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SHORT COMMUNICATION

Absence of Transgenerational Phenotypic Plasticity in Fecundity in the Parasitoid *Anagrus erythroneurae* (Hymenoptera: Mymaridae)

Felipe Andreazza[1] **and Jay A. Rosenheim**

Department of Entomology and Nematology, University of California, Davis, One Shields Avenue, Davis, CA 95616
[1]Corresponding author, e-mail: andreazzafelipe@yahoo.com.br

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ABSTRACT. To improve biological pest control, we would like to understand the main factors that limit the reproductive success of key biological control agents, including parasitoid wasps. Previous research with proovigenic parasitoids in the genus *Anagrus* collected from multiple field sites revealed positive correlations between parasitoid fecundity and local host density. In this article, we test whether this variation in fecundity is a reflection of transgenerational phenotypic plasticity. We exposed females in laboratory microcosms to either low or high host densities and then quantified the fecundity of their adult daughters. Mean fecundity of daughters did not differ across host density treatments, arguing against the operation of phenotypic plasticity. Significant variation in mean fecundity across different families instead suggested the possibility that genetic variation may underlie the observed fecundity differences.

Key Words: local adaptation, egg load, fitness, *Erythroneura elegantula*

To improve biological pest control, we would like to understand the main factors that limit the reproductive success of key biological control agents. Theory suggests that parasitoids should evolve life histories that balance the risk of having fitness constrained primarily by the time needed to locate hosts ("time limitation") versus the risk of exhausting their lifetime complement of eggs ("egg limitation") (Godfray 1994). Under this theory, it has been predicted that parasitoid fecundity should evolve to match the local availability of hosts, with more fecund, but short-lived parasitoids evolving in regions of higher host density (Ellers et al. 2000). Support for this prediction has recently been reported by Segoli and Rosenheim (2013): parasitoids collected from sites supporting higher densities of leafhopper hosts were more fecund than wasps collected from sites harboring lower density host populations. However, this pattern could be generated either by 1) local adaptation (i.e., genetic changes in parasitoid fecundity) or 2) by phenotypic plasticity.

For strictly proovigenic parasitoids, like *Anagrus erythroneurae* (S. Trjapitzin and Chiappinni), opportunities for adaptive phenotypic plasticity of fecundity in response to host availability appear to be limited relative to most parasitoids. *A. erythroneurae* neither matures nor resorbs eggs during the adult stage, instead, all eggs are matured during the pupal stage of the developing parasitoid. Thus, the opportunity for adult *A. erythroneurae* to adjust rates of egg maturation in response to perceived host availability, which is well developed in most adult synovigenic parasitoids (Heimpel and Rosenheim 1998, Papaj 2000), is lacking. The pupal stage of *A. erythroneurae* occurs within the leafhopper host egg, which is imbedded in *Vitis* leaves; from such a location, it is difficult to envision how cues revealing information about local host density could be perceived by developing pupal *Anagrus*. Nevertheless, our experiment (see Materials and Methods) should reveal the operation of such a mechanism, should one exist.

Phenotypic plasticity could still be important, however, despite the proovigenic pattern of egg maturation, through two different possible mechanisms. First, leafhopper hosts might produce larger volume eggs when their densities are higher, resulting in greater resource availability for developing *Anagrus* parasitoids and thus larger and more fecund females. No support for this possibility was found by Segoli and Rosenheim (2013), however, who showed that neither leafhopper egg size nor parasitoid size varied between high-density sites (in agricultural settings) and low-density sites (in natural settings). Second, the maternal environment might shape the phenotype of the offspring through transgenerational phenotypic plasticity. Recent work has demonstrated that a surprisingly broad array of traits in many insects may be modified through phenotypic plasticity, including traits related to egg size and number (Mousseau and Fox 1998, Stillwell and Fox 2009). The goal of this work was to test experimentally the possibility that the host density experienced by the mother (low vs. high) might shape the fecundity of the daughters produced, i.e., we wished to ask if a mother's perception of host availability shapes the process of egg maturation that occurs during the pupal stage of her offspring. Proovigenic species like *A. erythroneurae* offer an ideal opportunity to test this hypothesis, because transgenerational plasticity in offspring fecundity is less likely to be confounded with plasticity occurring within the adult stage of the offspring, as would be likely in a synovigenic species. The experimental protocol we employed would, however, also detect within-generation phenotypic plasticity occurring during the pupal stage of the offspring in response to local host density, if it were operating. A second goal was to assess the possibility that different females, collected from a variety of sites, might produce offspring differing in mean fecundity, as a first step toward assessing the possibility of genetic variation in fecundity in this species.

