Ecology, 97(5), 2016, pp. 1345–1356

Host size and spatiotemporal patterns mediate the coexistence of specialist parasitoids

Apostolos Pekas,[1] Alejandro Tena,[2] Jeffrey A. Harvey,[3] Ferran Garcia-Marí,[1] and Enric Frago[4,5]

[1]*Instituto Agroforestal Mediterráneo (IAM), Universitat Politècnica de València, Cami de Vera 14, 46022 València, Spain*
[2]*Instituto Valenciano de Investigaciones Agrarias, Unidad Asociada de Entomología UJI-IVIA, Moncada, València, 46113, Spain*
[3]*Department of Terrestrial Ecology, Netherlands Institute of Ecology, 6708 PB, Wageningen, The Netherlands*
[4]*Laboratory of Entomology, Wageningen University, Droevendaalsesteeg 1, Building 107, PO Box 16,*
6700AA, Wageningen, The Netherlands

Abstract. Many insect parasitoids are highly specialized and thus develop on only one or a few related host species, yet some hosts are attacked by many different parasitoid species in nature. For this reason, they have been often used to examine the consequences of competitive interactions. Hosts represent limited resources for larval parasitoid development and thus one competitor usually excludes all others. Although parasitoid competition has been debated and studied over the past several decades, understanding the factors that allow for coexistence among species sharing the same host in the field remains elusive. Parasitoids may be able to coexist on the same host species if they partition host resources according to size, age, or stage, or if their dynamics vary at spatial and temporal scales. One area that has thus far received little experimental attention is if competition can alter host usage strategies in parasitoids that in the absence of competitors attack hosts of the same size in the field. Here, we test this hypothesis with two parasitoid species in the genus *Aphytis*, both of which are specialized on the citrus pest California red scale *Aonidiella aurantii.* These parasitoids prefer large scales as hosts and yet coexist in sympatry in eastern parts of Spain. Parasitoids and hosts were sampled in 12 replicated orange groves. When host exploitation by the stronger competitor, *A. melinus*, was high the poorer competitor, *A. chrysomphali*, changed its foraging strategy to prefer alternative plant substrates where it parasitized hosts of smaller size. Consequently, the inferior parasitoid species shifted both its habitat and host size as a result of competition. Our results suggest that density-dependent size-mediated asymmetric competition is the likely mechanism allowing for the coexistence of these two species, and that the use of suboptimal (small) hosts can be advantageous under conditions imposed by competition where survival in higher quality larger hosts may be greatly reduced.

Key words: Aphytis; *California red scale; competitive exclusion; host quality; host–parasitoid interactions; interspecific competition; intraguild interactions; size-mediated interactions.*

Introduction

Interspecific competition is an important driver of niche differentiation and has long underpinned much ecological and evolutionary theory. Moreover, competition can drive species divergence and ultimately affect community structure and function (Morin 2011). According to the competitive exclusion principle, species sharing identical niches cannot coexist (Hardin 1960, DeBach 1966, Chesson 2000) because the stronger competitor will drive the weaker competitor to extinction by monopolizing habitat space and resources. In ecological communities, however, species exploiting similar resources are often able to coexist. Under these circumstances, natural selection may enable competing species to diverge in resource use and phenotype, ultimately leading to niche differentiation or character displacement (Hairston et al. 2005, Schoener 2011, Stuart and Losos 2013).

Insect herbivores are one of the most diverse groups of organisms in terrestrial ecosystems and many species, in turn, harbor specialized natural enemies, such as parasitoid wasps (Hymenoptera). These are insects whose larvae develop in or on the bodies of other insects, whereas the adults are free living (Godfray 1994). Unlike arthropod predators, which may require many prey to achieve maturity, the growth and development of parasitoids is dependent on the resources contained in a single host that is often not much larger than the adult parasitoid. For this reason, individual hosts usually sustain the development of a single parasitoid species, and when more than one parasitoid species attacks a host, one competitor ultimately dominates the host and eliminates all others (Harvey 2005, Harvey et al. 2013). However, different stages (e.g., eggs, larvae, pupae) of insect herbivores often harbor multiple parasitoid species, leading to the possibility of interspecific competition among

Manuscript received 19 January 2015; revised 5 October 2015; accepted 6 November 2015; final version received 8 January 2016. Corresponding Editor: J. A. Rosenheim.

[5]E-mail: enric.frago@gmail.com

them (Price 1972, Harvey et al. 2013). Parasitoids are therefore excellent organisms for exploring interspecific competition.

Many theoretical and laboratory studies have explored the mechanisms influencing interactions that facilitate coexistence among parasitoids. Theoretical studies have highlighted the role of interspecific competition in structuring parasitoid communities (May and Hassell 1981, Murdoch et al. 1996, Bonsall and Hassell 1999, Borer 2002, Borer et al. 2003) and their conclusions have been partially supported in laboratory experiments. Yet, at present, there is relatively little information explaining how parasitoids with broadly overlapping host niches coexist in nature (but see Tscharntke 1992, Bográn et al. 2002, Borer et al. 2004, Snyder et al. 2005). Mechanisms favoring coexistence include different levels of specialization in resource use, temporal and spatial (local or regional) resource partitioning, and processes related to environmental heterogeneity (Schoener 1974, Chesson 2000, Amarasekare 2003, Snyder et al. 2005). Interspecific competition among parasitoids has been reported in several host–parasitoid associations, but our understanding of the mechanisms involved is hampered by limited evidence of competitive interactions in the field (Magdaraog et al. 2012). Studies of spatial patterns have been commonly undertaken to explain parasitoid coexistence. For example, Borer et al. (2004) demonstrated that the use of hosts located on either leaves or stems reduced the intensity of competitive contests between species of *Encarsia* and *Aphytis*. A recent study has also demonstrated that an introduced parasitoid, *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), has displaced the native *Aphytis chrysomphali* (Mercet) (Hymenoptera: Aphelinidae) in Mediterranean regions where the native species is at a competitive disadvantage due to local environmental conditions (Sorribas et al. 2010). The authors also report that seasonal fluctuations in the abundance of these two species explain their coexistence in habitats where they coexist. These studies have helped us to unravel various spatial and temporal mechanisms allowing for parasitoid coexistence in the field, but how different species interact when they co-occur in the same location and at the same time still remains largely unexplored. Selection may drive parasitoids sharing the same host species and stage to partition host resources in some way that allows them to coexist by reducing niche overlap (Briggs 1993, Mills and Getz 1996). However, evidence for host-stage partitioning in the field is scarce.

Host selection models often predict that female parasitoids should oviposit in high-quality hosts, on which their fitness is optimized. Quality is defined as characteristics of the host that affect parasitoid growth, development, survival and reproduction and include host stage or size at parasitism (Godfray 1994, Harvey 2005). Host and parasitoid size are often positively correlated, and in turn these parameters are related to other traits such as longevity, fecundity, and host-finding ability (Godfray 1994). However, when larger, high-quality hosts are monopolized by a dominant competitor, adult females of an inferior competitor may be forced to oviposit in smaller hosts (i.e., of low quality) where their larval survival is higher. Hosts of low quality can therefore provide the weaker competitor with competition-free space (Winkler and Hall 1981, Briggs 1993). These interactions can ultimately lead to ecological character displacement if the weaker competitor evolves an innate preference for, or increased performance on hosts of smaller size.

