Determinants of Clutch Size and Seed Preference in a Seed Beetle, *Stator beali* (Coleoptera: Bruchidae)

CHARLES W. FOX AND TIMOTHY A. MOUSSEAU

Department of Biological Sciences, University of South Carolina, Columbia, SC 29208

ABSTRACT Many insects lay their eggs in clutches on discrete patches of resource that represent the only food available to progeny. The ability of females to distinguish resource patches of variable size and adjust their clutch size accordingly may enable these insects to reduce sibling conflict and exploit resources optimally. *Stator beali* Johnson is an unusual seed beetle because it lays eggs in clutches rather than singly. Within a natural population, clutch size varied from 1 egg to >19 eggs on seeds of Texas ebony, *Chloroleucon ebano* (Berlandier). We examined the heritability of clutch size in *S. beali*, and then determined the extent to which females can adjust clutch size in response to seed size. We obtained conflicting evidence as to whether clutch size variation is heritable; a standard parent–offspring regression analysis suggested that the heritability of clutch size was not different from 0 ($h^2 = 0.10 \pm$ SEM 0.18), whereas an analysis of full sibs suggested that it was substantially greater than 0 ($h^2 = 0.33 \pm 0.12$). This discrepancy is likely the result of large nongenetic effects on clutch size. When presented with both a large and a small *C. ebano* seed, females preferentially laid eggs on the large seed. When forced to lay eggs on seeds of a specific size, females presented with 5 large seeds laid larger clutches (mean $\pm$ SEM, 6.7 $\pm$ 0.4 eggs) than those presented with 5 small seeds (5.2 $\pm$ 0.5 eggs). Females also actively avoided superparasitism in the laboratory when alternative, nonparasitized seeds were available. However, most seeds collected in the field (56%) were superparasitized, and many (15%) had >2 clutches on them, suggesting that seeds are a limited resource for ovipositing females.

KEY WORDS *Chloroleucon ebano*, clutch size, heritability, seed size, superparasitism

MANY INSECTS LAY their eggs in clutches on discrete patches of resource (Godfray et al. 1991). If progeny are immobile, this resource patch is the only food available to them until after pupation. The size of the patch thus determines the amount of resources available for development and the extent of competition among siblings. The ability of females to distinguish resource patches of variable size and adjust clutch size in response to resource size may enable these insects to reduce sibling conflict and exploit their resources optimally (Hardy et al. 1992). For example, optimality models predict that larger clutches should be placed on higher quality or larger patches of resource (Godfray et al. 1991). Indeed, some seed beetles (Mitchell 1975) and many hymenopteran parasitoids (Schmidt and Smith 1985, Takagi 1986) measure the size of a host, and use this information to adjust the number of eggs laid on that host.

Stator beali Johnson is a specialist seed parasite that, unlike most seed beetles (which generally lay a single egg per oviposition bout), lays eggs in clutches on a seed (lays multiple eggs per oviposition bout). *S. beali* is a specialist on the seeds of Texas ebony, *Chloroleucon ebano* (Berlandier) L. Rico [=*Pithecellobium flexicaule* (Benth.) Coult.] (Fabaceae: Mimosoideae) (Johnson and Kingsolver

1976). Seeds of this species are large relative to beetle body sizes (seed size range ≈0.3–1.2 g; beetle size range ≈2.5–5.9 mg) and large relative to most other legume seeds. Upon hatching, larvae burrow into the seed on which they were laid, complete development, and pupate. Larvae are restricted to the seed their mother has chosen for them, and the amount and quality of resources available to them is thus determined entirely by seed size, seed quality, and the number of larvae developing within the seed.

Within a natural population of *S. beali*, clutch size varied from 1 egg per clutch (rare) to >19 eggs per clutch (see below). We describe a series of laboratory experiments designed to examine the genetic and environmental sources of this variation in clutch size. We 1st examine the heritability of clutch size in *S. beali*, and then determine the extent to which females adjust clutch size in response to seed size. We also examine female preferences for large seeds, and the tendency toward discrimination against seeds bearing previously laid clutches.

