-offs of maternal behaviors with maternal survival and fecundity was enhanced.

Until they laid eggs, females were housed individually in medium sized, 100×15 mm, disposable Petri dishes with moist sand, a small plastic shelter, and one metal beer-bottle cap (30 mm diameter, 5 mm deep) that contained nine mint nutlets (*Lamium purpureum)*. All dishes were monitored daily for moist sand and new egg clutches. A new beer-bottle cap with fresh food was replaced every other day. The Petri dishes containing the bugs were kept in incubators that followed a 13L:11D photoperiod with a day/night temperature of 24°/18°C (±1°C).

In all the treatments, the number of offspring and unhatched eggs were counted 1 day after hatching began. A sample of ten nymphs was taken from each clutch to obtain an estimate of first instar mass. In general, viable eggs in a clutch hatch synchronously (within a few hours) and we have found no evidence of cannibalism or consumption of inviable or unhatched eggs.

Treatments

Females in the "no care" treatment (NC) were permitted to lay a clutch of eggs, but not to provide any extended care. Each female was allowed to remain with her eggs for 2 days after egg-laying was first observed to ensure that she had finished laying her clutch. The female was then removed from her eggs and set up in a new 100×15 mm Petri dish to lay a new clutch. The eggs were placed in a beer-bottle cap and kept in a smaller disposable Petri dish (35×10 mm) that contained a moist paper towel. The eggs were then checked every day for hatching. Using a small camel hair paint brush, the eggs were gently rolled around in the beer-bottle cap, to inhibit mold growth, daily until hatching occurred.

In the "guard only" treatment (GO), females were allowed to lay their own eggs and then guard their clutch until hatching. The day after hatching, each female was removed from the clutch and set up in a new 100×15 mm Petri dish to lay and guard a new clutch.

Females in "guard and provision full clutch" treatment (GPF) were permitted to experience all aspects of care. Each female laid her own clutch, guarded the clutch, and then cared for her offspring by provisioning food. Each female was allowed to remain with her clutch in the 100×15 mm Petri dish until hatching. One day after hatching, female and offspring were chilled on ice to count offspring. While still partly immobilized by the cold, the female and offspring were transferred to an offspring rearing arena, a large 150×15 mm disposable Petri dish. As offspring typically reside under cover objects, mother and offspring were placed under a hut (curved piece of black polyethylene) that was near one side of the dish. Three beer-bottle caps, each containing nine nutlets, were on the opposite side of the dish. The female could thus travel to the beer-bottle caps to obtain food for her offspring. The beer-bottle caps were counted daily for the number of remaining nutlets and then the beer-bottle caps were removed and replaced with new beer-bottle caps containing nine fresh nutlets. The number of nutlets missing from a beer-bottle cap is a good measure of provisioning (Agrawal et al. 2001) for several reasons. First, mothers do not need to remove nutlets to eat their contents. Further, mothers were frequently observed transporting nutlets, but offspring were never observed doing so. Lastly, nutlets were usually only found in the beer-bottle caps or in areas where offspring had been (i.e., where the mother had delivered food to them). The mother was removed from the offspring rearing arena once her offspring reach the third instar, when provisioning is complete, and set up in a new 100×15 mm Petri dish to lay another clutch.

The females in the "guard and provision to a reduced clutch" treatment (GPR) experienced similar conditions to the females in GPF treatment, in that they laid eggs, guarded the eggs, and cared for offspring until the third instar. However, in this treatment, clutches were experimentally reduced to 30 offspring. Because most first-clutches are in the range 50–130 eggs, 30 offspring represents a substantially reduced number of offspring (but this number is not far below the natural range of variation).

The data collected from each female included: number of clutches produced, lay and hatch dates of each clutch, number of offspring in each clutch, mass of a sample of ~10 first instars from each clutch, and maternal death date. Additional data were taken on females that provisioned (i.e., GPR and GPF mothers). These data included: number of nutlets provisioned each day and development rate of offspring to third instar.

