Ovipositional preferences and progeny development of the egg parasitoid *Avetianella longoi*: factors mediating replacement of one species by a congener in a shared habitat


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Abstract

Populations of *Phoracantha recurva*, a cerambycid beetle that appeared in California around 1995, have increased rapidly while populations of the congener *Phoracantha semipunctata*, that arrived in California before 1985, have dramatically declined in the southwestern part of the state where the two species occur together. Both species colonize stressed Eucalyptus trees and fallen branches in the shared habitat. The proportion of *P. recurva* in the mixed population increased rapidly from 0.1% in 1995, the first year this species was detected, to 4.7% the following year, and 74% in 1997. To determine whether differential parasitization of eggs of the two beetle species by an egg parasitoid may be a contributing factor in this apparent ecological replacement of one species with a congener, we evaluated oviposition preference and host suitability of eggs of the two congeneric beetle species using strains of the egg parasitoid *Avetianella longoi* reared on eggs of either *P. semipunctata* (S-strain wasps) or *P. recurva* (R-strain wasps) for multiple generations. In both choice and no-choice bioassays, female parasitoids of both strains preferred to oviposit in *P. semipunctata* eggs, and survival rates were much higher in *P. semipunctata* eggs than in *P. recurva* eggs, for host eggs of two age classes (0.5 and 2.5 days old). Preference and survival of progeny of R-strain or S-strain females on *P. semipunctata* eggs were not significantly different, indicating that the preference for *P. semipunctata* eggs as a host resource was innate and had not been affected by >15 generations of selection. A substantial fraction of *P. recurva* eggs parasitized by wasps of either strain survived parasitization and produced neonate larvae, whereas no *P. semipunctata* eggs survived parasitization. Furthermore, a significantly larger percentage of parasitized *P. recurva* eggs produced neither a parasitoid nor a neonate larva than parasitized *P. semipunctata* eggs. The size and sex ratio of progeny of either parasitoid strain were minimally affected by host egg species. Although *P. recurva* eggs were smaller in diameter and weight than *P. semipunctata* eggs, eggs of both species were large enough to support the development of several parasitoids, so it is unlikely that insufficient nutrition was a contributing factor to survival of parasitoids in eggs of either host species. Cumulatively, these results suggest that eggs of both species contain adequate nutrition for developing parasitoids, but that ovipositing *A. longoi* females and their developing progeny frequently are not able to terminate *P. recurva* egg development.

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1. Introduction

*Eucalyptus* spp. were introduced into California in the late 1800s and are now ubiquitous landscape, shade, and windbreak trees throughout the state. During most of the first 100 years after their introduction, eucalypts remained virtually pest-free, with few native herbivores expanding their host range to include *Eucalyptus* spp. (Johnson and Lyon, 1988). However, some time before 1985, the eucalyptus longhorned borer, *Phoracantha semipunctata* (F.) (Coleoptera: Cerambycidae), an insect native to Australia, arrived in California (Scriven et al., 1986). Since that time, the beetle has spread throughout most of the state, killing large numbers of *Eucalyptus* trees.

In an effort to control the beetle, several of its natural enemies have been introduced into California, including the egg parasitoid *Avetianella longoi* Sisco
(Hymenoptera: Encyrtidae) (Hanks et al., 1996), and several larval parasitoids (e.g., *Syngaster lepidus* Brullé and *Jarra phoracantha* Austin, Quicke, and March) (Paine et al., 1995). *A. longoi* is now widely established in the state, and parasitization rates of >90% have helped bring *P. semipunctata* populations under control and minimize tree mortality (Hanks et al., 1996). In southwestern California, there is no conclusive evidence of permanent establishment of any of the larval parasitoids, and so *A. longoi* is the primary biological control agent attacking *P. semipunctata*.

In 1995, a congeneric species, *Phoracantha recurva* Newman, appeared in southwestern California, and since that time, its numbers have increased rapidly (Hanks et al., 1997). In 1995, *P. recurva* adults constituted 0.01% of adult *Phoracantha* beetles collected from naturally infested logs in southern California during our routine sampling operations (the rest of the beetles were *P. semipunctata*). The proportion of *P. recurva* in the *Phoracantha* population increased to 4.7% in 1996, and by 1998, exceeded 95%, with numerous sites yielding exclusively *P. recurva* (Hanks et al., 1997). The two *Phoracantha* species generally appear to have similar life histories and exploit the same biological niche. Eggs of both species are laid under loose bark or in bark cracks of freshly fallen logs or standing trees, particularly stressed trees, and the developing larvae bore into and consume the cambium layers, resulting in rapid tree death.