Materials and Methods

Study Populations. The beetle population used in these experiments was collected from infested

pods of *C. ebano* in Mont Meta Memorial Park, San Benito, Cameron County, Texas, in late July 1994. Fruits were collected from >50 *C. ebano* trees within an ≈1-ha² area. Infested fruits were separated from noninfested fruits within 24-h. Seeds in noninfested fruits were subsequently used for laboratory rearing and laboratory experiments. Approximately 400 adults emerged from the infested fruits, and were used to initiate the laboratory population. Beetles were maintained in laboratory growth chambers at large population sizes (>300–400 individuals per generation) on *C. ebano* before and during these experiments. The heritability experiment was initiated with adults reared from field collected seeds. The remaining experiments were initiated with beetles reared in the laboratory for 2–3 generations. All experiments were performed at 30–31°C, a photoperiod of 15:9 (L: D) h, and were initiated with virgin females and males collected within 24 h after adult emergence from their rearing seed.

Heritability of Clutch Size. Virgin females (*n* = 90) developing from eggs laid in the field were used to initiate the genetic analysis. Each virgin female was weighed within 24 h of emergence from her rearing seed, paired with a virgin male, and confined in a 60-mm petri dish with 15 *C. ebano* seeds of similar size (small and large seeds were discarded such that all seeds ranged from 0.7 to 1.0 g). Dishes were checked for eggs at 24-h intervals until the female died. The average size of all clutches laid in the 1st 24 h was recorded as the female's clutch size (clutches laid in the 2nd 24-h period tended to be smaller than those laid the 1st d). All eggs were reared to adult at 30°C and a photoperiod of 15:9 (L:D) h.

As with the parents, all emerging female offspring were weighed within 24-h of emergence from their rearing seed (mean = 2.4 females per family), paired with a nonsibling virgin male, and confined in a 60-mm petri dish with 15 *C. ebano* seeds. Dishes were checked at 24-h intervals until a female had laid eggs. Clutch size was defined as the average size of all clutches laid in this 24-h period.

Effect of Seed Size on Seed Selection. Virgin female *S. beali* (*n* = 25) were each paired with a single virgin male and confined in a 60-mm petri dish (1 pair per dish) with both 1 small and 1 large *C. ebano* seed (small seed, 0.42 ± SEM 0.01 g; large seed, 0.88 ± 0.02 g). Dishes were checked at 24-h intervals until a female laid her 1st clutch. One female that laid eggs on both the large and small seed was deleted from subsequent statistical analyses.

Effect of Seed Size on Clutch Size. Virgin female *S. beali* were each paired with a virgin male and confined in a 60-mm petri dish (1 pair per dish) with either 5 small seeds (0.41 ± 0.01 g) (*n* = 40 females) or 5 large seeds (1.04 ± 0.10 g) (*n* = 31 females). Dishes were checked at 24-h intervals until a female laid her first clutch.
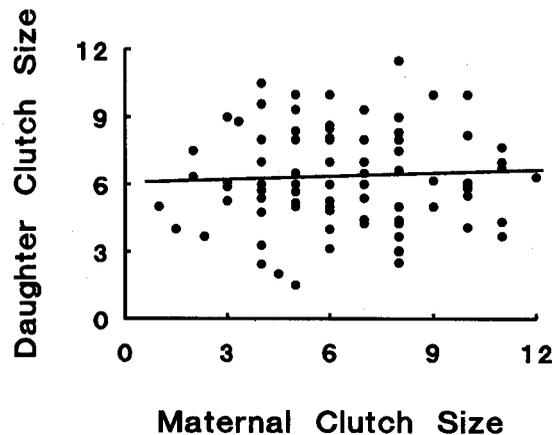

Maternal Clutch Size

Fig. 1. Relationship between maternal and daughter clutch sizes in *S. beali*. Daughter clutch size equals the mean clutch size of all daughters in the family; $y = 5.99 + 0.05x$, $R^2 = 0.01$, $h^2 = 0.10 \pm 0.18$, $P > 0.05$, $n = 90$ families.

Avoidance of Superparasitism. Although *C. ebano* produces an abundance of seeds, few of these seeds are available to *S. beali* females at any time (C.W.F., unpublished data); females can only oviposit on seeds of recently dehisced pods, or seeds in pods damaged by other organisms. Thus, superparasitism, in which females oviposit on hosts that already bear conspecific eggs or larvae, is common in natural populations of *S. beali*.

To determine whether females actively avoided seeds bearing *S. beali* eggs, virgin female *S. beali* (*n* = 39) were each paired with a virgin male and confined in a 60-mm petri dish (1 pair per dish) with 2 seeds of equal size—1 bearing eggs laid by another *S. beali* female <24 h previous to the introduction of the new female, and the other bearing no eggs.

