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## Why walnut flies superparasitize: time savings as a possible explanation

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**Abstract** This study evaluated a possible fitness advantage, specifically time savings, that might account for an unusual propensity in walnut flies (*Rhagoletis* spp.) to superparasitize their walnut hosts and to place eggs into existing egg-laying cavities. The first part of this study demonstrated that, in laboratory assays, females of two walnut fly species, *R. boycei* and *R. juglandis*, save time when cavities are reused and that in *R. juglandis*, where it was examined in detail with in vivo staining of eggs, time saving was not an artifact of differences in the size of clutches deposited at new versus existing sites. We further demonstrated that time savings reflected a reduction in the time required to generate the cavity itself. In the second part of the study, we evaluated the possibility that, in the field, time saved by reusing existing cavities is nullified by extra time spent mating associated with a previously described tendency for males to guard these cavities. Field observations of *R. juglandis* indicated that use of existing sites was, as expected, associated with increased mating. Yet, despite the added time spent mating, in observations of similar length females attempting to lay eggs at existing sites deposited clutches more often than females attempting to lay eggs at new sites. We discuss these results in the context of the more common pattern of superparasitism avoidance observed in host-specific insects.

**Key words** Tephritid fruit fly · Superparasitism · Host-marking pheromone · Clutch size · Parasitoid

### Introduction

Numerous host-specific insects such as entomophagous parasitoids and herbivorous insects avoid laying eggs

where others have been laid previously (reviewed in Prokopy and Roitberg 1987; Averill and Prokopy 1989; Papaj 1993; Godfray 1994), i.e., they avoid “superparasitizing” their hosts. Avoidance of superparasitism is commonly mediated by a host-marking pheromone laid down during egg deposition (reviewed in Prokopy and Roitberg 1987; Averill and Prokopy 1989) and is thought to reduce levels of competition between the progeny of one female. Although superparasitism avoidance is both common and reasonable, there are nevertheless conditions under which it “pays” to superparasitize (Alphen and Visser 1990; Visser et al. 1990). In fact, several species of tephritid flies, including walnut-infesting species of the genus *Rhagoletis*, display an extraordinary propensity to deposit clutches of eggs in already occupied fruit. Not only do females of these species often express a preference for parasitized fruit, but they deposit clutches directly into previously established egg-laying cavities (Papaj et al. 1989, 1992; Papaj 1993, 1994; Lalonde and Mangel 1994; Papaj and Messing 1996). Cavities in the field have been estimated to contain as many as 20 clutches (Back and Pemberton 1915; Papaj, unpublished observations), even though females engage in fruit-marking behavior which has been shown in at least one species to result in deposition of a pheromone that deters egg-laying (Prokopy et al. 1978; Papaj et al. 1992). Given the prominence of theoretical and empirical studies of superparasitism in behavioral ecology (reviewed in Godfray 1994), such seemingly paradoxical behavior demands close scrutiny. It would seem particularly worthwhile to document fitness advantages of superparasitism that are generalizable to taxa showing superparasitism avoidance.

A possibly generalizable advantage concerns time saved by laying eggs in parasitized hosts (Papaj 1993). An increasingly well-developed body of theoretical and empirical work on the egg-laying behavior of insects suggests that, under time limitation, individuals “manage” their time in functional ways (Stephens and Krebs 1984; Mangel and Clark 1988; Mangel and Roitberg 1989; Godfray 1994). Time limitation is not requisite for

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effective time management adaptations: even female insects described as egg limited can benefit by managing time effectively (Papaj 1990). Moreover, especially where egg deposition itself has costs in terms of reduced life expectancy, an egg-limited insect can simultaneously be a time-limited one (Roitberg 1989). On these grounds at least, behavior which "saves time" should be generally functional. In this study, we examine the possibility that time-saving accounts for superparasitism in walnut-infesting flies.

There is previous evidence for time saving as a benefit of superparasitism. Studies of the Mediterranean fruit fly, for example, suggest that a female saves time by depositing her eggs in an existing cavity (Papaj et al. 1992; Papaj 1993; Papaj and Messing 1996). A similar result was found for females of an encyrtid parasitoid of lepidopteran eggs which, despite clear losses in terms of larval fitness, have a preference for reusing holes drilled into the host egg by other females over drilling their own holes (Takasu and Hirose 1991).

There were two reasons in the present study for examining this issue further in walnut flies. First, walnut flies lay their eggs in clutches. What looks like time saving might actually reflect differences in clutch size (Papaj 1993), particularly when the second clutch is smaller than the first (cf. Papaj et al. 1990). Second, aspects of the mating system of walnut flies suggested to us that time saved by using existing egg-laying cavities, even if real, might be offset or even exceeded by time expended in additional matings. Walnut flies have a resource-based mating system in which males defend territories on fruit, even guarding existing egg-laying cavities (Papaj 1994). Since, as in other *Rhagoletis* flies (Prokopy et al. 1971; Smith and Prokopy 1980, 1982), matings tend to be initiated while a female is attempting to lay eggs, the propensity of males to guard existing sites may expose a female that prefers such sites to relatively more encounters with males. To the extent that matings are unwanted, use of existing sites may incur a time expenditure and possible fitness loss. That matings are, in fact, unwanted is suggested by three observations: (1) females arriving at fruit always contain live sperm (Alonso-Pimentel and Papaj, unpublished observation); (2) as in other *Rhagoletis* spp. studied, females are generally attempting to lay eggs (and not engaging in active courtship behavior) when mating occurs and often appear to physically resist matings and (3) egg loads of females mated in this way are as high as those of females that actually lay eggs (Alonso-Pimentel and Papaj, in press), suggesting that they are indeed highly motivated to lay eggs.

