Potential fitness consequences of associative learning in a parasitoid wasp

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Several studies have documented associative learning in insects, but the adaptive value of such learning is not yet well understood. To evaluate this issue, we quantified long-term fitness consequences of associative learning in the parasitoid wasp, *Biosteres arisanus*. We compared individual wasps that were allowed to choose host substrate based on experience (''learning'' wasps) to wasps that could only make random substrate choice (''random'' wasps) in an environment where only one out of two substrates contained host eggs. In two experiments, the average number of host eggs parasitized and offspring produced were significantly larger for learning than for random wasps. Our results allow detailed examination of the conditions under which learning would have positive fitness effects in ecological systems similar to ours. These conditions include relatively long search duration for hosts; the ability to remember a learned preference over extended periods of interfering activities; and large mean differences between alternatives, and small variances, which together allow rapid evaluation of alternatives and long duration of exploiting the superior one. *Key words: Biosteres arisanus,* decision making, fitness, learning, parasitoid wasps. *[Behav Ecol 11: 536–543 (2000)]*

O ver the past few decades, researchers have established that insects are capable of associative learning, defined as the ability to acquire a neural representation of a new association between a stimulus and an environmental state that may affect fitness (e.g., Dudai, 1989). Two well-studied model systems are fruit flies, *Drosophila melanogaster,* and parasitoid wasps. Under controlled laboratory settings, adult and larval stages of fruit flies use associative learning to seek better food sources and avoid electric shock or predation (e.g., Aceves-Pina and Quinn, 1979; Davis, 1996; Dukas, 1999; Tully, 1996). Parasitoid wasps can learn to associate their host with the more conspicuous cues emitted by the host substrate and can learn to seek novel odors associated with food (e.g., Lewis and Takasu, 1990; Turlings et al., 1993).

Flies and wasps appear to use associative learning to seek stimuli with positive fitness effects and to avoid stimuli with negative fitness effects; this suggests that learning in these species is an adaptive property maintained by natural selection. However, little direct evidence linking associative learning to fitness exists (Dukas, 1998; Papaj and Prokopy, 1989), and thus the alternative that associative learning is a nonadaptive, emergent property of nervous systems beyond a threshold level of complexity cannot be rejected. Ultimately, to demonstrate that learning in these insects is indeed an adaptive trait, one must document that it has significant positive effects on fitness under natural settings.

The link between associative learning and fitness in such insects is not an obvious one. Experiments documenting significant associative learning in flies and parasitoids have been conducted under restrictive settings, where subjects are usually exposed only to a minimal set of carefully chosen, distinct stimuli and environmental states, and a single-choice test is conducted immediately after training in an isolated environment. Hence it is unclear whether learning documented under such conditions would occur or have significant effects on

behavior under more realistic settings, where animals encounter numerous environmental stimuli and states and have to make many decisions, some of them long after experiencing a certain association. It is feasible that under such complex environmental conditions, individuals would revert to rely solely or mostly on their innate biases.

Even if individuals depict significant long-term use of learned experiences, it is not guaranteed that their fitness would be higher than that of nonlearning individuals. First, the nonlearning individuals may use alternative strategies resulting in an equal reproductive and survival rate. Second, limiting factors, such as egg-laying rate or mortality rate that cannot be reduced by learning may prevent learning from significantly increasing fitness. For example, even if learning increases encounter rate with hosts, nonlearners may be as successful if they can lay as many eggs as learners because of a physiological limit on the number of eggs laid per day. Similarly, positive effects of learning may be masked if survival rate from egg to adulthood is low and highly variable.

As a first step in evaluating fitness consequences of learning, we conducted laboratory experiments with *Biosteres arisanus* (Sonan), an egg parasitoid of tephritid fruit flies. Although these fruit flies are polyphagous, they typically show strong seasonal preferences for certain fruits (Wong et al., 1984). Hence in a given season lasting over a few weeks, one fruit type may receive most fruit fly eggs. Learning to seek a specific fruit species and bypass others may significantly increase egg-laying rate by the parasitoids (e.g., Turlings et al., 1993; Vet and Dicke, 1992). We tested this prediction by comparing the fitness of wasps that were allowed to express learning to the fitness of control wasps that were manipulated to make random choices.

