Limits to the reproductive success of two insect parasitoid species in the field

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Abstract. Debates on the relative importance of different factors in limiting the realized fitness of insect parasitoids and herbivores have continued for decades. One major reason for the duration of these debates is the paucity of empirical evidence regarding the reproductive success of minute insects under field conditions. We used a novel technique to estimate lifetime reproductive success in two *Anagrus* spp. parasitoids, whose hosts are eggs of leafhoppers that feed on grape leaves. Females were collected soon after they died naturally, and the number of eggs in their ovaries was counted. We used these data to estimate the lifetime oviposition success of individual females. We found that more than 10% of females from the field exhausted their entire supply of eggs before they died. The lifetime reproductive success of females was positively related to their body size and was higher at field sites with more abundant hosts, although we could not rule out a causal role for other site-specific factors. In addition, we found that females from habitats rich with hosts emerged with more eggs, suggesting that they might be adapted to local conditions. The results are consistent with theoretical predictions from models considering the risk of egg limitation.

Key words: Anagrus spp.; body size; egg limitation; fecundity; host density; proovigeny; realized fitness.

INTRODUCTION

The lifetime reproductive success of organisms may be limited by many factors, including food availability, mate availability, competition, and predation (Roff 2002). Debates concerning the relative importance of different potentially limiting factors for particular microbe, plant, and animal taxa pervade the evolutionary ecology literature. For example, is the reproductive success of birds limited by zygotes or by the production of fully provisioned offspring (Mock and Forbes 1995)? Is seed production by plants limited by the amount of available pollen or by resources for seed provisioning (Knight et al. 2005)? A theoretical treatment of a generic version of these questions predicted that organisms evolve life histories that balance the impacts of multiple limiting factors, rather than being consistently limited by any given factor (Rosenheim et al. 2010). Nevertheless, the relative importance of different limiting factors, and the way in which organisms balance their investments in coping with them is still poorly understood. One of the contributing reasons for this is that it is often difficult to estimate realized lifetime reproductive success for organisms in the field and to identify the factor(s) that prevented additional reproductive success. This is especially true for minute mobile organisms such as insect herbivores and parasitoids.

The lifetime reproductive success of parasitoids is considered to be limited by two main factors: (1) the finite number of hosts available to a female for oviposition during her lifetime (i.e., host limitation) and (2) the finite supply of mature eggs (i.e., egg limitation; Rosenheim [1996, 1999], Sevenster et al. [1998], Ellers et al. [2000], Rosenheim et al. [2010]). While there is a wide consensus regarding the importance of host limitation, egg limitation has sometimes been considered to be negligible, and as a consequence has been omitted from mathematical models predicting parasitoid population dynamics, life history, and behavioral traits (e.g., Charnov and Skinner 1985, Visser et al. 1992, Godfray 1994, Hassell 2000). Direct evidence for egg limitation can be obtained from egg loads of females that are captured in the field (Heimpel and Rosenheim 1998). However, these data are scarce and difficult to interpret, because females might have had the opportunity to lay more eggs if their reproductive lives had not been truncated by being captured. In addition, most parasitoid species are synovigenic, i.e., females continue to mature eggs throughout their life, and may also resorb eggs (Papaj 2000). Thus, if an eggless female is captured it is generally not possible to determine whether (1) it has not yet matured eggs, (2) it has matured and laid all of its eggs, or (3) it has matured and resorbed all of its eggs. These scenarios have fundamentally different implications for the occurrence and importance of egg limitation.

The relative importance of host vs. egg limitation may have strong implications for the selective forces acting on parasitoids in the field. For example, it may affect

selection on basic life history traits such as body size. Body size has been shown to increase the potential for parasitoid reproductive success via increasing fecundity, longevity, or host searching efficiency under laboratory conditions (e.g., Hardy et al. 1992, Rivero and West 2002, Bezemer and Mills 2003). However, the effect of body size on fitness in the field is likely to depend strongly on the main limiting factors. For example, if females rarely become egg limited, a positive effect of body size on potential fecundity may have little relevance to their realized reproductive success (Gotthard et al. 2007). Similarly, a positive effect of body size on potential longevity in the laboratory may be of small importance if intense predation sharply depresses female survival in the field. This highlights the importance of studying these relationships under natural conditions, while considering lifetime reproductive success rather than a particular fitness component (Ellers et al. 2001).