Interactions among parasitoids in the genus *Aphytis* Howard (Hymenoptera: Aphelinidae) and their scale insect hosts represent an excellent model of competitive displacement in insects (Luck and Podoler 1985). *Aphytis* species are idiobiont parasitoids that do not allow the host to grow after parasitism (Harvey 2005), and thus host size is a good proxy for parasitoid size. Parasitoid species in this genus are important biological control agents of the diaspidid California red scale *Aonidiella aurantii*, which is one of the most serious citrus pests worldwide. *A. chrysomphali*, *A. lingnanensis,* and *A. melinus,* have been long used against *A. aurantii* in augmentative biological control programs to control scale outbreaks (Moreno and Luck 1992). In California, *A. chrysomphali* was initially introduced in an attempt to control *A. aurantii* but the results were ineffective. A decade later *A. lingnanensis* was introduced and this species subsequently displaced *A. chrysomphali* in most of the areas sampled (DeBach and Sisojević 1960). *A. melinus* was introduced later and in turn displaced *A. lingnanensis* in many interior areas of California (DeBach and Sundby 1963). The difference in host-size-dependent sex allocation between *A. melinus* and *A. lingnanensis* was suggested as an explanation for competitive displacement (Murdoch et al. 1996). In contrast with *A. lingnanensis, A. melinus* prefers smaller hosts for the production of female progeny so that it exploits *A. aurantii* before it grows into the size suitable for the production of female *A. lingnanensis* (Luck and Podoler 1985).

A recent large-scale survey in eastern Spain showed that, although *A. melinus* (the stronger competitor) dominates over *A. chrysomphali* (the weaker competitor), varying environmental conditions and seasonal variation in parasitoid abundance facilitates coexistence of both species in a large part of the areas surveyed (Sorribas et al. 2010). *A. chrysomphali* is known to carry the parthenogenesis-inducing symbiotic bacterium *Wolbachia,* which allows the parasitoid to produce female progeny regardless of host size (Pina 2007, Pekas et al. 2010). In comparison to the interaction between *A. lingnanensis* and *A. melinus* in California*,* in Spain *A. chrysomphali* can therefore utilize smaller hosts to produce female progeny. This scenario offers an excellent opportunity to explore the factors that relax competition and allow coexistence between these two closely related species.

Here we examine interactions involving scales and parasitoids sampled in 12 replicated orange groves where both *Aphytis* species coexist in sympatry but at varying levels of host exploitation. We first assessed whether parasitism rates and the size of the hosts used by these two species varied, and whether this variation also differed in time (i.e., among seasons) and space (i.e., among substrates in the host plant). In each of the different seasons, we tested several hypotheses. First, we hypothesize that small hosts provide competition-free space for the weaker competitor. A high percentage of host exploitation by the dominant species should therefore lead to a shift in the size of the hosts attacked by the weaker one. More precisely, we hypothesize *A. chrysomphali* should develop in smaller hosts or instars under conditions of high host exploitation by the stronger competitor, *A. melinus*. Second, we tested whether competitive interactions were also affected by location within the host plant (i.e., substrate). In particular, we hypothesize that the weaker competitor *A. chrysomphali* should suffer greatly from competition on substrates where hosts are bigger and thus is more likely to be exploited by the stronger competitor.

METHODS

Study system

The host, *A. aurantii* is a diaspidid insect that reproduces sexually and is ovoviviparous. It can have up to four generations per year, and a single citrus tree can harbor more than 100 000 individuals (Murdoch et al. 1989). After hatching, first-instar nymphs disperse and, once settled, they remain fixed in the host plant during the rest of their life. *A. aurantii* is sexually dimorphic, females have three instars and males five. *Aphytis* spp. are ectoparasitoids that are highly specialized on diaspidid scales. *Aphytis* spp. are idiobionts (i.e., the host is paralyzed and arrests development once parasitized) and commonly solitary (i.e., a single parasitoid emerges from each host). Many species, however, can be facultatively gregarious. Species in the genus *Aphytis* usually reproduce sexually, but some (including *A. chrysomphali*) can reproduce parthenogenetically when infested with the bacterium *Wolbachia* (Pina 2007). *A. aurantii* size varies greatly among seasons, populations and host plant substrates (Pekas et al. 2010). Second-instar females and males, and third-instar virgin females are the *A. aurantii* stages commonly attacked by *Aphytis* parasitoids, because first instars are usually too small to allow the development of the parasitoid larvae.

Study sites, sampling design, and assessment of parasitism

Aonidiella aurantii scales were sampled in 12 citrus orchards in the Valencian citrus growing area (Appendix S1: Fig. S1), where scales were attacked in sympatry by both *A. melinus* and *A. chrysomphali*. Citrus trees were sampled from February until December 2007. Each orchard was sampled three to five times per season (winter, spring, summer, and autumn) depending on *A. aurantii* abundance. In each sampling date and site, 40 branches (<10 mm in diameter and bearing at least 10 leaves) and 50 fruits (when available) were sampled from a total of 10 different trees infested with scales. Twenty-four hours following collection, up to 50 scales per date, site, substrate (i.e., leaves, fruits, and branches) and instar susceptible to *Aphyti*s parasitism (i.e., second-instar males, second-instar females, and third-instar females) were randomly selected and their size estimated. *A. aurantii* scales have a flat body, and to estimate their size, scale (or body) length was multiplied by scale width. Presence of *Aphytis* eggs, larvae, prepupae, or pupae, was used to assess parasitism in these same insects. When *A. aurantii* parasitoids were collected at the egg stage, larva or prepupa, hosts were transferred into glass vials, maintained at 22–25°C, 60–70% relative humidity and 16:8h light:dark, and inspected every 2 d until parasitoid identification was possible. Species identification was based on the coloration of the pupae (Pekas et al. 2010). Parasitism rate, and proportion of host exploitation by either parasitoid, were estimated as the proportion of parasitized individuals. The sampling procedure used, prevented post-collection parasitism, and might have underestimated parasitism rates. Multiple parasitism events, however, are not common in this system because the two parasitoids studied are idiobionts and paralyze the hosts at oviposition.

Statistical analyses

All analyses were performed in R (R 3.1.3; R Development Team 2015). Several mixed effects models were fitted with a Gaussian error distribution with the lmer function in the lme4 package (Bates et al. 2014). Model fit was assessed by visual inspection of the residuals, and with a chi-squared goodness-of-fit test. In some models, model fit was improved by transforming the response variable. The following models were fitted: (1) differences in the size of *A. aurantii* hosts (expressed as log[surface + 0.5]) were tested by including season, plant substrate and their interaction as categorical fixed factors; (2) differences in the size of parasitized *A. aurantii* hosts (also log-transformed) were tested by including season, plant substrate, parasitoid species, and their pairwise interactions as categorical fixed factors; (3) differences in mean size among scale stages were tested by including stage as a categorical fixed factor; (4) differences in parasitism rates (expressed as their square root) were tested by including season, substrate, parasitoid species, and their pairwise interactions as categorical fixed factors. To account for the nested sampling design, study site, and date nested within study site were included as random factors. In the models with parasitism rate as a response variable, only study site was included as random factor because for each parasitoid and sampling date a single value was obtained. To test for specific differences between parasitoid species or among plant substrates and seasons, the following post hoc pairwise comparisons were also performed: (1) mean host size on different plant substrates and seasons and (2) parasitism rates and (3) the size of the hosts attacked by the two parasitoid species. These comparisons were performed with nonparametric bootstrapping techniques by drawing 10 000 mean difference pseudo-samples with the boot function in the boot package (Davison and Hinkley 1997). In these comparisons, the significance level was corrected with the Bonferroni adjustment.