Results

The average size of *S. beali* clutches collected on *C. ebano* seeds at Mont Meta Memorial Park was 7.6 ± SEM 0.9 eggs (range, 1–19; *n* = 61). This was slightly higher than the average clutch size of females in the laboratory genetic experiment (6.4 ± 0.2 eggs; range, 1–12; *n* = 90; Mann–Whitney *U* test, *P* < 0.05), possibly the result of reduced environmental effects on clutch size in the laboratory. The heritability of clutch size estimated from full sibs (90 families) (Becker 1992) was 0.33 ± 0.12 (*P* < 0.05) (estimated using SAS PROC Varcomp, method REML; SAS Institute 1985, Becker 1992). However, the estimate of clutch size heritability using a parent–offspring regression analysis (Falconer 1989) was substantially lower, and not significantly different from zero (*h²* = 0.10 ± 0.18, *P* > 0.05, *n* = 90; Fig. 1). Similar patterns were detected for offspring body size; the heritability estimate estimated from full sibs was large
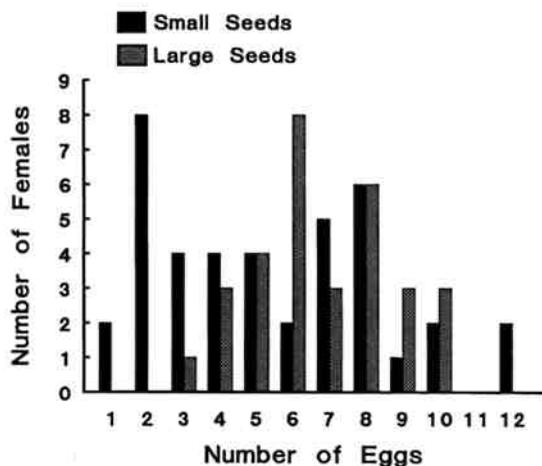
Fig. 2. Distribution of *S. beali* clutch sizes on small and large *C. ebano* seeds.

($h^2 = 0.57 \pm 0.27$, $P < 0.05$, $n = 90$) while the estimate from a parent–offspring regression was not ($h^2 = 0.02 \pm 0.12$, $P > 0.05$, $n = 90$). However, there was no relationship between female body size and clutch size in either mothers or daughters (mothers, $r^2 = 0.01$; daughters, $r^2 = 0.03$; $P > 0.05$ for each; $n = 90$ for each).

When presented with both a large and a small seed, 22 of 24 female *S. beali* laid eggs on the large seed (sign test, $P < 0.001$). When forced to lay eggs on seeds of a specific size, females varied the size of their clutch in response to seed size; females presented with 5 large seeds laid significantly larger clutches (6.7 ± 0.4 eggs, $n = 31$) than those presented with 5 small seeds (5.2 ± 0.5 eggs, $n = 40$) (Mann–Whitney U test, $P < 0.001$; Fig. 2). This seed size effect was not caused by a difference in female body size between treatments; there was no significant difference between body weight of females in the 2 treatments (Mann–Whitney U test, $P > 0.05$).

Female *S. beali* avoided superparasitism in the laboratory experiment when a nonparasitized seed was available; 77% of females (30 of 39) laid eggs only on the seed that was not parasitized (sign test, $P < 0.001$). Of the 9 females that laid on previously parasitized seeds, 7 laid on both seeds, and only 2 laid solely on the previously parasitized seed. However, despite this avoidance of superparasitism in the laboratory, most seeds collected in the field (56% of seeds bearing clutches) were superparasitized, and many (15%) had >2 clutches ($n = 99$).

Discussion

Our experiment provides conflicting evidence as to whether clutch size variation in *S. beali* is heritable; a standard parent–offspring regression analysis indicated that clutch size was at best weakly heritable, whereas an analysis of full sibs suggested

that the heritability of clutch size is significantly larger than zero. Similarly, the estimated h^2 of body size was statistical different from zero when estimated from full sibs, but not when estimated from parent–offspring regression. These discrepancies likely indicate large common environment or maternal effects on progeny size and clutch size. However the observed common environment/maternal effect on clutch size does not appear to be a direct result of nongenetic effects on body size; clutch size was not correlated with body size in either the parental or offspring generations. Similar maternal/common environment effects on clutch size were evident in a hybridization experiment in which *S. beali* (which lays clutches) was crossed with the congener *S. limbatus* (Horn) (which lays eggs singly); hybrid offspring laid clutches that resembled their mother's clutch (Fox and Mousseau 1995, see also Nilsson and Johnson 1993). Further experimentation is needed to explain the observed common environment effect on clutch size, and thus to estimate accurately the genetic contribution to clutch size variation.