In the first part of this study, we examined whether clutch deposition time was different for first versus second clutches deposited in the same cavity for each of two walnut-infesting species, *R. boycei* and *R. juglandis*. We then stained eggs in vivo to determine whether any observed time difference between first and second clutches was due to a difference in "setup" time involved in piercing the walnut husk and generating the cavity or simply to a difference in clutch size. In the second part of this

study, we conducted field observations aimed at evaluating the presumed trade-off between time saved by laying eggs in existing sites and time expended in additional matings incurred by the use of such sites.

## Materials and methods

### Controlling for effects of clutch size on time-savings

#### General protocol

Details of the natural history and distribution of *R. boycei* and *R. juglandis* can be found in Boyce (1934) and Bush (1966). Both fly species are readily available locally. *R. boycei* flies used in laboratory experiments originated from infested *Juglans major* fruit collected at several sites in the Pinaleno Mountains in Graham County, Arizona. *R. juglandis* flies originated from infested fruit collected either in the town square of Patagonia, Santa Cruz County, Arizona, or in lower Garden Canyon in Cochise County, Arizona. Pupae (with puparia) were stored in darkness at about 5°C until they were retrieved and warmed at room temperature. Flies were maintained in 1-gallon (3.79-l) plastic containers supplied with protein hydrolysate food strips, free sucrose, and water. Flies used for testing generally varied from 14 to 21 days in age. Experiments were run from 10 a.m. to 4 p.m.

#### Laboratory experiment 1

This experiment assessed the time spent depositing a first and second clutch. Eight to ten females of one species were placed in a 17 × 17 × 17 cm clear acrylic-frame cage, and an equal number of females of the other species were placed in another cage. A walnut was placed on top of a pedestal within each cage. Observations began when females arrived on the walnut. We measured the time spent laying eggs, noting whether oviposition was completed at a new or an existing site. Timing with a stopwatch began when a female inserted its ovipositor into the fruit. Deposition of a clutch was inferred from the occurrence of ovipositor-dragging behavior. This behavior is typical for the genus and appears to involve deposition of a host-marking pheromone. Females were not permitted to mark, being brushed gently off the fruit as soon as they began to drag their ovipositors.

After deposition of a second clutch at a given site, the site was sealed with athletic tape to prevent females from depositing a third clutch at the same site. After accumulation of several double clutches on each fruit, fruits were removed and exchanged between cages. As part of a larger comparative study of these two species, we were interested in comparing time savings directly. We therefore attempted to generate double clutches by each species in equal numbers on each fruit.

#### Laboratory experiment 2

In experiment 1, any time difference between first and second clutches could reflect time saved in "setting up" the clutch or a difference in clutch size. In this experiment, an in vivo stain, acridine orange, was used to distinguish first from second clutches. When taken up in sucrose solutions by adults, eggs fluoresce green at the appropriate wavelengths (Brenner 1984; Strand et al. 1990). Owing to fly availability, this experiment was conducted only with *R. juglandis*.

Female flies were allocated in groups of eight to ten to cages consisting of 16-fl oz (473-ml) clear, plastic cups mated to plastic petri dish bottoms. In half of the cages, females were provided with acridine orange in three forms: acridine orange/sucrose solutions (1 vol acridine orange: 10 vol sugar: 100 vol water), sugar cubes soaked with several drops of the acridine orange/sucrose so-

lution, and protein hydrolysate food strips dipped briefly in the acridine orange/sucrose solution.

At least 48 h before testing, females were individually marked by chilling them on ice and placing one- to -three dots of tempera paint on their thorax. On the test day, two  $17 \times 17 \times 17$  cm clear acrylic-frame, screen observation cages were set up in an observation room maintained at ambient indoor temperature (about 23°C) and ambient (usually low) humidity. Eight to ten marked females from one treatment were placed in one cage, and an equal number of marked females of the other treatment were placed in another cage. A walnut was placed on top of a pedestal within each cage. Individual flies were then placed on the walnut and observations begun. Occasionally females flew to the fruit without being placed on it and observations were made of these females as well. Whether females were placed on the fruit or flew there did not in any obvious way affect their propensity to use existing sites or make new ones.

We measured the time spent laying eggs with a stopwatch started when a female inserted its ovipositor into the fruit. Clutch deposition was judged as described above. We further distinguished two phases in egg-laying behavior and measured the duration of each phase for first and second clutches, where possible. The first phase, termed the circling phase, is characterized by the pivoting of the female about the point at which her ovipositor is inserted in the fruit. The circling phase is followed by a quiescent phase in which the female is motionless save for subtle movements of the ovipositor. During the quiescent phase, the abdominal segments (and especially segment 7) are conspicuously more extended than during the circling phase.