Because these two beetle species share the host tree resource and utilize similar oviposition sites, *A. longoi* females encounter egg masses of the two borer species at similar rates while foraging for hosts on Eucalyptus host material in the field (Luhring et al., 2000). However, in the space of three years, *P. recurva* populations largely replaced *P. semipunctata* in their shared habitat. There are at least three possible causes of this apparent replacement. First, *P. recurva* may have a greater inherent rate of increase (e.g., higher fecundity, faster development, or more annual generations). Second, *P. recurva* may be a better direct competitor for the host resource than *P. semipunctata* when both species colonize host material simultaneously, or may be a better indirect competitor as a result of earlier seasonal activity and access to available host material before *P. semipunctata* emerges in the spring. Third, the egg parasitoid may have differential rates of successful parasitization of the two borer species, resulting in decreased survival and reduced population size of *P. semipunctata*, while concomitantly enhancing *P. recurva* populations by reducing competition for their shared host resource. Studies to test the first two hypotheses are currently under investigation and the results will be reported elsewhere. The third hypothesis, that *A. longoi* is more successful in parasitizing *P. semipunctata* than *P. recurva*, is the subject of the work presented here.

The results of a recent study confirmed that the host–parasitoid interactions between *A. longoi* and the two *Phoracantha* spp. are not equal (Luhring et al., 2000). Although wasps located sentinel egg masses of each species equally well in field trials, the percent parasitization within *P. semipunctata* egg masses was significantly higher than in *P. recurva* egg masses. Laboratory studies with both choice and no-choice oviposition tests confirmed that *A. longoi* exhibited a marked preference for *P. semipunctata* eggs. Furthermore, parasitoid survival rates were much higher in *P. semipunctata* than in *P. recurva* hosts.

However, Luhring et al. (2000) conducted studies in 1997 with *A. longoi* that had been reared primarily or exclusively on *P. semipunctata* hosts. That is, the laboratory colonies of *A. longoi* used in that study had been reared solely on *P. semipunctata*, while in the field, *A. longoi* could only have been exposed to *P. recurva* for a few generations since the beetle’s arrival. Consequently, the differences in oviposition and survival seen in the previous study may have been influenced by local selection of *A. longoi* during the approximately five year period when *P. semipunctata* eggs were the only hosts available. Our goal in the work described here was to determine whether the host preferences and host suitability reported in Luhring et al. (2000) could be modified by forced rearing of *A. longoi* on *P. recurva* for multiple generations, or whether these traits were relatively resistant to selection. To address this goal, two strains of parasitoids were developed. The first, designated the S-strain, had been reared since its introduction into California in 1992 exclusively on *P. semipunctata* eggs. The second, termed the R-strain, was started from the S-strain, but subsequently had been reared for >15 generations exclusively on *P. recurva* eggs. Our specific objectives were to compare: (1) the physical characteristics (size and weight) of eggs of the two host species; (2) the ovipositional rates of *A. longoi* of each strain on eggs of the two host species, as a measure of host preference; and (3) the size and percent survival of progeny of each wasp strain from eggs of each beetle host, as measures of host suitability.

2. Methods

2.1. Laboratory colonies

Colonies of *P. recurva* and *P. semipunctata* were maintained as described by Hanks et al. (1993, 1995). Adult beetles were caged in cylindrical hardware cloth cages with 14.5 cm diameter plastic petri dish tops and bottoms. Beetles were fed 10% honey–water solution and eucalyptus pollen (Wando and Jarrah Tree Pollen, Great Health, Brea, CA 92621). The bottom of each cage was lined with filter paper. An oviposition
substrate was prepared by covering the bottom of a 9 cm diameter petri dish with filter paper and placing this dish on the filter paper lining the bottom of the cage. Adult females of both Phoracantha species lay egg masses of 10–200 eggs at night (Hanks et al., 1993; unpublished data) between the 2 layers of filter paper. For parasitization trials, freshly laid beetle eggs were collected from colonies daily between 07:00 and 08:30h. Therefore, the youngest Phoracantha eggs used in parasitization bioassays were no more than 12 h old. Eggs were held in covered petri dishes at 22 °C with a 14:10 h (L:D) photoregime until used in the bioassays. Egg masses were divided to achieve egg mass sizes of 10–20 eggs. Phoracantha spp. eggs were either 0.5 or 2.5 days old when they were used in bioassays. Host eggs within this age distribution are most suitable for parasitization by A. longoi and those most likely to be parasitized in the field (Luhring et al., 2000).

The parasitoid rearing procedures followed those described by Hanks et al. (1995). Two Avetianella longoi strains were used: (1) R-strain parasitoids had been reared continuously on P. recurva eggs since July 1996 (>15 generations) before being used in experiments; (2) S-strain parasitoids had been reared continuously on P. semipunctata eggs since 1992 (>70 generations).