Although we have not formally quantified resource distributions in the field, our field observations suggest that the abundant resources present in our experiments were a reasonable reflection of field conditions. Burrower bugs typically nest in large, densely packed stands of their host plant. In our experiment, the foraging distance for provisioning mothers was approximately 9 cm. At our study site, a radius of 9 cm would include 10–20 plants each producing ~192–384 nutlets over a 3- to 4-week period that encompasses the time when first clutches are laid. Based on these numbers, approximately 1,900 to 7,700 nutlets are expected within a 9 cm radius. As mothers typically provision ~80 nutlets to a full size clutch, a single mother in such an area may experience an average foraging distance that is less than 9 cm. The density of mothers varies considerably among patches in the field with no obvious relationship to nutlet availability. Foraging distances are likely to vary among patches because of the variation in competition intensity. Nonetheless, our knowledge to date on this system suggests that food availability in this experiment was within the realm of biological reality. Of course, our experimental conditions differed from natural conditions in a variety of other respects that are potentially important (see Discussion).

Counting hatches

Petri dishes containing newly hatched eggs were placed on ice for about an hour to reduce the movement of the bugs for ease of counting. The clutches were counted using dissecting microscopes. A sample of ten offspring was collected at this time. The sample was carefully examined under the scope to prevent the inclusion of sand or other debris. The sampled offspring were placed in an eppendorf tube lined with aluminum foil and stored at −30°C. The remaining offspring in the Petri dish were allowed to warm up at room temperature.

Measuring mass of first instars

The first instar samples were fully desiccated in a drying oven, set between 57–60°C, for about one day before weighing. The dry mass (±1 μg) of each sample of offspring was measured using a Sartorius MC5 Microbalance. The mass of the sample divided by the number of individuals was taken as the average individual mass for a clutch.

Data analyses

Fecundity

Some females in the NC treatment produced a very small clutch 3–4 days after laying a larger clutch. The eggs of these smaller clutches typically hatched less than 2 days after, and often on the same day as, the preceding larger clutch. In all of our analyses, we treated these small clutches as part of the preceding larger clutch. Treating the data in this way was always conservative with respect to identifying differences between the NC treatment and the other three treatments.

Fecundity was analyzed as the number of live hatched offspring. In general, there were few unhatched eggs and we did not record counts of these unhatched eggs on the first clutches. These unhatched eggs were counted for most of the subsequent clutches. Hatching success was high (>95%) in most clutches and those clutches with reduced hatching success tended to be small (i.e., a small fraction of a female's total egg production). When we performed analyses on total eggs laid, the results were qualitatively similar (not shown).

We investigated costs of guarding and provisioning by examining differences between treatments. We compared the treatments with respect to total cumulative number of hatched offspring, number of clutches, and mass of offspring in the second clutch by ANOVA using the GLM procedure in SAS (1999). To avoid creating differences among treatments in resource availability, mothers in all treatments always had access to a plentiful supply of mint nutlets. Consequently, it was possible for mothers in the GO, GPR, and GPF treatments to provision offspring in the few hours between hatching and collection of offspring samples for weighing. Thus, when testing for differences in offspring mass we included the number of nutlets provisioned during this interval as a covariate.

We used a Kruskal-Wallis test to examine among-treatment heterogeneity in the time between laying first and second clutches. Post-hoc comparisons were performed using a nonparametric Tukey-type test as suggested by Zar (1984).

Survival

Differences in the maternal survival function where examined using the product limit method (SAS 1999). This type of survival analysis takes into account censoring of data. Though our data set is not 100% complete, missing data were not biased towards longer-lived individuals: no individuals were allowed to 'leave' the study and we did not terminate the study until the last mother died. Therefore, we were able to confirm our survival function analysis with a Kruskal-Wallis test on death date, again using a nonparametric Tukey-type post-hoc test (Zar 1984). In addition to a mother's death date, we were also interested in a mother's last lay date because it marks the last day she is reproductively active (i.e., gaining fitness through fecundity). We performed similar analyses on last lay date to those described for death date.

For reasons explained below, we examined the variation in the frequency of mothers surviving to produce three (or more) clutches among the GO, GPR, and GPF treatments using a χ^2-test.

