Density-Dependent Development in *Wyeomyia smithii* (Diptera: Culicidae): Intraspecific Competition Is Not the Result of Interference

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ABSTRACT Density-dependent development in the pitcher plant mosquito, *Wyeomyia smithii* (Coquillett), indicates variation in competitive ability both within and between populations. We tested 2 disparate populations of *W. smithii* for 3 types of interference competition: chemical and encounter competition and cannibalism. We found no significant effects of any of these interference mechanisms or variation in food consumption within or between populations. We suggest that asymmetric competition in *W. smithii* is more likely the result of differences in assimilation or use of assimilated resources than to larval interference or differential ability to consume food.

KEY WORDS *Wyeomyia smithii*, competition, interference, mosquito

INTRASPECIFIC COMPETITION AMONG larval mosquitoes results in prolonged development, reduced survivorship, lower pupal weight, and, consequently, lower fitness in nature (Mogi 1984, Hawley 1985, Bradshaw and Holzapfel 1986, 1992), in quasi-natural microcosms (Frank and Curtis 1977, Livdahl 1982, Bradshaw and Holzapfel 1989), and in laboratory populations reared on natural substrates (Carpenter 1982, Fish and Carpenter 1982, Hard et al. 1989, Fisher et al. 1990). In addition to the depletion of food by scramble or resource competition, competitive interference may occur through direct physical contact (Shannon and Putnam 1934, Dye 1984, Broadie and Bradshaw 1991), through chemical inhibition by metabolites or growth retardants (Moore and Fisher 1969, Ikeshoji and Mulla 1970, Dye 1984, Broadie and Bradshaw 1991), and/or through cannibalism among otherwise non-predatory larvae (Mogi 1978, Koenekoop and Livdahl 1986). Thus, all 3 modes of interference competition have been clearly demonstrated in mosquitoes.

In the pitcher-plant mosquito, *Wyeomyia smithii* (Coquillett), increased larval density results in the expected loss of fitness (Istock et al. 1975; Bradshaw and Holzapfel 1986, 1990, 1992) as a consequence of increased generation time and reduced survivorship and replacement rates. When food and density are varied in a full-factorial design (Istock et al. 1975), there is a significant food-by-density interaction, implying some form of intrapopulation interference or facilitation. When single populations are reared at a variety of densities in intact pitcher-plant leaves under near-natural conditions in the laboratory, there is no significant correlation between density tolerance and latitude or altitude of origin (Bradshaw and Holzapfel 1989). When different populations are forced to encounter each other in the same leaf at high densities, however, southern populations achieve a higher rate of increase than northern populations. Thus, in *W. smithii*, there is an implication of interference competition within at least 1 northern population (Istock et al. 1975) and evidence for differential competitive ability among populations (Bradshaw and Holzapfel 1989). In our current article, we set aside the question of modes of exploitation competition and address the question of whether interference competition affects survivorship and development within and between a northern and a southern population of *W. smithii*.

Materials and Methods

Source of Populations. Laboratory stocks were established from collections in northern Florida (30° N latitude) and eastern Massachusetts (42.5° N latitude) (localities WI and FV of Bradshaw and Holzapfel 1989, 1990). These stocks were reared in the laboratory for 5 generations before the start of experiments, under conditions given in Bradshaw (1986). All experiments used individuals from each stock population. Competition experiments evaluated the effect on a test cohort of competition from the same or different population.

Lifetime Fecundity. Larvae were reared as uniform-aged cohorts from either the southern or northern population in the leaves of intact pitcher plants under near-natural conditions of light, temperature, and food as described in Bradshaw (1986). These experiments were carried out in a

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controlled-environment room maintaining 80% RH, a long-day photoperiod of 18:6 (L:D) h, including 2, 0.5-h dim twilights, and a smooth sine-wave thermoperiod with a mean of 21°C and a daily flux of 15°C, lagging the light cycle by 3 h. Larval cohorts from each population separately were reared at low (10 larvae per leaf) and high (40 larvae per leaf) densities with 200 freeze-dried, adult *Drosophila melanogaster* Meigen added as food. Female pupae were weighed to the nearest 0.01 mg and individual pupae were transferred to a 0.95 liter small cohort cage made from polyethylene freezer containers (1 qt). A pesticide-free raisin was provided as a carbohydrate source. Each cage contained a cut-off scintillation vial (2.5 by 4.0 cm) with 15 ml water and a 2.0 cm disk cut from a leaf of *Sarracenia purpurea* L. to stimulate oviposition.