Materials and Methods

The parasitoid collecting sites used for the experiments were 1) the University of California Cold Canyon Preserve located west of Winters, CA (38° 30′27.40″ N, 122°05′49.10″ W), a natural habitat with historically low leafhopper densities (Population 1); 2) a low-input commercial vineyard in Pope Valley, CA (38° 39′02.62″ N, 122°27′56.53″ W) with historically moderate leafhopper densities (Population 2); and 3) two adjacent high-input commercial vineyard blocks in Napa Valley, CA (38° 27′15.46″ N, 122° 25′36.32″ W) with historically high leafhopper densities (Populations 3 and 4). Leafhopper density at each site was estimated by collecting a sample of 40 leaves from the middle third of each cane, randomly with respect to damage or leafhoppers present. One half of each leaf (top and bottom) was inspected under a stereomicroscope to count: 1) unemerged eggs without any sign of parasitism; 2) unemerged eggs showing signs of parasitism; 3) empty eggs from which a leafhopper had emerged; and 4) empty eggs from which a parasitoid had emerged.

Leaves collected from each site and containing parasitized leafhopper eggs were held in cardboard emergence cages (12.7 cm in diameter and 24 cm in height) fitted with a funnel at the top leading emerging wasps to a glass collecting vial supplied with a strip of honey-saturated filter paper. Emerging female wasps were collected each morning between 07:00 and 10:00 h, allowed to mate with males from the same population, and then released singly into a rearing cage. Rearing cages contained a single grapevine, *Vitis vinifera* (L.) cv. Chardonnay hosting either high or low densities of leafhoppers, *Erythroneura elegantula* (Osborn). The vines were grown from dormant cuttings in a greenhouse free of pests and pesticides.

The experiment was run from 28 June 2014 to 14 August 2014. Leafhoppers were collected as adults from a high-density population in a small private vineyard in Davis, CA (38° 32′58.86″ N, 121° 46′48.03″ W). We added either 10 adult leafhoppers per vine (low density treatment) or 30 adults per vine (high density treatment) 4 d prior to introducing a single mated female parasitoid per cage. Cages were held at ambient laboratory temperature (24–26°C) and natural day length. The rearing cages were cylindrical, 12.7 cm in diameter and 33 cm in height, with fabric-covered vents on the side and top. Ten days after introducing parasitoid females, vines were transferred into emergence cages to collect the progeny of the experimental parasitoids. Emergence cages were of the same design as used in the original parasitoid collections but with the bottoms removed to allow them to be fitted over the potted grapevines. Emerging parasitoids were collected daily. All parasitoids were sexed, and females were dissected under a stereomicroscope to establish egg load. All females were slide mounted, the length of a hind tibia measured with an ocular micrometer as an index of body size, and species identity confirmed using a phase-contrast microscope and antennal and wing morphology (Triapitsyn 1998). To evaluate if the leafhopper egg density treatments were established successfully, we quantified egg densities in five half-leaves taken per cage for five replicates of each treatment. Note that under our experimental design, the hypothesis of transgenerational phenotypic plasticity could be supported either by 1) an increase in fecundity of daughters whose mothers were exposed to higher host densities or 2) a decrease in fecundity of daughters whose mothers were exposed to lower host densities. For this reason, we suggest that our hypothesis can be tested with any starting parasitoid population.

The experiment was analyzed as a mixed-model analysis of variance with JMP Pro 11.0 (SAS Institute 2013), with random effects for population and family, and family nested within population; hind tibia length as a covariate; host density as a fixed effect; and fecundity of daughters as the dependent variable.

Results

Leafhopper host densities at the time leaves were collected to rear parasitoids were 0.048 ± 0.008 ($N = 40$), 0.083 ± 0.015 ($N = 40$), and 0.089 ± 0.025 ($N = 40$) eggs per cm^2 of leaf surface for Populations 1, 2, and 3–4 combined, respectively. Host density treatments were established as desired in the laboratory: leaf samples revealed mean leafhopper egg densities of 0.053 ± 0.021 ($N = 5$) and 0.163 ± 0.048 ($N = 5$) eggs per cm^2 of leaf surface for the low and high density cages, respectively, when the parasitoids were introduced. Our experimental host density treatments fall comfortably within the range of leafhopper egg densities recorded in the field (Muphy et al. 1998).

The host density treatments experienced by the mother parasitoids had no effect on the fecundity their daughters (Fig. 1; $F = 0.09$, df = 14.9, $P = 0.77$). The slight, and nonsignificant, difference between the treatments was the opposite of what would be expected under adaptive plasticity: mothers experiencing lower host availability produced daughters with slightly higher fecundities (25.5 ± 2.9 eggs) than did mothers experiencing higher host availability (24.8 ± 2.0 eggs).

