

SUPERPARASITISM AND INTRASPECIFIC COMPETITION BY
THE SOLITARY LARVAL-PUPAL PARASITOID *ARCHYTAS*
MARMORATUS (DIPTERA: TACHINIDAE)

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ABSTRACT

Superparasitism and intrinsic larval competition by the solitary larval-pupal parasitoid *Archytas marmoratus* (Townsend) were studied *in vivo*. In superparasitized hosts, when two parasitoids entered a host pupa, only one parasitoid completed development. The surviving *A. marmoratus* maggot eliminated the conspecific competitor through physiological suppression during the second stadium of the supernumerary maggot. Supernumerary parasitoids never survived beyond the second instar, regardless of the time interval between initial parasitism and subsequent superparasitism. Physical combat was not evident because the parasitoid eliminated did not show signs of physical injuries. Scramble competition for host resources was not a probable mechanism of elimination because puparial weights and adult eclosion rates from superparasitized host pupae, and those from singly parasitized pupae, were not significantly different.

Key Words: Parasitoid competition, intraspecific interactions.

RESUMEN

El parasitismo y la competencia larval intrínseca del parasitoide larvo-pupal *Archytas marmoratus* (Townsend) fueron estudiados *in vivo*. En hospedantes superparasitados, cuando dos parasitoides entraron en una pupa hospedante, solamente uno completó el desarrollo. La larva sobreviviente de *A. marmoratus* eliminó al competidor conespecífico mediante supresión fisiológica durante el segundo estadio de la larva supernumeraria. Los parasitoides supernumerarios nunca sobrevivieron más allá del segundo instar, independientemente del intervalo entre el parasitismo inicial y el superparasitismo subsecuente. El combate físico no fue evidente debido a que el parasitoide eliminado no mostró señales de daño físico. La competencia por recursos de

hospedante no fue un mecanismo probable de eliminación debido a que los pesos pupales y las tasas de eclosión de adultos de las pupas superparasitadas y de las parasitadas por una sola larva no fueron significativamente diferentes.

Archytas marmoratus (Townsend) (Diptera: Tachinidae) is a solitary larval-pupal parasitoid of numerous species of Noctuidae (Lepidoptera). Included in its host range are many important pest species in the genera *Helicoverpa*, *Heliothis*, *Pseudaletia*, and *Spodoptera* (Arnaud 1978, Ravlin & Stehr 1984).

A. marmoratus has a complex life history that allows it to parasitize a wide range of host instars. Females do not oviposit directly on hosts; instead they deposit numerous eggs in the vicinity of potential host larvae. The eggs soon hatch into planidia-type larvae (Wood 1987). Parasitism occurs when a host contacts a planidium which then burrows between the host cuticle and epidermis where it resides (Bratti et al. 1993). The first instar of *A. marmoratus* begins feeding on the host larva, but it does not molt until after the host pupates. The first instar must reenter the host following each larval-larval molt of the host. After the host undergoes its larval-pupal molt, the first-instar parasitoid penetrates the hemocoel under the host wing pad, where it induces the formation of a respiratory tunnel. *A. marmoratus* development within the host pupa is rapid. The maggot molts to the second instar 1 - 2 days following host pupation; the second and third stadia last 2 - 4 days each, with pupariation occurring within the host remains (Vickery 1929).

Because female *A. marmoratus* deposit multiple eggs at one time (Vickery 1929), and more than one female may oviposit in the same location, considerable potential for superparasitism exists. Despite this potential, only one *A. marmoratus* maggot completes development in a host (Vickery 1929, Hughes 1975). Among the possible mechanisms for the elimination of supernumerary parasitoids are physical combat, scramble competition for host resources, or physiological suppression (Salt 1961, Fisher 1971, Vinson & Iwantsch 1980). In this study, I examine aspects of superparasitism and intrinsic larval competition, including parasitism rates, elimination of supernumerary parasitoids, and effects on parasitoid development and emergence.