Optimality models generally predict that females oviposit larger clutches on higher-quality hosts (Godfray et al. 1991). For parasitoids, and likely *S. beali*, host quality (for example, seed quality) is in part determined by host size (for example, seed size), because large hosts can successfully produce more and larger offspring (Hardy et al. 1992, Siemens and Johnson 1992). In the laboratory, *S. beali* preferentially oviposited on large seeds. When forced to lay on either large or small seeds females adjusted clutch size in response to seed size, laying larger clutches on larger seeds. This is consistent with results from experiments with many hymenopteran parasitoids (for example, Hardy et al. 1992, Vet et al. 1993). An examination of the distribution of clutch sizes laid by females on the 2 sizes of seeds, however, indicates substantial variance in clutch size on both large and small seeds (Fig. 2). This suggests that only some females modified clutch size in response to seed size; although there was an increase in the number of small clutches laid by females on small seeds, some large clutches were still laid on small seeds. We suspect that future experiments will detect substantial among-female variation, and likely genetic variation, in the ability to modify clutch size. Alternatively, there may be other sources of variation in clutch size (other than seed size) that we have not examined in these experiments.

Many parasitic insects discriminate between previously parasitized and nonparasitized hosts (Ikawa and Suzuki 1982), and either avoid parasitized hosts or modify clutch size according to the extent of prior parasitism (Dijken and Waage 1987, King and Skinner 1991, Hooker and Barrows 1992). Superparasitism is generally considered a behavior that is expressed when unparasitized hosts are rare (Roitberg and Mangel 1988, Wilson and Lessels 1994; but see Papaj et al. 1989, Lalon-

de and Mangel 1994); females accept the reduced offspring fitness associated with superparasitism rather than risk dying before laying their eggs (Parker and Courtney 1984). Data for another seed beetle support this hypothesis; *Callosobruchus maculatus* (F.) are more likely to add eggs to an egg-laden seed if they have been deprived of hosts (Wilson 1988, Messina et al. 1992). For *S. beali*, identification of previously parasitized hosts is likely simple because eggs are laid on the surface of the seed, and thus, easily encountered during exploration of the seed (the egg chorion remain on the seed even after the larvae enter the seed). Also, females likely chemically mark seeds during oviposition (as in other bruchids; for example, Messina and Renwick 1985). In the laboratory, when presented with both parasitized and unparasitized seeds, female *S. beali* preferentially oviposited on unparasitized seeds. However, in field collections of *C. ebano*, >50% of all parasitized seeds were superparasitized. Although these field data represent only a single census at a single location and seed availability may vary temporally and spatially, they suggest that seeds are a limited resource for ovipositing females. Seeds are produced in abundance by *C. ebano*, and are abundant for much of the year (either on the ground or on the trees), but most of these seeds are unavailable as an oviposition substrate; *S. beali* oviposits directly onto host seeds, and is thus restricted to fruits that have either dehisced or been damaged by other organisms (Nilsson and Johnson 1993).

We suspect that this limited availability of *C. ebano* seeds, in combination with the large size of these seeds (relative to beetle size), likely explains the evolution of clutch size in *S. beali*. The small size of most bruchid hosts results in intense larval competition within seeds bearing >1 egg, such that fitness of offspring decreases with increasing numbers of eggs per seed (Wilson 1988, Siemens and Johnson 1992). Thus, for most bruchids, selection favors small clutches or laying eggs individually. However, the large size of *C. ebano* seeds allows large *S. beali* clutches with little reduction in fitness of progeny (C.W.F., unpublished data). That seed size is likely a major determinant of clutch size evolution is indicated by another seed beetle. Female *C. maculatus* tend to distribute eggs uniformly among seeds to reduce competition among larvae. However, populations associated with larger seeds more readily superparasitize or lay larger clutches than populations associated with small seeds (Messina and Mitchell 1989). In *S. beali*, limitation in suitable oviposition substrates theoretically should select for large clutches, but, unlike other bruchids, larval competition does not select against large clutches because of the large size of their host. However, further exploration is necessary to examine the effects of seed availability and seed size on clutch size evolution in *S. beali*.

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