The relationship between the size of the hosts available and the size of those parasitized by either parasitoid species was analyzed in two different ways. A linear model was built with mean size of the parasitized hosts at a particular date and site included as a response variable; as predictors mean size of all scales sampled at a particular date and site, and parasitoid species were included as continuous and categorical fixed factors, respectively. Manly's selectivity measure (Manly et al. 2002) was also estimated for each parasitoid species by dividing the size of the hosts parasitized by the size of the hosts available at a particular date and site. A ratio > 1 would reveal the parasitoid was using hosts larger than the average in the environment, and the opposite would be true for ratios lower than one. For each parasitoid species this difference was tested with a *t* test.

Interspecific parasitoid interactions were modeled with generalized linear mixed effects models assuming a binomial error distribution with a log-link function with the glmer function in the lme4 package (Bates et al. 2014). As a response variable, each host was therefore considered either parasitized or not by a given *Aphytis* species. By doing so, the proportion of scales sampled in each season or substrate was accounted for in the models. A model for each parasitoid species and season was built including the following fixed factors: (1) proportion of host exploitation by the competing parasitoid, a continuous fixed factor that represented the intensity of the antagonistic interaction between the two parasitoids in a given date and site; (2) plant substrate as a categorical fixed factor; and (3) host size as a continuous fixed factor. As in the previous models, to account for the nested sampling design, study site and date nested within study site were included as a random factors. In these models, we were particularly interested in the interaction terms as they would indicate how the competitive interactions affected parasitism on different substrates or host sizes. In particular, we expected that a given parasitoid species would be reared from smaller hosts or from hosts on specific plant substrates at high proportion of host exploitation by the competing congener. The main terms, therefore, do not necessarily explain interspecific parasitoid interactions, but a correlation between parasitism rates by the two competing parasitoids. In these analyses, model reduction was conducted by removing nonsignificant interactions ($P > 0.05$). All binomial models showed no sign of overdispersion with dispersion factors < 1. In all mixed models, the absence of data points with high influence was checked by calculating Cook's distances. Significance of fixed terms in linear mixed models was tested with the likelihood ratio test.

RESULTS

Aonidiella aurantii size and abundance

Considering all *A. aurantii* instars together, mean insect size was significantly different among seasons and substrates (mixed model effect for season $\chi^2_3 = 47.2$, $P < 0.001$; substrate $\chi^2_2 = 146.5$, $P < 0.001$), but differences among substrates were not consistent among seasons (significant mixed model interaction term, $\chi^2_6 = 146.5$, $P < 0.001$; Fig. 1). Post hoc tests among seasons revealed that scales were largest in spring, intermediate in autumn, and smallest in summer and autumn ($P < 0.008$, after Bonferroni correction). Among plant substrates, scales were largest on branches, intermediate on fruits, and smallest on leaves ($P < 0.017$, after Bonferroni correction). In spring, scales were smaller on fruits and larger on branches; in summer and autumn, scales were smaller on leaves; and in winter, scales were larger on

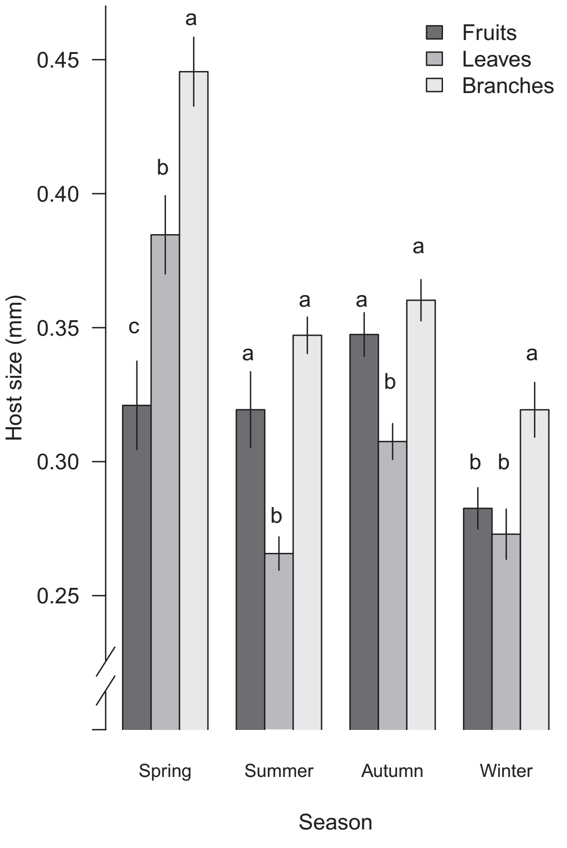

FIG. 1. Size (mean ± SE) of the collected *Aonidiella aurantii* scales at different seasons and plant substrates. Different letters among means in the same season indicate significant differences ($P < 0.05$) according to the Tukey post-hoc test.

branches (pairwise comparisons in Fig. 1, $P < 0.017$, after Bonferroni correction). *A. aurantii* instars also differed in size: third-instar females were the largest (0.524 ± 0.004 mm² [mean ± SE]), followed by second-instar males (0.228 ± 0.001 mm²) and second-instar females (0.177 ± 0.001 mm²). These differences were significant (mixed-model effect for stage, $\chi^2_2 = 19187.2$, $P < 0.001$; post hoc test $P < 0.001$; inset Fig. 6). The abundance of the different instars collected also differed among seasons and substrates (Appendix S1: Fig. S2).

Aonidiella aurantii *parasitism at different seasons and plant substrates*

The two parasitoid species imposed different rates of parasitism on *A. aurantii* (mixed-model effect $\chi^2_1 = 24.2$, $P < 0.001$). Parasitism also differed among seasons and plant substrates (mixed-model effect for season $\chi^2_3 = 10.5$, $P = 0.014$; substrate $\chi^2_2 = 6.1$, $P = 0.047$). Only the interaction between parasitoid species and season was significant, which revealed seasonal differences in parasitism rates by the two parasitoids studied (season × substrate interaction $\chi^2_6 = 4.4$, $P = 0.617$; season × parasitoid interaction $\chi^2_3 = 20.4$, $P < 0.001$; substrate × parasitoid interaction $\chi^2_2 = 0.9$, $P = 0.643$). These differences were mainly due to *A. melinus* having greater rates of parasitism relative to *A. chrysomphali*, on all substrates in summer (pairwise comparisons in Fig. 2, $P < 0.017$, after Bonferroni correction).

Size of parasitized Aonidiella aurantii *at different seasons and plant substrates*

For each sampling date and study site, the mean size of parasitized scales was significantly correlated with the mean size of non-parasitized scales (linear model size effect, $F_{1,57} = 86.8$, $P < 0.001$). In this model, parasitoid species was also a significant explanatory variable. *A. melinus* was reared from hosts 24.6% larger than *A. chrysomphali* (linear model parasitoid species effect with *A. chrysomphali* as reference category, $\beta = 0.09$, $F_{1,57} = 15.7$, $P < 0.001$; Fig. 3). Manly's selectivity measure revealed that *A. melinus,* but not *A. chrysomphali,* used hosts larger than the average found in the environment. The ratio was significantly larger than one in *A. melinus* (1.19 ± 0.04, $t_{31} = 4.30$, $P < 0.001$), but it did not differ from one in *A. chrysomphali* (0.92 ± 0.05, $t_{27} = -1.61$, $P = 0.119$). The size of the hosts from where both parasitoids were reared differed significantly (mixed model effect for parasitoid species $\chi^2_1 = 17.0$, $P < 0.001$; Fig. 4), and differences were also significant among seasons and plant substrates (mixed model effect for season $\chi^2_2 = 6.9$, $P = 0.032$; substrate $\chi^2_2 = 19.2$, $P < 0.001$; Fig. 4). In this model, only the interaction between