Both A. longoi strains were held in 10-cm diameter, cylindrical plastic cages with small, screen-covered holes on the top for ventilation. Adult parasitoids were fed honey applied to the surface of one screened hole. Parasitoids were added to the colonies by placing filter paper slips containing A. longoi pupae in the colony cage 1–2 days before adult eclosion. Adult parasitoids emerged and mated, and females begin to oviposit ≈1 day after eclosion. Filter paper slips containing freshly laid Phoracantha egg masses were placed in the A. longoi colony cages where they remained for 1.5–2 days. At the end of this oviposition interval, the egg masses were removed and held in covered petri dishes until the developing parasitoid pupae darkened. Surviving Phoracantha spp. neonates were removed as they emerged from the dishes containing the parasitized eggs. The parasitoids developed from egg to adult in ≈14 days at 22 °C, under ambient light conditions in a well-lit laboratory with north-facing windows.

2.2. Host egg and neonate size

To test whether eggs of the two beetle species might represent different weights or volumes of host resources for the parasitoids to exploit, eggs and neonate beetle larvae were weighed and measured. Beetle eggs were collected from both colonies between 07:30 and 09:00 h. Sixty eggs of each species of two age classes (0.5 days old, an age suitable for parasitization, and 3.5 days old, past the optimum age for parasitoid oviposition) were weighed individually with an electronic microbalance and measured (length and width) using an ocular micrometer in a stereoscopic microscope. Phoracantha eggs hatch in 5–6 days at 22 °C and parasitization rates decline significantly in older eggs (Luhring et al., 2000). A separate set of beetle eggs was used to determine neonate larval weights. Sixty neonates of each borer species were weighed <1 day after eclosion and before being allowed to feed. The data sets were compared between the two species by ANOVA, using Proc GLM in PC-SAS for Windows version 6.12 and an α ≤ 0.05 (SAS Institute, 1996).

2.3. Female progeny size relative to parasitoid strain and host species

To test whether the host from which a female wasp had been reared affected her size or the size of her progeny, four parasitoid strain–host combinations were tested: (1) S–R: progeny from S-strain wasps developing in P. recurva eggs; (2) R–R: progeny from R-strain wasps developing in P. recurva eggs; (3) S–S: progeny from S-strain wasps developing in P. semipunctata eggs; and (4) R–S: progeny from R-strain wasps developing in P. semipunctata eggs. Hind tibial length was used as a measure of female wasp size. Beetle eggs (2.5 days old) were presented to the wasps in no-choice situations. Singly parasitized beetle eggs (determined by the presence of a single parasitoid egg pedicel protruding from the host egg) were separated from the egg masses, and held in shell vials at 22 °C until the parasitoid emerged. The hind tibial length was measured for 30 randomly selected females from each treatment group. Differences among treatment means were determined by two-way ANOVA on square root (x + 0.5) transformed data (Sokal and Rohlf, 1981) using Proc GLM with Least Squares Means separation and an α ≤ 0.05 (SAS Institute, 1996).

2.4. Choice and no-choice oviposition bioassays—general methods

Avetianella longoi ovipositional preferences and the percentages of successful development of progeny were tested using mated adult female parasitoids (<5 days old) with no prior experience with Phoracantha eggs. Wasps were used only once, and host eggs of two age classes (0.5 and 2.5 days old) were tested in separate experiments. All oviposition trials were conducted in 5 cm petri dishes with locking lids for 3 h (09:00–12:00 h), after which the wasps were separated from the egg masses. Egg masses were labeled, and held together in a 10-cm petri dish at 22 °C. Avetianella longoi egg pedicles protruding through the chorions of the host eggs were counted 1–2 days after exposure of the eggs to female wasps to determine percent parasitization in each egg mass. For all trials, one of four potential fates was
recorded for each parasitized egg: (1) beetle larvae eclosed; (2) parasitoid(s) emerged; (3) both host and parasitoid died; and (4) parasitoids developed, but then entered diapause. Emerging parasitoids were sexed to determine the sex ratio obtained from each treatment.

Differences among treatment means were determined by ANOVA using Proc GLM with Least Squares Means separation and an $x \leq 0.05$ (SAS Institute, 1996). Replicates were blocked in each experiment to control for potential changes in levels of parasitization for replicates conducted at different times. Analyses of ovipositional rates, survival of the parasitoids, eclosion of beetle larvae, egg death, and parasitoid sex ratio were conducted on arcsine square-root transformed data, whereas analyses of the number of parasitoid eggs per host egg were conducted on square root ($x + 0.5$) transformed data (Sokal and Rohlf, 1981).

2.5. No-choice oviposition bioassays

To test the behavioral response of females to host eggs of a single species, and the successful development of the resulting progeny, two naïve A. longoi females of the same strain were placed in a 5 cm petri dish and presented with a mass (10–20 eggs per mass) of either P. recurva or P. semipunctata eggs on a filter paper slip. The same four treatment combinations of female wasp strain and host species eggs were used as described above for female size experiments (S–R, R–R, S–S, and R–S).

2.6. Choice oviposition bioassays

To test the behavioral responses of females to simultaneous presentation of eggs of both host species and the successful development of the resulting progeny, four naïve A. longoi females of the same strain were placed in a 5 cm petri dish and presented with one mass each of P. recurva eggs and P. semipunctata eggs (10–20 eggs per mass).