Maternal performance over consecutive clutches

To determine if the performance of individual mothers changed over consecutive clutches, we used paired *t*-tests to compare the sizes of first and second clutches within each treatment. We examined only the change between the first and second clutch because sample size shrinks at later clutches due to maternal mortality. We also tested whether the size of a mother's first clutch was related to the size of her second clutch by examining the correlation between the sizes of first and second clutches. A positive correlation would indicate individual consistency (i.e., mothers that have larger than average first clutches also tend to have larger than average second clutches), whereas a negative correlation would indicate a possible trade-off between clutches (i.e., mothers that invested into producing larger than average first clutches tended to produce smaller than average second clutches).

Similar analyses were performed with respect to provisioning for GPR and GPF mothers. Because GPR mothers always reared 30 offspring, the analysis was performed on total number of nutlets provisioned. This analysis would not be appropriate for GPF mothers because they raised variable numbers of offspring and provisioning is known to depend on offspring number (Agrawal et al. 2001). To account for variation in offspring number, we analyzed the residuals from the linear regression of provisioning on clutch size. We combined data from both first and second clutches to calculate the regression equation. For both treatments, we included only those mothers that survived until offspring reached third instar of the second clutch (i.e., we excluded mothers that died during the period when care is provided). To demonstrate that mothers rearing full clutches provisioned more than mothers rearing experimentally reduced clutches, we compared the provisioning done for first clutches by GPR mothers and GPF mothers using the normal approximation of the Wilcoxon-Mann-Whitney rank test.

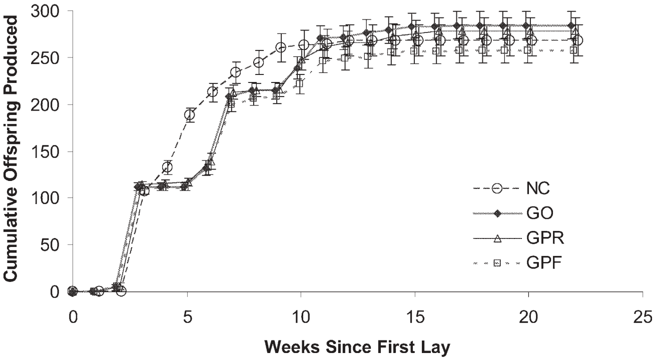

Fig. 1 Cumulative offspring produced by the burrower bug (*Sehirus cinctus*) measured as number of life hatchlings over the entire experiment (i.e., lifetime reproduction). Weekly estimates for each treatment are slightly offset from one another for clarity. The last point represents total fecundity after all mothers had died. *Bars* represent standard errors

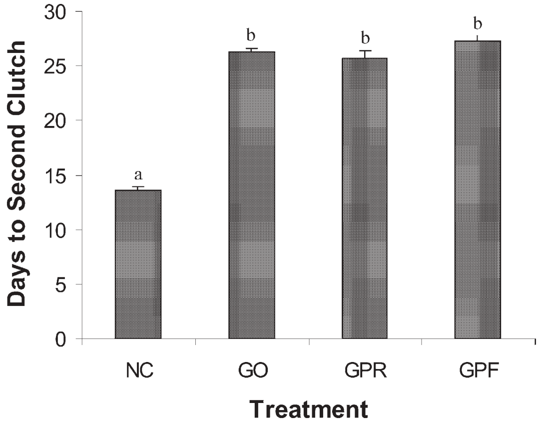

Fig. 2 Time between the lay dates of first and second clutches. *Letters* denote statistically distinguishable groups

Results

Among-treatment differences in fecundity

Total lifetime fecundity was not significantly different among the four treatments (ANOVA: $F_{3,192}$=0.62, P=0.60). There were obvious differences among the treatments in their trajectories leading to this end point (Fig. 1). One important source of this heterogeneity was the time between the lay dates of successive clutches. Significant heterogeneity among treatments in the time between the first and second clutches was found (Kruskal Wallis: χ^2=103.3, *df*=3, P<0.0001, Fig. 2). Nonparametric post-hoc comparisons indicate that NC mothers had a significantly shorter inter-clutch interval than mothers in the other treatments (Nonparametric Tukey: NC vs GO: Q=7.88, P<0.001; NC vs GPR: Q=6.89, P<0.001; NC vs GPF: Q=8.43, P<0.001). No significant differences were observed among the remaining three treatments. NC mothers laid second clutches on average ~13 days after laying the first clutch whereas mothers in the other treatments took about twice as long (Fig. 2). Perhaps as