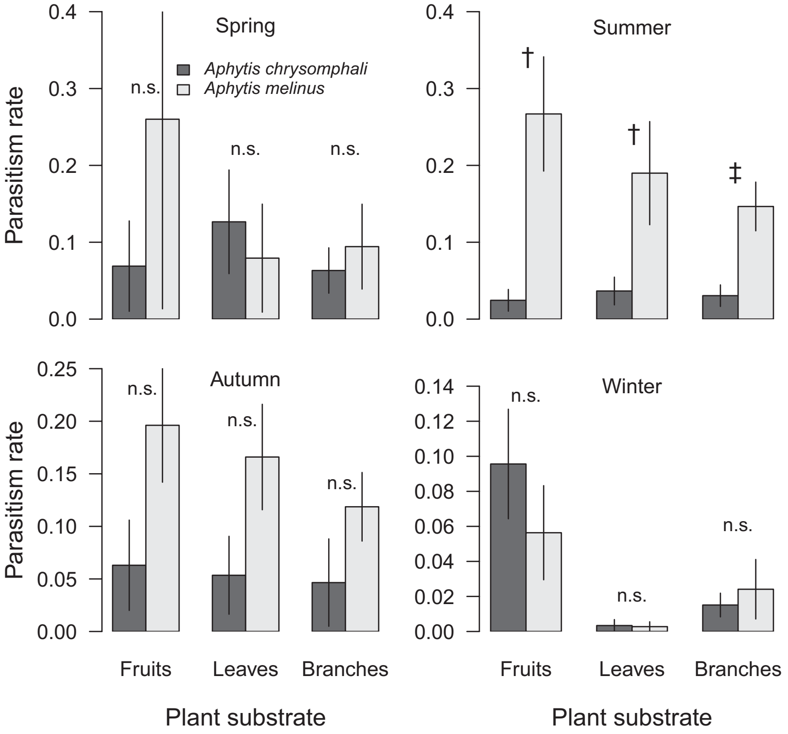

FIG. 2. Parasitism rates (mean ± SE) by *Aphytis chrysomphali* (dark gray) and *Aphytis melinus* (light gray) on *Aonidiella aurantii* scales collected on different substrates and seasons. Individuals sampled from a specific date and population were considered as replicate units. Pairwise comparisons for each season and substrate combination are presented at the top of the bars. P values were considered significant after Bonferroni correction (†$P < 0.017$; ‡$P < 0.003$; n.s., not significant).

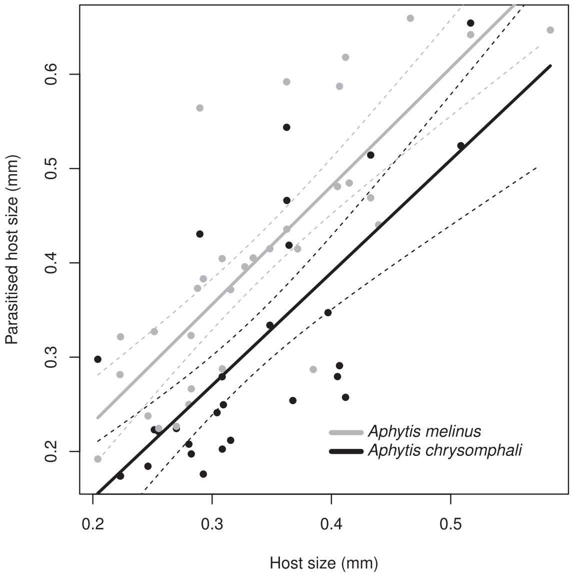

FIG. 3. Relationship between the size of the hosts available and size of the hosts attacked by *Aphytis chrysomphali* (black) or *Aphytis melinus* (light gray) at each sampling date. Lines represent independent linear regression models with 95% confidence intervals for each parasitoid species (for *A. chrysomphali*, $R^2 = 0.73$, and for *A. melinus*, $R^2 = 0.81$).

parasitoid species and season was significant, which revealed seasonal differences in the size of the hosts attacked by the two parasitoids (season × substrate interaction $\chi^2_4 = 5.9$, $P = 0.212$; season × parasitoid interaction $\chi^2_2 = 6.3$, $P = 0.042$; substrate × parasitoid interaction $\chi^2_2 = 1.6$, $P = 0.438$). Pairwise comparisons revealed that *A. melinus* used larger hosts on leaves in autumn, but *A. chrysomphali* used larger hosts on leaves and branches in summer (pairwise comparisons in Fig. 4, $P < 0.017$, after Bonferroni correction). In these analyses, winter samples were excluded due to the low number of parasitized hosts obtained.

Interspecific parasitoid interactions

In spring, *A. chrysomphali* parasitism was not significantly affected by any of the variables included in the models, whereas *A. melinus* parasitism was positively related with host size (Table 1). In spring, and for both parasitoids, none of the interactions in the models were significant. In summer, parasitism by both parasitoids was related to host size and proportion of host exploitation by the competing congener. In this season, interactions were only significant in the models for *A. chrysomphali* parasitism. When *A. melinus* host exploitation was low, parasitism by *A. chrysomphali* was greater on larger *A. aurantii* scales. At high proportions of host exploitation by *A. melinus*, however, parasitism by *A. chrysomphali* was larger on small hosts (Table 1, Fig. 5, top panel). In summer, *A. melinus* parasitism intensity also affected *A. chrysomphali* parasitism on the different plant substrates. High *A. melinus* proportion of host exploitation led to low *A. chrysomphali* parasitism on leaves, but this pattern was not found on fruits and branches (Table 1, Fig. 5, middle panel). In autumn, parasitism by both parasitoids was again related to host size. Similar to what was found in summer, no significant interactions were found in the models for *A. melinus,* but proportion of host exploitation by *A. melinus* negatively affected *A. chrysomphali* parasitism on the different substrates. This effect was significantly stronger on branches than on either leaves or fruits (Table 1, Fig. 5, lower panel). Similar models were also built to explore the effect of interspecific parasitoid interactions on the host instar attacked (Table 2). Results were similar to those exploring the effect on host size: significant interactions were only found in the models for *A. chrysomphali* but not for *A. melinus*. In summer, the *A. aurantii* instar from which *A. chrysomphali* was recovered was influenced by *A. melinus* host exploitation. Parasitism by *A. chrysomphali* was negatively related to *A. melinus* host exploitation on larger hosts (i.e., third-instar females and males), but not on smaller ones (i.e., second-instar females; Fig. 6).

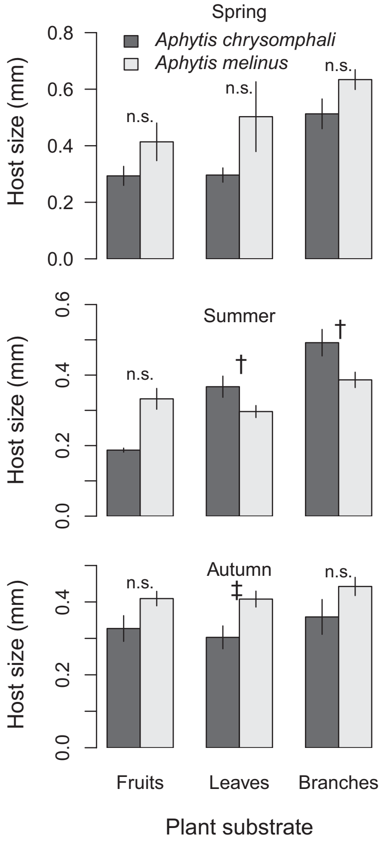

FIG. 4. Size (mean ± SE) of *Aonidiella aurantii* hosts attacked by *Aphytis chrysomphali* (dark gray) and *Aphytis melinus* (light gray) on different substrates and seasons. Pairwise comparisons for each season and substrate combination are presented at the top of the bars. *P* values were considered significant after Bonferroni correction († $P < 0.017$; ‡ $P < 0.003$; n.s., not significant).

