

# Performance of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) Reared on Factitious Hosts, Including the Target Host, *Ostrinia nubilalis* (Lepidoptera: Crambidae)

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The performance of the parasitoid *Trichogramma ostriniae* (Pang et Chen) on eggs of four factitious hosts, *Ostrinia nubilalis* (Hübner), *Sitotroga cerealella* (Olivier), *Trichoplusia ni* (Hübner), and irradiated *Ephesthia kuhniella* (Zeller), is presented. We measured parasitoid longevity, individual and population levels of parasitism, survival of progeny to emergence, and progeny sex ratio for 9 to 11 generations in laboratory colonies. Wasps reared from each source were tested on the source host and on the target host, European corn borer (*O. nubilalis*). Under the conditions tested, *E. kuhniella* was a poor host, *T. ni* and *O. nubilalis* were good hosts, and *S. cerealella* was intermediate. We found no evidence that being reared for many generations on an alternate host decreased the performance on the target host, *O. nubilalis*. Sex ratio was female biased and did not change over time. There may have been an increase in performance of wasps reared on *S. cerealella* and *E. kuhniella* over time. We discuss the information gained from different measures of parasitoid performance and the possible causes for variation in performance over time under laboratory conditions. © 2001 Academic Press

**Key Words:** biological control; *Ephesthia kuhniella*; European corn borer; factitious host; laboratory selection; mass rearing; *Ostrinia nubilalis*; parasitoid; *Sitotroga cerealella*; *Trichogramma ostriniae*; *Trichoplusia ni*.

## INTRODUCTION

Since the introduction of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), to the United States in the early 1900s, several *Trichogramma* (Hymenoptera: Trichogrammatidae) species have been investigated for use in its biological control. Only *Trichogramma nubilale* (Ertle et Davis)

and *Trichogramma maidis* (Pint et Voeg) have attracted much attention for the control of *O. nubilalis* (Ertle and Davis, 1975; Burbutis and Goldstein, 1983; Prokrym *et al.*, 1992), and neither is currently used commercially in the United States. Although native and introduced species of *Trichogramma* are found in sweet corn in the United States (Baker *et al.*, 1949; Ertle and Davis, 1975; Andow, 1992), rates of parasitism of *O. nubilalis* are low and contribute little to pest suppression. In Europe, augmentative biological control of *O. nubilalis* by use of several species of *Trichogramma* that are commercially available has been successful (Hassan, 1993; Pavlik, 1993; Ravensberg and Berger, 1988; Bigler and Burnetti, 1986).

Investigators in the United States have looked to the closely related Asian corn borer, *Ostrinia furnacalis* (Guenée), for additional sources of natural enemies for augmentative control of *O. nubilalis* (Volden and Chiang, 1982; Hoffmann *et al.*, 1995). One species, *Trichogramma ostriniae* (Pang et Chen), is widely used in China and Taiwan and has generated considerable interest because of its potential efficacy against *O. nubilalis* (Hassan and Guo, 1991; Pavlik, 1993). *T. ostriniae* has been imported into the United States from Jilin Province in northern China and is being developed for use in augmentative biological control programs (Hoffmann and Shelton, 1994; Hoffmann *et al.*, 1995; Wang and Ferro, 1998; Hoffmann, 1999). Commercial rearing of *T. ostriniae* will be necessary to implement augmentative biological control of *O. nubilalis*.

Often insects other than the target hosts are used to reduce the cost or increase the efficiency of mass production of parasitoids. However, even for a polyphagous parasitoid, the suitability of these factitious hosts may vary greatly. Characteristics such as host egg volume, chorion thickness, nutritional content, age, and egg distribution can affect rates of parasitism as

well as the number, quality, and sex ratio of parasitoids reared from these host eggs (Flanders, 1935; Clausen, 1939; Stinner *et al.*, 1974; Lewis *et al.*, 1976; Hohmann *et al.*, 1988; Ram and Irulandi, 1989; Kazmer and Luck, 1991; Baorong *et al.*, 1992; Corrigan and Laing, 1994; Hoffmann *et al.*, 1995; Greenberg *et al.*, 1998). In addition to the direct suitability of a factitious host, there is also the potential for an evolutionary response by the parasitoid to being reared for many generations on an alternate host. Laboratory selection for physiological, morphological, or behavioral characteristics that are adaptive when the factitious host is used may cause a parasitoid to perform less well on the target host (van Bergeijk *et al.*, 1989; Leppla and Fisher, 1989; Hopper *et al.*, 1993).

The biology of *T. ostrinae* reared on *O. nubilalis* and studies of field releases in the northeastern United States are reported elsewhere (Hoffmann and Shelton, 1994; Hoffmann *et al.*, 1995; Hoffmann, 1999). Because parasitoid performance is related to host quality (Flanders, 1935; Ram and Irundi, 1989; Baorong *et al.*, 1992; Schmidt, 1994), and host species differ in various qualities (e.g., size, chorion thickness, and distribution) likely to be related to wasp fitness, we investigated the effect of host species on components of wasp fitness over several generations in the laboratory. The performance of *T. ostrinae* was measured as individual female longevity, number of eggs parasitized, number of progeny per egg, survival of progeny, and the sex ratio of progeny. We ask (1) whether wasp performance differed among host egg species and (2) whether the rearing of parasitoids on one host species for several generations would influence performance on the target host species. Specifically, we asked whether being reared on hosts other than *O. nubilalis* would influence performance on *O. nubilalis*.