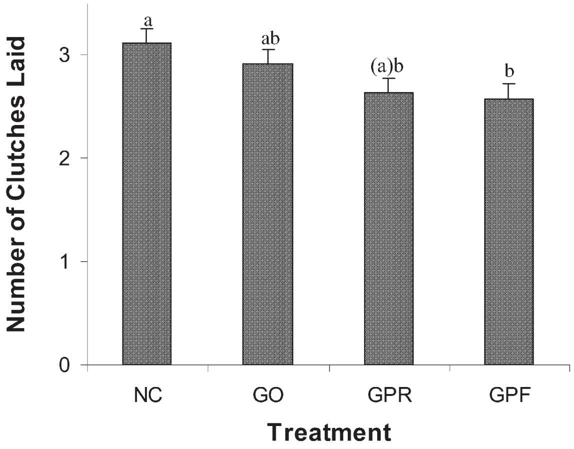

Fig. 3 Number of clutches laid. Significant heterogeneity was detected among the treatments. *Letters* denote statistically distinguishable groups

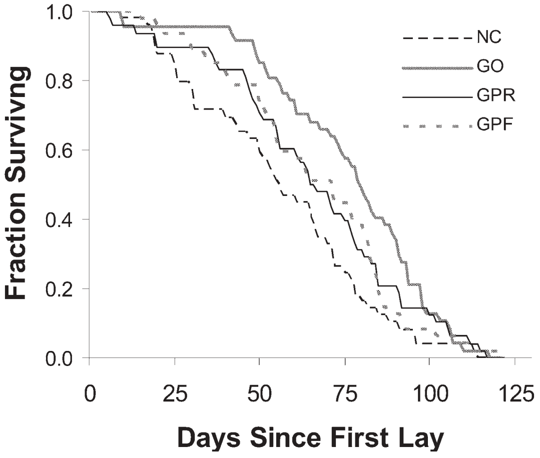

Fig. 4 Survival curves. Significant heterogeneity was observed among the four survival curves. GO mothers tended to live longer than other mothers whereas NC mothers tended to die sooner than other mothers

result of this difference in the time between clutches, there was significant heterogeneity in the number of clutches laid by mothers in different treatments (ANOVA: $F_{3,194}$=3.20, *P*=0.02). The NC mothers laid significantly more clutches than GPF mothers (Tukey-Kramer: *P*=0.04). The comparison between NC and GPR mothers was non-significant (Tukey-Kramer: *P*=0.07; see Fig. 3). Examining second clutches, we were unable to detect any differences among treatments in the mass per offspring (ANOVA: $F_{3,166}$=0.341, *P*=0.34; number of offspring included as a covariate) or in the calculated total mass of hatched offspring (ANOVA: $F_{3,167}$=1.35, *P*=0.26).

Among-treatment differences in survival

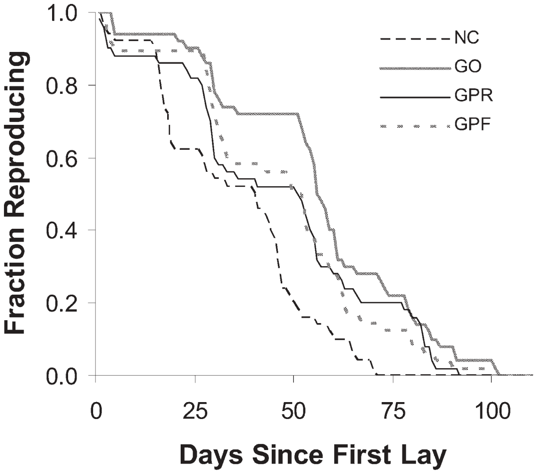

Fig. 5 Reproductive activity curves. The fraction of mothers that were still reproducing is plotted against time. Each mother was considered as reproductively active until the day she laid her last clutch. Significant heterogeneity was observed among the four activity curves. GO mothers remained reproductively active the longer than other mothers whereas NC mothers became reproductively inactive sooner than other mothers