Table 1. Effect of host size, host plant substrate, and proportion of host exploitation by the competing parasitoid on parasitism by *Aphytis chrysomphali* and *Aphytis melinus* on *Aonidiella aurantii* scales.

	Spring (*n* = 641)			Summer (*n* = 1656)			Autumn (*n* = 1813)		
Parameters	β	*F*	*P*	β	*F*	*P*	β	*F*	*P*
Parasitism by *A. chrysomphali*									
Intercept	−2.67	−4.55	**<0.001**	−5.96	−5.73	**<0.001**	−1.75	−1.48	0.138
Host size	−0.28	−0.38	0.706	3.86	4.74	**<0.001**	−2.39	−3.37	**0.001**
A. melinus host exploitation	3.57	1.36	0.175	11.37	2.73	**0.006**	−8.91	−1.25	0.212
Substrate (leaves)	−0.23	−0.66	0.512	1.78	1.84	0.065	−0.24	−0.58	0.564
Substrate (branches)	−0.67	−1.60	0.110	0.99	1.04	0.297	0.27	0.59	0.556
Host size × *A. melinus* host exploitation				−25.73	−2.36	**0.018**			
Substrate (leaves) × *A. melinus* host exploitation				−11.23	−2.16	**0.031**	−4.02	−1.10	0.270
Substrate (branches) × *A. melinus* host exploitation				−7.44	−1.77	0.078	−10.08	−2.30	**0.022**
Parasitism by *A. melinus*									
Intercept	−6.72	−4.95	**<0.001**	−1.79	−5.07	**<0.001**	−2.39	−7.07	**<0.001**
Host size	3.51	3.45	**0.001**	1.58	−3.68	**<0.001**	2.84	8.21	**<0.001**
A. chrysomphali host exploitation	13.37	1.62	0.104	−19.43	3.79	**<0.001**	−15.12	−2.52	**0.012**
Substrate (leaves)	−0.28	−0.36	0.718	−0.17	−0.71	0.477	−0.01	−0.04	0.966
Substrate (branches)	0.19	0.27	0.790	−0.27	−1.23	0.220	−0.31	−1.85	0.064

Notes: A different mixed-effects model with binomial error distribution was built for each parasitoid species and season of the year. For the categorical variable substrate, fruit was the reference category. Significant *P* values are presented in boldface type. Significant interactions are graphically represented in Fig. 5.

Discussion

The results of this investigation reveal that competition between two sympatric parasitoids attacking the same herbivore host in the field has generated divergence in their use of hosts based on size and stage. We show that, when there is high host exploitation by the dominant competitor, *A. melinus*, the inferior competitor, *A. chrysomphali*, switches from using large, high-quality hosts to exploiting smaller hosts of lower quality. In orange groves, when host exploitation by *A. melinus* was high*, A. chrysomphali* predominated in smaller scales, but we did not find any effect in the opposite direction, revealing the asymmetrical nature of this antagonistic interaction. This suggests that hosts of lower quality can provide *A. chrysomphali* with competition-free space. For the weaker competitor, being able to exploit a host earlier in its development is likely to be an important strategy that dilutes competition. This is particularly relevant because both parasitoids are idiobionts and because laboratory experiments revealed, that when multiparasitizing hosts of different size, *A. melinus* always wins (Alejandro Tena, *unpublished results*). Under field conditions, temporal and spatial changes in host use are among the most commonly cited strategies used by parasitoids to avoid competition (e.g., De Moraes et al. 1999). Both intra- and interspecifically, preference for different host stages by parasitoids can also increase resource heterogeneity (e.g., Price 1972, Frago et al. 2012). Theoretical models also suggest that under certain conditions host stage-structure can allow for parasitoid coexistence (Briggs 1993). However, there is little evidence demonstrating that coexistence among competing parasitoids can be driven by a shift in host use to smaller hosts of lower quality by the inferior competitor that nevertheless enables it to survive and persist. In herbivorous insects, the enemy-free space hypothesis has been demonstrated in many systems where preference for plants of low nutritional quality is compensated through escape from natural enemies (Stamp 2001). Our study provides an example of this same principle in the third trophic level, and reveals that parasitoids within the same guilds (e.g., which attack the same host stage when they are alone) are able to partition resources in ways that facilitate their coexistence.

When host exploitation rates by the stronger competitor are high, the weaker competitor becomes more prevalent in smaller hosts. This was especially apparent in summer when *A. melinus* was most abundant and exploited a high percentage of hosts, thus significantly affecting host selection by *A. chrysomphali*. The stronger effects in summer might be explained by high levels of competition because, in this season, differences in rates of parasitism between the two species were the highest (*A. melinus* had 5- to 10-fold higher parasitism rates depending on the substrate). Even with this strong disadvantage, the weaker competitor was reared from larger hosts on two plant substrates in summer, which suggests that when suffering strong competition, *A. chrysomphali* has evolved strategies to survive at low densities but on high-quality hosts. The greater antagonisitc interactions between the studied parasitoids in summer is agreement with Sorribas et al. (2010), who found a higher proportion of *A. melinus* wasps in July and August. These