3. Results

3.1. Host egg and neonate size

Mean lengths of 0.5-day-old eggs of the two Phoracantha species were not significantly different ($P$. semipunctata, $2.45 \pm 0.02$ mm; $P$. recurva, $2.47 \pm 0.02$ mm; $F = 1.74$, $df = 1$, 9, $P = 0.22$), but $P$. semipunctata eggs were larger in diameter ($P$. semipunctata, $0.72 \pm 0.008$ mm; $P$. recurva, $0.56 \pm 0.008$ mm; $F = 274.9$, $df = 1$, 9, $P < 0.0001$) and heavier ($P$. semipunctata, $566 \pm 7.8 \mu$g; $P$. recurva, $405 \pm 4.6 \mu$g; $F = 2125.2$, $df = 1$, 9, $P < 0.0001$). The distributions of egg diameter and weight show significant non-overlapping regions (Fig. 1A and B) for the two beetle species. Eggs $\leq 0.64$ mm in diameter were exclusively $P$. recurva, and eggs $\geq 0.79$ mm wide and 590 $\mu$g in weight were exclusively $P$. semipunctata.

The same pattern held for 3.5-day-old host eggs, with mean egg lengths being not significantly different between the two species ($P$. semipunctata, $2.41 \pm 0.02$ mm; $P$. recurva, $2.44 \pm 0.02$ mm; $F = 1.82$, $df = 1$, 9, $P = 0.21$), whereas $P$. semipunctata eggs were larger in diameter ($P$. semipunctata, $0.71 \pm 0.01$ mm; $P$. recurva, $0.57 \pm 0.008$ mm; $F = 192.23$, $df = 1$, 9, $P < 0.0001$) and heavier ($P$. semipunctata, $511 \pm 8.2 \mu$g; $P$. recurva, $370 \pm 4.9 \mu$g; $F = 945.42$, $df = 1$, 9, $P < 0.0001$) than $P$. recurva eggs. The distributions of egg diameters and weights also showed significant non-overlapping areas (Figs. 2A and B). At 3.5-day-old, eggs $\leq 0.54$ mm wide were exclusively $P$. recurva, and those $\geq 0.79$ mm wide or $\geq 590 \mu$g in weight were exclusively $P$. semipunctata eggs.

Phoracantha semipunctata neonate larvae (437.33 $\pm$ 9.97 $\mu$g) were significantly heavier than $P$. recurva neonates (318.67 $\pm$ 7.64 $\mu$g; $t = 10.18$, $df = 118$.

![Fig. 1. Distribution of diameters (A) and weights (B) of 0.5-day-old eggs of Phoracantha semipunctata and P. recurva.](image-url)
P < 0.0001). From the distributions of neonate larval weights (Fig. 3), 87% of *P. recurva* neonates weighed <350 µg, whereas 95% of *P. semipunctata* neonates weighed >400 µg.

3.2. Female size

Host species significantly affected the size of emerging progeny females ($F = 6.77, df = 1,116, P = 0.003$), but the effect was not uniform across strains. Host species did not significantly affect the size of progeny of S-strain females (Fig. 4). However, progeny of R-strain females emerging from *P. semipunctata* eggs were significantly larger than wasps reared from any of the other treatment groups.

3.3. No-choice oviposition bioassays

Parasitoids of both strains oviposited significantly more in *P. semipunctata* eggs than *P. recurva* eggs for 0.5-day-old host eggs (Table 1). Ovipositional rates of each wasp strain in the eggs of a single host species were not significantly different. When presented with 2.5-day-old host eggs, R-strain females did not show an ovipositional preference between *P. semipunctata* and *P. recurva* eggs, whereas S-strain females showed a marked preference for *P. semipunctata* eggs. Ovipositional rates by females of the two strains were equal in *P. recurva* eggs, but S-strain females oviposited significantly more in *P. semipunctata* eggs than did R-strain females (Table 1).

Parasitoid strain did not affect progeny survival in either 0.5- or 2.5-day-old host eggs of a given species, but for both strains, progeny survival in *P. recurva* eggs was significantly lower than in *P. semipunctata* eggs (Table 1). When host eggs were 0.5 days old, only 19–25% of the progeny of both parasitoid strains survived to adulthood in *P. recurva* eggs, compared to survival rates of >90% in *P. semipunctata* eggs. For 2.5-day-old...
As expected, survival rates of parasitoid progeny were similar to the trends found in the no-choice tests. For both strains and both host egg age classes, significantly more parasitoid progeny survived in *P. semipunctata* eggs than in *P. recurva* eggs. Within host species, survival rates of progeny of both R-strain and S-strain females of either age class were uniformly high and not significantly different within an age class. Sex ratio of progeny was not affected by any host species–parasitoid strain combination for the older age classes, but there were significantly higher proportions of female progeny of the S-strain emerging from either host species than R-strain progeny emerging from *P. semipunctata* hosts. The proportion of female R-strain progeny from *P. recurva* hosts was not different from S-strain sex ratios from either host or from R-strain progeny emerging from *P. semipunctata* hosts (Table 2).