Females were maintained singly and were provided with 3 male consorts chosen haphazardly from a continuously developing colony. Cages were checked 3 times per week for the life of the females. At each check, eggs were counted and removed and then observed for 1 wk to score hatching and assure fertility. Lifetime fecundity was then calculated for individual females from the northern and southern population from the lifetime number of eggs produced subject to the restriction that, to be entered into the analysis, a female had to have produced at least 1 fertile egg.

Chemical Competition. These experiments were designed to test for diffusible chemical effects on 1st instars imposed by potentially competing 1st or 4th instars of the same or different population. Glass experimental containers were constructed with the 2 sides separated by a plastic partition. Three, 2.5 cm disks of 0.22-μ pore membrane filters (Nuclepore Corporation, Pleasanton, CA) were glued into the partition to allow molecular flow between the 2 sides, but blocked the passage of bacteria or protozoa. Each side of the container was filled with 15 ml distilled water. On 1 side was placed the 10 first instars <36 h from time of hatch; on the other side were placed either 10 first instars (control), 10 fourth instars, or 80 fourth instars from the same or different population. All containers were maintained in a single incubator at 18.0 ± 0.5°C and a photoperiod of 16:8 (L:D) h. Twenty-five dead adult *D. melanogaster* were placed on each side of the container on the day of mosquito hatch and then 100, 50, and 25 dead adult *D. melanogaster* after 1, 2, and 3 wk, respectively. Each experimental cohort was checked 3 times per week until all larvae had developed or died. At each check, moribund 4th instars were replaced to maintain a constant competitor density, experimental pupae were removed and weighed and their development times and weights recorded. For the experimental larvae, percentage pupation (arcsine transformed), pupal weight, and pre-adult development time were used as separate indices of fitness. These traits were also combined into a composite index of fitness,

$$r_c' = \frac{\ln[(\text{female pupal weight})(\%\ \text{pupation})]}{(\text{days from oviposition to adult eclosion})}$$

Female pupal weight is positively correlated with egg number and serves as an estimate of fecundity; percentage of pupation is an estimate of survivorship; and together they provide an analogue of cohort replacement rate. Cohort mean generation time is the time from oviposition to mean egg $\tau = \Sigma x E_x / \Sigma E_x$ where E_x = number of eggs produced by the cohort on day x after oviposition of the cohort. Time from adult eclosion to oviposition of mean egg varies little among populations of *W. smithii* (Bradshaw and Holzapfel 1990). Development time from oviposition to adult eclosion thus provides a good analogue of generation time.

Each index of fitness was analyzed using type III sums of squares in the GLM procedure (SAS Institute 1985) in a replicated full-factorial design with independent variables (1) population of experimental 1st instars (south, north), (2) population of competitor larvae (south, north), (3) density of competitors (10 first, 10 fourth, and 80 fourth instars), and their 2- and 3-way interactions. In the case of pupal weight and development time, sex (male, female) was added as another level in the analysis.

Encounter Competition. Encounter competition was assessed by measuring food consumption of southern or northern larvae at low and high densities in the presence of competing larvae from the same or different population using dried, dyed food as in Broadie and Bradshaw (1991) with the following modifications. Food consisted of ground *D. melanogaster*. Food consumption was measured as the number of abdominal segments filled with dyed food (Dadd 1968), to the nearest 0.5 segment. To establish the experimental feeding time, 4 fourth instars from day 3 were fed 0.05 g dyed food in a 60-mm petri dish containing 15 ml distilled water. Based on the resulting time–dependent feeding curve (Fig. 1), 20 min was selected as the fixed experimental feeding time. All experiments were conducted in an incubator set at 21.0 ± 0.5°C in the light, used a 60-mm petri dish with 15 ml distilled water and 0.05 g food, and used 4th instars from day 3. Experimental densities were 4, 8, 16, 32, and 64 larvae per dish with 5–10 replicates at each density. Single population (north or south) experiments were run at all densities. Because of difficulties in obtaining large numbers of synchronously molting larvae, mixed populations (north : south = 1:1) were run at densities of 8 and 64 per dish only. As in Broadie and Bradshaw (1991), feeding rates were determined at low density (4 per dish single population; 8 per dish mixed population) before and after each experimental replicate. Feeding rates at these low densities were tested with 1-way analysis of variance (ANOVA) to confirm that food availability had not diminished and that chemical inhibitors were not accumulating during experiments.