MATERIALS AND METHODS

All tests were conducted at the Istituto di Entomologia "Guido Grande", Università degli Studi di Bologna, Bologna, Italy. *A. marmoratus* adults were reared in plexiglass cages (40 × 40 × 40 cm) in an environmental chamber maintained at 27 ± 2° C, 60 ± 10% R.H. and 14:10 (L:D) photoperiod (fluorescent light). To obtain *A. marmoratus* planidia for parasitization, pieces of pleated filter paper were placed on the bottom of cages as oviposition substrate the day prior to parasitism. Thus, all planidia were less than 24 h old at the time of parasitization. Larvae of the factitious host, *Galleria mellonella* L. (Lepidoptera: Galleriidae), were reared on artificial medium (Campadelli 1973) in plastic containers (23 × 11 × 8 cm) held at 30 ± 2° C, 60 ± 10% R.H., and 0:24 (L:D) photoperiod.

Penultimate-instar larvae of *G. mellonella* in apolysis were isolated in containers with fresh diet. The following morning, these groups were reexamined for newly-molted ultimate-instars. Each *G. mellonella* larva was infested by gripping it behind the head capsule with a soft forceps and transferring *A. marmoratus* planidia to the

larval thorax with a fine camel hair brush. Each larva was held until the planidia burrowed into the cuticle. Then it was placed in a new plastic container with fresh diet.

Six parasitism treatments were used, with three groups of *G. mellonella* larvae being superparasitized and three groups being singly parasitized. The three superparasitized groups were: (A) Newly molted (day 1) ultimate-instar *G. mellonella* larvae parasitized with two *A. marmoratus* planidia (Superparasitized - Day 1), (B) Day 1 larvae parasitized once and superparasitized two days later (Superparasitized - Day 1, 3), and (C) Day 1 larvae parasitized and superparasitized four days later (Superparasitized - Day 1, 5). Only larvae with visible planidia were superparasitized. The three corresponding singly parasitized control groups were parasitized on day 1 (Day 1 Control), day 3 (Day 3 Control), or day 5 (Day 5 Control) of the ultimate stadium, respectively.

G. mellonella were weighed upon pupation and individually isolated until *A. marmoratus* pupariation, or *G. mellonella* eclosion or death. *A. marmoratus* puparia were weighed one day after pupariation and held individually until eclosion. Host remains were dissected to determine the number and status of *A. marmoratus* maggots. To verify that all parasitoid remains had been recovered during the initial inspection, the host remains were macerated in 10% KOH and reexamined. No additional *A. marmoratus* were recovered by this procedure. All parasitoid remains (bodies or exuviae) were identified to larval instar (Ravlin & Stehr 1984), and bodies were carefully inspected for signs of physical injury. The size and degree of sclerotization of the cephalopharyngeal skeletons of maggots from superparasitized hosts were compared at the stage at which the first maggot died. The *A. marmoratus* in each superparasitized host were classified according the developmental stage to which they survived. If neither parasitoid survived longer than the other, the competitive interaction was considered a "tie". True winners of competitive interactions were those that actually survived to adult eclosion.

Chi square tests were used to examine differences in parasitism rates and parasitoid survival among the different treatments. To determine if parasitoid size and development were affected by parasitism treatment, data were analyzed by analysis of covariance (ANCOVA) with host pupal weight serving as a covariate. Pairwise comparisons between superparasitized treatments and their corresponding controls were made by least squares means *t*-tests.

RESULTS

Because first-instar parasitoids had to reenter the host successfully after it pupated, not all *G. mellonella* pupae contained *A. marmoratus* (Table 1). However, parasitoid entries into host pupae were independent events because the proportion of superparasitized hosts across superparasitism treatments (19.8%) was approximately equal to the square of the proportion of the parasitized pupae in all control treatments (44.5%, $X^2 = 0.001$, $df = 1$, $P > 0.9$). The first planidium that entered the host pupa did not exclude the entry of the other planidium because superparasitism rates were not significantly less than the values expected had entries been independent events. Even though approximately 20% of host pupae were superparasitized (Table 1), a maximum of one *A. marmoratus* survived per host.