Any clutches deposited by females of a given treatment were sealed with athletic tape to prevent females of the same treatment from depositing another clutch at the same site. Females were removed and replaced after they had deposited a clutch. After several single clutches had been accumulated on each fruit, both fruits were removed from their cages, the tabs were removed and the fruits exchanged between cages. Females were again permitted to deposit eggs in the test fruit. When a second clutch was deposited at a site (as indicated by the initiation of host-marking behavior), the site was sealed with tape to prevent deposition of further clutches. We attempted to generate equal numbers of double clutches in each dye treatment sequence (acridine orange then untreated versus untreated then acridine orange) for a given test fruit.

At the end of a test day, oviposition sites were dissected carefully under a stereoscope. Single clutches were counted in saline. Eggs from double clutches were mounted in Fluoromount mounting medium on a depression slide and the number of eggs in each clutch were counted under a fluorescent microscope. Specimens were illuminated at 480 nm because, contrary to Brenner's (1984) report for the screwworm fly, stained walnut fly eggs did not fluoresce more in the UV range than did unstained eggs. At the longer wavelength, acridine-orange-stained eggs fluoresced bright green, while unstained eggs appeared dark gray.

### Laboratory experiment 3

In an effort to assess when in the oviposition sequence eggs were actually laid, we permitted some females to initiate sites but then interrupted them after the circling phase was deemed to have ended and the quiescent phase to have commenced. At the same time, we permitted other females to initiate new sites and complete oviposition at those sites. In each case, we made time measurements and sealed the site with adhesive tape to prevent further use by females. At the end of the experiment, sites were dissected and eggs counted under stereoscopy.

### Statistical analysis

Although the time data distribution was skewed to the left, the skew was not severe and so ought not to have jeopardized the interpretation of significance levels. For this reason and because

there is no clear-cut nonparametric equivalent of a repeated-measures analysis of variance on continuous data, we used repeated-measures analyses of variance. In the analysis, clutch position was treated as a repeated measure and fly species (experiment 1) or acridine orange treatment (experiment 2) as an independent variable. In relating clutch size to measurements of the duration of egg-laying phases, we constructed a series of simple linear regression models.

### Time spent in extra male encounters

#### Field observations

Observations were made of *R. juglandis* females foraging on fruit at a number of southern Arizona sites: Box Canyon, Sonoita Creek, and Harshaw in Santa Cruz County, as well as lower Gardner Canyon and upper Carr Canyon in Cochise County. Our basic protocol was as follows. We patrolled the tree until a female was sighted on a fruit. We then recorded her behavior on the fruit using the Observer 3.0 software (Noldus Technology; Noldus 1991) on a Psion Organizer II Model LZ64. We recorded the occurrence and duration of walking and ovipositor-boring attempts. We also noted whether egg-laying attempts occurred at sites of damaged tissue indicative of existing egg-laying sites or at undamaged sites. We also recorded any interactions between the focal female and males or other females that occurred during the observation period. Interactions with males included both failed and successful mounting attempts by males. Finally, we recorded whether egg-laying attempts ended spontaneously, were interrupted by interactions with males or other females or attacks by predators, or resulted in clutch deposition. Clutch deposition was inferred from the occurrence of ovipositor-dragging behavior, as described above. An observation continued until a female flew from the fruit.

While this protocol permitted us to assess adequately the consequences of mating for use of new versus existing sites, deposition of clutches at new sites was too rare in this set of observations to permit us to evaluate adequately the time saved in nature by depositing clutches at existing versus new sites. In an effort to make this evaluation, we included in our analysis, data on oviposition times from observations made at other sites in other years. Made in the context of another study (Papaj 1994), these observations were conducted under a protocol in which a fruit, not a female, was the focal unit. In all other respects, data collection was similar to that described above; thus, estimates of oviposition time ought to be unbiased and not radically different from estimates made using the focal-female protocol. Most importantly, the data added were not biased towards clutches at new or existing sites.

### Statistical analysis

Time data again tended to be skewed to the left. Given the univariate nature of the designed comparisons, nonparametric statistics, namely Mann-Whitney tests, were appropriate. Frequency data were analyzed with *G*-tests with appropriate corrections (Sokal and Rohlf 1995).