The suitability of a host may also depend in part of the origin of the wasp because selection within populations reared on different hosts may lead to genetic adaptation over time. The wasps used in our study originated from a laboratory colony maintained on *O. nubilalis*. We hypothesized that parasitoid performance would depend on host egg species used and that over 9 to 11 generations wasps may evolve to perform better on a new host.

## MATERIALS AND METHODS

About 1000 *T. ostrinae* reared on *O. nubilalis* were obtained from the USDA APHIS Mission Biological Control Center, Mission, Texas to establish a laboratory colony in Ithaca, New York 5 months prior to this study. *T. ostrinae* had been reared at Mission, Texas for approximately 1 year before we initiated our colony. We maintained the colony at 16L:8D h, 25:23°C, and ~80% RH. Freshly laid eggs of *O. nubilalis* were supplied approximately three times per week as hosts for

*T. ostrinae*. Water and undiluted honey were provided *ad libitum* to the colony. The colony was propagated by exposure of *O. nubilalis* eggs to parasitism for 48 h. The parasitized eggs were allowed to develop and then returned to the wasp colony upon hatching.

*O. nubilalis*, *Sitotroga cerealella* (Olivier), *Ephestia kuhniella* (Zeller), and *Trichoplusia ni* (Hübner) were chosen as experimental hosts because their eggs are commonly available and differ in physical characteristics that might affect parasitism. A standard method was developed by Flander (1929) to rear *Trichogramma* species on eggs of the angoumois grain moth, *S. cerealella*. This species has been the most widely used factitious host for mass rearing *Trichogramma* (Marston and Ertle, 1973; Smith, 1996). *S. cerealella* eggs are small, dark, and ovoid, are laid singly, and have relatively thick chorions. The Mediterranean flour moth, *E. kuhniella*, is another widely used factitious host for *Trichogramma* spp. (Hohmann *et al.*, 1988; Schmidt, 1994; Smith, 1996). Its eggs are also laid singly but are slightly larger than those of *S. cerealella* and have thin chorions (Schmidt, 1994). The cabbage looper, *T. ni*, is not commonly used as a host for rearing *Trichogramma*, but has large eggs that have been shown to support the development of several individuals per egg (Pak and Oatman, 1982; Hohmann *et al.*, 1988, Hoffmann *et al.*, 1995) and may be chemically attractive to *Trichogramma* females (Nordlund *et al.*, 1977). *T. ni* eggs are also laid singly and have a thick chorion. The eggs of the target pest, *O. nubilalis*, are laid as flat disks in shingled clusters. The egg volume of *O. nubilalis* is moderate, and the chorion is relatively thin. Eggs of this species are not commonly used for rearing of *Trichogramma* species (Smith, 1996).

The *O. nubilalis* used for this study came from the USDA Corn Insects Research Unit, Ames, Iowa, where they were reared on a wheat germ diet. Ovipositing adults were held at 16L:8D h, 25:20°C, and ~70% RH in 0.63-mesh hardware cloth cylinders (25 × 38.1 cm). Egg masses were oviposited onto wax paper wrapped around the cylinders. The other factitious host eggs were obtained from commercial sources as needed. *S. cerealella* eggs were supplied by Rincon-Vitova Insectaries, Inc., Ventura, California; *T. ni* eggs were obtained from the Boyce-Thompson Institute, Ithaca, New York; and irradiated (killed) *E. kuhniella* eggs were received from Ciba Bio-Logicals, Guelph, Ontario, Canada.

This study took place from mid February to late August in 1994. Separate *T. ostrinae* cultures were maintained on each of these hosts for 9 to 11 generations at 16L:8D h, 25:23°C, and ~80% RH. To avoid superparasitism in the individual cultures, we attempted to always have an excess number of host eggs available to female wasps. The individual performances of 20 randomly selected females from each

colony were measured by use of eggs of their natal hosts. The performances of another 20 individuals were measured by use of eggs of the target host, *O. nubilalis* (wasps reared on *O. nubilalis* were tested only on *O. nubilalis*). Measurements were made every other generation.

The seven treatment combinations were designated OO for wasps from the colony maintained on *O. nubilalis* tested on *O. nubilalis* eggs, SO for wasps from *S. cerealella* tested on *O. nubilalis* eggs, EO for wasps from *E. kuhniella* tested on *O. nubilalis*, TO for wasps from *T. ni* tested on *O. nubilalis*, SS for wasps from *S. cerealella* tested on *S. cerealella*, EE for wasps from *E. kuhniella* tested on *E. kuhniella*, and TT for wasps from *T. ni* tested on *T. ni*. Initiation of the colonies on hosts other than *O. nubilalis* was staggered so that the generations were not synchronized between treatments.