The risk of egg limitation in the field may also interact with the effect of host availability on parasitoid reproductive success. Laboratory studies characterizing the functional response of parasitoids to increasing host densities often demonstrate elevated rates of oviposition as host density increases (Fernandez-Arhex and Corley 2003). When hosts are sufficiently abundant, this increase may be constrained by egg supply, or may be reduced due to increased choosiness by females (Mangel and Heimpel 1998, Casas et al. 2004). High costs of egg limitation under field conditions may further lead to an evolutionary response in fecundity as a local adaptation to spatial variation in host density (Kraaijeveld and van Derwel 1994, Ellers et al. 2000). However, the relationship between host density and parasitoid potential or realized fecundity has rarely been characterized (Heimpel and Casas 2007).

Our research goal was to obtain direct estimates of the lifetime reproductive success of parasitoids in the field. To circumvent some of the key logistical difficulties, we studied proovigenic species, in which adult females emerge with their full egg load and do not mature or resorb eggs after emergence. We used a novel technique to capture females soon after they die naturally in the field, rather than at an arbitrary point during their lives. This allowed us to estimate the realized lifetime oviposition success of females and the proportion of females that become egg limited in the field. Based on theory, we predicted that some of the females in the population would become egg limited, and that the lifetime reproductive success of females will be positively affected, at least to some extent, by body size and by host density.

METHODS

Parasitoids and host insects

We studied two species of wasps of the genus *Anagrus*, *A. daanei* Triapitsyn and *A. erythroneurae* Triapitsyn Chiappini (Hymenoptera, Mymaridae), which parasitize eggs of leafhoppers in the genus *Erythroneura* (Homoptera: Cicadellidae). *Erythroneura* spp. are major herbivores of grapes, *Vitis* spp., including both cultivated grapes and wild grapes growing in riparian habitats in California. *Anagrus* spp. are minute insects (~0.8 mm in length). They complete their entire development (egg to adult) inside the leafhopper egg, consuming the egg as they develop. They are solitary (develop singly inside the leafhopper egg), proovigenic (emerge with their full egg load), and do not resorb eggs (Jepsen et al. 2007). *Anagrus* parasitoids are short lived, even under the most benign laboratory conditions. *Erythroneura* spp. females deposit eggs singly or in clusters under the leaf epidermal tissue or along leaf veins. We found three species of leafhoppers in our field sites: (1) the western grape leafhopper *Erythroneura elegantula* Osborn, which is native to California; (2) the variegated leafhopper *Erythroneura variabilis* Beamer; and (3) the Virginia creeper leafhopper *Erythroneura ziczac* Walsh.

Wasp sampling

We sampled parasitoids from five vineyards and one natural site in central California several times from mid-June to mid-September 2010. For exact locations and characteristics of field sites, see Appendix A.

Newly emerged wasps.—To characterize the distribution of initial parasitoid egg loads, we brought fresh leaves with leafhopper damage from the field to the laboratory several times along the season (three to six collections depending on population density) and placed them inside emergence cages. Emerging wasps were collected daily and dissected immediately or stored at −30°C until dissection. Females were dissected in a drop of water under a dissecting microscope to count the number of eggs in the abdomen (see Plate 1; for a full description of the dissection method see Appendix A). Following dissection we slide mounted females in Hoyer's solution and measured the length of a hind tibia as an estimate of body size. We used these data to characterize the relationship between initial egg load and parasitoid body size for each of the study sites. Species were identified using a phase-contrast microscope (for additional details on species identification see Appendix A).

