Mating Frequency and Fecundity in *Agrilus anxius* (Coleoptera: Buprestidae)

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ABSTRACT Bronze birch borers (*Agrilus anxius* Gory) (Coleoptera: Buprestidae), a key pest of birches in North America, have the potential to be a major threat to Eurasian birch forests. Therefore, the consequences of single versus multiple mating on the longevity, fecundity and fertility of female *A. anxius* were examined. There were three treatments: females observed mating one time and 1) then housed alone, 2) kept with their mate, and 3) females paired and housed with a randomly chosen male. Another group of females were observed mating once, and then dissected, 47 of 48 of them had received spermatophores. Weight and longevity of females did not differ among mating treatments. Fecundity, but not fertility, was positively correlated with longevity in all treatments. A high percentage of females in all three mating treatments laid eggs, and there was not a significant difference among treatments. The fecundity of females that laid eggs also did not differ among treatments. These results suggest that a single successful mating may be sufficient to ensure maximal fecundity for females, and that potential for failure of any mating is low. However, there is no apparent cost to multiple mating. Thus, multiple mating may increase fecundity for female bronze birch borers. Our results suggest that laboratory rearing protocols need not ensure females mate multiple times. At the population level, our results suggest that a mate-finding Allee effect at low population densities is unlikely. We also compare the results to an earlier study with the emerald ash borer, *Agrilus planipennis* Fairmaire.

KEY WORDS multiple mating, fecundity, fertility, Allee effect

One of the important population processes affecting the establishment of invasive species is the Allee effect (Yamanaka and Liebhold 2009). The Allee effect is the positive relationship between population density and per capita growth rate (Allee et al. 1949, Stephens et al. 1999). The most well-known, and intuitive, component Allee effect is mate-finding. Roughly put, at low population densities, individuals may not be able to locate mates, and so below a critical population threshold, the population will not establish (Stephens et al. 1999). The contribution of the mate-finding component to the Allee effect has been extensively modeled (Taylor and Hastings 2005, Courchamp et al. 2008, Yamanaka and Liebhold 2009). Among the factors shown to exacerbate the mate-finding Allee effect is the need for multiple mates to reach optimal fitness, and mate choice (Allee et al. 1949, Taylor and Hastings 2005, Gascoigne et al. 2009).

As well as the potential demographic costs of multiple mating at low population densities, there are potential individual fitness costs to multiple mating for females; such as physical damage from the mating process (Morrow and Arnqvist 2003, Jones et al. 2010), and exposure to toxins or predators (Magnhagen 1991, Reinhardt et al. 2005, Siva–Jothy 2006, Eady et al. 2007, Lafaille et al. 2010). However, multiple mating is common in female insects. In most cases where the fecundity of single and multiply mated females have been compared, females benefited from mating more than once (Arnqvist and Nilsson 2000). Multiple mating by females may have several benefits; direct material benefits (e.g., nuptial gifts, fresh sperm, and gonadotropins) (Arnqvist and Nilsson 2000, Dunn et al. 2005, Edvardsson 2007, Yamane and Miyatake 2008, Avila et al. 2011), avoiding costly harassment from males (Arnqvist and Rowe 2005) or genetic benefits (Jennions and Petrie 2000, Slatyer et al. 2011).

Bronze birch borer, *Agrilus anxius* Gory (Coleoptera: Buprestidae), is endemic to North America (Bright 1987) and feeds on most members of the genus *Betula.* Larvae feed on the cambium and phloem of the tree and can rapidly kill a tree by girdling it. Most of the *Betula* spp. that are native to North America are resistant to bronze birch borer until they are under physiological stress, such as that associated with drought, or mature age (Anderson 1944, Barter 1957, Ball and Simmons 1986, Nielsen et al. 2011). The beetle is found in low densities in the forest on native trees, but has the potential to be an outbreak pest when birches are under stress. Several large outbreaks oc-

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curred in the last century during periods of birch decline (Nash 1943, Anderson 1944, Barter 1957, Ball and Simmons 1986).