Based on the condition of the parasitoid remains in host pupae, supernumerary parasitoids were almost always eliminated during their second stadium (test for differences among *A. marmoratus* instars, $X^2 = 133$, $df = 2$, $P < 0.001$, Table 2); this difference was consistent across superparasitism treatments (test for differences among superparasitism treatments, $X^2 = 3.4$, $df = 2$, $P = 0.18$, Table 2). Three of the *A. mar-*

TABLE 1. PARASITISM AND SUPERPARASITISM OF *G. MELLONELLA* PUPAE BY *A. MARMORATUS* WHEN PARASITIZED AT DIFFERENT INTERVALS DURING THE ULTIMATE LARVAL STADIUM.

Treatment ¹	Number of <i>A. marmoratus</i> Maggots per <i>G. mellonella</i> Pupa		Total Number of Pupae Tested
	1 Maggot	2 Maggots	
Superparasitized Day 1, 1	93 (36%)	47 (18%)	261
Day 1 Control	48 (41%)	—	118
Superparasitized Day 1, 3	87 (44%)	40 (20%)	196
Day 3 Control	56 (62%)	—	91
Superparasitized Day 1, 5	47 (44%)	25 (23%)	108
Day 5 Control	44 (36%)	—	123
Total	375	112	897

¹See text for details of each treatment.

marmoratus maggots that died as first instars (17%, $n = 18$) had failed to penetrate the host cuticle following host pupation. The remainder that did enter their host pupa grew (when compared with newly hatched planidia), but did not become successfully established in the host and molt. Only four (22%) of these first-instars showed signs of physical injury, such as melanized wounds. Few of those maggots that died as second instars had signs of physical injury (5%, $n = 94$), but they typically had smaller and/or less sclerotized cephalopharyngeal skeletons than those that survived to a later stage (75%, $n = 94$, $X^2 = 17.7$, $df = 2$, $P < 0.001$). In two superparasitized pupae (2%, $n = 112$), the supernumerary *A. marmoratus* died before completing their final larval ecdysis.

Adult eclosion of *A. marmoratus* did not differ across treatments ($X^2 = 10.1$, $df = 5$, $P > 0.07$, Table 2), or when considering superparasitism versus single parasitism treatments ($X^2 = 3.6$, $df = 1$, $P = 0.06$, Table 2). Overall, development times of *A. marmoratus* (from host pupation to *A. marmoratus* adult eclosion) from singly and superparasitized host pupae were not significantly different (overall $\bar{x} \pm SE = 13.4 \pm 0.2$ days, $F = 0.1$, $df = 1$, 128, $P = 0.80$). In pairwise comparisons between superparasitized groups and their respective control groups, the only significant difference was between the Day 1, 3 superparasitized and Day 3 control groups; the controls emerged one day earlier (12.7 ± 0.3 days) than the parasitoids from the superparasitized group (13.9 ± 0.4 days) ($t = 3.1$, $P = 0.0024$).

The size of *A. marmoratus* puparia increased significantly with host weight (puparia from superparasitized hosts: $y = 0.3 + 0.46x$, $r^2 = 0.82$; puparia from singly parasitized hosts: $y = 1.3 + 0.42x$, $r^2 = 0.73$). However, puparia from singly parasitized hosts were not significantly heavier (71.1 ± 1.9 mg) than those from superparasitized hosts (68.2 ± 2.5 mg, $F = 1.7$, $df = 1$, 136, $P = 0.19$, test for homogeneity of intercepts). Host weight alone accounted for over 74% of the variation in *A. marmoratus* weights.

TABLE 2. DEVELOPMENTAL STAGE OF *A. MARMORATUS* IN *G. MELLONELLA* PUPAE SUPERPARASITIZED AT DIFFERENT TIME INTERVALS DURING THE ULTIMATE LARVAL STADIUM. TABLE VALUES REFLECT THE FINAL DEVELOPMENTAL STAGE REACHED BY COMPETING PARASITIDS. NO HOSTS PRODUCED PARASITIDS THAT SURVIVED TO THE SAME STAGE.