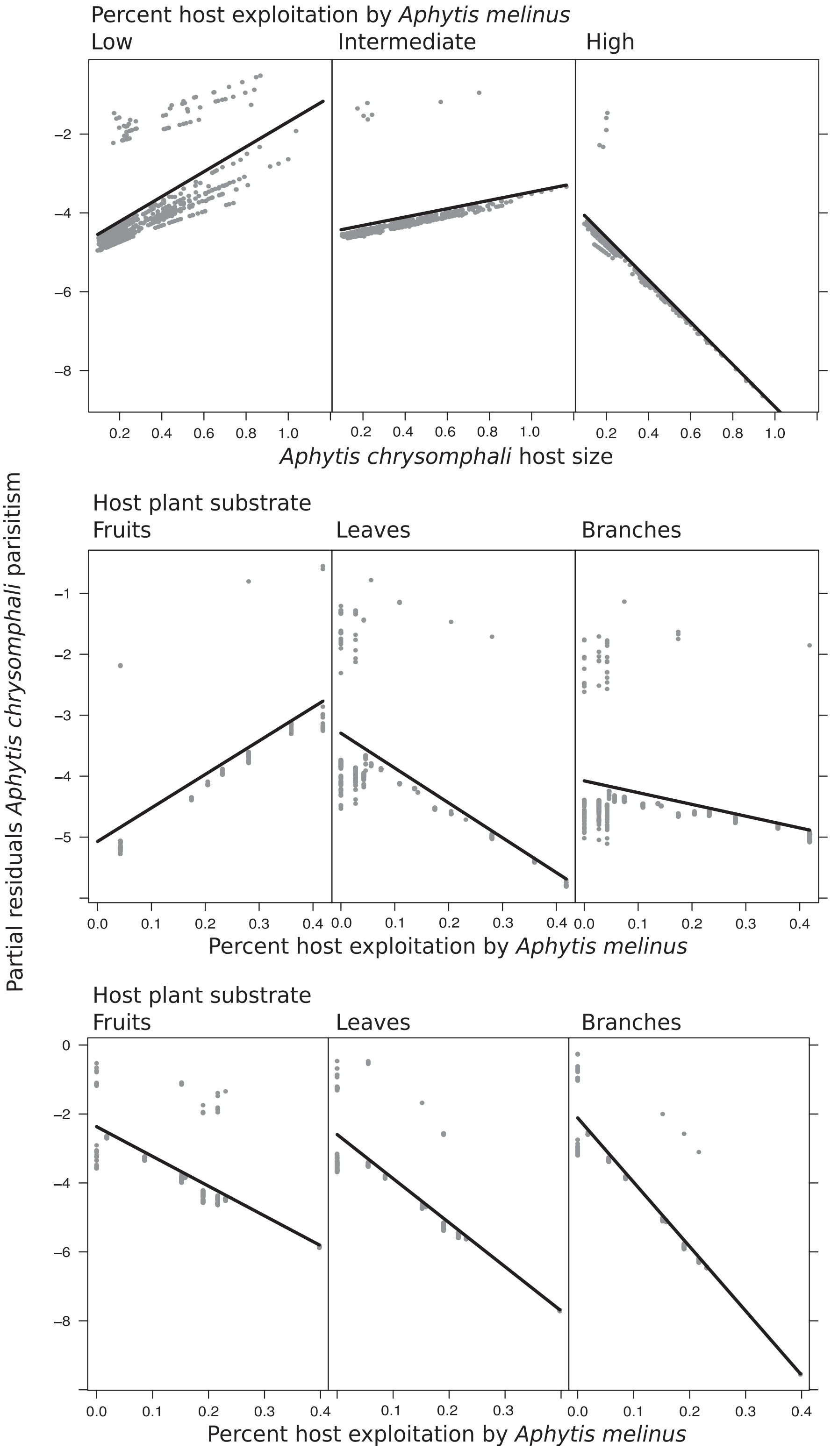

FIG. 5. Plots showing partial residuals from the models on the effect of *Aphytis melinus* percentage host exploitation on the probability of *Aphytis chrysomphali* parasitism on different *Aonidiella aurantii* sizes and plant substrates. The top panel shows the effect of *A. melinus* density on the size of the hosts attacked by *A. chrysomphali* in summer. Middle and lower panels show the effect of *A. melinus* density on *A. chrysomphali* parasitism for different substrates in summer (middle panel) and autumn (lower panel). In summer, the interaction is only significant between fruits and leaves and in autumn only between fruits and branches. The fitted lines were estimated from the generalized mixed effects models, and the points represent the partial residuals from the models, based on the visreg package in R.

TABLE 2. Effect of host stage, host plant substrate, and proportion of host exploitation by the competing parasitoid on parasitism by *Aphytis chrysomphali* and *Aphytis melinus* on *Aonidiella aurantii* scales.

	Spring (n = 641)			Summer (n = 1656)			Autumn (n = 1813)		
	β	F	P	β	F	P	β	F	P
Parasitism by *A. chrysomphali*									
Intercept	−3.37	−5.96	**<0.001**	−2.09	−2.65	**0.008**	−3.74	−2.99	**0.003**
Host stage (males)	1.50	4.10	**<0.001**	0.09	0.25	0.804	2.00	5.81	**<0.001**
Host stage (L2)	−0.10	−0.19	0.846	−3.19	−4.39	**<0.001**	1.33	3.55	**<0.001**
A. melinus host exploitation	4.85	1.90	0.058	−19.04	−2.78	**0.005**	−9.25	−1.22	0.222
Substrate (leaves)	−0.26	−0.72	0.471	0.03	0.05	0.964	−0.54	−1.22	0.221
Substrate (branches)	−0.57	−1.30	0.195	−0.32	−0.57	0.569	0.42	0.87	0.384
Host stage (males) × *A. melinus* host exploitation				5.19	0.75	0.455			
Host stage (L2) × *A. melinus* host exploitation				25.41	3.68	**<0.001**			
Substrate (leaves) × *A. melinus* host exploitation							−3.05	−0.82	0.412
Substrate (branches) × *A. melinus* host exploitation							−10.43	−2.32	**0.020**
Parasitism by *A. melinus*									
Intercept	−4.52	−3.58	**<0.001**	−1.23	−3.74	**<0.001**	−0.91	−2.80	**0.005**
Host stage (males)	−0.82	−1.13	0.259	0.06	0.27	0.788	−0.80	−4.81	**<0.001**
Host stage (L2)	−17.06	−0.01	0.991	−0.05	−0.27	0.789	−0.85	−4.92	**<0.001**
A. chrysomphali host exploitation	14.20	1.61	0.108	−19.04	−3.64	**<0.001**	−14.19	−2.41	**0.016**
Substrate (leaves)	−0.38	−0.49	0.627	−0.30	−1.26	0.206	−0.20	−0.34	0.730
Substrate (branches)	−0.10	−0.14	0.887	−0.25	−1.15	0.249	−0.24	−2.15	**0.031**

Notes: A different mixed-effects model with binomial error distribution was built for each parasitoid species and season of the year. For the categorical variable substrate, fruits were the reference category, and for host stage the reference category was third-instar females. Significant P values are presented in boldface. The significant interactions between *A. melinus* density and the host stage attacked by *A. chrysomphali* is graphically represented in Fig. 6.

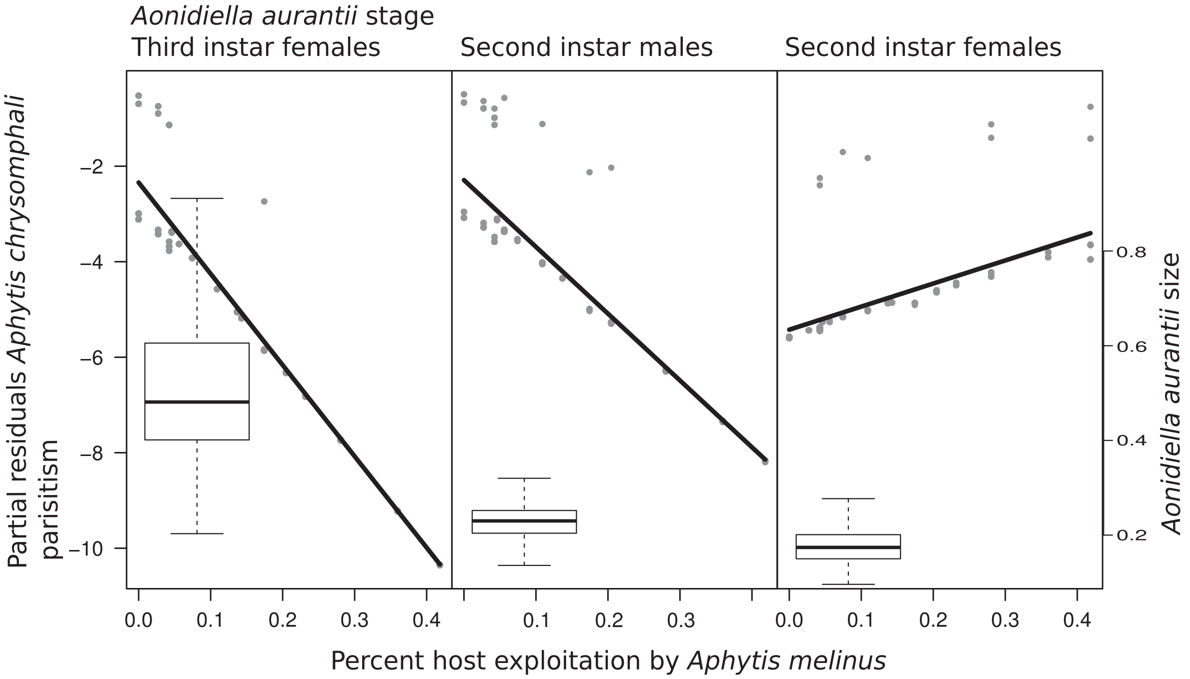

FIG. 6. Plot showing partial residuals from the models on the effect of *Aphytis melinus* density on the probability of *Aphytis chrysomphali* parasitism on different stages of *Aonidiella aurantii* in summer (left y-axis). The fitted lines were estimated from the generalized mixed effects models, and the points represent the partial residuals from the models, based on the visreg package in R. The inset shows box plots for the size of the different A. aurantii stages (right y- axis); the median is represented by a line inserted between the first and the third quartiles (box limits), and whiskers represent the 95% confidence interval of the median.

authors also showed that dominance of *A. melinus* over *A. chrysomphali* is positively correlated with temperature. In California, Luck and Podoler (1985) suggest that a greater tolerance of extreme temperatures is one feature that may enable *A. melinus* to outcompete *A. lingnanensis*. It is therefore possible that, in our system, temperature plays a key role in modulating parasitoid antagonistic interactions during the warmest months.