To isolate individual adult *T. ostrinae* females for measurement, 50 to 100 parasitized host eggs from each colony were placed in plastic shell vials ( $2.6 \times 4.8$  cm). Newly emerged adults were allowed 24 h to mate. Mated females were then placed singly in  $2.6 \times 4.8$ -cm vials containing 20–30 host eggs. Eggs were exposed to a wasp for 24 h and then placed in a gelatin capsule (size 000). If the wasp was still alive after 24 h, fresh eggs were placed in the vial and the experiment was continued. Host eggs were replaced daily until each wasp died. Exposed eggs from each day were kept separate and allowed to develop in gelatin capsules under the environmental conditions used for the maintenance of the parasitoid colonies. Emerged host larvae were removed daily to prevent them from feeding on remaining eggs.

Each wasp was allowed to parasitize eggs until she died, after which her longevity was recorded. Because wasps were not fed they lived only a short time. However, unfed longevity is an important aspect of wasp fitness because wasps may not always find water or a food source in the wild. Several days after each trial, host eggs that turned black were scored as parasitized. Female, male, and unemerged (dead but fully developed) wasps per egg were subsequently counted. We calculated the number of eggs parasitized, the number of progeny developing from each egg, the survival of progeny, and the sex of surviving progeny.

To predict the success of a population of parasitoids released for biological control, or to optimize a mass rearing strategy, it is important to know the number of eggs parasitized by individual wasps and the fraction of wasps in the population that actively parasitized hosts. We estimated the population-wide level of oviposition using the sample of 20 wasps tested each generation from each treatment.

Comparisons among treatments (colony host and test host) and over time (generation) were made using mixed-model analysis of variance and logistic regres-

sion (SAS PROC MIXED and PROC GENMOD; SAS Institute, 1989, 1996). The statistical models incorporated repeated measures and specified simultaneous contrasts where appropriate. The specific attributes of individual and colony fitness analyzed were (1) individual longevity, (2) the number of eggs parasitized per wasp (parasitism by active wasps), (3) the number of eggs parasitized per wasp tested (population parasitism), (4) the fraction of wasps in a treatment that parasitize, and (5) the number of progeny per host egg parasitized. Because the equality of a wasp includes attributes of her progeny, we also analyzed (6) the survival of progeny to adulthood and (7) the sex ratio of the progeny. Longevity was included as a covariate in the analyses of number of eggs parasitized because wasps that lived longer had more opportunity to parasitize. Logistic regression (SAS PROC GENMOD; SAS Institute, 1996) was used for the analysis of the fraction of wasps parasitizing. The logistic regression model included treatment, longevity, and generation as predictors of parasitism.

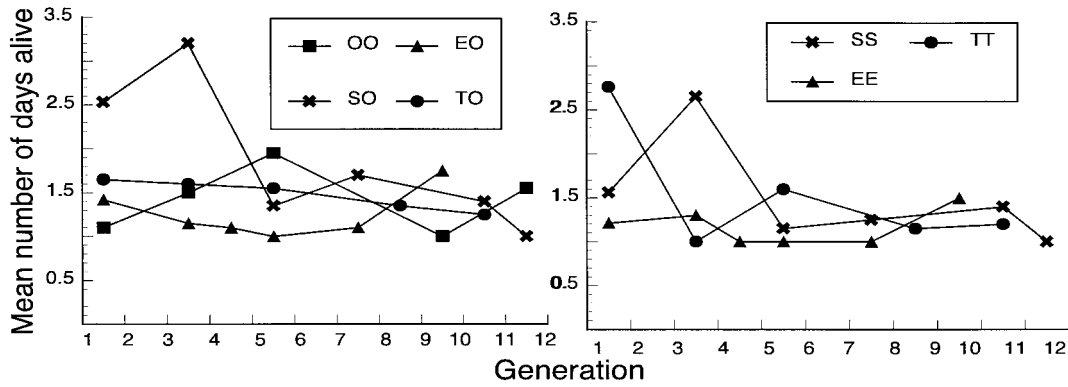
## RESULTS

### *Longevity of Female T. ostrinae*

In the absence of food, most of the female *T. ostrinae* died within 2–3 days of emergence, but some survived for up to 6 days. Wasps from non-*O. nubilalis* hosts tested on *O. nubilalis* eggs lived longer on average than those tested on the hosts from which they came (Fig. 1; Table 1). On average, wasps from *S. cerealella* tested on *O. nubilalis* lived the longest (most lived for 2 days, mean 1.89 days, SD 1.10,  $N = 109$ ) and wasps from *E. kuhniella* tested on *E. kuhniella* lived the shortest amount of time (most lived for 1 day, mean 1.17 days, SD 0.74,  $N = 108$ ). Throughout the experiment the average longevity of wasps decreased in all colonies with the exception of *E. kuhniella*, which started out low and did not decrease over the duration of the experiment when tested on both *E. kuhniella* and *O. nubilalis* host eggs (Fig. 1).