There was significant heterogeneity among treatments in maternal survival curves (Fig. 4; log-rank: χ^2=10.8, *df*=3, *P*=0.01). A Kruskal-Wallis test on death date confirmed the survival analysis (Kruskal-Wallis: χ^2=14.3, *df*=3, *P*=0.002). NC mothers died sooner than GO mothers (Nonparametric Tukey: *Q*=3.78, *P*<0.001). but GPR and GPF mothers were intermediate and indistinguishable from either NC or GO mothers. There were also significant differences among treatments in maternal reproductive activity curves based on last lay date (Fig. 5; log-rank: χ^2=26.7, *df*=3, *P*=0.01). A Kruskal-Wallis test on last lay date confirmed this analysis (Kruskal Wallis: χ^2=20.2, *df*=3, *P*=0.0002). Post-hoc comparisons indicated that GO and GPF mothers were reproductively active for significantly longer than NC mothers (GO vs NC—Nonparametric Tukey: *Q*=4.45, *P*<0.001; GPF vs NC—Nonparametric Tukey *Q*=2.73, *P*<0.05). Though GPR mothers followed a similar reproductive schedule to GO and GPF mothers, the comparison with NC mothers was marginally non-significant (GPR—Nonparametric Tukey: *Q*=2.40, *P*<0.10).

There are several clear differences between the NC treatment and other treatments (e.g., time between clutches, survival). As reflected by Fig. 1, the NC treatment appeared qualitatively different than the other three treatments. We sought to distinguish among the remaining three treatments by examining the frequency of mothers producing three (or more) clutches. This test was chosen for two reasons. First, GO, GPR, and GPF mothers all tended to lay clutches on similar schedules (as opposed to NC mothers). Second, this test examined differences at a time point when the effects of the different treatments had been able to accumulate but when a reasonable fraction of mothers were still alive (very few mothers in any of these three treatments survive to produce a fourth clutch). Differences among the treatments in the frequency of mothers producing a third clutch were non-

Table 1 Difference between sizes of first and second clutches of the burrower bug (*Sehirus cinctus*). Values in parentheses show results from the analysis excluding females laying abnormally small clutches ($\leq$15 eggs)

Treatment	Size of first clutch ± SE	Size of second clutch ± SE	Mean difference ± SE	Paired *t*	*n*	*P*
NC	106.3±3.1	90.7±5.1	15.6±5.0	3.09	46	0.003*
	(105.6±3.1)	(92.6±4.8)	(13.0±4.5)	(2.92)	(45)	(0.005*)
GO	113.3±3.6	111.3±5.1	2.0±4.8	0.42	45	0.68
	(114.4±3.5)	(113.6±4.7)	(0.8±4.7)	(0.17)	(44)	(0.87)
GPR	113.1±3.7	108.3±4.2	4.8±4.7	1.03	44	0.31
	(112.3±3.7)	(110.6±3.7)	(1.7±3.6)	(0.47)	(43)	(0.64)
GPF	112.2±2.8	107.8±4.9	4.5±5.5	0.81	42	0.42
	(111.6±2.8)	(110.0±4.5)	(1.5±4.8)	(0.32)	(41)	(0.75)

* $P<0.05$ after sequential-Bonferroni correction

Table 2 Pearson product-moment correlation between sizes of first and second clutches. Values in parentheses show results from the analysis excluding females laying abnormally small clutches ($\leq$15 eggs)

Treatment	Correlation	*t*	*n*	*P*
NC	0.32 (0.44)	2.27 (3.25)	46 (45)	0.028 (0.002*)
GO	0.44 (0.36)	3.24 (2.52)	45 (44)	0.002* (0.016*)
GPR	0.31 (0.52)	2.10 (3.94)	44 (43)	0.042 (0.0003*)
GPF	0.06 (0.20)	0.38 (1.27)	42 (41)	0.71 (0.21)

* $P<0.05$ after sequential-Bonferroni correction

significant (Kruskal Wallis: $\chi^2=5.75$, *df*=2, *P*=0.057) though GO differed from the two provisioning treatments in the predicted direction: GO (72%) > GPR (52%) $\geq$ GPF (51%).