Live wasps.—To characterize egg loads of actively foraging females, we collected live females from the field by shaking grape canes above a white plastic cafeteria tray at each site several times during the season (three to six sampling days per site, depending on population density). We stored, dissected, and measured these females in the same way as newly emerged females. We could not obtain parasitoids from the natural site using this method because population densities were too low. For additional details on this sampling method see Appendix A.

Dead wasps.—To quantify parasitoid lifetime realized oviposition success, we collected females from the field upon their death. This was done using two methods. (1)

While shaking grape canes to get live females, we occasionally also found dead parasitoids falling onto our trays. These wasps were probably already dead and lodged on the grape foliage. (2) We placed modified capture trays under dense vegetation in the field for up to 24 hours several times during the season (two to eight times; see Appendix A for details). Capture trays were made from a "sandwich" of two plastic cafeteria trays, with the top tray having many holes and a plastic grid that served as a wind baffle on top. Dead wasps falling off the vegetation landed initially mostly on the upper tray and eventually fell through the holes and into the lower tray, where they were fully protected from the wind. Capture trays were left for up to 24 hours in the field and were brought back to the laboratory to be searched carefully for dead *Anagrus* parasitoids (for additional details and justification for this sampling method see Appendix A). Dead females collected by these two methods were brought to the laboratory and immediately dissected and measured. Females that could not be identified to species level were excluded ($n = 22$). In addition, we excluded: females that had been partially consumed, with major body regions missing ($n = 5$); females that exhibited fungal growth ($n = 2$); females with their internal soft tissues consumed by predators that employ extra-oral digestion (spiders and insects with piercing-sucking mouthparts, $n = 15$); and females with no soft wet tissues inside their body ($n = 5$). We estimated the initial egg load of dead females using the relationships between egg load and hind tibia length observed for newly emerged females collected from the same site. We estimated the lifetime oviposition success of a female as its estimated initial egg load minus its observed residual egg load at death. This calculation occasionally resulted in a negative estimate of lifetime reproductive success in cases where the egg load of a dead female was higher than the estimated initial egg load for that female. Mean lifetime oviposition success did not differ among dead wasps that were collected via shaking or via trays, and thus we pooled data for these two methods. We estimated the risk of egg limitation as the proportion of females that had no eggs left in their ovaries upon their death. We were not able to obtain dead wasps from fields with extremely low wasp densities. Sample sizes of dissected parasitoids from the different field sites and sampling methods are indicated in Appendix A. Sample sizes were unequal across sites because of strong inter-site variation in leafhopper and parasitoid abundance.

Leafhopper density

To quantify leafhopper density we collected 30 leaves from each site monthly from July to September 2010, 90 leaves per site in total. In two sites (SJ and PC) we found no leafhopper feeding damage during July, and thus we collected just two leaf samples ($n = 60$). We selected one leaf per grape plant (at least 2 m apart) without regard to foliar damage (the leaf was chosen with eyes shut).

We counted the number of leafhopper eggs (both hatched and unhatched) on half of each leaf (top and bottom) and measured the leaf area examined using a leaf area meter. Using these data we estimated the mean number of *Erythroneura* spp. eggs per square centimeter of leaf for each field site.

Statistical analysis

Analyses were performed using JMP 10.0 pro for Windows. To examine changes in egg loads of females throughout life, we used a mixed model with sampling method (upon emergence, alive, dead) and parasitoid species as fixed effects and with field site ($N = 6$) as a random effect. We tested the possibility of a nonlinear effect of body size on lifetime reproductive success using a generalized additive model (GAM). However, the nonlinear effect was not significant; thus, we used a general linear model instead, with species and field site ($N = 4$) as cofactors. Similarly, we used GLM to test the effect of leafhopper density variation across fields on the lifetime reproductive success of parasitoids, with species and tibia length as cofactors. Because host density is confounded with field site, we compared the fit of this model to a model including field site ($N = 4$) as a nominal factor rather than host density as a continuous factor, using AIC_c-based model selection. Finally, we used logistic regressions to test the effect of body size and host density on female's probability of becoming egg limited.