### *Individual Wasp Rate of Parasitism*

The number of eggs parasitized by wasps that parasitized at least once varied among treatments (Fig. 2; Table 1). Wasps reared from *E. kuhniella* parasitized the fewest eggs when tested on *O. nubilalis* (mean 8.81 eggs, SD 7.04; least squares estimate for EO treatment significant at  $P = 0.02$ ) even when it was taken into account that they lived for the shortest time. Wasps reared from *S. cerealella* tested on *S. cerealella* parasitized the most eggs. Wasps reared from *S. cerealella* and from *E. kuhniella* tended to parasitize fewer eggs when tested on *O. nubilalis* than did wasps reared from *O. nubilalis* (Table 1).



**FIG. 1.** Longevity of *T. ostrinae* over time. Each point represents the mean of 20 wasps. No wasps were tested in the generations without points. Each line is a different treatment. Treatment OO is wasps from the colony maintained on *O. nubilalis* tested on *O. nubilalis* eggs. SO is wasps from *S. cerealella* tested on *O. nubilalis*. EO is wasps from *E. kuhniella* tested on *O. nubilalis*. TO is wasps from *T. ni* tested on *O. nubilalis*. SS is wasps from *S. cerealella* tested on *S. cerealella*. EE is wasps from *E. kuhniella* tested on *E. kuhniella*. TT is wasps from *T. ni* tested on *T. ni*.

The average number of hosts parasitized decreased significantly over time for wasps from *S. cerealella* when tested on *S. cerealella* and for wasps from *T. ni* when tested on *T. ni* (Fig. 2; Table 1). The least squares estimates for the interactions of each of these treatments with generation was significantly negative at  $P < 0.05$ . There was no statistically significant change in parasitism rate over time in any of the other treatments.

#### Estimate of Population Rate Parasitism

The number of eggs parasitized per wasp (active and not active) varied with treatment (Table 1). Wasps

from *T. ni* when tested on *O. nubilalis* and wasps from *O. nubilalis* when tested on *O. nubilalis* had the highest mean parasitism (mean 15.61 eggs, SD 13.66 and mean 12.15 eggs, SD 12.67, respectively). Wasps from *E. kuhniella* parasitized the fewest eggs when tested on *E. kuhniella* or *O. nubilalis* (mean 6.60 eggs, SD 10.81 and mean 4.03 eggs, SD 6.48, respectively). Overall, the number of eggs parasitized per wasp was higher among wasps tested on *O. nubilalis* than among those tested on other egg species (Table 1).

The number of eggs parasitized in some treatments changed over time (Table 1). Specifically, parasitism by wasps from *O. nubilalis* when tested on *O. nubilalis*

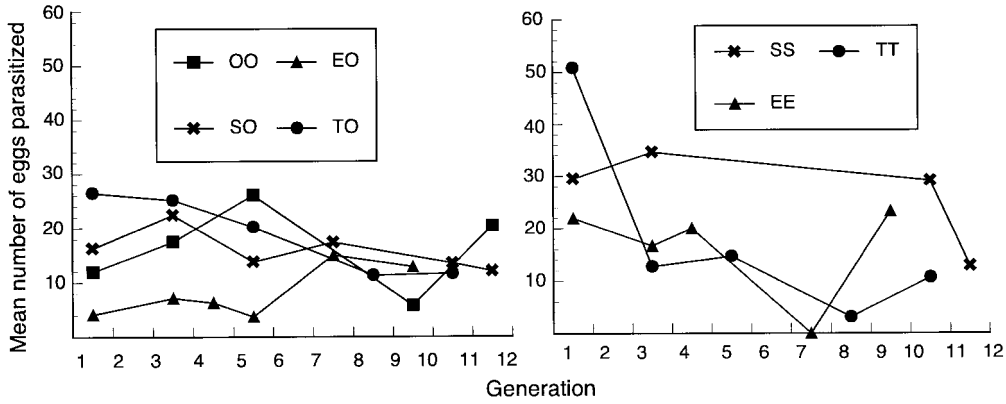
**TABLE 1**

Analysis of Variance of *Trichogramma ostrinae* Longevity and Level of Parasitism as Affected by Colony Host and Test Host Species

Source	Longevity ( $N = 764$ )		Parasitism by active wasps ( $N = 442$ )		Population parasitism ( $N = 743$ )	
	$F$	$P$	$F$	$P$	$F$	$P$
Intercept	14.45	0.0001	1.98	0.04	8.57	0.0001
Longevity	—	—	25.44	0.00	120.04	0.0001
Treatment	22.28	0.0001	2.93	0.01	7.97	0.0001
Generation	38.59	0.0001	1.69	0.19	2.98	0.08
Generation $\times$ treatment	12.07	0.0001	3.5	0.00	6.41	0.0001
Longevity $\times$ generation	—	—	3.47	0.06	0.04	0.85
Longevity $\times$ treatment	—	—	3.81	0.00	4.53	0.0001
Contrasts <sup>a</sup>						
OO vs all other	6.37	0.01	6.72	0.01	5.15	0.02
OO vs SO EO TO	5.79	0.02	9.90	0.00	0.52	0.47
EO vs EE	15.65	0.0001	—	—	—	—
SO vs SS	8.76	0.0001	—	—	—	—
TO vs TT	3.66	0.01	—	—	—	—

Note. Longevity and generation are included as ordered categorical variables.