Maternal performance in successive clutches

For NC mothers, the number of offspring in the first clutch was significantly greater than in the second clutch (Table 1). There was no significant difference observed in any of the other treatments. There was no significant correlation between the size of the first and second clutch for GPF mothers (Table 2). The other three treatments showed evidence of a positive correlation, but only the correlation for GO remained significant after a sequential Bonferroni adjustment at $\alpha=0.05$. When the data were re-analyzed excluding females that laid an abnormally small clutch ($\leq$15 eggs), significant positive correlations were observed for NC, GO, and GPR (Table 2). Only a single female was excluded from each treatment in this analysis.

Only the GPR and GPF treatments could be analyzed with respect to provisioning. As expected, mothers rearing full clutches provisioned more than mothers rearing experimentally reduced clutches. For example, with first clutches, GPF mothers did almost twice as much provisioning as GPR mothers (GPF mean±S.E=79.2±4.8 nutlets, GPR=45.8±3.8 nutlets; Wilcoxon-Mann-Whitney Rank: *Z*=4.77, *P*<0.0001). Within the GPR treatment, mothers provisioned the first clutch less than the second clutch (Table 3). There was a significant positive correlation in the number of nutlets provisioned by a mother to her first versus second clutch (Table 4). Similar results were observed in the analysis of provisioning in the GPF treatment after removing the effects of variation in clutch size. GPF mothers provisioned less to their first clutches than to their second clutches (Table 3). The level of provisioning for the first clutch was correlated with the level of provisioning for the second clutch (Table 4).

Discussion

The costs of parental care depend on the ecological context in which care is expressed. Only field experiments have the potential to reveal the true costs of care realized in nature. Unfortunately, such experiments are impossible for many organisms because of their behavior and natural history (including the semi-fossorial habits of burrower bugs). In such cases, laboratory experiments can provide valuable insights into the relative size and form of costs under controlled conditions. When costs measured in a simplified laboratory arena differ from those expected in the field, then costs are likely to be mediated by an environmental factor that differs between these settings. In this case, candidate factors can be manipulated in subsequent laboratory experiments as means of identifying which environmental factors mediate costs. Bearing in mind the limitations of a laboratory study, we were able to gain a number of insights into possible costs of care in burrower bugs, along with some unexpected insights into

Table 3 Difference between in provisioning to first and second clutches. Values for GPF are adjusted for clutch size by using residuals from the regression of nutlets provisioned on clutch size combining data from first and second clutches; values in parentheses are unadjusted numbers of nutlets provisioned

Treatment	Mean nutlets		Mean difference ± SE	Paired *t*	*n*	*P*
	First clutch	Second clutch				
GPR	44.5±4.6	64.6±5.2	−20.1±5.1	−3.92	31	0.0005*
GPF adjusted	−10.3±5.7	−10.3±8.9	−20.6±8.3	−2.49	31	0.02*
Unadjusted	(77.8±5.7)	(98.3±8.9)	(−20.5±8.6)	(−2.39)	(31)	(0.02)

* $P<0.05$ after sequential-Bonferroni correction with two comparisons (GPR and GPF adjusted)

Table 4 Pearson product-moment correlation between provisioning to first and second clutches

Treatment	Correlation	*t*	*n*	*P*
GPR	0.45	2.73	31	0.01*
GPF adjusted	0.43	2.53	31	0.02*
Unadjusted	0.38	(2.19)	(31)	(0.04)

* $P<0.05$ after sequential-Bonferroni correction with two comparisons (GPR and GPF adjusted). See Table°3 for details on GPF adjusted vs unadjusted

maternal behavior when successive clutches were produced.

