

ASYMMETRICAL REPRODUCTIVE ISOLATION
BETWEEN *STATOR LIMBATUS* (HORN) AND
S. BEALI JOHNSON (COLEOPTERA: BRUCHIDAE)

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ABSTRACT

Stator limbatus (Horn) and *S. beali* Johnson are closely related seed beetles (Coleoptera: Bruchidae) that overlap in distribution in southern Texas and northern Mexico. We examine directional biases in the ability of these species to hybridize. In laboratory crosses, only *S. limbatus* ♀ × *S. beali* ♂ crosses produced developing eggs. However, over one-third (37.5%) of all developing eggs failed to hatch, and 22% of all hatched eggs failed to produce an adult beetle. When mated to either hybrid or *S. beali* males, all emerging hybrid females produced eggs, but none of these eggs developed.

Stator limbatus (Horn) and *S. beali* Johnson are closely related seed beetles (Coleoptera: Bruchidae) that overlap in distribution in southern Texas and northern Mexico (Johnson and Kingsolver 1976). The two species are morphologically very similar, suggesting that they are sister species, although they differ in body size (*S. beali* is generally larger), diet breadth (*S. limbatus* uses approximately 60 host species in nature, while *S. beali* uses only *Chloroleucon ebano* (Berlandier) L. Rico), and clutch size (*S. limbatus* lays eggs singly, while *S. beali* lays clutches) (Johnson and Kingsolver 1976; Nilsson and Johnson 1993). Recent hybridization experiments demonstrate that *S. limbatus* and *S. beali* can hybridize in the lab, although hybrid females produce only infertile eggs (Nilsson and Johnson 1993). Here, we extend the observations of Nilsson and Johnson (1993) by examining directional biases in the ability of *S. limbatus* and *S. beali* to hybridize. We also examine the effect of hybridization on clutch size.

MATERIALS AND METHODS

Specimens of *Stator limbatus* were collected from *Acacia greggii* A. Gray approximately 10 miles south of Van Horn, Texas, in late July 1994. Specimens of *S. beali* were collected from *C. ebano* in Mont Meta Memorial Park, San Benito, Texas, in late July 1994. Both populations were reared in the lab for one generation prior to the hybridization experiment to ensure acclimation to laboratory conditions.

Virgin females of each species were collected from isolated seeds within 24 h after adult emergence from the seed and paired with a single virgin male of the other species. Pairs were confined in a 60 mm petri dish with either 15 *C. ebano* seeds (*S. beali* ♀ × *S. limbatus* ♂) or 15 *A. greggii* seeds (*S. limbatus* ♀

× *S. beali* ♂). Dishes were checked for eggs at 24 h intervals until the female died.

All emerging female hybrid offspring were weighed, paired with either a virgin *S. beali* male or hybrid male, and confined in a 60 mm petri dish with 15 seeds of *C. ebano*. Because initial crosses using *S. limbatus* males did not produce developing eggs (see below), *S. limbatus* males were not mated to hybrid females. Dishes were checked at 24 h intervals until a female laid her first clutch.

RESULTS AND DISCUSSION

24 of 49 (49%) *S. limbatus* ♀ × *S. beali* ♂ crosses produced eggs, while only 9 of 45 *S. beali* ♀ × *S. limbatus* ♂ crosses produced eggs. Of the females that produced eggs, 18 of 24 (75%) *S. limbatus* ♀ × *S. beali* ♂ crosses produced developing eggs (11 of which produced surviving adult offspring reared at 30°C, L:D 15:9), while none of the *S. beali* ♀ × *S. limbatus* ♂ crosses produced developing eggs. We suspect that none of the *S. beali* ♀ × *S. limbatus* ♂ crosses successfully mated and that eggs produced by *S. beali* females represent egg dumping by unfertilized females: although dishes were not observed continuously to record matings, *S. limbatus* males were never observed mating with *S. beali* females.

The failure of most hybrid pairs to produce eggs indicates large prezygotic barriers to hybridization between these species. The asymmetry of the reproductive isolation may be caused by body size differences between the species: male *S. limbatus* are generally much smaller than female *S. beali* and may be incapable of successfully copulating, due either to female rejection of small males or morphological incompatibilities between the large female and small male. Alternatively, *S. beali* females may be more choosy in mate selection than *S. limbatus* females, as has been demonstrated for sister species of picture-winged *Drosophila* in Hawaii (Kaneshiro and Boake 1987). Further experiments are required to disentangle these hypotheses.

Post-zygotic reproductive isolating mechanisms are likely also present between *S. limbatus* and *S. beali*, as evidenced by relatively high mortality of developing eggs in the *S. limbatus* ♀ × *S. beali* ♂ crosses (37.5% of developing eggs failed to hatch). In addition to developmental errors due to hybridization, mortality due to differences in adaptation to host plants may result in intense selection against hybrids. Individuals heterozygous at all loci influencing their ability to use *A. greggii* (half of their alleles having come from an *A. greggii* adapted beetle, and half from a *C. ebano* adapted beetle) may have poor survivorship on this host relative to *A. greggii* adapted beetles. Our data support this hypothesis; hatch to adult survivorship of hybrids was low compared to that of non-hybrid beetles (78% for hybrids, >95% for non-hybrid beetles; *e.g.*, Fox *et al.* 1994, 1995).

The sex ratio of emerging offspring was highly female skewed (2.4 females to each male; sign test, $P < 0.05$). This is consistent with Haldane's rule, in which the heterogametic sex is often absent, rare, or sterile (Coyne 1985). Although the sex ratio was female skewed, it is unknown whether emerging males were sterile. All emerging hybrid males successfully mated with hybrid females, and all of these females laid eggs, though none of the eggs developed. Also, all hybrid females produced eggs, although, regardless of male type (*S. beali* or hybrid), none of the eggs developed ($N = 24$ females).

Mean clutch size of *S. beali* females, as estimated from 140 virgin females collected from the laboratory population, was 6.4 ± 0.2 (range 1 to 11). *Stator*

limbatus females, in comparison, always laid eggs individually in laboratory experiments (Fox *et al.* 1994, 1995). Only 5 of 24 hybrid females laid distinct clutches of greater than 1 egg. The remaining females scattered eggs within and among seeds. Even females that laid distinct clutches laid an average clutch of only 3.2 eggs. Thus, as observed by Nilsson and Johnson (1993), hybrids laid clutches that were similar to *S. limbatus* clutches. This may be explained by dominance of *S. limbatus* clutch size alleles. However, because only *S. limbatus* ♀ × *S. beali* ♂ crosses laid eggs, this may also reflect a non-genetic maternal effect in which all offspring resemble their mother's phenotype. We have detected large common environment/maternal effects on clutch size in *S. beali*, and similar effects may influence clutch size of hybrids.

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