<i>A. marmoratus</i> Stage ¹		Number and Percent of Outcomes for Each Treatment ²			
(A)	(B)	Superparasitized Day 1, 1	Superparasitized Day 1, 3	Superparasitized Day 1, 5	Total
Adult	II Instar	18 (40%)	19 (42%)	8 (18%)	45
Adult	I Instar	8 (57%)	3 (21%)	3 (21%)	14
Pupae	II Instar	10 (36%)	12 (43%)	6 (21%)	28
Pupae	I Instar	1 (50%)	0 (0%)	1 (50%)	2
III Instar	II Instar	9 (41%)	6 (27%)	7 (32%)	22
III Instar	I Instar	1 (100%)	0 (0%)	0 (0%)	1
Total		47 (42%)	40 (36%)	25 (22%)	112

¹(A) Stage reached by parasitoid surviving the longest, (B) Stage reached by first parasitoid that died.

²See text for details of each treatment.

DISCUSSION

A variety of mechanisms for the elimination of supernumerary larvae exist among the Tachinidae. Physical combat has been observed among first instars of *Marquartia chalconota* Meigen (Mellini & Baronio 1971). Anoxia is responsible for elimination of older supernumerary maggots of *Lixophaga diatraeae* (Townsend) (King et al. 1976). Superparasitism results in reduced body size for several potentially gregarious tachinids (Pschorn-Walcher 1971, Ziser et al. 1977, Reitz 1994). This variation in parasitoid size is attributed to resource depletion in superparasitized hosts. However, these species have significantly different life histories from *A. marmoratus*. Because of the high potential of superparasitism occurring in the field, and the relationship between parasitoid and host size (*A. marmoratus* puparia are > 50% of the weight of host pupae), *A. marmoratus* would be expected to have evolved an effective mechanism for eliminating conspecific competitors.

Given the consistent stage at which supernumerary maggots of *A. marmoratus* are eliminated and the lack of demonstrable physical injuries to these "losing" maggots, physiological suppression of conspecific competitors cannot be excluded as a mechanism of intraspecific competition. If competition were based solely on physical attacks, all "losing" maggots should show signs of injury (Mellini & Baronio 1971, Mellini 1990). Also, some encounters, especially in hosts superparasitized on the same day, could be expected to be resolved when both parasitoids were third instars.

In fact, if direct physical combat was responsible, it should occur most often among third instars. Unlike many solitary hymenopteran parasitoids that have free-roaming larvae adapted for fighting (Vinson 1985, Kfir & van Hamburg 1988, McBrien & Mackauer 1990), first and second instars of *A. marmoratus* reside in respiratory tunnels (Mellini 1990) that form along the wing pad margins of host pupae. Only third instars become mobile (Hughes 1975, Bratti et al. 1992). However, no host pupae contained two third instars. While second and third instars of *A. marmoratus* possess sickle-shaped mandibles that could inflict serious damage, any observed damage could have occurred after a competitor had already died from other causes. In addition, second instars are considerably smaller than *G. mellonella* pupae; therefore, unless parasitoid respiratory attachments are in close proximity, second instars would not encounter one another, further increasing the occurrence of encounters among third instars.

The possibility that supernumerary maggots are eliminated through scramble competition for host resources is also not supported by the present data. If scramble competition was operating, greater variation in the stage at which competitors are eliminated would be expected. In particular, larger hosts should more frequently support multiple third instars. Additionally, if scramble competition was responsible for elimination of supernumerary maggots, the size of *A. marmoratus* should vary with the number of maggots present in a host pupa. However, the relationship between *A. marmoratus* weights and host pupal weights did not vary with respect to whether a host was singly or superparasitized. Also, adult eclosion rates did not differ significantly as might be expected as a result of scramble competition.

A possible scenario for physiological suppression of supernumerary *A. marmoratus* is that older maggots (i.e., those parasitizing the host larva first) are more developmentally advanced, and initially have faster development rates in the host pupa, thus molting to the second and third instar sooner than subsequent maggots (Bratti et al. 1992, 1993). Because the host pupa dies by the time an *A. marmoratus* maggot molts to the third instar (Allen 1926), the maggot that molts to its final instar first could make the host environment unsuitable for younger maggots to continue their development. Therefore, maggots reaching their final instar first could suppress com-

petitors by production of proteolytic enzymes (Mellini 1990) or degradation of the host. Further *in vitro* studies of intrinsic competition should elucidate the specific means of physiological suppression used by *A. marmoratus*.

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