<sup>a</sup> OO, wasps from the colony maintained on *O. nubilalis* tested on *O. nubilalis* eggs; SO, wasps from *S. cerealella* tested on *O. nubilalis*; EO, wasps from *E. kuhniella* tested on *O. nubilalis*; TO, wasps from *T. ni* tested on *O. nubilalis*; SS, wasps from *S. cerealella* tested on *S. cerealella*; EE, wasps from *E. kuhniella* tested on *E. kuhniella*; TT, wasps from *T. ni* tested on *T. ni*.



**FIG. 2.** Number of eggs parasitized per active wasp over time. Each point represents the mean of the wasps that parasitized from each 20-wasp sample per treatment per generation tested. No wasps were tested in the generations without points. Each line is a different treatment. The labels are the same as those in Fig. 1.

decreased, and parasitism by *E. kuhniella* when tested on *E. kuhniella* increased (least squares estimates for each of these interactions significant at  $P < 0.05$ ).

*Fraction of Wasps That Parasitized*

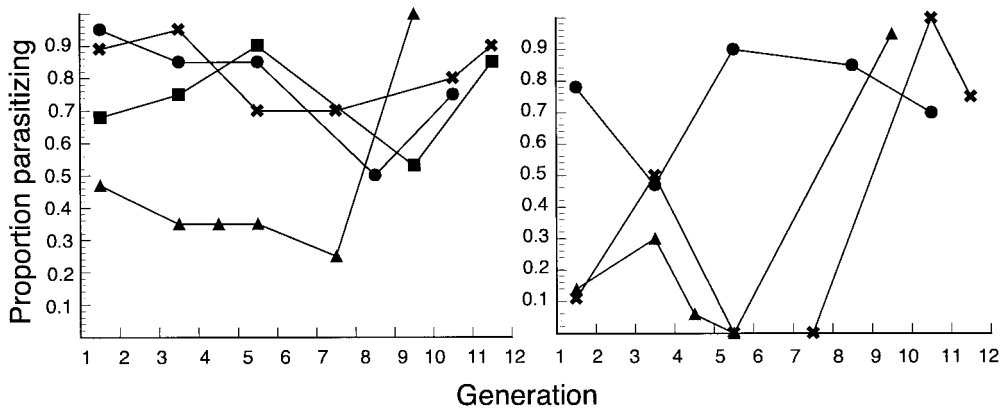
A total of 442 of 742 *T. ostrinae* parasitized host eggs. The fraction of wasps parasitizing at least one host egg, the active proportion of the population, was quite variable over generations and among treatments (Fig. 3). The colony reared on *E. kuhniella* had the smallest active population until its ninth generation when it became comparable to that of other colonies. The fractions of wasps from the *S. cerealella* colony that parasitized hosts also increased significantly over time when tested on *S. cerealella* host eggs (Fig. 3; logistic regression, significant increase in likelihood of parasitism with generation *E. kuhniella*  $\chi^2 = 10.53$ ,  $P < 0.0001$  and *S. cerealella*  $\chi^2 = 18.64$ ,  $P < 0.0001$ ).

We found by use of a logistic regression model that included the effect of generation and wasp longevity

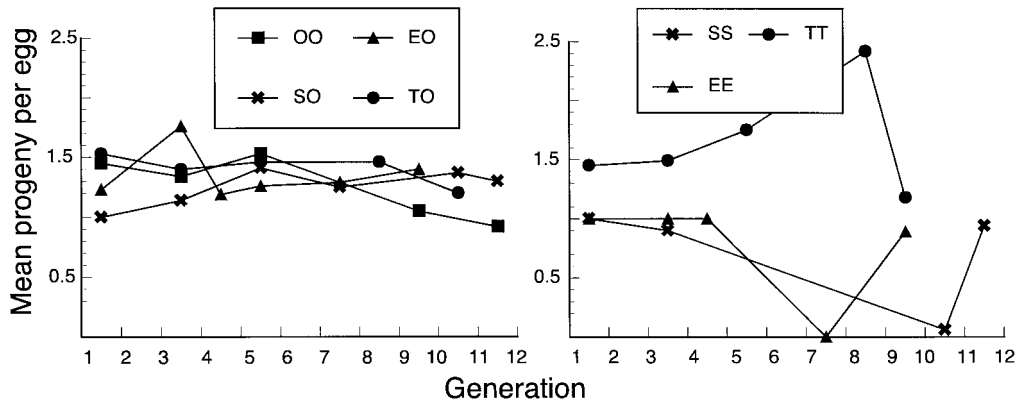
that wasps from *S. cerealella* and *T. ni* were more likely to parasitize hosts when tested on *O. nubilalis* than when tested on their original host (SO vs SS  $\chi^2 = 47.00$ ,  $P < 0.0001$  and TO vs TT  $\chi^2 = 60.79$ ,  $P < 0.0001$ ). Wasps from the *E. kuhniella* colony showed a nonsignificant trend in the same direction.