The fate of parasitized eggs also followed the patterns seen in the no-choice tests, with a significant proportion of parasitized *P. recurva* eggs of both age classes surviving parasitization and producing neonate beetle larvae, whereas none of the *P. semipunctata* eggs survived parasitization (Table 2). There was no difference between the proportions of *P. recurva* eggs within an age class that produced neonate larvae for either parasitoid strain. Substantial and equal proportions of *P. recurva* eggs of both age classes failed to produce either a parasitoid or a neonate beetle larva, regardless of parasitoid strain. Mortality rates were significantly lower for *P. semipunctata* eggs of either age class parasitized by females of either wasp strain. Finally, unlike what was

### Table 1

Mean number of host eggs parasitized and fate of *A. longoi* parasitoid progeny from no-choice oviposition trials

<table>
<thead>
<tr>
<th>A. longoi strain</th>
<th>Host species (0.5-day-old eggs)</th>
<th>P. recurva</th>
<th>P. semipunctata</th>
<th>Significance</th>
<th>Host species (2.5-day-old eggs)</th>
<th>P. recurva</th>
<th>P. semipunctata</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Host eggs per mass</td>
<td>R-strain</td>
<td>29.9 ± 5.7*a</td>
<td>47.4 ± 6.0*b</td>
<td><em>F</em>₂,₁₀ = 4.80</td>
<td>22.0 ± 4.3*b</td>
<td>35.7 ± 6.7*b</td>
<td><em>F</em>₂,₁₀ = 26.41</td>
<td></td>
</tr>
<tr>
<td>parasitized by A. longoi</td>
<td>S-strain</td>
<td>31.5 ± 6.2*a</td>
<td>54.7 ± 6.3*b</td>
<td><em>P</em> = 0.002</td>
<td>16.6 ± 3.9*b</td>
<td>74.5 ± 3.9*b</td>
<td><em>P</em> = 0.0001</td>
<td></td>
</tr>
<tr>
<td>% A. longoi survival</td>
<td>R-strain</td>
<td>18.7 ± 5.8*a</td>
<td>91.1 ± 2.2*b</td>
<td><em>F</em>₂,₁₀ = 62.89</td>
<td>42.8 ± 7.5*b</td>
<td>86.1 ± 3.4*b</td>
<td><em>F</em>₂,₁₀ = 13.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S-strain</td>
<td>25.0 ± 6.4*a</td>
<td>92.8 ± 3.7*b</td>
<td><em>P</em> = 0.0001</td>
<td>47.5 ± 8.7*b</td>
<td>86.6 ± 2.7*b</td>
<td><em>P</em> = 0.0001</td>
<td></td>
</tr>
<tr>
<td>% A. longoi females</td>
<td>R-strain</td>
<td>65.7 ± 12.7*a</td>
<td>67.9 ± 4.3*a</td>
<td><em>F</em>₂,₁₀ = 0.48</td>
<td>56.9 ± 10.6*a</td>
<td>56.0 ± 7.9*a</td>
<td><em>F</em>₂,₁₀ = 0.71</td>
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<tr>
<td></td>
<td>S-strain</td>
<td>58.6 ± 12.0*a</td>
<td>73.0 ± 2.9*a</td>
<td><em>P</em> = 0.70</td>
<td>50.0 ± 10.6*a</td>
<td>67.6 ± 4.4*a</td>
<td><em>P</em> = 0.55</td>
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<tr>
<td>% Parasitized eggs with closed beetles</td>
<td>R-strain</td>
<td>39.4 ± 7.4*a</td>
<td>0*b</td>
<td><em>F</em>₂,₁₀ = 26.81</td>
<td>15.3 ± 5.7*a</td>
<td>0*b</td>
<td><em>F</em>₂,₁₀ = 9.05</td>
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<tr>
<td></td>
<td>S-strain</td>
<td>27.1 ± 7.2*a</td>
<td>0*b</td>
<td><em>P</em> = 0.0001</td>
<td>13.3 ± 4.2*a</td>
<td>0*b</td>
<td><em>P</em> = 0.001</td>
<td></td>
</tr>
<tr>
<td>% Parasitized eggs that died</td>
<td>R-strain</td>
<td>42.7 ± 7.5*a</td>
<td>5.4 ± 1.9*b</td>
<td><em>F</em>₂,₁₀ = 21.52</td>
<td>37.5 ± 7.6*a</td>
<td>9.6 ± 3.2*b</td>
<td><em>F</em>₂,₁₀ = 6.29</td>
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<td>S-strain</td>
<td>46.4 ± 7.4*a</td>
<td>6.7 ± 3.9*b</td>
<td><em>P</em> = 0.0001</td>
<td>36.5 ± 7.8*a</td>
<td>10.8 ± 2.8*b</td>
<td><em>P</em> = 0.0007</td>
<td></td>
</tr>
<tr>
<td>% Parasitized eggs with diapaused parasitoids</td>
<td>R-strain</td>
<td>0*a</td>
<td>2.0 ± 1.0*a</td>
<td><em>F</em>₂,₁₀ = 0.87</td>
<td>0*a</td>
<td>4.8 ± 2.2*a</td>
<td><em>F</em>₂,₁₀ = 4.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S-strain</td>
<td>1.8 ± 1.8*a</td>
<td>1.1 ± 0.9*a</td>
<td><em>P</em> = 0.46</td>
<td>0.3 ± 0.3*a</td>
<td>3.7 ± 1.1*a</td>
<td><em>P</em> = 0.005</td>
<td></td>
</tr>
</tbody>
</table>