We chose four treatments that span a wide range with respect to extent of maternal care. At the extremes, the average difference between treatment groups was 24 days of attention to eggs and offspring. Despite these dramatic differences in the amount of care exhibited, we were unable to detect any significant differences in lifetime fecundity among the four treatments despite having quite large sample sizes ($n\approx 50$ per treatment). Contrary to our expectations, these data suggest that the costs of care with respect to lifetime fecundity under laboratory conditions are, at most, small.

On the other hand, there were some obvious differences among the treatments. In most cases, the NC treatment was easily distinguished from the other treatments. The clearest benefit of providing no care was a reduction in the time between clutches. NC mothers produced a second clutch almost twice as fast as mothers in the other treatments (Fig. 2). Although NC mothers in our experiment reproduce more rapidly, they have no advantage in total lifetime fecundity because these mothers stop reproducing and die sooner than other types of mothers (Figs. 4, 5). The rapid reproductive effort by these mothers may be the cause of their earlier mortality. Such negative effects of reproduction are commonly documented in studies of senescence (Rose 1991). In birds, this pattern is evident over a broad phylogenetic context (Bennett and Owens 2002).

The dramatic reduction in the time to produce a second clutch could be beneficial under natural conditions for two reasons, despite the lack of benefit with respect to lifetime fecundity observed in our experiment. First, in the field there are external sources of mortality (e.g., predators, parasitoids) that reduce the expected reproductive lifetime, putting a premium on faster reproduction. Second, the quality of the environment (e.g., overall abundance of resources) may decline over time so that it may be difficult for offspring produced later in the season to survive. Therefore, we interpret the observed increase in inter-clutch interval among the GO, GPR and GPF treatments relative to the NC treatment as a potentially large ecological cost of egg guarding.

The overall similarity of the GO, GPR, and GPF treatments combined with their differences from the NC treatment indicates that the primary cost of care is due to egg-guarding. In the handful of studies that have measured costs of care in arthropods, a cost of egg-guarding in the form of increased inter-clutch interval is common. Similar effects on inter-clutch interval have been observed in the lace bug *Gargaphia solani* (Tallamy and Denno 1982), the woodroach *Cryptocercus punctulatus* (Nalepa 1988), the earwig *Euborellia annulipes* (Rankin et al. 1996), and the maternal subsocial spider *Coelotes terrestris* (Gundermann et al. 1997). Reductions in the inter-clutch interval by the removal of eggs are sometimes interpreted as indicating that the presence of eggs inhibits the onset of the next gonadotrophic cycle (Nalepa 1988; Rankin et al. 1996). Our data add to existing examples (Tallamy and Denno 1982; Kaitala and Mappes 1997) that support the assumption of Tallamy and Schaefer (1997) that egg guarding in Hemipterans is costly.

As in our own work, these studies compared mothers that were experimentally prevented from guarding eggs to mothers that were not. Comparisons between the two treatments are intended to reveal the effects of guarding. From these experiments it is not possible to know whether the differences between treatments are a direct or indirect consequence of the egg guarding manipulation. For example, the reduced survival of guarding mothers is unlikely to be a direct consequence of the experimental manipulation on guarding, but rather an indirect consequence arising from the increased rate of reproduction. We interpret the change in the rate of reproduction as directly reflecting the costs of guarding but, as with any experimental manipulation, we cannot be certain that this change is the result of some indirect effect of the manipulation.

Surprisingly, we did not find any effect of provisioning on fecundity or inter-clutch interval. The only hint of a cost of provisioning was with respect to survival (see Results and Figs. 4, 5). However, our statistical analysis did not support a cost of provisioning; the observed differences were not significant. In contrast, a survivorship cost associated with a quantitative increase in the level of provisioning effort has recently been documented in the dung beetle *Onthophagus taurus* (Hunt et al. 2002).