Results

Egg loads throughout life

Mean egg loads were, reassuringly, highest for newly emerged wasps, intermediate for wasps collected live from the field, and lowest for wasps collected dead ($P < 0.001$; see Appendix B: Table B1). Egg load for *A. daanei* was: newly emerged females, 32.2 ± 0.77 (mean $\pm$ SE, $n = 186$), alive 25.5 ± 0.66 ($n = 248$), and dead 13.0 ± 1.1 ($n = 68$); and for *A. erythroneurae* newly emerged females, 26.8 ± 0.92 ($n = 84$), alive 19.8 ± 0.86 ($n = 78$), and dead 10.7 ± 1.42) ($n = 33$), indicating that on average females laid ~60% of their eggs during their lives.

Egg limitation

Twelve percent of all *Anagrus* females collected from the capture trays had completely exhausted their lifetime supply of eggs (13% for *A. daanei* and 9% for *A. erythroneurae*; Fig. 1).

Relationship between body size and lifetime reproductive success

Lifetime oviposition success for female parasitoids collected from four vineyards was positively correlated with their body size (Fig. 2; $P < 0.001$; Appendix B: Table B2). However, body size had no effect on the probability that females would become egg limited ($P = 0.436$; Appendix B: Table B3).
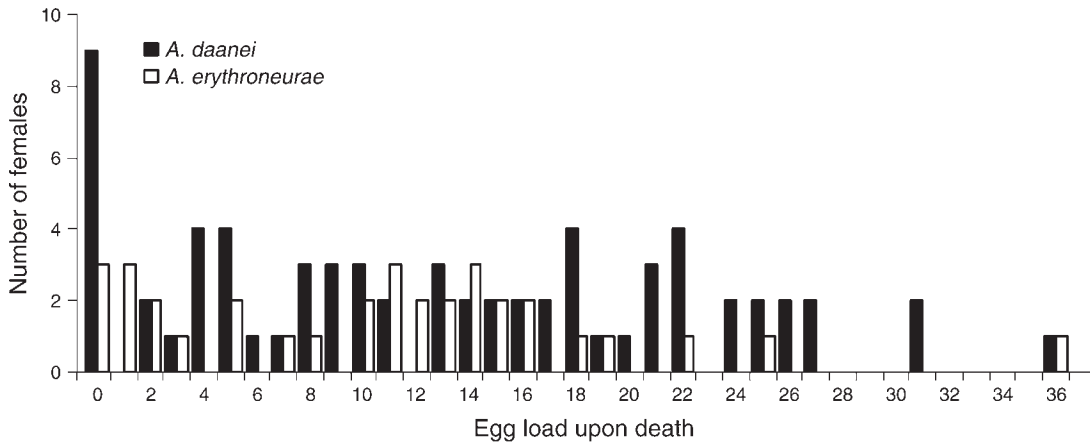
FIG. 1. Distribution of egg loads of dissected *Anagrus daanei* and *A. erythroneurae* females collected dead from the field.

Relationship between leafhopper density and lifetime reproductive success

Lifetime oviposition success for parasitoids collected from four vineyards was positively correlated with leafhopper density in those fields (Fig. 3, $P = 0.003$; Appendix B: Table B4). However, the AIC_c value for this model (731) was greater than the AIC_c value for a model including field site as a nominal variable instead of leafhopper density as a continuous variable (728; Appendix B: Table B2). Thus, despite the suggestive trend, we were unable to distinguish the effect of leafhopper density per se from that of the field site. Host density had no effect on the probability of females becoming egg limited ($P = 0.96$; Appendix B: Table B5).

Relationship between leafhopper density and initial egg load

Egg loads of newly emerged parasitoids increased with leafhopper density observed at their collection site, even after controlling statistically for body size, suggesting that fecundity was, through some mechanism, matching local reproductive opportunities. The positive relationship between initial egg load and mean host density was not linear. Rather, fecundity increased rapidly for parasitoids collected at sites with low to moderate leafhopper densities, but then showed little further increase (see Appendix C).