## Results

### Evaluation of time savings with controls for clutch size

#### Laboratory experiment 1

For both species, second clutches were deposited in less time than were first clutches (Table 1). Repeated-measures ANOVA indicated a strong effect of clutch position on the duration of clutch deposition ( $F_{1,33} = 12.08$ ,  $P < 0.001$ ). By contrast, there was no significant effect of

**Table 1** Total time of deposition of first and second clutches at same sites on a fruit and difference in deposition time for first vs. second clutches, arranged according to fly species. Standard errors of means shown in parentheses

Fly species	Clutch position	<i>n</i>	Mean time of deposition (s)	Mean Time difference (s)
<i>Rhagoletis boycei</i>	First	21	476 (±68.3)	215.2 (±74.3)
	Second	21	257.9 (±37.7)	
<i>Rhagoletis juglandis</i>	First	14	532.4 (±190.1)	408.5 (±190.5)
	Second	14	123.9 (±24.4)	

**Table 2** Effect of dye treatment and clutch position on the total time spent ovipositing at a site and mean clutch size. The mean differences are also shown. Standard errors of means are shown in parentheses

Dye treatment	Clutch position	Mean total time (s)	Mean difference (s)	Mean clutch size	Mean difference
Acridine orange	First	470.8 (±68.3)	268.0 (±109.5)	19.7 (±2.2)	-0.5 (±3.5)
	Second	210.1 (±18.3)		20.1 (±2.3)	
None	First	564.7 (±160.3)	347.6 (±157.2)	16.2 (±2.3)	-1.9 (±3.7)
	Second	217.1 (±35.8)		18.1 (±2.7)	

species on the duration of clutch deposition ( $F_{1,33} = 0.14$ , n.s.) and no interaction between species and clutch position ( $F_{1,33} = 1.16$ , n.s.).

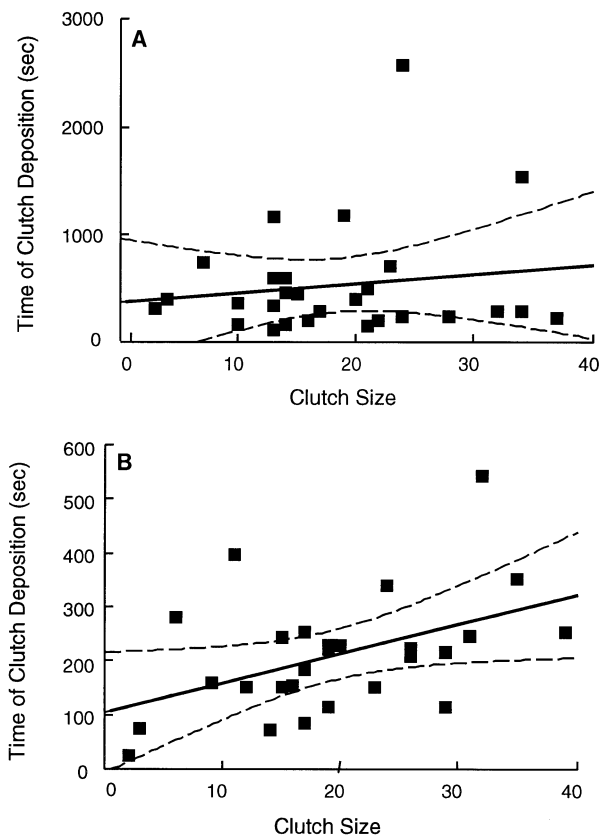
*Laboratory experiment 2*

Results for first and second clutches deposited at the same site are shown in Table 2. The median total time spent depositing eggs at a site is higher for first than for second clutches, regardless of acridine orange treatment. Repeated measures ANOVA indicated that clutch position had a highly significant effect on the total time spent laying eggs (clutch position effect,  $F_{1,27} = 10.05$ ,  $P = 0.004$ ). In the same analysis, dye treatment had no significant effect on the total duration of clutch deposition (dye treatment effect,  $F_{1,27} = 0.21$ , n.s.).

Acridine orange treatment had no obvious effect on fly vigor or behavior. This is most apparent in the data on the size of single clutches (i.e., those clutches at sites that never received additional eggs). The size of such clutches did not depend significantly on acridine orange treatment [mean for acridine orange flies =  $17.5 \pm 2.4$  (SE) eggs; mean for untreated flies =  $23.7 \pm 2.9$  eggs;  $t$ -test,  $t = -1.65$ ,  $df = 38$ , n.s.]. The same lack of effect was found for the total duration of clutch deposition (mean for acridine orange flies =  $413.0 \pm 46.1$  s; mean for untreated flies =  $426.8 \pm 68.1$  s;  $t$ -test,  $t = -0.165$ ,  $df = 40$ , n.s.).

In contrast to total time spent laying eggs, mean clutch size was lower for first than for second clutches, regardless of acridine orange treatment. However, the differences were small and, in a repeated-measures ANOVA (Table 2), neither clutch position ( $F_{1,26} = 0.20$ , n.s.) nor dye treatment ( $F_{1,26} = 1.57$ , n.s.) had a significant effect on clutch size. Neither was there a significant interaction between clutch position and dye treatment ( $F_{1,26} = 0.08$ , n.s.).