We present results of three experiments: the first tested whether *B. arisanus* is capable of associative learning, the second examined the effect of such associative learning on egg production, and the third quantified the effect of learning on the number of offspring produced. We focus here on insect learning because the notion that many insects other than social bees can learn is somewhat recent (Papaj and Lewis, 1993), and its adaptive value seems less obvious than learning

in long-lived mammals and birds. However, the general topic we address is the adaptive value of learning, an issue little studied in any species. Hence our work is relevant for other organisms as well.

General methods

Wasps and fruits

B. arisanus parasitizes eggs of Mediterranean and oriental fruit flies and is the dominant parasitoid of these fruit flies in Hawaii (Wong and Ramadan, 1987). Wasps used in the experiments were reared at the USDA-ARS Tropical Fruit and Vegetable Research Laboratory in Honolulu, Hawaii, according to the methods of Wong and Ramadan (1992). Parasitoids were reared on oriental fruit fly eggs placed in oviposition units containing papaya juice (Ramadan et al., 1994). Parasitized fly puparia were shipped from the rearing laboratory to Kauai, Hawaii, where the puparia were placed in 30 × 30 × 30 cm wood and screen cages containing water and undiluted honey. The cages were held in a room devoid of fruit at 22°C and 80% relative humidity, with natural light augmented with fluorescent light on a photoperiod of 12 h light:12 h dark. Adult wasps eclosed within 1–3 days after arrival and were tested at ages of 5–11 days.

We used two fruit types in each experiment, but due to seasonal changes in availability of appropriate fruit, we used a total of four fruit types. The fruits used were kumquat, orange, lemon, and guava; all fruits were uninfested and thoroughly washed and dried before an experiment. We conducted a series of preliminary training and testing sessions to determine the types and optimal states (e.g., size, color, and ripeness) of fruit used. A precondition for using a certain pair of fruits was no significant preference for one over the other by experimentally naïve wasps (see below). Every morning, half of the fruits were introduced individually into a cage containing approximately 50 mature female oriental fruit flies; a fruit was removed after the flies laid four egg clutches. Fruits with host eggs were kept in a screen container until provided to the wasps within a few hours. The other half of the fruits were host free and were kept in a separate screen container until use.

Test chamber

The test chamber consisted of a 30 × 30 × 30 cm Plexiglas and screen cage with sides darkened with black cardboard. The wasp was placed on a small piece of filter paper (the "launch") near the screen door; at the far screen wall of the box were two dishes, one at each side of the chamber, and each contained one fruit type. Fruits in the test chamber contained no host eggs and were needle-punctured once before each test to increase odor emission. The position of each fruit type (right or left) was randomly determined each session. Behind the screen wall was a small fan, which was turned on for 5 s every minute. The fan provided air flow directed toward the wasp, but keeping it off most of the time enabled the small wasp to fly and orient toward the fruit more easily (Messing et al., 1997). The ambient light (from 2 3.5-m² glass windows) was augmented with light from a 250-watt lamp positioned above the far side of the cage; overall, each fruit dish was illuminated with identical light intensities of 6500 lux.

Experiment 1

Methods

In the first experiment, our aim was to verify that *B. arisanus* can learn. First, we tested experimentally naïve wasps for an innate preference between orange and guava. Each wasp was placed on the launch in the test chamber and allowed to land
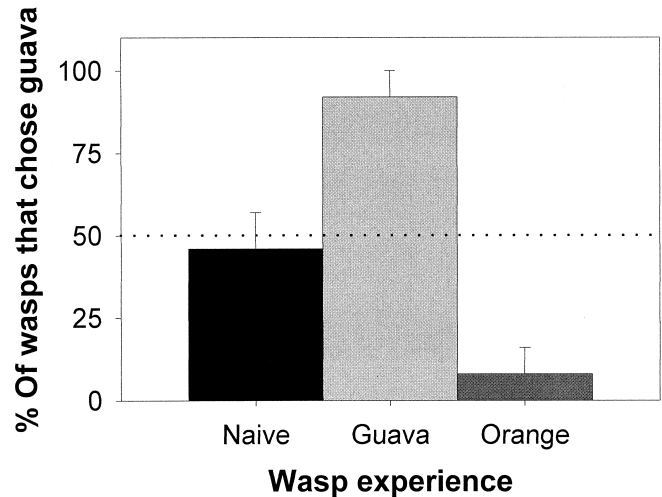

Figure 1
The percentages of wasps that chose guava as a function of their experience. Naïve wasps ($n = 24$) had no prior experience with fruit, 12 wasps had experienced guava with host eggs and orange devoid of eggs, and 12 wasps had experienced orange with host eggs and guava devoid of eggs. The dotted line depicts the expected random choice of 50%.

on one of the two fruits. Wasps that did not land on fruit within 5 min were removed and not included in the analysis. We tested 24 wasps in 3 blocks of 8 trials, with half the trials in each block having orange on the left side and guava on the right side, and the other half having the fruit sides reversed. We then proceeded with a quantification of associative learning.