Significant variation in offspring fecundity was observed across families collected from the four source populations (random effect covariance parameter estimate for family term: 40.9 ± 20.6 [SE]; the 95% confidence interval [0.42–81.3] did not overlap zero; Fig. 2). Too few parasitoids were collected from Populations 1 and 2 to provide a formal test of differences between populations.

Discussion

We found no evidence for transgenerational phenotypic plasticity in fecundity for *A. erythroneurae* parasitoids: mother parasitoids that experienced low versus high host densities when ovipositing did not produce daughters differing in mean fecundity. Our experimental design also should have detected changes in fecundity triggered by daughter parasitoids having some mechanism of detecting host availability while they developed as pupae in a *Vitis* leaf. Thus, we see no evidence for phenotypic plasticity in fecundity for these solitary and strictly proovigenic parasitoids, whose egg maturation is completed prior to adult emergence. To our knowledge, this is the first test for transgenerational phenotypic plasticity in parasitoid fecundity that is not mediated by changes

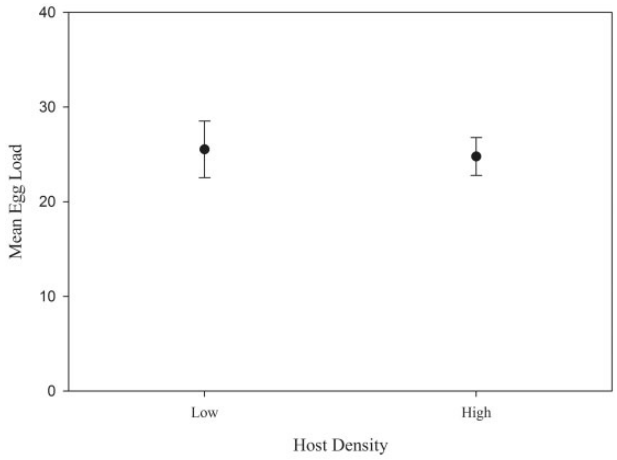

Fig. 1. Mean (±1 SE) fecundity of *A. erythroneurae* offspring produced by mothers given access to low (25.5 ± 2.9 eggs, $N = 9$) versus high (24.8 ± 2.0 eggs, $N = 11$) densities of hosts (eggs of the western grape leafhopper, *Erythroneura elegantula*).

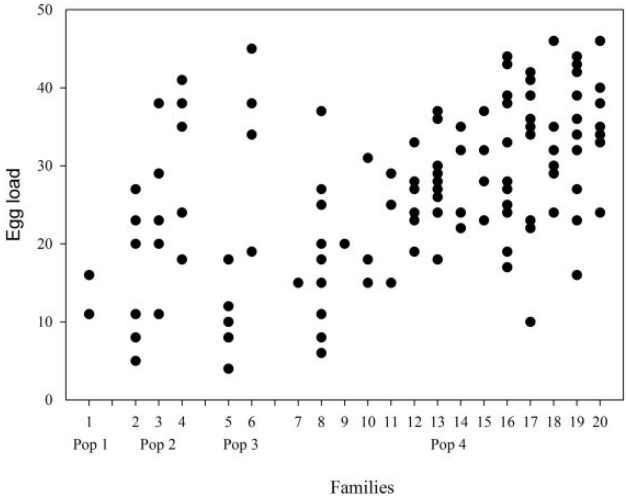

Fig. 2. Fecundity of individual *A. erythroneurae* females ($N = 122$) reared from twenty different mothers collected from four populations. Population 1: wild *Vitis californica* (Benth.) site; Population 2: low input agriculture site; Populations 3 and 4: high input agriculture sites.

in clutch sizes produced per host by mother parasitoids (*A. erythroneurae* is strictly solitary, with only one parasitoid offspring developing per host individual). Our finding of significant between-family differences in mean fecundity suggests instead that the differences in fecundity documented by Segoli and Rosenheim (2013) for parasitoids collected from populations with varying host availability may have a genetic basis.

Transgenerational plasticity in parasitoids has been demonstrated to shape other important life history traits, including development time and the proliferation of clonal offspring in a polyembryonic wasp (Morag et al. 2011a,b). Thus, assessing its possible operation in *A. erythroneurae* was an important step in assessing different mechanisms that might underlie positive associations of parasitoid fecundity with local variation in host availability. Future work will now focus on the hypothesis of local adaptation, which opens up the intriguing possibility that parasitoid fecundity could evolve as part of an eco-evolutionary feedback between host availability and parasitoid traits linked to the exploitation of host populations.

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