*The Number of Progeny per Host Egg*

The number of fully developed progeny in a host egg varied among treatments (Fig. 4; Table 2, number of progeny per egg). The greatest number of progeny per host egg was produced by wasps from *T. ni* colonies tested on *T. ni* eggs (Fig. 4; mean 1.69 progeny per egg parasitized, SD = 0.75) and on *O. nubilalis* eggs (Fig. 4; mean 1.41 progeny per egg parasitized, SD 0.35). Wasps from *S. cerealella* on average yielded only 0.69 (SD 0.42) developed progeny per *S. cerealella* egg parasitized (this number is less than one because the parasitoid egg failed to develop in some host eggs that were scored as parasitized).



**FIG. 3.** Proportion of the *T. ostrinae* tested that parasitized at least one egg. Each point is the proportion of 20 wasps from one treatment in one generation that laid eggs. No wasps were tested in the generations without points. Each line is a different treatment. The labels are the same as those in Fig. 1.



**FIG. 4.** Number of *T. ostrinae* progeny that developed in eggs parasitized by *T. ostrinae* over time. Each point is the mean number of progeny per egg parasitized by the active wasps from each 20-wasp sample per treatment per generation tested. No wasps were tested in the generations without points. Each line is a different treatment. The labels are the same as those in Fig. 1.

The number of progeny per egg was quite variable over time for all of the treatments (Fig. 4). There was a decline in the mean number of progeny per host egg for wasps from *O. nubilalis* tested on *O. nubilalis* and for wasps from *S. cerealella* tested on *S. cerealella* (Table 2).

#### Survival of Progeny to Adulthood

The fraction of eggs parasitized from which adult wasps emerged differed among treatments, ranging from a mean of 98% (wasps from *S. cerealella* tested on *S. cerealella*) to 58% (wasps from *E. kuhniella* tested on *O. nubilalis*) (Table 2). For wasps reared from *E. kuhniella* and *S. cerealella*, the mortality of developed progeny was greater on *O. nubilalis* than on their

original host species (Fig. 5; Table 2). On average, the survival to emergence decreased with time (Fig. 5; Table 2).

#### Sex Ratio of Progeny

The sex ratio of the offspring was generally female biased, ranging from 55% female (TT treatment, first generation) to 100% female (EO treatment, third generation). Variation of sex ratio between treatments was statistically significant (Fig. 6; Table 2). Wasps from colonies maintained on *S. cerealella* and tested on *O. nubilalis* produced a significantly higher fraction of progeny that were female than the other treatments (mean 0.87, SD 0.026; least squares estimate for the SO treatment significantly positive at  $P < 0.0001$ ). On

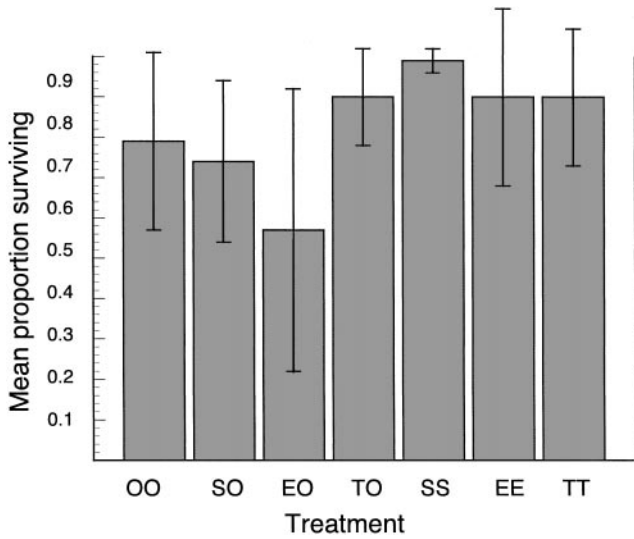
**TABLE 2**

Analysis of Variance of *Trichogramma ostrinae* Quality of Progeny as Affected by Colony Host and Test Host Species

	No. of progeny per egg ( $N = 421$ )		Progeny survival ( $N = 426$ )		Progeny sex ratio ( $N = 410$ )	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Source						
Intercept	13.08	0.00	3.68	0.00	7.34	0.00
Longevity	5.03	0.02	—	—	—	—
Treatment	3.39	0.00	11.82	0.00	3.19	0.01
Generation	4.00	0.05	12.56	0.00	0.72	0.40
Generation $\times$ treatment	3.09	0.01	4.19	0.00	0.68	0.67
Contrasts <sup>a</sup>						
OO vs all other	—	—	—	—	5.42	0.02
EO vs EE	2.58	0.11	45.01	0.00	0.79	0.37
SO vs SS	0.36	0.55	5.04	0.02	9.44	0.00
TO vs TT	3.75	0.05	0.10	0.75	0.04	0.85

Note. Longevity and generation are included as ordered categorical variables.