DISCUSSION

Parasitoids traditionally have been considered to be host rather than egg limited. Although a growing body of theoretical and empirical work suggests that instead, females evolve to balance these opposing risks (Godfray 1994, Rosenheim et al. 2010), the actual occurrence of egg limitation in the field is still poorly characterized. In our study we found that 9–13% of the females laid all of their eggs before they died. The ecological importance of egg limitation is likely to be higher than inferred merely
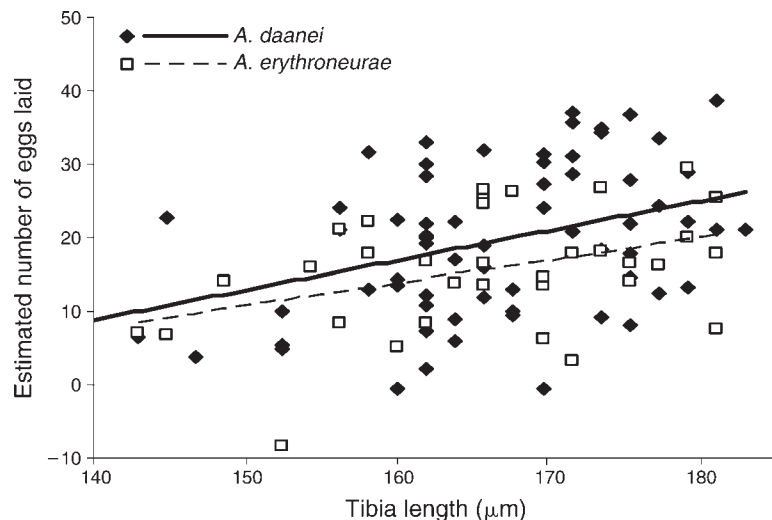

FIG. 2. Estimated lifetime oviposition success of female *Anagrus daanei* and *A. erythroneurae* collected dead from the field vs. body size (hind tibia length).
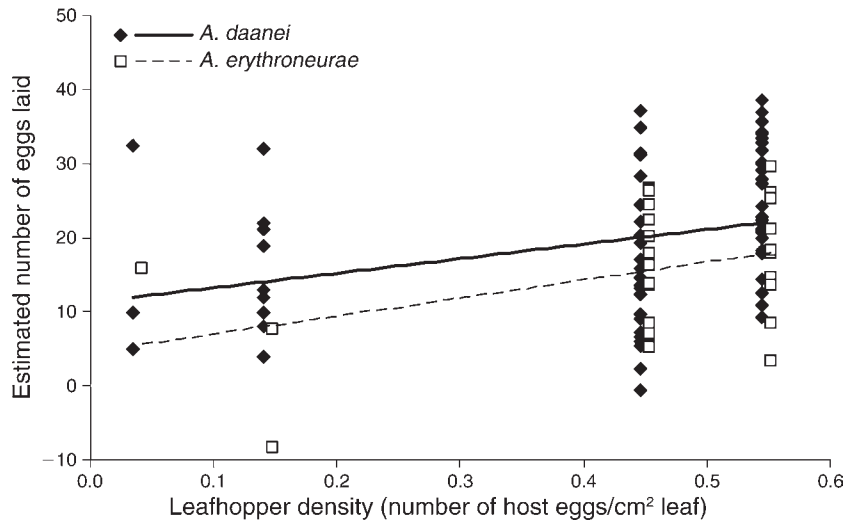
Fig. 3. Estimated lifetime oviposition success of female *Anagrus daanei* and *A. erythroneurae* collected dead from the field vs. cumulative leafhopper density per field. For better visibility, data points for *A. daanei* were moved slightly to the left and for *A. erythroneurae* slightly to the right.