Individual wasps were tested for their abilities to associate fruit properties (odor and visual appearance) with the availability of host. In each experimental session, an experimentally naïve wasp was subjected to two training trials of 15 min each, followed by the learning test. During training, a wasp was held individually inside a regular 30 × 30 × 30 cm wood and screen cage. During one training trial, the wasp was placed on one fruit type containing host, and during the other trial, the wasp experienced the other fruit type with no host. Trial duration was determined in preliminary experiments in which we tested for the optimal duration that allows wasps sufficient time for detecting hosts but relatively little time for egg laying. After we placed a wasp on the fruit, she typically initiated searching, which involved slow scanning of the fruit surface using her antennae, and probing with her ovipositor inside fruit wounds and punctures in search for eggs. Wasps on host-containing fruit were also engaged in egg-laying attempts, but we did not document the number of successful attempts.

Experimental sessions were conducted in three blocks of eight sessions, each block consisting of a random presentation of the eight possible combinations of (1) type of fruit presented first (two options), (2) fruit containing host presented first (two options), and (3) spatial position of fruit types at the test chamber (two options). Presentation of all these combinations allowed us to control for alternatives to associative learning, such as side preference or preference for fruit presented first or second. We released wasps to the wild after testing.

Results

In the preliminary test for innate preference, experimentally naïve wasps showed no fruit preference: 46% of the wasps chose guava, and 54% chose orange (Figure 1). In the tests

Table 1

An illustration of the protocol used in experiment 2

Day	Time	Session	Learning wasp	Random wasp
1	0800	1	Sampling: 15 min on lemon⁻	Placed in cage with kumquat⁺
1	0815		Sampling: 15 min on kumquat⁺	
1	0830		Choice test between lemon and kumquat	
1	0840		Placed in cage with fruit type chosen (kumquat⁺ or lemon⁻)	
1	1100	2	Choice test between lemon and kumquat	Placed in cage with lemon⁻
1	1110		Placed in cage with fruit type chosen (kumquat⁺ or lemon⁻)	
1	1400	3	Choice test between lemon and kumquat	Placed in cage with kumquat⁺
1	1410		Placed in cage with fruit type chosen (kumquat⁺ or lemon⁻)	
2	0800	4	Choice test between lemon and kumquat	Placed in cage with lemon⁻
2	0810		Placed in cage with fruit type chosen (kumquat⁺ or lemon⁻)	
2	1100	5	Choice test between lemon and kumquat	Placed in cage with lemon⁻
2	1110		Placed in cage with fruit type chosen (kumquat⁺ or lemon⁻)	
2	1400	6	Choice test between lemon and kumquat	Placed in cage with kumquat⁺
2	1410		Placed in cage with fruit type chosen (kumquat⁺ or lemon⁻)	

Depicted are one learning and one random wasp, both randomly assigned to receive kumquat with host eggs and host-free lemon, indicated by + and − signs, respectively. The learning wasp received a fresh fruit item of her choice each session. The fruit contained host eggs when the wasp chose kumquat, or the fruit was host free when the wasp chose lemon. By contrast, by assignment, the random wasp received a fresh kumquat with host eggs in three out of the six sessions and a host-free lemon in the remaining three sessions. See Methods for the full protocol.

of experienced wasps, 92% of the wasps alighted on the fruit that had contained host eggs during their training (log-linear model, $\chi^2 = 19.5$, df = 1, $p < .001$; Figure 1). The effects of fruit type, presentation order, and spatial positions of fruit were not significant ($p > .4$). These results indicate that *B. arisanus* wasps can show good learning ability, at least under the restricted conditions tested.