<sup>a</sup> OO, wasps from the colony maintained on *O. nubilalis* tested on *O. nubilalis* eggs; SO, wasps from *S. cerealella* tested on *O. nubilalis*; EO, wasps from *E. kuhniella* tested on *O. nubilalis*; TO, wasps from *T. ni* tested on *O. nubilalis*; SS, wasps from *S. cerealella* tested on *S. cerealella*; EE, wasps from *E. kuhniella* tested on *E. kuhniella*; TT, wasps from *T. ni* tested on *T. ni*.



**FIG. 5.** Fraction of *T. ostrinae* progeny surviving to adulthood. Each bar is the mean ( $\pm 1$  SD) of the progeny of all of the wasps sampled per treatment over the entire experiment. Treatment OO is wasps from the colony maintained on *O. nubilalis* tested on *O. nubilalis* eggs. SO is wasps from *S. cerealella* tested on *O. nubilalis*. EO is wasps from *E. kuhniella* tested on *O. nubilalis*. TO is wasps from *T. ni* tested on *O. nubilalis*. SS is wasps from *S. cerealella* tested on *S. cerealella*. EE is wasps from *E. kuhniella* tested on *E. kuhniella*. TT is wasps from *T. ni* tested on *T. ni*.

average, wasps from *O. nubilalis* tested on *O. nubilalis* had a lower female bias than other treatment combinations (Table 2). There was no significant change in sex ratio over time, and the sex ratio was not related to the longevity of the wasp (Table 2).

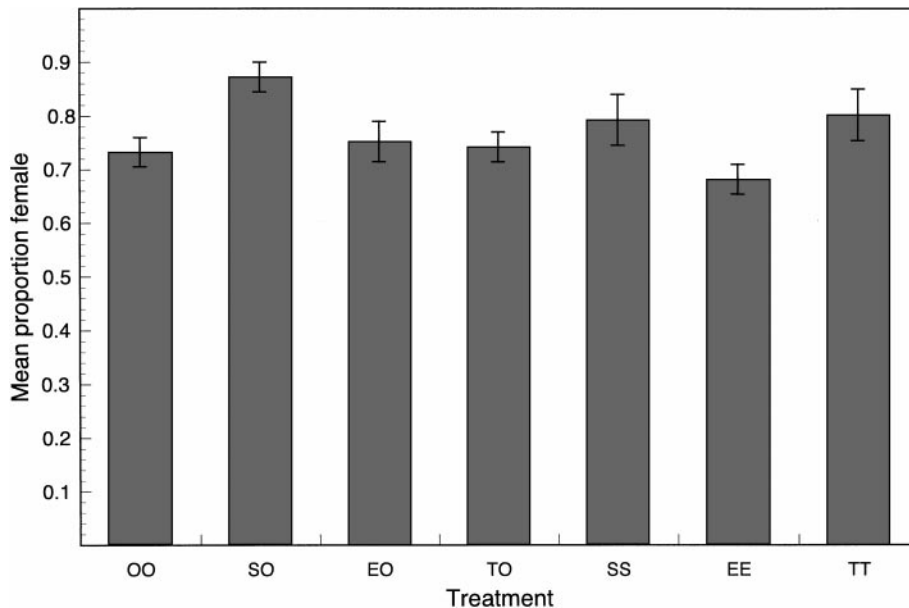
## DISCUSSION

### *Differences among Hosts*

On average, wasps reared from *E. kuhniella* lived the fewest days, and the fraction of those wasps that parasitized a host was lower than that for wasps reared from other hosts. Additionally, there was relatively high mortality of progeny produced by wasps from *E. kuhniella*. The poor performance of wasps tested on *E. kuhniella* may have been the result of the use of irradiated eggs, which may be a poorer substrate for *Trichogramma* development than live eggs (Goldstein *et al.*, 1983; Smith, 1996; Romeis *et al.*, 1997). However, wasps reared from *E. kuhniella* also performed poorly on *O. nubilalis*, with a 42% mortality level. This suggests that wasps that did manage to emerge from the poor-quality hosts performed poorly even when presented with a higher-quality host.

*S. cerealella* has been a commonly used host for laboratory rearing of *Trichogramma* (Marston and Ertle, 1973; Smith, 1996). In these experiments its suitability was erratic. On average, its longevity, rate of parasitism, and progeny survival were high but variable. Wasps from *S. cerealella* tested on *O. nubilalis* had the highest female-biased sex ratio. However, the rate of parasitism was variable and declined over the duration of the experiment along with the number of progeny per host egg parasitized.

*T. ni* is not commonly used as host for the mass rearing of *Trichogramma*, probably because it is not economical to use a species that produces relatively few large eggs (Smith, 1996). However, these large host



**FIG. 6.** Sex ratio of the progeny of *T. ostrinae*. Each bar is the mean ( $\pm 1$  SD) of the proportion of female progeny per treatment per generation tested. The treatment labels are the same as those in Fig. 5.

eggs yielded the most progeny per host egg, and wasps reared from the large eggs parasitized many hosts on average.