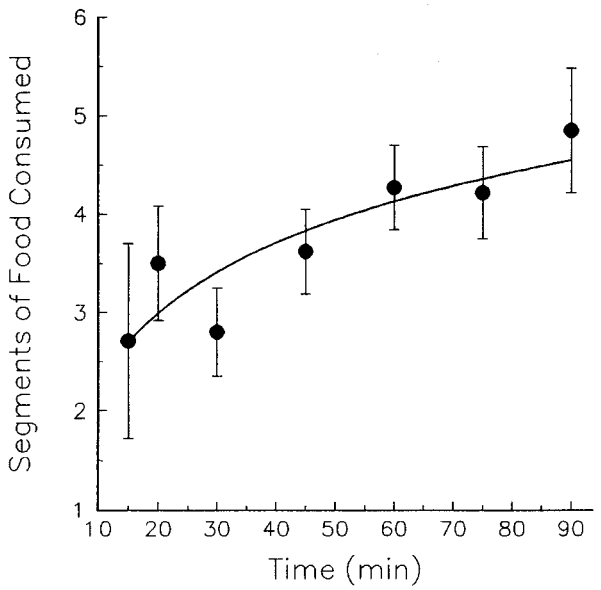

Fig. 1. Dependence of food consumption (number of abdominal segments containing dyed food) on feeding time of 4, 3-d-old 4th instars (mean ± 2 SEM).

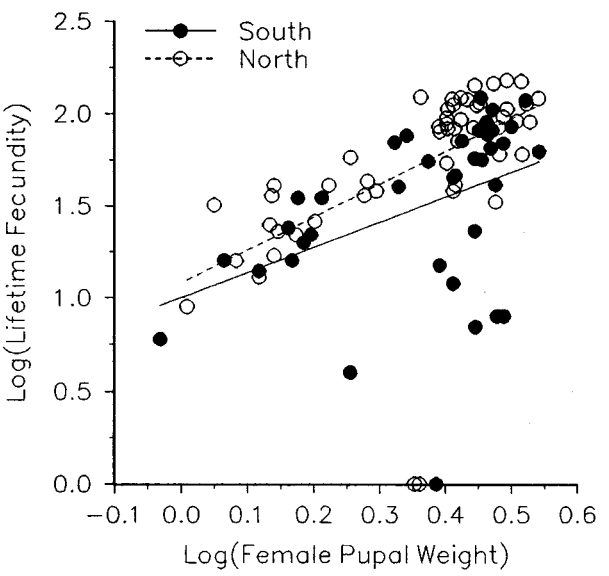

Fig. 2. Effect of female pupal weight on lifetime fecundity by geographic origin of the population (north or south).

Feeding rates were analyzed using type III sums of squares of the GLM procedure (SAS Institute 1985) using a full-factorial design with independent variables population of origin (north, south), encounter (intra-, interpopulation), density (8, 16, 32, 64 per dish), and their 2- and 3-way interactions. The mean number of segments of food consumed in the 1st and last low density trials were compared by ANOVA to confirm that food level had not significantly diminished through intermediate trials.

Cannibalism. Cannibalism was assessed by exposing cohorts of test 1st instars from the southern or northern population at high and low density to competing 4th instars from the same or different population. Test 1st instars alone (without 4th instars) served as the control. Trials were run at densities of either 20 or 50 untanned 1st instars per 15 ml of distilled water in a 60-mm petri dish. At each density, for each population, and for the presence of competitors from the same or different population, 10 trials were conducted with 10 fourth instars and 10 trials with no 4th instars present. After 72 h, the remaining 1st instars were counted to determine percentage of survivorship. All possible combinations of 4th and 1st-instar populations were tested. Percentage of survivorship (arcsine transformed) was analyzed using type III sum of squares of the GLM procedure (SAS Institute 1985) using a full-factorial (2 × 2 × 2) design with independent variables: density of 1st instars (20, 50), density of 4th instars (10, 0), origin of 1st instars (south, north), and their 2- and 3-way interactions.