The absence of a clutch size difference between first and second clutches implies that the difference in the total duration of clutch deposition is not a difference in



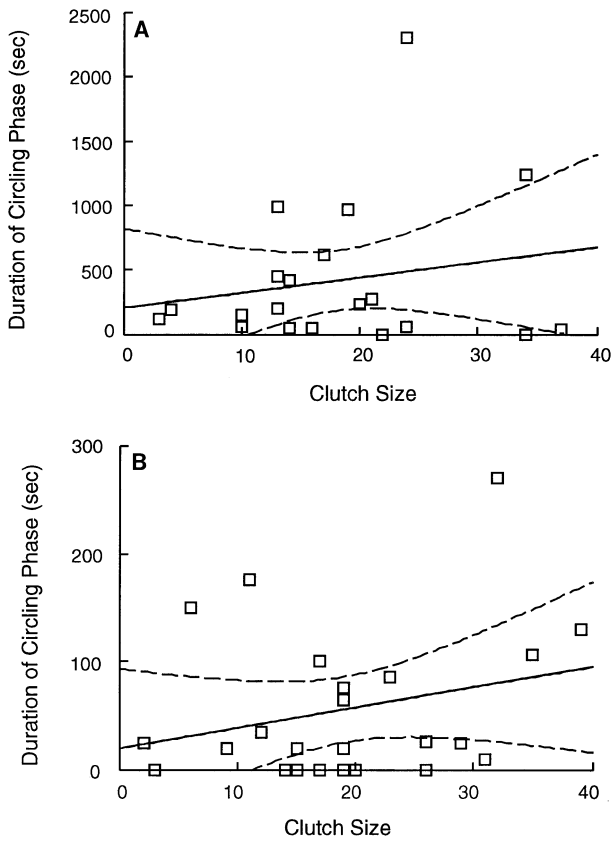
**Fig. 1** Regression of total duration of clutch deposition on *R. juglandis* clutch size. Data were pooled over dye treatments. **A** First clutches (regression coefficient = 8.65,  $t = 0.75$ ,  $df = 26$ , n.s.,  $R^2 = 2.1\%$ ). **B** Second clutches at the same sites (regression coefficient = 5.55,  $t = 2.66$ ,  $df = 26$ ,  $P = 0.01$ ,  $R^2 = 21.4\%$ )

time required to deposit the eggs themselves into the fruit. Rather, the time difference likely reflects a difference in “setup time”, i.e., the time required to penetrate the husk surface and sculpt the cavity into which eggs are placed. This interpretation received support when to-

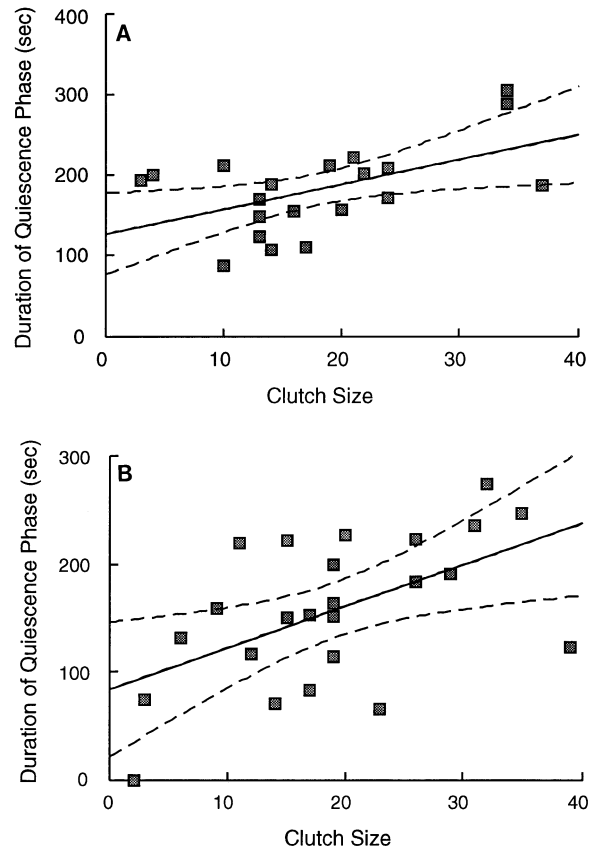
**Table 3** Effect of acridine orange treatment and clutch position on the duration of two components of oviposition, circling and quiescence, in *R. juglandis*. Differences in duration for first vs.

second clutches deposited at the same site are also shown. Standard errors are shown in parentheses

Dye treatment	Clutch position	Mean duration of circling phase (s)	Mean difference (s)	Mean duration of quiescent phase (s)	Mean difference (s)
Acridine orange (n=14)	First	348.3 (±109.3)	308.6 (±112.6)	193.5 (±14.9)	17.2 (±16.4)
	Second	39.7 (±15.3)		176.3 (±12.3)	
None (n=15)	First	506.5 (±277.7)	450.3 (±266.2)	168.3 (±18.6)	5.0 (±14.9)
	Second	69.2 (±23.3)		144.1 (±25.4)	



**Fig. 2** Regression of duration of the circling phase on *R. juglandis* clutch size. Data were pooled over dye treatments. **A** First clutches (regression coefficient = 11.66,  $t = 0.82$ ,  $df = 18$ , n.s.,  $R^2 = 3.6\%$ ). **B** Second clutches at the same sites (regression coefficient = 1.88,  $t = 1.28$ ,  $df = 22$ , n.s.,  $R^2 = 6.9\%$ )