A primary purpose of this experiment was to compare the influences of various rearing hosts on the ability of *T. ostriniae* to control *O. nubilalis* in the field. We found that on average wasps reared on species other than *O. nubilalis* lived longer when tested on *O. nubilalis* than when tested on the host on which they were reared. In addition, a higher fraction of wasps from the *T. ni* and the *S. cerealella* eggs successfully parasitized one or more hosts when tested on *O. nubilalis* than when tested on eggs of the species on which they had been reared. It is possible that the use of *O. nubilalis* eggs is somehow less metabolically costly than the use of other hosts or that parasitism might be facilitated by the configuration of the eggs because *O. nubilalis* eggs are laid in clumps rather than singly.

### *Changes in Performance over Time*

We found great variation in important aspects of parasitoid fitness across generations. Attributes of an animal that are related to behavior, such as rate of parasitism, are expected to be variable because many factors influence behavior (Vet *et al.*, 1990; Godfray, 1994). However, some of the characteristics that we measured did not vary randomly, but rather increased or decreased over the duration of the experiment.

The performance of parasitoids kept in the laboratory for many generations can change over time as a result of changes in the laboratory environment or as a consequence of genetic changes in the wasp colony (Hopper *et al.*, 1993). Changes in the laboratory, such as food, temperature, light, and disease levels, or changes in the bioassay used to measure wasp performance are likely to affect all colonies kept in the same laboratory similarly. Changes in wasp performance in a single healthy colony, over many generations when external factors are constant, may be a genetic response to selection on the wasp population. A decline in performance over time may possibly be due to inbreeding or disease.

We have no reason to believe that there were any systematic changes over time in experimental protocol or average host quality (size, age, and distribution) within any of the host species that we used. However, because the hosts for each wasp colony in this experiment were reared in different environments, the changes over time that we detected in individual colonies could be due to temporal variation in host quality.

In addition to possible environmental variation, the interpretation of the changes over time in this experiment are limited by the lack of replicate colonies of each treatment. Colonies could become genetically different over generations as the result of genetic drift. However, because characters that we have measured

are closely related to fitness, and we measured them over only a few generations, genetic drift is not likely to have caused measurable differences among colonies (Falconer, 1989; Hopper *et al.*, 1993).

*Changes over time in some treatments.* The fraction of wasps that parasitized at least one host egg increased over time for wasps from the *E. kuhniella* and the *S. cerealella* colonies. However, the changes occurred suddenly and there was high variability in the fraction of wasps parasitizing hosts. Consequently, fitness parameters of these colonies would have to be measured over more generations to be sure that the sudden increases were not just aberrations. The increase in the population-level rate of parasitism by wasps from the *E. kuhniella* colony was most likely the result of a higher proportion of the wasps actively parasitizing one or more hosts. This was not the case for wasps from the *S. cerealella* colony, probably because as more of the wasps parasitized one or more hosts, each wasp parasitized fewer total hosts and produced fewer progeny per host parasitized. For the same reason, the overall rate of parasitism by wasps from *O. nubilalis* tested on *O. nubilalis* also declined.

*Declines in all treatments.* In all of the treatments, there was a general decline over time in wasp longevity and in the survival of progeny to adulthood. Each of the colonies on the non-*O. nubilalis* hosts was initiated at different dates. For example the first generation of wasps reared on *T. ni* was contemporary with the ninth generation of wasps reared on *E. kuhniella*. Therefore the decline over time is not related to date of inception but to the number of generations in rearing. These results suggest that the conditions within each colony independently declined over time. The decline of fitness of parasitoids reared for many generations in the laboratory is commonly observed and can be due to genetic inbreeding, disease, or suboptimal conditions (Leppla and Fisher, 1989; Hopper *et al.*, 1993; Penn *et al.*, 1998). Surprisingly, this decline occurred even in the colony maintained on *O. nubilalis* even though these wasps came from a colony successfully maintained on *O. nubilalis* for many generations in the same laboratory.

Ideally a population of parasitoids mass reared for biological control would include females that all actively parasitize many eggs, live for a long time, and produce mostly female offspring on the target hosts. Explanations for why this does not happen are intrinsic to the parasitoid (such as life history trade-offs) or the host, or they may be due to laboratory techniques used. Many aspects of wasp fitness must be assessed to judge the suitability of a host, along with such practical issues as the ease of mass rearing of particular host species under laboratory conditions.

In this experiment important attributes of wasp fitness varied among host species and over time. We



found that both *T. ni* and *O. nubilalis* were suitable hosts, that *S. cerealella* was moderately suitable, and that irradiated *E. kuhniella* eggs were poor hosts. Wasps reared from *O. nubilalis* did not perform significantly better than wasps reared from the other hosts in all respects, and there is some evidence that over time the performance of wasps from *E. kuhniella* and *S. cerealella* improved. Based on the results of this experiment, the most appropriate host species for the laboratory rearing of *T. ostrinae* would be whichever species, among those tested here, that could be mass reared most economically, with the exception of *E. kuhniella*, which is a poor host.

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