1 All values are untransformed means ± SEM. Statistics are based on the transformed data. Letters indicate significant differences within *A. longoi* strain (*P* < 0.05; least squares means comparisons).

2 ANOVA statistics apply to comparison of means among strains and host species in the four cells immediately to the left of the values.

host eggs, the survival of parasitoids in *P. recurva* eggs increased to 42–47%, but this was still significantly lower than the ~86% survival rates in *P. semipunctata* eggs (Table 1). Parasitoid strain and host egg had no effect on the sex ratio of progeny, with sex ratios varying from 58.6 to 73.0% female in 0.5-day-old host eggs, and from 50.0 to 67.6% female in the 2.5-day-old host eggs (Table 1).

However, the outcome of parasitization was distinctly different for eggs of the two beetle species (Table 1). Regardless of parasitoid strain, significant fractions of parasitized *P. recurva* eggs survived parasitization and produced neonate beetle larvae, for both the 0.5- and 2.5-day-old host egg age classes. In contrast, no *P. semipunctata* eggs of either age class parasitized by females of either strain, produced neonate larvae. Furthermore, a significantly larger proportion of *P. recurva* eggs parasitized by females of either strain died, failing to produce either a wasp or a neonate larva, irrespective of age class. Although a small proportion of parasitoid progeny of both strains entered diapause before emergence from 0.5-day-old eggs of other host species, there were no significant differences among strain–host combinations. In 2.5-day-old host eggs, however, significantly more individuals from either strain entered diapause if they were developing in *P. semipunctata* eggs than if they were in *P. recurva* eggs (Table 1).

### 3.4. Choice oviposition bioassays

The patterns of oviposition observed in the choice experiments were similar to the results from the no-choice trials (Table 2). Parasitoids of both strains oviposited significantly more in *P. semipunctata* eggs than in *P. recurva* eggs when given either 0.5- or 2.5-day-old host eggs. Within host species, levels of oviposition by females of each strain were not significantly different.
observed in the no-choice test, there were no significant differences among host egg species–parasitoid strain combinations for either age class of eggs in the percentage of parasitoids that entered diapause before emerging from host eggs.

4. Discussion

The increase in the proportion of *P. recurva* in the *Phoracantha* populations in southwestern California has occurred extraordinarily rapidly, particularly in light of the fact that each species produces, at most, only 1–2 generations per year in southern California climatic conditions (Bybee et al., submitted; Hanks et al., 1993, 1997). *Phoracantha semipunctata* populations have declined precipitously, while at the same time, numbers of *P. recurva* have increased dramatically. Thus, the overall increase in the proportion of *P. recurva* in their ecological niche, and not just a rapid proliferation of *P. recurva*. Although there are several potential mechanisms that could contribute to the change in the balance of the populations, the differential selection pressure exerted by a parasitoid that preferred one of the two species could be a critical factor.

*Avetianella longoi* was identified and described relatively recently (Longo et al., 1993; Siscaro, 1992), and its host preferences and host location mechanisms in its native habitat in Australia are unknown. The results of field trials indicate that differential parasitization of the eggs of the two *Phoracantha* species was not the result of failure of *A. longoi* to locate *P. recurva* eggs because egg masses of both species were located at equal rates in the field (Luhring et al., 2000). However, having located eggs, both laboratory and field bioassays indicated that the wasps were more likely to utilize *P. semipunctata* eggs than *P. recurva* eggs as hosts. These results suggest that the long range host habitat or host location cues used by *A. longoi* females are similar for eggs of both *Phoracantha* species, and that the parasitoid uses short range or contact cues to differentiate between potential hosts. These cues could include physical or chemical cues associated with the egg surfaces or contents.