Results

Lifetime Fecundity. Lifetime fecundity of females from both the south and the north was positively correlated with pupal weight (Fig. 2). Analysis of covariance of lifetime fecundity with female pupal weight as a covariate and density (10, 40 larvae per leaf) and locality of origin (south, north) as treatments revealed significant effects of pupal weight ($F = 16.59$; df = 1, 90; $P < 0.001$) but not locality or density or any 2- or 3-way interactions among pupal weight, locality, and density (max $F = 3.86$; df = 1, 90; $P > 0.05$).

The common regression of log(lifetime fecundity) on log(female pupal weight) did not differ significantly from 1.0 ($b \pm s_b = 1.59 \pm 0.31$).

These results show that gross lifetime fecundity of the southern and the northern population was an increasing, isometric function of female pupal weight and this function did not differ between the 2 localities. Consequently, pupal weight serves as a good analogue of lifetime fecundity in both populations and was entered into equation 1 without adjustment for population of origin.

Chemical Competition. ANOVA of larval survivorship showed no significant effects of population of origin, competitor density, or their interaction (Model $F = 2.40$, df = 8,40; $P > 0.05$; Fig. 3A). ANOVA of pupal weight (Fig. 3B) revealed significant effects of sex ($F = 1105.08$; df = 1, 82; $P < 0.001$) and population of origin ($F = 8.99$; df = 1, 82; $P < 0.01$), but not competitor density ($F = 2.74$; df = 2, 82; $P > 0.05$) or any interactions (max $F = 2.42$; df = 1, 82; $P > 0.05$). Similarly, ANOVA of development time (Fig. 3 C and D) revealed significant effects of sex ($F = 11.56$; df = 1, 82; $P < 0.01$) and population of origin ($F = 10.56$; df = 1, 82; $P < 0.01$) but not competitor density ($F = 2.00$; df = 2, 82; $P > 0.05$) or any interactions (max $F = 0.59$; df = 2, 82; $P > 0.05$). Males were lighter than females but developed

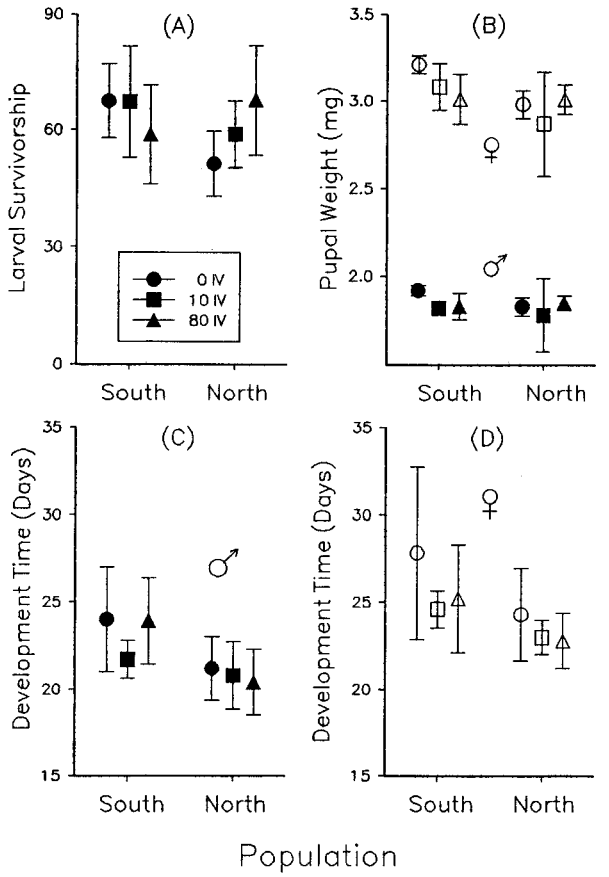

Fig. 3. Chemical competition: the transmembrane effect of competitor density on (A) larval survivorship (arcsine transformed), (B) pupal weights, and (C) male or (D) female development times. Plots show means ± 2 SEM and competitor densities of 0, 10, or 80 fourth instars (IV).