There are several potential explanations for why we did not find larger costs of provisioning. It is possible that provisioning by burrower bug mothers is always relatively inexpensive. Alternatively, experimental conditions may have lacked the ecological factors responsible for generating costs that could occur in the field. For example, food may be more difficult to collect in the field than in this experiment because of its abundance (see Methods) and/or because the terrain in the field is more rugged. A recent study in the European beewolf *Philanthus triangulum* suggested that when the effort required to provision offspring was increased, provisioning offspring became more expensive with respect to future reproduction (Strohm and Marliani 2001). Another potentially important factor was the lack of natural enemies in our experiment. Such enemies are of importance in generating costs of parental care if they reduce the expected future reproduction of a provisioning mother more so than a non-provisioning mother. That is, natural enemies must be more likely to encounter and attack provisioning mothers than non-provisioning mothers. If provisioning is expensive in nature, our results imply that these costs must be mediated by factors that increase the complexity of the environment (e.g., spatial ruggedness, natural enemies). Our study provides a solid benchmark for future experiments that manipulate such factors and, in doing so, will be able to measure costs as well as identify the ecological factors responsible for generating them.

Although it is conceptually appealing to view the costs identified in this experiment as lower bounds on the costs in nature, this interpretation may be misleading. Tallamy and Brown (1999) argue that many insects showing maternal care are semelparous, or are at least functionally so under natural conditions. If mothers are unlikely to produce a second clutch in the field regardless of whether they cared for their first clutch, any reduction in future reproduction due to care observed in the laboratory is irrelevant. The extent to which *Sehirus cinctus* is capable of producing multiple clutches in natural conditions in unknown. Even for semelparous organisms a cost of maternal care could still exist. For example, mothers could produce a larger clutch rather than save energy to care for a smaller clutch. Such a within-clutch trade-off has been observed in birds (Heaney and Monaghan 1995, 1996; Monaghan et al. 1998).

In addition to examining costs, our experiment allows us to examine individual maternal performance over successive clutches. We found no evidence for a trade-off between investment into first versus second clutches. Point estimates of the correlation between first and second clutch size were positive rather than negative (Table 2). Furthermore, we found positive correlations in the levels of provisioning across the two clutches (Table 4). These results indicate that individual mothers are consistent throughout their lifetimes, rather than showing evidence of trade-offs. In their study of lace bugs, Tallamy and Denno (1982) also found that first clutch size did not trade-off with future reproduction but rather showed a positive effect (at least in one treatment). Many authors (Rendel 1967; van Noordwijk and de Jong 1986; Charlesworth 1990; Houle 1991) have noted that variation in overall condition (due to acquisition ability) will contribute to a positive correlation among characters that might be expected to show trade-offs for functional/energetic reasons.

Mothers provision their second clutches more than their first clutches (Table 3). One possible explanation is that mothers are selected to invest heavily into second clutches because they are unlikely to produce a third clutch under natural conditions. Another possibility is that mothers become better at provisioning with experience. Several studies in birds suggest that improved provisioning ability from experience may contribute to increases in breeding success with age (e.g., Emslie et al. 1992; Green 2001).

To conclude, egg guarding appeared to be more expensive than provisioning in our experiment, though this pattern could change with different ecological conditions. In general, there is no clear pattern across taxa as to whether pre- or post-hatching care is more costly. In the lace bug *Gargaphia solani*, mothers guard eggs as well as offspring. The experiment by Tallamy and Denno (1982) suggests that guarding eggs is more expensive than guarding offspring. In the maternal subsocial spider *Coelotes terrestris*, mothers guard egg sacs and provision offspring. In their study on the costs of these two phases of care, Gundermann et al. (1997) argue that provisioning offspring is very expensive relative to guarding. Egg guarding merely delayed the production of a second clutch whereas provisioning greatly reduced the probability of ever producing a second clutch. In his parental care book, Clutton-Brock (1991 stated, “In birds and mammals, feeding young has heavy energetic costs that typically exceed those of egg production or gestation by a substantial margin...” However, more recent studies in birds challenge this view by finding that incubation costs can be substantial (Heaney and Monaghan 1995, 1996; Williams 1996; Cichon 2000; Visser and Lessells 2001; Engstrand and Bryant 2002; Ilmonen et al. 2002).

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