**Fig. 3** Regression of duration of the quiescent phase on *R. juglandis* clutch size. Data were pooled over dye treatments. **A** First clutches (regression coefficient = 3.04,  $t = 2.27$ ,  $df = 18$ ,  $P < 0.02$ ,  $R^2 = 26.9\%$ ). **B** Second clutches at the same sites (regression coefficient = 3.84,  $t = 3.07$ ,  $df = 22$ ,  $P = 0.006$ ,  $R^2 = 30.0\%$ )

tal duration of clutch deposition was regressed against clutch size. For first clutches, there was no significant relationship between overall time spent ovipositing and clutch size (Fig. 1a). For second clutches, however, overall duration of clutch deposition was significantly and positively related to clutch size (Fig. 1b). The difference in significance levels squares with the notion that females at new sites are spending significant amounts of time penetrating the husk and forming the cavity, whereas females using existing sites are spending time primarily placing eggs within the already formed cavity.

Further support for this interpretation emerges when the total duration of clutch deposition is broken down into the duration of circling and quiescent phases (Table 3). The median duration of the circling phase was greater for first than for second clutches, whereas the median duration of the quiescent phase differed little between first and second clutches. Separate repeated-measures ANOVAs indicated that clutch position had a very highly significant effect on the duration of the circling phase ( $F_{1,19} = 9.12$ ,  $P = 0.007$ ), but no significant effect on the duration of the quiescent phase ( $F_{1,19} = 0.85$ , n.s.). For

**Table 4** Results of the experiment in which females were interrupted at the end of the circling phase and beginning of the quiescent phase, while control females were uninterrupted. Standard errors are shown in parentheses

Treatment	<i>n</i>	Mean duration of circling phase (s)	Mean total duration of oviposition (s)	Mean clutch size	Mean ratio of eggs number: time in oviposition
Interrupted	13 <sup>a</sup>	54.5 (±10.9)	54.5 (±10.9)	0.7 (±0.6)	0.018 (±0.017)
Uninterrupted	11	58.9 (±14.9)	199.2 (±21.1)	17.5 (±2.5)	0.097 (±0.019)

<sup>a</sup> *n*=12 for clutch size and ratio data

**Table 5** Consequences of use of new versus existing sites for matings in *R. juglandis*. Shown are field data for focal females that made at least two egg-laying attempts during an observation

Attribute	Statistic	At new sites	At existing sites
Proportion of egg-laying attempts interrupted by male	Median	0	0.375***
	SE	0.03	0.07
	<i>N</i>	17	8
Number of matings	Median	1	3*
	SE	1.2	3.1
	<i>N</i>	17	8
Mating duration (s)	Median	50	54.5 <sup>ns</sup>
	SE	47.7	20.4
	<i>N</i>	13	7

\*  $P < 0.05$ ; \*\*\*  $P < 0.001$  according to a Mann-Whitney *U*-test of values at new versus existing sites

neither duration variable was there a significant effect of dye treatment.

Regressions of duration on clutch size further supported the notion that the circling phase involved establishment of the cavity whereas the quiescent phase involved the placement of eggs in the cavity. The duration of the circling phase was not significantly related to clutch size for either first or second clutches and  $R^2$  values (measures of the proportion of variation in circling duration explained by regression on clutch size) were small (Fig. 2). In contrast, for both first and second clutches, the duration of the quiescent phase was significantly and positively related to clutch size (Fig. 3).

### Laboratory experiment 3

In a final effort to demonstrate that circling behavior involved piercing the husk and generating a cavity but not the actual placement of eggs within the cavity, we interrupted some females after the circling phase was deemed to have ended and the quiescent phase to have commenced. Other females were permitted to complete oviposition at new sites. The interruption treatment was alternated with the control treatment systematically over time. The results are shown in Table 4. The duration of the circling phase was not significantly different for interrupted versus uninterrupted females (*t*-test,  $t = -0.237$ ,  $df = 19$ , n.s.). Yet uninterrupted females laid a mean of over 15 eggs, compared to almost no eggs for interrupted females (*t*-test,  $t = -6.58$ ,  $df = 21$ ,  $P < 0.001$ ). In fact, just 2 of 13 interrupted females laid any eggs at all. Finally, the mean ratio of number of eggs laid to overall duration of clutch deposition was significantly lower for interrupted than for uninterrupted females (*t*-test,  $t = -3.48$ ,  $df = 20$ ,  $P = 0.002$ ).

Extra time spent in additional male encounters

### Field observations

Owing in part to interruptions by males, few clutches were actually deposited during our observations and direct field evidence for time savings was therefore scant. When field data from other sites and years were included in the analysis, the median overall ovipositor-boring time for clutches deposited at punctures was  $150.5 \pm 74.3$  s (SE) ( $n = 8$ ), whereas the median time for clutches deposited at new sites was  $363 \pm 47.3$  s ( $n = 5$ ). This difference was significant (Mann-Whitney *U*-test,  $U = 35.00$ ,  $P < 0.03$ ) and, while we could not rule out differences in clutch size as a factor in this difference, fully consistent with laboratory data.