Although many egg parasitoids (*e.g.*, *Trichogramma* spp.) use host size and shape to assess host quality and determine clutch size (Kloppe and Teerink, 1962; Schmidt and Smith, 1987), and host size can affect the size, survival, and fitness of the resulting progeny (*e.g.*, Bai et al., 1992; Flanders, 1935; Holmman et al., 1988; Marston and Ertle, 1973), it appears unlikely that host size plays a major role in *A. longoi* oviposition decisions. Although *P. recurva* eggs are smaller in diameter and volume than *P. semipunctata* eggs, size is probably not an important host selection factor for *A. longoi* because *Phoracantha* eggs of either species are large enough to support the successful development of several parasitoids. For example, as many as five parasitoids have been observed emerging from a single *P. semipunctata* egg (Hanks et al., 1996). Furthermore, it may be difficult for *A. longoi* to assess the dimensions of individual eggs because *Phoracantha* females lay eggs in dense overlapping masses sandwiched tightly between bark layers.

Consequently, for *A. longoi*, chemical or tactile cues associated with the egg shell or contents may provide more reliable indicators of host quality than size or shape. The use of chemical cues in recognition of host eggs by egg parasitoids has been well documented. For
example, accessory gland secretions on the chorion have been shown to mediate host recognition in *Trichogramma* and *Telenomus* spp. (De Jong and Pak, 1984; Nordlund et al., 1987; Strand and Vinson, 1983), whereas amino acids or salts inside the egg stimulate *Trichogramma* oviposition (Kainoh and Brown, 1994; Wu and Qin, 1982; Nettles et al., 1985).

Nevertheless, the differences in *Phoracantha* egg diameters and weights may be useful for identifying field collected eggs because the largest eggs (≥0.79 mm diameter and ≥590 µg) were exclusively *P. semipunctata*, whereas the smallest eggs (≤0.54 mm diameter) were exclusively *P. recurva*, regardless of egg age. Neonate larval weight may also provide a good rule-of-thumb estimator of species because 76% of *P. recurva* neonate weighed ≤380 µg and 97% of *P. semipunctata* neonates weighed ≥380 µg. Our efforts to identify other morphological characters by which the eggs and larvae of the two species can be distinguished reliably have failed. Thus, the only alternative to using egg weights and/or diameters for species determination is to rear larvae through to adults for unambiguous identification, a labor-intensive process which can take six months or more.

Our results clearly indicate that *P. semipunctata* eggs are a better host resource for *A. longoi* than are *P. recurva* eggs. The parasitoid readily oviposited and had a high rate of survival in *P. semipunctata* eggs of both age classes, reflecting the fact that *A. longoi* is an excellent biological control agent for *P. semipunctata* (Hanks et al., 1996; Longo et al., 1993). In contrast, *A. longoi* oviposition rates were significantly lower in *P. recurva* eggs in both choice and no-choice assays, and when oviposition did occur, survival rates of the developing parasitoid were poor. This difference in suitability of the eggs of the two host species was demonstrated dramatically by two further pieces of data. Specifically, no *P. semipunctata* eggs survived parasitization, whereas many *P. recurva* eggs survived parasitization and produced a neonate beetle larvae. Furthermore, another large fraction of parasitized *P. recurva* eggs died, producing neither host larvae nor parasitoids. These results suggest that the parasitoid is not well adapted to *P. recurva* as a host, even after rearing on *P. recurva* for multiple generations, and that in most cases, neither the ovipositing female nor her developing progeny were able to subvert the development or defenses of *P. recurva* eggs successfully.

The preference of *A. longoi* females for *P. semipunctata* eggs and the suitability of *P. semipunctata* eggs as hosts appears to be innate, and was not shifted noticeably even after rearing *A. longoi* on *P. recurva* eggs for >15 generations (see baseline data in Luhring et al., 2000, for *A. longoi* ovipositional preferences and host suitability before exposure to and rearing on *P. recurva*). Nor did there appear to be any conditioning of progeny based on the species of host in which they had developed. In choice and no-choice bioassays, R-strain females oviposited in *P. recurva* eggs no more frequently than did S-strain females, and survival rates of progeny of both strains were low and equal. Conversely, when given the opportunity to oviposit in *P. semipunctata* eggs in both choice and no-choice tests, R-strain *A. longoi* readily oviposited, and survival of progeny was high. This is in contrast to several studies with *Trichogramma* spp. in which colonies reared for a number of generations on certain factitious hosts were less successful on their original hosts when presented with their original hosts again (e.g., Bai et al., 1992; van Bergeijk et al., 1989).

From a practical viewpoint, our results suggest that in mixed populations of the two *Phoracantha* spp., differential parasitization by *A. longoi* represents a mortality factor that acts much more strongly on *P. semipunctata* than *P. recurva*. Other effects being equal, this differential parasitization increasingly would favor *P. recurva*, as appears to be happening in southwestern California, where the proportion of *P. semipunctata* in the *Phoracantha* population has declined rapidly since 1995. Several other studies have implicated parasitoids as driving the success or failure of an exotic herbivore species as it invades a previously occupied ecological niche (see Lawton, 1986). For example, Settle and Wilson (1990) found that an egg parasitoid preferentially eliminated a native grape leafhopper, thus allowing an invading leafhopper to become the dominant species in California vineyards. Although the subject of much speculation in the literature, well documented examples of parasitoid-mediated species replacement are rare (Denno et al., 1995).