from the ~10% of females that completely exhaust their egg supply. The reason is that females with low egg loads may become choosier, in a way that decreases their oviposition rate. This may reduce the probability of females actually becoming egg limited (Mangel and Heimpel 1998). The more selective host choice behavior of females foraging under a strong risk of egg limitation may also result in offspring with higher expected survival and quality, because their mothers oviposit in higher quality hosts. Finally, females that lay all of their eggs are likely to make a disproportionately large contribution to total reproduction, compared with their mere proportions in the population (Rosenheim 2011). Indeed, by summing the number of eggs laid by eggless females, compared to the total number of eggs laid by all females that were collected dead, we estimate that 13–23% of all eggs laid in the field are laid by egg-limited females (representing a 50–80% increase relative to the raw proportions of egg limited female). Thus, egg limited females are predicted to produce disproportionately more and higher quality offspring, and thereby to contribute more to the next generations than predicted by their mere proportions in the population.

Body size is assumed to be a major determinant of fitness in many organisms (Messina and Fox 2001). Our study provides direct evidence for a positive effect of body size on the lifetime reproductive success of parasitoids in the field. The occurrence of egg limitation in our study system may suggest that body size can potentially affect oviposition success via its effect on initial fecundity. This would be true if smaller females with fewer eggs were more prone to egg limitation. However, body size had no effect on the probability that females would become egg limited. These results suggest that the positive effect of body size is not generated by

fecundity effects. Instead, large females emerge with more eggs and also appear to be capable of laying more eggs in the field. Whether body size affects oviposition success via increasing foraging efficiency, longevity, or both, is yet to be determined.

We found that wasps collected from sites with higher leafhopper densities laid more eggs during their lifetime. However, we could not rule out a causal role for other site-specific factors. Even if we assume a true positive effect of host density on oviposition success, the variation in reproductive output was modest (approximately twofold) relative to the variation in leafhopper density among field sites (~15-fold). The occurrence of egg limitation for *Anagrus* in the field may provide a possible explanation for a subdued effect of host density on oviposition success. An alternative explanation may be that a positive effect of host density on oviposition rates is compromised by the effect of intraspecific competition. This may be the case if parasitoids aggregate in host-rich patches, as predicted under the ideal free distribution (Heimpel and Casas 2007). Further investigation is required to confirm the positive effect of leafhopper density on parasitoid reproductive success observed in this study and to learn about the relative importance of the above mechanisms.

The risk of egg limitation may impose selection on females to produce more eggs in host rich habitats. Indeed, we found a positive relationship between leafhopper density and initial egg loads of females across field sites. The relationship was not linear, suggesting a possible constraint on the maximum number of eggs that a female can produce and carry, or diminishing returns from increasing oviposition rate as leafhopper density increases (see previous paragraph). If egg production has a genetic component in this

PLATE 1. *Anagrus* female, dissected under the microscope, and its eggs. Photo credit: M. Segoli.

system, as has been found in several other parasitoid species (reviewed in Jervis et al. 2008), the results would suggest a rapid evolution of egg loads in response to local host availability. This may also explain the lack of effect of host density on the probability of females becoming egg limited.

In summary, we have presented direct evidence for the occurrence of egg limitation, and for the effects of both individual and environmental variation on the fitness of two parasitoid species in the field. Additionally, we presented observations that hint at the possibility that parasitoid life histories may be evolving in this system under the selective influence of variable host availability and a shifting balance between the risks of egg vs. host limitation. The results are consistent with theoretical developments suggesting that organisms evolve life histories that balance the impacts of multiple limiting factors. Further work is needed to explore the associated

possibility of eco-evolutionary feedbacks between oviposition opportunities and fecundity, a possibility that has been little considered. Our findings provide one step toward revealing factors that limit the realized lifetime reproductive success of insects in the field, with implications for insect reproductive behavior, life history evolution, and population dynamics.

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SUPPLEMENTAL MATERIAL

Appendix A

Additional details on field sites and methods (*Ecological Archives* E094-229-A1).

Appendix B

Full results for statistical analyses (*Ecological Archives* E094-229-A2).

Appendix C

The effect of host density on the initial egg load of female parasitoids (*Ecological Archives* E094-229-A3).