The antagonistic impact of *A. melinus* on *A. chrysomphali* was also affected by the substrate from which hosts were collected. On leaves in summer months, *A. chrysomphali* parasitism was significantly affected by *A. melinus* percentage host exploitation. In summer, the smallest *A. aurantii* scales were collected from leaves, and hence our a priori expectation that competition would be more intense in high-quality hosts was not met. This can be explained because *A. melinus* prefers hosts on leaves, as shown by Borer et al. (2004), or by increased levels of competition on those substrates where large hosts of higher quality are limited. In autumn, however, the proportion of host exploitation by the dominant parasitoid did not affect the size of the hosts from where *A. chrysomphali* was obtained, but it did affect its rates of parasitism on branches. In autumn, branches contained the largest hosts, which contrasts with what was observed in summer. In combination, these results show that competitive interactions vary throughout the year with strong antagonistic interactions in summer, less in autumn (*A. chrysomphali* suffers competition on leaves), probably little in spring, and almost nonexistent in winter. Studies with other organisms show that interspecific competition is often episodic, and its intensity depends on temporal resource overlap and abundance. As far as we are aware, few published studies reporting seasonal variation in parasitoid competition exist (Harvey et al. 2013), but many examples have been reported in other animal groups. For example, in desert lizards, competition between *Sceloporus merriami* and *Urosaurus ornatus* is highly asymmetrical. The former species has a strong negative effect on the latter, but only in years when resources are limiting (Dunham 1980). As the stronger competitor, *A. melinus* might outcompete *A. chrysomphali* through either exploitative or interference competition. As shown in other parasitoid species (Harvey et al. 2013), for example, *A. melinus* might be able to locate and exploit larger hosts more efficiently than *A. chrysomphali* (DeBach and Sisojević 1960). Alternatively, *A. melinus* females may be able to detect and kill *A. chrysomphali* eggs (A. Tena, *unpublished data*) or else the larvae of the superior competitor kill those of the inferior congener.

In citrus agroecosystems, host size has traditionally been seen as an important trait explaining why *A. melinus* dominates over other *A. aurantii* parasitoids. For example, in California, *A. melinus* parasitizes hosts earlier in their development so that they do not reach the size required for *A. lingnanensis* to exploit them (Luck and Podoler 1985). Pekas et al. (2010) also showed that host size has important effects on parasitism by *A. melinus* and *A. chrysomphali*. In Spain, *A. melinus* has displaced *A. chrysomphali* in some areas where the former was introduced 40 years ago, but in other regions both species do coexist in sympatry. In these areas, we have found that density-dependent size-mediated asymmetric competition is the most likely mechanism allowing the two species to coexist. It is increasingly recognized that species traits, and in particular body size, can be more important than species identity in mediating species interactions (Schneider et al. 2012). Specialist parasitoids cannot utilize alternative host species so that host-size-mediated interactions may importantly facilitate use by multiple specialists. How prevalent and strong these interactions are in parasitoid trophic webs, however, is a question that has been largely unexplored. In the area of our study, the two *Aphytis* species are known to attack more hosts than *A. aurantii* (Pina 2007). For example *A. chrysomphali* has been reared from other diaspidid hosts in the genus *Chrysomphalus,* which can also provide a competition-free space for *A. chrysomphali*. These hosts, however, are found in very small numbers in citrus groves (Pina 2007) and small *A. aurantii* hosts (less preferred by *A. melinus*) may constitute a more reliable competition-free space, even if this incurs costs in terms of adult fitness (i.e., small adult size; Pekas et al. 2010).

Changes in resource use or morphology among competitors are commonly perceived as a response to avoid competitive exclusion, and are central in understanding the role of competition in character displacement. Field studies on character displacement usually report greater trait differences in populations where competing species are found in sympatry rather than in allopatry (Brown and Wilson 1956). How these differences are affected by the density of the interacting species, however, is thus far poorly understood. Host size often correlates with adult fitness (e.g., reproductive success) in parasitoids (Godfray 1994, Harvey 2005). Consequently, it can be argued that selection for utilizing smaller hosts by parasitoids will be avoided in most situations. However, as we have shown, selection can favor the use of suboptimal (small) hosts under conditions imposed by competition where survival in higher quality larger hosts is greatly reduced. Other studies have also shown that host selection or sex allocation behavior in parasitoids sometimes contradicts theory based on models of host size or quality. For instance, parasitoids are halplodiploid and female wasps can determine the sex of their progeny at oviposition (Godfray 1994). Female progeny are often allocated to large, high-quality hosts and male progeny to small, low-quality hosts, because eggs are more costly to produce than sperm and larger females produce more eggs than smaller conspecifics (Charnov et al. 1981). However, Harvey et al. (2004) found that the solitary parasitoid *Microplitis demolitor* preferentially laid fertilized (female) eggs in early (L1 and L2) instars of its host, *Chrysodeixis includens*, and unfertilized male eggs in later (L3 and L4) instars, even though the size of progeny was greater when wasps developed in later instars. This is because the premature death of parasitoid larvae was higher in larger hosts.

In the field, parasitoid adults might display complex behaviors to avoid previously parasitized hosts. Given the seasonal disparities in host density patterns, *A. chrysomphali* adults may evolve refined behavioral strategies to predict when *A. melinus* host exploitation intensity will favor a switch from large to smaller hosts or instars. Further studies manipulating host exploitation intensity by *A. melinus*, are therefore required to determine how *A. melinus* affects *A. chrysomphali* host size selection. These experiments will determine whether host size selection is a trait that has been shaped by selective forces, or whether it is a plastic response that varies depending on *A. melinus* density. While several field studies have explored how interspecific competitive interactions shape niche overlap and coevolution in several animal taxa, our understanding of these interactions in insects, and parasitoids in particular, is still primarily based on laboratory experiments (Harvey et al. 2013). This knowledge will provide new insights into niche differentiation and coexistence in this diverse group of animals, as well as into the natural control that parasitoids impose on their hosts.

Acknowledgments

We would like to thank Amparo Aguilar for her valuable help in the field and in the laboratory, and Camille Ponzio for her comments on an earlier version of the manuscript. We are also grateful to three anonymous reviewers and Jay Rosenheim for their helpful comments. This work was supported by the project AGL2005-07155-C03-03 assigned to F. Garcia-Mari from the Ministerio de Educación y Ciencia of Spain. E. Frago was funded by Marie Curie Intra-European Fellowship within the 7th European Community Framework Programme (FP7-PEOPLE-2012-IEF #329648).

Literature Cited

Amarasekare, P. 2003. Competitive coexistence in spatially structured environments: a synthesis. Ecology Letters 6:1109–1122.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7. http://CRAN.R-project.org/package=lme4

Bográn, C., K. Heinz, and M. Ciomperlik. 2002. Interspecific competition among insect parasitoids: field experiments with whiteflies as hosts in cotton. Ecology 83:653–668.