Bronze birch borer is also a key pest of birch in landscape settings, especially of old world species (Johnson and Lyon 1991). Old world species of birch have been shown to have low or no resistance to attack by bronze birch borer (Nielsen et al. 2011). The situation is almost the mirror image of that seen with a congener of bronze birch borer, the invasive emerald ash borer, *A. planipennis* Fairmaire. The beetle, which has a similar ecological role to bronze birch borer in its native Far Eastern Asia, has been devastating to North American species of ash (*Fraxinus* spp.), which appear to have no resistance to emerald ash borer (Rebek et al. 2008). Recently, the European Plant Protection Organization has become concerned that bronze birch borer would have an impact on European birches similar to the impact of emerald ash borer on North American ashes if bronze birch borer were to be introduced to Europe (Herms and Muilenburg 2011).

The general reproductive biology of bronze birch borer is typical of *Agrilus* spp. (Barter 1957, Akers 1985). Males emerge shortly before females. Both males and females need 1 to 3 d of maturation feeding before becoming reproductively active (Barter 1957, Akers 1985). Mating lasts from 5 to 12 min, and there is no mate guarding or pair bonding (Barter 1957, Akers 1985). Although Barter contended that females avoided a second mating, no evidence of that has been seen in the laboratory (Akers 1985, Akers and Nielsen 1992). Given the lack of pair bonding, it is likely that subsequent matings are with new partners. Females lay eggs 6–7 d after emergence (Barter 1957, Williams and Neiswander 1959) over a 3–6 wk period. Females have been found to lay up to 25 eggs (Barter 1957).

A study on the effect of multiple mating in bronze birch borer by Akers and Nielsen (1992) found that mating frequency did not affect fecundity or fertility. However, in that study, females produced many fewer eggs (mean = 3.5 eggs/females) than seen in previous studies (Barter 1957), suggesting that benefits of multiple mating might have been masked by overall unfavorable conditions for the females. Rutledge and Keena (2012) found that in emerald ash borer, a single mating resulted in either a full complement of fertile eggs, or no eggs at all. The percentage of females that had no eggs dropped steeply when females were allowed to mate multiple times. This suggested that multiple mating is the optimal strategy for female emerald ash borer to ensure fecundity. Female emerald ash borer housed with a previous mate laid more eggs more often than females housed with a randomly chosen male, suggesting that emerald ash borer is 'choosy' when it comes to mating partners. To determine if the earlier work by Akers and Nielson (1992) represented a real difference between the congeners, we followed the protocols of Rutledge and Keena (2012) with bronze birch borer. If bronze birch borer can reach optimal fitness with one mating, this could have implications for population dynamics, as well as for laboratory rearing efforts.

In this study, we determined the lifetime fecundity and fertility of females in three mating treatments. In the first, we allowed beetles to choose mates, and then housed the mated females individually, ensuring that she had only one mating opportunity. In the second treatment, we allowed males and females to choose partners and mate. These females were then housed with their mates to allow them subsequent mating opportunities. In the third treatment, arbitrarily chosen males and females were housed as pairs, ensuring them the opportunity to mate, but denying them the ability to choose their own partner. Finally, to determine the frequency of successful spermatophore transfer, a fourth group of females was frozen and dissected immediately after mating.

Materials and Methods

Insects. Two heavily infested specimens of *Betula utilus* variety *jacquemontii* (Spach) were collected in February 2011. The trees were located in the landscapes of two private homes on the same block in Portland, CT (41.5739 N, 72.5741 W) and showed evidence of multiple years of infestation. The trees were cut into bolts and brought back to the laboratory, where they were stored in an unheated screened shed. We began checking for emergence in the third week of May, and once emergence started, beetles were collected daily before 10:00 a.m. Collected beetles were sexed on site by examining the color of the frons (female beetles have a copper colored frons, while the males are green) and by looking for a groove on the first and second abdominal sternite, which is found only on the male (Bright 1987). They were then placed in single-sex vials and transported directly to the U.S. Department of Agriculture Forest Service quarantine facility in Ansonia, CT. Individuals were placed into single-sex 7.6 liters containers for at least 3 d of maturation feeding before use in experiments. The containers (22.2 liters × 21.1 w × 22.2 h cm) have a single 7.5 cm hole cut in the tight-fitting lid and in two opposite sides of the container. The side holes have white, no-see-um, polyester netting hot glued over the holes and a square of the same fabric over the container opening. Beetles were supplied hardened-off foliage of *B. utilus* variety *jacquemontii* and water. The ends of a branch with 10 leaves were placed through a 5 mm hole in the lid of a 236.5 ml plastic container (Berry Plastics T31408CP, Evansville, IN) with water in it before being put in the 7.6 liters containers. Water was supplied by inserting a 5 cm long piece of dental wick through a hole in the lid of a 29.6 ml squat plastic container (Solo Cup Company P100 cup with a PL1 lid, Lake Forest, IL). A paper towel was placed in the bottom of the container to absorb excess moisture. Beetles were held at 25 ± 2°C, a photoperiod of 16:8 L:D h and 65 ± 5% relative humidity (RH). These conditions have been shown to be ideal for *A. planipennis* Fairmaire, a close relative of *A. anxius* (Keena et al. 2009). Foliage was changed