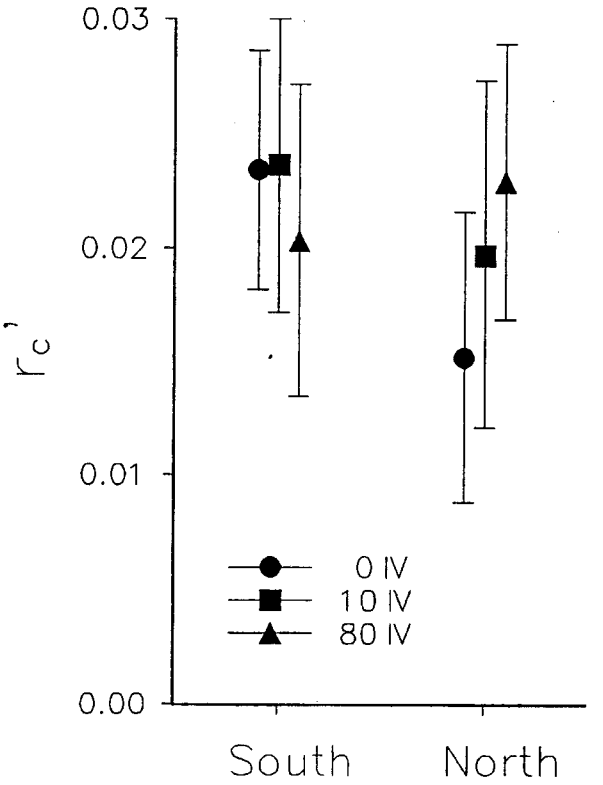

Fig. 4. Chemical competition: the transmembrane effect of competitor density on a composite index of fitness, r_c' (equation 1). Symbols as in Fig. 3.

faster; pupae from the south were heavier than those from the north but the heavier pupae developed more slowly.

When survivorship, pupal weight, and development time were combined into a composite index of fitness (Fig. 4), ANOVA of r_c' showed no significant effect of competitor density ($F = 0.26$; df = 2, 40; $P > 0.05$) or locality of origin ($F = 0.44$; df = 1, 40; $P > 0.05$); there was, however, a significant locality of origin by density interaction ($F = 3.91$; df = 2, 40; $P < 0.05$). When the zero competitor density was dropped from the ANOVA (because competitor locality of origin is undefined when competitor density is zero), the preliminary ANOVA incorporating origin of the 1st instars (south, north), competitor origin (south, north), and competitor density (10, 80 per dish) was not significant (model $F = 1.51$; df = 7, 19; $P > 0.2$) when testing for the main effects and all possible interactions.

These results show that there are differences between the sexes and between the northern and the southern population in pupal weight and development time but reveal no significant effects of chemical competition on survivorship, pupal weight, development time, or their combination, r_c'.

Encounter Competition. The feeding rate of 4th instars (Fig. 5) showed no effect of population of origin (north, south), mixture (intrapopulation,

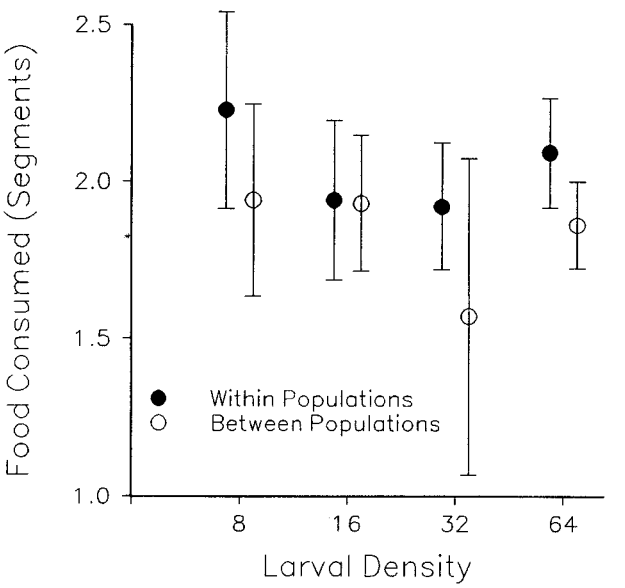

Fig. 5. Encounter competition: effect of larval density on food consumption (mean ± 2 SEM number of abdominal segments with food) of within- and between-population encounters.