Any given female tended either to attempt to use existing sites or to establish new ones, but rarely used both types of sites. In analyzing data with respect to matings, we therefore grouped females according to their preferred site. As predicted, given that males guard egg-laying sites, females attempting to deposit eggs at existing sites were proportionately more frequently interrupted by males than were females attempting to establish new sites (Table 5). In addition, the overall number of matings (whether occurring in the context of egg-laying or not) was significantly higher for females initiating egg-laying at existing egg-laying sites. Since the duration of individual matings did not depend significantly on site status in this set of observations (Table 5), it seems likely that females attempting to lay eggs in existing sites spent more time overall engaged in mating than females attempting to lay eggs at new sites.

The apparent difference in time spent mating notwithstanding, females initiating egg-laying in existing sites deposited proportionately more clutches during a given

**Table 6** Patterns of egg-laying attempts and clutch deposition for *R. juglandis* females at new vs. existing sites. Time data are shown for that subset of focal females for which accurate time records were made

Attribute	Statistic	At new sites	At existing sites
Percentage of females depositing clutches	%	9.7	42.1**
	<i>N</i>	34	19
Percentage of females attempting egg-laying	%	50	42.1 <sup>ns</sup>
	<i>N</i>	34	19
Time spent attempting to lay eggs (s)	Median	90	147.5 <sup>ns</sup>
	SE	50.6	50.1
	<i>N</i>	27	14
Total time on fruit (s)	Median	330	426 <sup>ns</sup>
	SE	197.1	393.8
	<i>N</i>	29	14

\*\*  $P < 0.01$  according to a *G*-test

observation than females initiating egg-laying at new sites (Table 6). This difference was due neither to differences in the proportion of females that attempted to lay eggs at each type of site nor to differences in time spent attempting to lay eggs (Table 6). Rather, the difference was due to the tendency for an egg-laying attempt to result in egg deposition: 100% of females ( $n = 8$ ) that attempted to lay eggs at existing sites actually did so, compared to just 18% of females ( $n = 17$ ) that attempted to lay eggs at new sites.

Given that the median overall time that a focal female was observed at a new versus an existing site was not significantly different (Table 6), it seems likely that females using existing sites were laying eggs at higher rates than females establishing new sites, at least in terms of the time spent on the fruit. It also seems likely that the time spent in mating by females attempting to use existing sites, whether or not it constituted "wasted" time, was more than offset by the time saved in "setup" time for clutch deposition. While our data do not permit us to make a direct, statistically robust comparison, the extra time spent mating when attempting to reuse sites was in the order of 100 s (Table 5), whereas the estimated time saved in the field by reusing a site for egg-laying was on the order of 200 s. Females that reuse sites thus appear to gain a net advantage in terms of the time required to deposit clutches.

## Discussion

### Time saved by reusing egg-laying sites as a benefit of superparasitism

Time saving for superparasitizing walnut flies essentially involves reduction in "handling time," specifically time spent in penetrating the husk and generating the cavity in the walnut husk within which eggs are placed. In other host-specific insects, time savings in terms of host handling time may or may not be an issue. For those herbivorous insects or entomophagous parasitoids that deposit eggs on the outside of host surfaces, for example, savings in handling time associated with the reuse of sites would presumably be negligible. In entomophagous endoparasitoids where superparasitism avoidance has per-

haps been best studied, time saving probably depends on the kind of host attacked. Where hosts are capable of escaping or defending themselves from attack (reviewed in Godfray 1994), for instance, selection to minimize the ovipositor penetration time of an endoparasitoid might be very strong and "setup" times for parasitization, even of unparasitized hosts, consequently short. Some parasitoids, for instance, inject venom rapidly into hosts, retreat, and return to lay eggs when the host is unable to defend itself (Vinson and Iwantsch 1980). In this case, superparasitism involving reuse of oviposition sites may provide little in the way of time savings in a process that is already relatively rapid.

Similarly, where hosts are relatively easy to penetrate (e.g., caterpillars), time saved by reusing sites may be marginal. Both with respect to active host defense and host penetrability, it is interesting to note that the only two instances in which entomophagous parasitoids have been shown to reuse oviposition sites involve, in one case, lepidopterous eggs and, in the other, silk-cocooned sawfly pupae (Price 1970; Takasu and Hirose 1991). Both hosts are relatively incapable of escape or active defense, and both possess relatively tough outer coverings.

### Fitness consequences of reusing egg-laying cavities

There are several reasons to believe that the saving of time by the reuse of existing egg-laying cavities has even more significance in terms of the overall reproductive success of a walnut fly female than is reflected in our assays. First, savings in time spent ovipositing may confer reductions in predation risk. Predation on walnut flies on fruit appears to be intense and, as noted for related species (Papaj 1993), female walnut flies seem particularly vulnerable to attack by predators when ovipositing. If so, a saving in clutch deposition time stands to increase female fitness over and above any advantage in terms of the rate at which clutches are laid. Second, in most assays, we used ripe to very ripe fruit. The time required to bore through a fruit surface depends on its penetrability, which depends in turn on the degree of ripeness (Papaj and Messing 1996). Where attacked fruit are less ripe and therefore less penetrable than fruit used in our as-

says, time savings might be significantly greater than measured in our assays.