twice a week. Voucher specimens were deposited at the Entomology Collection, Department of Entomology, The Connecticut Agricultural Experiment Station, New Haven, CT.

Mating Procedure. Up to 20 males and 20 females were placed together in a 7.6 liters plastic container with birch foliage and water, as previously described. Only males and females that had at least 3 d of maturation feeding were used (mean = 4.64 ± SE 0.08 d, range 3–6 d). The mating cage was placed in direct sunlight coming through a window to encourage mating. As natural pairs formed, they were removed from the cage and held separately in petri dishes (90 diameter × 15 d mm) to monitor time in copula. After mating, individuals were assigned numbers and weighed.

Background Level of Mating Success. To assess the success rate of spermatophore transfer in matings, 48 pairs of beetles were allowed to mate as described above. Individuals in each pair were assigned numbers and weighed. Females were then frozen, dissected, and inspected under a dissecting scope for the presence of a spermatophore in the common oviduct.

Mating Frequency Treatments and Pair Maintenance. Beetles were placed in one of three mating treatments. Two mating treatments were comprised of beetles that had been observed mating in the mating arena described above. For the 'observed together' treatment, pairs that had been observed mating were housed together until the female died. In the 'observed separate' treatment, mated females were housed alone and followed until the female died. Finally, in the 'unobserved together' treatment, an arbitrarily chosen male and female were housed together and followed until the female died. After mating or pairing, the beetles were assigned numbers and weighed.

Beetles were housed in wide-mouth 0.9 liters glass jars with a mesh lid (same mesh as used for the larger containers), foliage, water, and an egg-laying substrate. The stem of the foliage, 1–2 leaves of *B. utilus* variety *jacquemontii* prepared as previously described, and a piece of dental wick were placed through a hole in the lid of a 29.6 ml squat plastic container (Solo Cup Company P100 cup with a PL1 lid, Lake Forest, IL). The egg-laying substrate was a 20 cm long piece of 1.9 cm diameter solid PVC bolt wrapped first with white butcher paper, and then with a strip of 1.9 cm wide purple curling ribbon (spaced 1 cm apart so it did not overlap). One-third of a white tri-fold paper towel or a 9 cm diameter filter paper was placed in the bottom of the jar to soak up excess water but was also used by females for oviposition. Beetles were checked 2–3 times a week for mortality. The foliage was changed twice a week, and the bolts and filter paper were changed and checked for eggs once a week. Any eggs were removed and checked daily (except weekends) for hatching. The eggs were held in petri dishes placed on a platform over water in the bottom of a transparent plastic box (30 × 70 × 20 cm) to maintain high humidity, and they were held at the same conditions as the adults. At the end of 3 wk, any unhatched eggs were assessed to see if they were fertilized (eggs turn brown as the larva develops) or not (unembryonated eggs remain yellow in color and often desiccate). For both the unobserved together and the observed together treatments, any dead males were replaced with an arbitrarily chosen male so that a male was present throughout the life of the female. Females that did not survive at least 2 wk after the mating date were not included in the analyses because they may not have had time to start laying eggs, a critical parameter in this study.

Statistics. Linear regression was used to evaluate if the males and females that were allowed to choose their partners were using size as a factor in choosing their mates. Data from the two treatments where beetles had a choice of partner (observed together, observed separate) were pooled for this analysis.