Bonsall, M. B., and M. P. Hassell. 1999. Parasitoid-mediated effects: apparent competition and the persistence of host–parasitoid assemblages. Researches on Population Ecology 41:59–68.

Borer, E. T. 2002. Intraguild predation in larval parasitoids: implications for coexistence. Journal of Animal Ecology 71:957–965.

Borer, E. T., C. J. Briggs, W. W. Murdoch, and S. L. Swarbrick. 2003. Testing intraguild predation theory in a field system: does numerical dominance shift along a gradient of productivity? Ecology Letters 6:929–935.

Borer, E., W. Murdoch, and S. Swarbrick. 2004. Parasitoid coexistence: linking spatial field patterns with mechanism. Ecology 85:667–678.

Briggs, C. J. 1993. Competition among parasitoid species on a stage-structured host and its effect on host suppression. American Naturalist 141:372–397.

Brown, W., and E. Wilson. 1956. Character displacement. Systematic Zoology 5:49–64.

Charnov, E. L., R. L. Los-den Hartogh, W. T. Jones, and J. van den Assem. 1981. Sex ratio evolution in a variable environment. Nature 289:27–33.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics 31:343–366.

Davison, A. C., and D. V. Hinkley. 1997. Bootstrap methods and their applications. Cambridge University Press, Cambridge, UK.

De Moraes, C. M., A. M. Cortesero, J. O. Stapel, and W. J. Lewis. 1999. Intrinsic and extrinsic competitive interactions between two larval parasitoids of *Heliothis virescens*. Ecological Entomology 24:402–410.

DeBach, P. 1966. The competitive displacement and coexistence principles. Annual Review of Entomology 11:183–212.

DeBach, P., and P. Sisojević. 1960. Some effects of temperature and competition on the distribution and relative abundance of *Aphytis lingnanensis* and *A. chrysomphali* (Hymenoptera). Ecology 41:153–160.

DeBach, P., and R. A. Sundby. 1963. Competitive displacement between ecological homologues. Hilgardia 34: 105–166.

Dunham, A. 1980. An experimental study of interspecific competition between the iguanid lizards *Sceloporus merriami* and *Urosaurus ornatus*. Ecological Monographs 50:309–330.

Frago, E., J. Pujade-Villar, M. Guara, and J. Selfa. 2012. Hyperparasitism and seasonal patterns of parasitism as potential causes of low top-down control in *Euproctis chrysorrhoea* L. (Lymantriidae). Biological Control 60:123–131.

Godfray, H. C. J. 1994. Parasitoids: behavioral and evolutionary ecology. Princeton University Press, Princeton, New Jersey, USA.

Hairston, N. G., S. P. Ellner, M. A. Geber, T. Yoshida, and J. A. Fox. 2005. Rapid evolution and the convergence of ecological and evolutionary time. Ecology Letters 8:1114–1127.

Hardin, G. 1960. The competitive exclusion principle. Science 131:1292–1297.

Harvey, J. A. 2005. Factors affecting the evolution of development strategies in parasitoid wasps: the importance of functional constraints and incorporating complexity. Entomologia Experimentalis et Applicata 117:1–13.

Harvey, J. A., T. M. Bezemer, J. A. Elzinga, and M. R. Strand. 2004. Development of the solitary endoparasitoid *Microplitis demolitor*: host quality does not increase with host age and size. Ecological Entomology 29:35–43.

Harvey, J. A., E. H. Poelman, and T. Tanaka. 2013. Intrinsic inter- and intraspecific competition in parasitoid wasps. Annual Review of Entomology 58:333–351.

Luck, R., and H. Podoler. 1985. Competitive exclusion of *Aphytis lingnanensis* by *A. melinus*: potential role of host size. Ecology 66:904–913.

Magdaraog, P. M., J. A. Harvey, T. Tanaka, and R. Gols. 2012. Intrinsic competition among solitary and gregarious endoparasitoid wasps and the phenomenon of "resource sharing." Ecological Entomology 37:65–74.

Manly, B. F. J., L. Mcdonald, D. L. Thomas, T. L. Mcdonald, and W. P. Erickson 2002. Resource selection by animals: statistical design and analysis for field studies. Cambridge University Press, Kluwer Academic Publishers. Dordrecht, Netherlands.

May, R., and M. Hassell. 1981. The dynamics of multiparasitoid–host interactions. American Naturalist 117:234–261.

Mills, N. J., and W. M. Getz. 1996. Modelling the biological control of insect pests: a review of host–parasitoid models. Ecological Modelling 92:121–143.

Moreno, D. S., and R. F. Luck. 1992. Augmentative releases of *Aphytis melinus* (Hymenoptera : Aphelinidae) to suppress California red scale in California lemon orchards. Journal of Economic Entomology 85:1112–1119.

Morin, P. J. 2011. Community ecology. Second Edition. Wiley-Blackwell, Hoboken, New Jersey, USA.

Murdoch, W. W., R. F. Luck, S. J. Walde, J. D. Reeve, and D. S. Yu. 1989. A refuge for red scale under control by *Aphytis*: structural aspects. Ecology 70:1707–1714.

Murdoch, W., C. Briggs, and R. Nisbet. 1996. Competitive displacement and biological control in parasitoids: a model. American Naturalist 148:807–826.

Pekas, A., A. Aguilar, A. Tena, and F. Garcia-Mari. 2010. Influence of host size on parasitism by *Aphytis chrysomphali* and *A. melinus* (Hymenoptera: Aphelinidae) in Mediterranean populations of California red scale *Aonidiella aurantii* (Hemiptera: Diaspididae). Biological Control 55:132–140.

Pina, T. 2007. Control biologico del piojo rojo de California, *Aonidiella aurantii* (Maskell) (Hemiptera?: Diaspididae) y estrategias reproductivas de su principal enemigo natural *Aphytis chrysomphali* (Mercet) (Hymenoptera?: Aphelinidae). Dissertation. University of Valencia, Valencia, Spain.

Price, P. 1972. Parasitiods utilizing the same host: adaptive nature of differences in size and form. Ecology 53:190–195.

R Development Team. 2013. R version 3.0.2. R Project for Statistical Computing, Vienna, Austria. www.r-project.org

Schneider, F. D., S. Scheu, and U. Brose. 2012. Body mass constraints on feeding rates determine the consequences of predator loss. Ecology Letters 15:436–443.

Schoener, T. 1974. Resource partitioning in ecological communities. Science 185:27–39.

Schoener, T. W. 2011. The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. Science 331:426–429.

Snyder, R. E., E. T. Borer, and P. Chesson. 2005. Examining the relative importance of spatial and nonspatial coexistence mechanisms. American Naturalist 166:75–94.

Sorribas, J., R. Rodríguez, and F. Garcia-Mari. 2010. Parasitoid competitive displacement and coexistence in citrus agroecosystems: linking species distribution with climate. Ecological Applications 20:1101–113.

Stamp, N. 2001. Enemy-free space via host plant chemistry and dispersion: assessing the influence of tri-trophic interactions. Oecologia 128:153–163.

Stuart, Y. E., and J. B. Losos. 2013. Ecological character displacement: glass half full or half empty? Trends in Ecology & Evolution 28:402–408.

Tscharntke, T. 1992. Coexistence, tritrophic interactions and density dependence in a species-rich parasitoid community. Journal of Animal Ecology 61:59.

Winkler, D. W., and C. Hall. 1981. The dynamics of multi-parasitoid–host interactions. American Naturalist 117:526–543.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1890/15-0118.1/suppinfo