Experiment 2

Rational

In the second experiment, we compared the performance of wasps that were allowed to express learning (the "learning wasps") to some null model (the "random wasps") in a setting where only one of two fruit types contained host eggs. We allowed wasps belonging to the learning treatment to sample each of the two fruit types and to later use their experience to choose the fruit type on which they search for host eggs. In contrast, we allowed individuals of the random wasps treatment to spend equal amounts of time on each fruit type. The random assignment was appropriate because wasps showed no preference for one fruit type over the other (see Figure 1 and results below). However, the decision of allowing the random wasps to spend equal times on each fruit consisted of a convenient but somewhat arbitrary null model, which effectively implied that a relatively long time was spent on searching for host eggs compared to parasitizing eggs. In the field, the proportion of time spent searching in relation to the time spent egg laying depends on factors such as fruit and fly-egg densities, the spatial distributions of these resources, the wasps' ability to fly long distances, and weather conditions. Under some conditions, which we simulated in this experiment, search duration may be relatively long.

In this experiment, the random wasps were constrained to depict random choice, while the learning wasps were free to show anything from random choice to 100% preference for one fruit type or the other. There were at least three possible outcomes for such an experiment. First, the learning wasps might show random or close to random choice over time and hence would not have a higher fitness than the random wasps. Second, the learning wasps might depict significant long-term preference for the fruit type containing host eggs, but this would not translate into higher fitness due to constraints such

as host-egg finding rate or egg-laying rate. Finally, a third possible outcome is that the learning wasps would show significant preference for the fruit type containing host eggs and that this would translate into fitness higher than that of the random wasps.

Methods

Individual wasps of identical ages were randomly assigned into the learning or random treatment groups and placed individually in cages identical to the rearing cages and containing water and undiluted honey. The experiment lasted 2 days and consisted of six sessions of approximately 3 h each. The environment consisted of one fruit type containing host eggs and another that was host free. We replicated the experiment three times, each replicate including four learning and four random wasps. In each replicate, two wasps of each treatment group experienced kumquat with host eggs and two wasps experienced lemon with host eggs. A preliminary experiment revealed no preference for either fruit by experimentally naïve wasps (46% of 24 wasps chose kumquat and 54% chose lemon). We released wasps to the wild at the end of the experiment. The sections below detail the experimental protocol for each of the two treatments.

Learning wasps. In session 1, a wasp from the learning group began with two 15-min sampling periods (Table 1). During one sampling period, the wasp experienced one fruit type (either lemon or kumquat) containing host eggs, and during the other period, the wasp was placed on the other fruit type, which contained no host eggs. After we placed a wasp on a fruit, she typically initiated searching and probing within several seconds. Usually, wasps on fruit with host eggs were engaged in egg laying. The order of presentation of fruit types (lemon or kumquat) and host availability (fruit with or without host eggs) was random. That is, a wasp was randomly assigned to one of the four possible treatments: lemon⁺ then kumquat⁻, lemon⁻ then kumquat⁺, kumquat⁺ then lemon⁻, or kumquat⁻ then lemon⁺, where the plus and minus signs depict fruit with and without host eggs, respectively.

At the end of the 30-min sampling period, we transferred each wasp into the test chamber. A wasp typically left the launch and landed on a fruit within a few minutes. Then we transferred the wasp back to her cage and placed her on the fruit type of her choice; the fruit contained host eggs if the

wasp had experienced that fruit type with eggs during the preceding sampling (i.e., the wasp chose correctly), or the fruit was host free if the wasp had experienced that fruit type with no eggs during the preceding sampling (i.e. the wasp made a wrong choice). The wasp remained undisturbed in the cage for 3 h. Then the fruit was removed from her cage, marked, and refrigerated and the wasp placed back into the test chamber for the start of session 2.

In session 2, each learning wasp was allowed to choose a fruit type as in session 1. That is, the wasp could use her experience from session 1 to choose a fruit type. After choosing one of the fruit types, the wasp was returned to her cage and provided with a fresh fruit item of her choice (Table 1). Again, this fruit item contained host eggs if the wasp chose the same type that had had eggs in the first part of session 1. The item was host free if the wasp chose the same type that had been host free in the first part of session 1. Three hours later, the fruit was removed from the cage, marked, and refrigerated, and the wasp put into the test chamber for the start of session 3.

In session 3, the protocol was identical to the one for session 2 except that after 3 h, in the late afternoon of day 1, the fruit was removed from each wasp's cage, marked, and refrigerated; wasps remained with no fruit overnight. For sessions 4, 5, and 6, the protocol was identical to the one for session 2. Sessions 4–6 were conducted on the second day of the experiment.