A chi-squared test was used to assess if the number of females who laid at least one egg differed among the observed together, observed separate, and unobserved together treatments. The three mating treatments were compared further using analysis of variance (ANOVA) with treatment and egg laying status (whether or not the female had laid at least one egg or no eggs) as independent factors. The dependent factors tested were female weight, male weight, longevity, fecundity, and fertility. Fecundity was measured as the total number of eggs laid. To normalize the fecundity data, which was strongly skewed, it was ranked in all analyses. Fertility was considered to be the proportion of viable eggs (embryonated, whether they hatched or not), and the values were transformed by taking the arcsine square root of the proportions to normalize the data.

Finally, general linear models (GLM) were used to test for correlations between longevity and fecundity of egg-laying females, longevity and fertility of egg-laying females, weight and fecundity of egg-laying females, weight and fertility of egg-laying females and between fecundity and fertility of egg-laying females. All analyses were completed using SYSTAT nine (SPSS 1999).

Results

Partner Choice. In the mating treatments where male and female beetles were allowed to choose their partners from a large group of individuals, there was no correlation between male and female weights ($R^2 = 0.000$; $F_{1, 161} = 0.039$; $P = 0.844$).

Background Level of Mating Success. Forty-seven out of 48 of the females dissected after mating had spermatophores in their reproductive tracts, while one female had no spermatophore, a success rate of 98%. With only one nonsuccessful mating attempt, it is not possible to statistically compare the weights of participants in successful versus nonsuccessful pairings, but the nonsuccessful male and female were both within the normal range (Table 1). The length of the nonsuccessful mating was also well within range of successful matings (nonsuccessful 8 min; successful mean = 11.33 ± 0.80 min, range 4–45 min).

Table 1. Background success rate of spermatophore transfer and wt (±SE) on mating pairs of *Agrilus anxius* dissected immediately after mating, and wt (±SE), lifespans (±SE), fecundity (±SE), and fertility of *A. anxius* subjected to three different mating treatments

Treatment	Laying status[a]	N	Lifespan (d)[b]	Weight (g)	Fecundity[c]	Fertility (%)[d]	Male weight[e]
Dissected	Sperm	47	NA	0.0281 (±0.0006)	NA	NA	0.0189 (±0.0003)
	No sperm	1	NA	0.0301	NA	NA	0.0225
Observed together	Layers	80	37.45 (±2.20)	0.0296 (±0.0005)	71.49 (±6.92)	93.7	0.0219 (±0.0018)
	Nonlayers	4	22.93 (±1.26)	0.0273 (±0.0018)			0.0189 (±0.0022)
Observed separate	Layers	69	33.37 (±1.63)	0.0296 (±0.0006)	59.62 (±5.95)	90.0	0.0196 (±0.0004)
	Nonlayers	10	23.40 (±2.02)	0.0273 (±0.0012)			0.0194 (±0.0011)
Unobserved together	Layers	73	35.96 (±2.02)	0.0296 (±0.0005)	62.29 (±6.01)	92.1	0.0198 (±0.0003)
	Nonlayers	3	21.00 (±2.75)	0.0237 (±0.0045)			0.0238 (±0.0020)

[a] Laying status indicates whether the female laid any eggs during her lifetime. For background success rate, females were dissected to determine if sperm was successfully transferred to the female.
[b] Lifespan is days since emergence.
[c] Fecundity is no. of eggs laid over the course of a lifetime by laying females only.
[d] Fertility is percent of viable eggs. Eggs damaged during transfer or damaged by mold were excluded from the calculation.
[e] Weight of first male partner, subsequent male weights are not incorporated into averages.

Treatment, Fecundity, and Fertility. There was no significant effect of treatment on how many females laid eggs ($\chi^2 = 5.53$; df = 2; $P = 0.063$) (Table 1). When females that laid no eggs were removed from the analyses, there was not a significant difference in fecundity among the three treatments ($F_{2,220} = 1.35$; $P = 0.261$). The proportion of viable eggs also did not differ among treatments ($F_{2,215} = 0.515$; $P = 0.598$). There was no correlation between the number of eggs and the percentage of eggs that were viable ($R^2 = 0.015$; $F_{1,219} = 0.216$; $P = 0.071$).