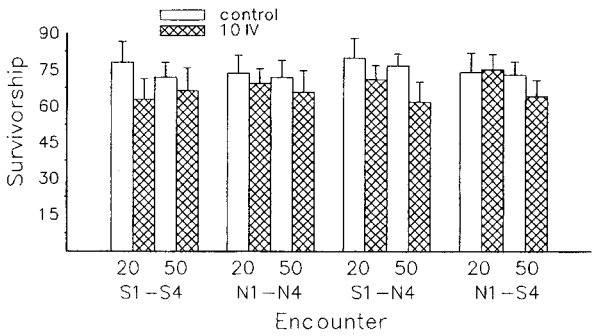

Fig. 6. Cannibalism: survivorship (mean ± 2 SEM) of 20 or 50 first instars after 72 h without (control) and with (10 IV) 10 4th instars in the same container. S1, N1, S4, and N4 represent southern or northern 1st instars encountering southern or northern 4th instars, respectively.

interpopulation encounter), larval density (8, 16, 32, or 64 larvae per container), or any of their 2- or 3-way interactions (ANOVA: model $F = 0.79$; df = 15, 100; $P > 0.5$). Fourth-instar feeding rate in the presence of abundant food is therefore similar for the southern and the northern population and is not affected by larval density, regardless of whether they encountered larvae from the same or other population. These results provide no evidence for interference by encounter competition or for density-dependent variation in food consumption within or between populations.

Cannibalism. Survivorship of freshly hatched 1st instars (Fig. 6) was not significantly affected by the density of 1st instars ($F = 3.44$; df = 1, 152; $P > 0.05$), by the density of putative cannibalistic 4th instars ($F = 0.01$; df = 1, 152; $P > 0.05$), or by any 2- or 3-way interactions (max $F = 1.25$; df = 1, 152; $P > 0.05$). Because there was no significant effect ($P = 0.93$) of 4th-instar density (10, 0), a test for independent effects of northern and southern 4th instars was not appropriate. There was a significant effect of origin of 1st instars ($F = 10.48$; df = 1, 152; $P < 0.01$). Southern 1st instars had higher survivorship than northern 1st instars, but there was no evidence for cannibalism in *W. smithii*.

Discussion

Increased density of larval encounter in *Wyeomyia smithii* leads to prolonged pre-adult development, lower survivorship to pupation, lower pupal weight, and lower fitness (Istock et al. 1975; Bradshaw and Holzapfel 1989, 1990, 1992). None of these fitness traits is significantly affected by chemical competition (Figs. 3 and 4), encounter competition (Fig. 5), or cannibalism (Fig. 6) within or between a southern and a northern population. All of these forms of interference competition have been identified in the genus *Aedes*: chemical and encounter competition in *A. sierrensis* using the same procedures in the same laboratory as the present study (Broadie and Bradshaw 1990) and cannibalism in *A. triseriatus* (Koenekoop and Livdahl 1986). We therefore conclude that interference competition is weak or absent in *W. smithii* and that larval competition involves primarily resource competition. At high densities within populations, female pupal weights become positively skewed (Bradshaw and Holzapfel 1992), indicating asymmetric competition among larvae. But herein we found no evidence for any effect of density on food consumption within populations (Fig. 5). These results suggest that in *W. smithii* asymmetric fitness among competing individuals probably results from differential ability to assimilate or to use assimilated resources.

In head-to-head encounter at limiting densities, southern populations of *W. smithii* achieve higher fitness than northern populations (Bradshaw and Holzapfel 1989). Herein we found no interpopulation interference mechanisms and no effect of density, population, or density by population interaction on feeding rate. The differential competitive ability of northern and southern populations of *W. smithii* may therefore also involve assimilation or use efficiency or some aspect of larval competition that transcends metamorphosis and affects male mating success or lifetime female fecundity.

Interference competition has been identified in the genus *Aedes*: in *A. aegypti* and the 2 treehole-breeding species, *A. triseratus* and *A. sierrensis* (Dye 1984; Broadie and Bradshaw 1990; Koenekoop and Livdahl 1986). The lack of interference mechanisms in *W. smithii* is then unlikely the result of the circumscribed nature of their habitat. Whether the presence or absence of interference competition reflects taxonomic affinity awaits its determination in a broader group of mosquitoes.

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