Predators and parasitoids may have significant indirect impacts on the interactions of herbivores through apparent competition (Holt, 1977, 1984). The presence of one herbivore may increase the abundance of a shared natural enemy and result in a significant reduction in the population size of a second herbivore (Hamback and Bjorkman, 2002; Karban et al., 1994; Pope et al., 2002; Settle and Wilson, 1990). Apparent competition could result in the elimination of a species of herbivore from an assemblage sharing natural enemies if there is an amensal relationship between competing herbivores (Bonsall and Hassell, 1997).

Apparent competition does not seem to be a critical factor in maintaining the relationships between the *Phoracantha* species in California. The parasitoid is marginally successful in *P. recurva* and the preference for *P. semipunctata* remains strong despite intense selection in laboratory colonies. Ovipositional preference for *P. semipunctata* is also highly significant in field populations of *A. longoi* (Luhring et al., 2000), and survival of *A. longoi* eggs and larvae have been previously shown to be reduced in *P. recurva* eggs in comparison to
**References**


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Hanks, L.M., Gould, J.R., Paine, T.D., Millar, J.G., Wang, Q., 1995. Biology and host relations of *Avetianella longoi* (Hymenoptera: Phoracantha congeneric analysis, it is not unreasonable to predict that the two sources. Using the predictions generated from their boring guild of insects and suggested that competition was frequently asymmetrical within the wood- Denno et al. (1995) reviewed the literature for patterns of competitive abilities between the two differences in fecundity, developmental rates, or com- exacerbates or ameliorates by other factors, such as competitive relationships that are distinctly different from the co- observed that populations of *P. semipunctata* declined sharply in both northern and southern Cali- a parasitoid can develop in either of the beetle hosts in no- choice situations, the ovipositional preference behavior and differences in egg suitability functionally limits the extent to which the association of herbivores can be characterized as sharing a natural enemy. The effects of differential parasitization may be exacerbated or ameliorated by other factors, such as differences in fecundity, developmental rates, or competitive abilities between the two *Phoracantha* species. Denno et al. (1995) reviewed the literature for patterns in interspecific competition and concluded that competition was frequently asymmetrical within the wood-boring guild of insects and suggested that competition was more likely between closely related, introduced, aggregative, and sessile species feeding on discrete res- sources. Using the predictions generated from their analysis, it is not unreasonable to predict that the two congeneric *Phoracantha* beetle species that are attracted to stressed host trees and which feed within concealed and limited resources under the bark would be subject to significant interference competition (Denno et al., 1995). Studies of direct interspecific *Phoracantha* larval competition in host logs in laboratory and natural settings are in progress (TDP and JGM, unpublished data).

Thus, the relative contributions of interspecific com- petition and differential rates of successful parasitization on the population dynamics of the two species are not yet clear. It is also likely that the interactions that we are observing in California reflect a very simplified ecological relationship that is distinctly different from the co- evolved communities within the native range. However, we have observed that populations of *P. semipunctata* declined sharply in both northern and southern Cali- fornia following introduction of *A. longoi* (Hanks et al., 1995; Paine et al., 2000). In contrast, *P. recurva* arrived in southern California at about the same time that *A. longoi* populations were abundant in the field. Despite the ubiquitous presence of the parasitoid, *P. recurva* numbers increased rapidly in southern California. However, the geographic distribution of *P. recurva* has not expanded to northern California, where populations of *P. semipunctata* remain at reduced levels. This natural experiment suggests a parasitoid-mediated replacement in southern California, as suggested by the results of the studies described herein.

In terms of the biological control program against *Phoracantha* beetles, our results indicate that *A. longoi* may be ineffective as a biological control agent for *P. recurva*, and that control of this abundant pest may require introduction of more efficacious and host-spe- cific natural enemies. However, although the prospects for management of *P. recurva* with *A. longoi* appear unlikely in the near term, it will be interesting to follow the situation as it unfolds in the field over time. As the proportion of *P. semipunctata* in mixed *Phoracantha* populations dwindles, *A. longoi* will face increasing se- lection pressure to adapt successfully to *P. recurva* as an alternate host. Our results indicate that this may take a considerable number of generations, if it happens at all, because percent parasitization and survival of progeny of R-strain females did not increase even after rearing for multiple generations on *P. recurva* eggs. Thus, at least in the short term, *P. recurva* eggs may act as a sink for *A. longoi* eggs, with the cumulative effects of poor oviposition and low survival of progeny decreasing *A. longoi* populations. Applying the model proposed by Heimple et al. (2003) which proposes that the presence of a marginal host could benefit a more suitable host, the presence of *P. recurva* over the long term could ease the pressure on *P. semipunctata*, and possibly allow for some rebound of *P. semipunctata* populations.

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