Beetle Size and Fecundity. *Female Weight.* Overall, females that laid eggs were significantly heavier than those that did not ($F_{1,233} = 7.299$; $P = 0.007$). However, there was no significant difference among treatments in the weights of females ($F_{2,233} = 0.693$; $P = 0.501$), nor was there significant interaction between treatment and laying status (whether or not a female had laid any eggs at all) ($F_{2,233} = 0.727$; $P = 0.484$) (Table 1). There was a slight, but significant correlation between fecundity and female weight among laying females ($R^2 = 0.038$; $F_{1,220} = 8.649$; $P = 0.004$). There was no correlation between weight and fertility (% egg viability) ($R^2 = 0.005$; $F_{1,215} = 1.009$; $P = 0.316$).

Male Weight. There was no significant difference among treatments in the weights of males ($F_{2,233} = 1.234$; $P = 0.792$), nor was there a significant difference between the weights of males who were partnered with females who laid eggs and those that did not ($F_{1,233} = 0.009$; $P = 0.926$). There was also no significant interaction between treatments and partners' laying status in male weights ($F_{2,233} = 0.438$; $P = 0.646$). Paternal weight was not correlated with fecundity ($R^2 = 0.008$; $F_{1,220} = 1.841$; $P = 0.176$), or fertility ($R^2 = 0.002$; $F_{1,215} = 0.529$; $P = 0.468$).

Longevity. Treatment did not impact longevity ($F_{2,233} = 0.038$; $P = 0.963$). There was a significant difference between the lifespan of females that laid eggs and those that did not ($F_{1,233} = 7.649$; $P = 0.006$), with laying females living an average of 12.1 d longer than females who did not lay eggs. There was no interaction between treatment and laying status, indicating that the relationship between laying status and lifespan was similar among all three treatments ($F_{2,233} = 0.195$; $P = 0.823$). Among laying females, there was a significant, positive correlation between lifespan and fecundity ($R^2 = 0.348$; $F_{1,220} = 117.437$; $P = 0.000$), although there was no correlation between longevity and fertility ($R^2 = 0.000$; $F_{1,215} = 0.006$; $P = 0.938$). Weight and longevity were not correlated ($R^2 = 0.000$; $F_{1,237} = 0.031$; $P = 0.859$).

Discussion

Multiple mating did not increase the fecundity or the fertility of bronze birch borer, which conforms to the findings of Akers and Nielson (1992). The rate of successful spermatophore transfer also did not differ significantly between our study (47 successful transfers in 48 matings) and Akers and Nielson's study (13 successful transfers in 15 matings) ($\chi^2 = 3.19$; df = 1; $P = 0.074$). However, in our results, percentage of females ovipositing and observed fecundity were considerably higher than those observed in Akers and Nielson (1992) (Table 2). The results were particularly stark when comparing the numbers for females fed on *B. pendula* (Roth): only 23% of females laid eggs (with an average of five eggs apiece), whereas in our study, 93% of females laid eggs (with an average of 54 eggs apiece). Part of that difference is attributable to the shorter lifespan of bronze birch borer feeding on *B. pendula* as lifespan is positively correlated with fecundity. However, in Akers and Nielson's study, even in the treatment in which bronze birch borer were fed the preferred *Populus deltoides* Bartram ex Marsh. foliage, the percentage of females ovipositing and their fecundity was still much lower than in our study (Table 2). How much of this difference in fecundity is because of diet, and how much is because of experimental conditions is unknown. Barter (1957) also found that adult bronze birch borer preferred poplar to *Betula* spp. (unspecified), but recorded a similar lifespan and fecundity to what we found. In our study, we casually observed that bronze birch borer did not feed on *B. populifolia* Marsh. but readily fed on *B. jaquemontii* foliage, even though both are acceptable larval hosts.

Table 2. A comparison of fertility and fecundity of *Agrilus anxius* in this study and in Akers and Nielson (1992)

	N[a]	No. laying females[b]	Foliage species	Lifespan[c]	Fecundity[d]	Fertility[e]
Rutledge and Keena (2012)	283	242a	*Betula jaquemonttii*	29.7 (±1.06)a	54.8 (±3.52)a	0.91a
Akers and Nielson (1992)	58	14b	*Betula pendula*	17.1 (±1.19)b	5 (±1.17)b	0.17b
	55	32c	*Populus deltoides*	31.0 (±3.14)a	14 (±3.72)c	0.16b

[a] This includes 44 females that were not included in the main statistical analyses because they died at 14 d or younger. This was done to make the data more comparable as Akers and Nielson (1992) included all females in their data.
[b] Number of females who had any eggs.
[c] Lifespan is days since emergence.
[d] Fecundity is no. of eggs laid over the course of a lifetime by laying females only.
[e] Fertility is percent of viable eggs. Eggs damaged during transfer or damaged by mites or mold were excluded from the calculation.