That time savings should depend on fruit ripeness is consistent with the behavior of the taxonomically related walnut husk fly, *R. completa*. Lalonde and Mangel (1994) noted that penetrability of *Juglans* fruit changed markedly over the course of a season and that use of existing sites by *R. completa* declined in association with that change. However, at least three other explanations for use of existing sites have been advanced (Papaj 1993, 1994; Lalonde and Mangel 1994), the first two of which are also consistent with Lalonde and Mangel's findings. First, females may save energy as well as time when existing sites are exploited; energy may actually be limiting. Second, tephritid ovipositors are subject to physical wear, and use of existing sites may relieve such wear. Time savings and ovipositor wear are not necessarily independent of one another. Females with worn ovipositors may take relatively longer to establish new sites and so benefit more in terms of savings by reusing sites than females with less worn ovipositors.

Finally, larvae may benefit in some way by being placed in existing sites, possibly growing faster as members of a second clutch by taking advantage of symbiotic bacteria injected by the female depositing the first clutch (reviewed in Papaj 1993). Evaluating this possibility requires data relating larval survival to number of clutches. While such data are lacking, we suspect that larvae compete within fruit to a degree that more than offsets any social facilitation. Infestation levels in fruit in the field, for instance, are positively correlated with fruit size, as expected if larvae were competing (C. Nufio and D.R. Papaj, unpublished data). For the taxonomically related Mediterranean fruit fly which also reuses sites, there is good evidence for larval competition (reviewed in Papaj 1993). Moreover, Mediterranean fruit fly females are deterred by a host-marking pheromone laid down after egg-laying and thus behave as though larvae compete (reviewed in Papaj 1993; Papaj and Messing 1996).

#### Why don't all *Rhagoletis* flies reuse existing sites?

Few studies of superparasitism avoidance have considered the possibility that there are advantages associated with superparasitism. Nevertheless, the benefits of superparasitism enumerated above are surely broadly applicable. Evaluating possible benefits for species that avoid superparasitism would provide useful confirmation of inferences made about the functional significance of such behavior. If avoidance of superparasitism, where observed, is truly functional, then the costs of superparasitism in terms of competition must exceed these benefits. In this context, it is worth asking why most *Rhagoletis* species studied avoid superparasitism (reviewed in Averill and Prokopy 1989). In particular, why don't more *Rhagoletis* species reuse egg-laying cavities? Reuse of existing sites appears to be host related. The genus *Rhagoletis* consists of species which choose fruit of members

of numerous families including Ericaceae, Rosaceae, Solanaceae, and Juglandaceae (Bush 1966), yet only those attacking juglandaceous hosts appear to reuse cavities (Papaj 1993, 1994; Lalonde and Mangel 1994). Unfortunately, since all walnut-infesting species belong to a single clade within the genus (the *suavis* group; Berlocher and Bush 1982), it is possible that this behavior arose just once within the genus and it is therefore not possible to state with confidence that the trait depends critically on host taxon or any other trait common only to members of the clade.

Even if host taxon were important, variation among species in reuse of sites might involve variation in the costs and/or benefits of this behavior. We have no reason to believe that time savings associated with reuse of oviposition sites on walnut husks are significantly greater than those associated with fruit attacked by other species, but data are lacking. Alternatively, the restricted distribution of the reuse trait within the genus may have to do with its possible cost, specifically competition incurred by progeny placed in occupied fruit (Lalonde and Mangel 1994). The fruit attacked by other members of the genus (e.g., hawthorn berries, blueberries, dogwood berries, snowberries, and cherries) seem to offer less material for growth and development than does a walnut husk. For example, hawthorn berries rarely yield more than three *R. pomonella* pupae, even when considerably more eggs have been deposited in them (Averill and Prokopy 1987; Feder et al. 1995). In contrast, walnut fruit commonly yield dozens of *R. juglandis* and/or *R. boycei* pupae (Papaj, unpublished data).

That use of existing cavities constitutes a trade-off between benefits such as time savings, on the one hand, and costs of larval competition, on the other, is illustrated by the behavior of the taxonomically related Mediterranean fruit fly, *Ceratitis capitata*. Under experimental conditions, females of this species were found to use existing sites when benefits in terms of time savings are high, but to avoid use of existing sites when benefits in time savings are low and costs of competition high (Papaj and Messing 1996). Similarly, *Ooencyrtus nezarae* parasitoids reused oviposition holes in host eggs when inexperienced or when deprived of the opportunity to oviposit for several days, but preferred unparasitized hosts after a recent oviposition (Takasu and Hirose 1991). In noting this pattern, Visser (1992) speculated that parasitoids were attending to time savings (and thus reusing sites) when time limited but were attending to larval competition (and thus using unparasitized hosts) when egg limited. It would add rigor to the functional analysis of superparasitism to evaluate the possible dynamics of a handling time/larval fitness trade-off in such a system.

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