We see both similarities and differences in the impact of multiple mating on the fecundity of bronze birch borer and the impact of multiple mating on emerald ash borer seen in Rutledge and Keena (2012). In both species, females were able to reach their full reproductive potential, both in terms of fecundity and fertility, with one successful mating. Additionally, for females of both species, at least under laboratory conditions, there were no negative consequences to being housed with a male (we are assuming that multiple mating took place when females were housed with males as we frequently observed matings while changing foliage and collecting eggs). Females in treatments with males lived as long and had as many eggs as females that were housed alone. In addition, both species showed positive correlations between longevity and fecundity, and weight and fecundity.

However, there were some significant differences between the species. Bronze birch borer males were more successful at transferring a spermatophore than were emerald ash borer males, with bronze birch borer males succeeding in 47 of 48 attempts, while emerald ash borer succeeded in only 43 of 52 attempts. As there is a low chance that a single bronze birch borer mating will fail, it is likely that a female will be able to successfully reproduce after a single mating. Indeed, while we found that 45% of singly mated emerald ash borer females failed to lay any eggs, the percentage of singly mated bronze birch borer females that failed to produce any eggs after a single mating (12%) was lower. Interestingly, in both species it seems that a successful mating may not be the only determining factor in whether or not the female produces eggs. In both species, the rate of failure to produce eggs after a single mating was 3–6 times as high as the observed rate of failure to pass a spermatophore.

Another difference between the two species was seen in the unobserved together treatment, in which females were housed with a random male instead of with one with which she had already mated. In emerald ash borer, the proportion of females not laying any eggs after being paired with a 'stranger' (12 of 31 females laid no eggs) was as high as that of females allowed only one mating opportunity (14 of 31 females laid no eggs) and much higher than when they were paired with a male with whom they had already mated (4 of 31 females laid no eggs). In bronze birch borer, the proportion of failures after being paired with a stranger (3 of 76 females laid no eggs) was the same as for females in the observed together mating treatment (4 of 84 females laid no eggs) where females were paired with a male whom they had already mated. This implies that bronze birch borer, under these circumstances, shows less mate discrimination than does emerald ash borer. When randomly chosen emerald ash borer couples were paired for 90 min, only 12% of the pairs copulated (12/97; C.E.R., unpublished data), while bronze birch borer under similar circumstance had a higher rate of success, with 62% copulating (15/24) (C.E.R., unpublished data).

Taken together, these two differences (a higher rate of success in passing a spermatophore and a lower degree of choosiness) suggest that bronze birch borer females may have a wider range of acceptable partners and may be less reliant on multiple mating to ensure fecundity and fertility than emerald ash borer. This will facilitate rearing under laboratory conditions as successful eggs production will occur under a variety of situations. This also could have implications for the establishment and spread of bronze birch borer in non-native habitats. In Rutledge and Keena (2012), we speculated that in low density populations, emerald ash borer may face a mate-finding Allee effect. As a result, their reproductive success, and therefore, population growth might be limited by their ability to find sufficient, acceptable partners (Allee et al. 1949, Kokko and Rankin 2006, Gascoigne et al. 2009, Rhainds 2010). However, if bronze birch borer females are able to reach high fecundity with a single mating with any male, they are more likely to find sufficient acceptable partners and are less likely to face an Allee effect. As a consequence, invasive populations may have a lower critical population threshold, adding to EPPO concerns about bronze birch borer (Herms and Muilenburg 2011).

However, we should note that we are not examining analogous populations. The emerald ash borer used in Rutledge and Keena (2012) were from Michigan, at the core of the invasive population, whereas the bronze birch borer used in this study were from a native population. Emerald ash borer is less genetically diverse in North America than in its native range (Bray et al. 2011), and may differ from native populations in other life history traits (Diamantidis et al. 2008). Whether the differences between these two

species are because of the current genetic diversity within the populations, to their status (native or invasive) or to some other difference needs to be further evaluated.

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