Random wasps. Each wasp of the random group was randomly assigned to one of two classes; one class of wasps received lemon with host eggs and kumquat with no host, and the other class received kumquat with host eggs and host-free lemon. Each session, a wasp from one class had a 0.5 probability of receiving a lemon with host eggs and a 0.5 probability of encountering a host-free kumquat. A wasp from the other class had a 0.5 probability of encountering kumquat with host eggs and a 0.5 probability of receiving host-free lemon. Wasps typically initiated searching and probing within several seconds after being placed on fruit, and wasps on fruit with host eggs were engaged in egg laying.

Overall, a random wasp's probability of encountering a fruit item with eggs was 50%, with the constraint that a wasp encountered at least one fruit item with eggs each day. This treatment simulates a hypothetical situation in which individuals lacking learning ability cannot notice the association between fruit type and host eggs and thus randomly search for host eggs.

At the start of session 1, each wasp was randomly assigned to a fruit item in the manner just detailed (Table 1). The wasp then was placed in her cage with that fruit for 3 h. At the end of the session, the fruit was removed from the cage, marked, and refrigerated.

Session 2 began immediately after the end of session 1. Each random wasp was transferred to a new fruit item. Again, that fruit item had a 0.5 probability of being a type containing host eggs. The wasp then experienced that randomly chosen fruit item for 3 h. At the end of the session, the fruit was removed from the cage, marked, and refrigerated.

The protocol for session 3 was identical to the one for session 2, except that at the end of session 3, in the late afternoon of day 1, the fruit was removed from each wasp's cage, marked, and refrigerated; wasps remained with no fruit overnight.

The protocol for sessions 4, 5, and 6 was identical to the one for session 2. Sessions 4–6 were conducted on the second day of the experiment.

Fitness. The fitness measure was the number of host eggs parasitized by each wasp during the experiment. We removed all fly eggs from each fruit, dissected them under a micro-scope, and counted the number of eggs containing wasp eggs. Except for one ambiguous case, no fly egg contained more than 1 parasitoid egg. For unknown reasons, two wasps in each treatment group laid no eggs and were excluded from the analysis. Hence the data set for each treatment group included 10 wasps: 6 experiencing kumquat with host eggs and 4 experiencing lemon with host eggs.

Statistical analyses included the effects of replicate and fruit type in addition to treatment effect. Preliminary analysis revealed no significant interactions, which were not included in the final analyses.

Results
Background information. The average number of fly eggs per fruit was identical for each of the two treatment groups (ANOVA, $F_{1, 15} = 0.14$, $p > .5$), although lemon contained twice as many fly eggs as kumquat (Figure 2a). Similarly, there was no difference between the learning and random treatment in the number of fly eggs parasitized per fruit ($F_{1, 15} = .16$, $p > .5$). In this case, however, the number of parasitoid eggs per fruit was similar for lemon and kumquat ($F_{1, 15} = 1.4$, $p > .1$; Figure 2b). The proportion of parasitized eggs per fruit was larger for kumquat than for lemon ($F_{1, 15} = 5.2$, $p < .05$) but similar between the learning and random treatments ($F_{1, 15} = 0.02$, $p > .5$; Figure 2c).

Behavioral observations. During the initial test, 9 out of the 10 wasps in the learning treatment chose the type of fruit that had contained eggs during the preceding sampling. Subsequent choices were also 80–90% correct over the six sessions spanning over 2 days (Figure 3). The wasps did not forget overnight, as indicated by the high percentage of correct choices at the first session on day 2 (session 4). The overall scores of individual wasps ranged between 4/6 to 6/6, or 67–100% correct choices, with an overall mean ($\pm$ SE) of 87 $\pm$ 3%.

Fitness. Wasps in the learning group parasitized significantly more eggs than wasps in the random group over the 2-day experimental period ($F_{1, 15} = 11.2$, $p < .005$; Figure 4a). Here fruit effect was nonsignificant ($F_{1, 15} = 0.26$, $p > .5$).

Experiment 3

Methods
The protocol for experiment 3 was similar to that of experiment 2, except for the following. First, the fitness measure was the number of offspring. This is probably a more appropriate fitness measure than the number of host eggs parasitized because a high level of egg mortality or a negative correlation between egg laying rate and an egg's probability of producing an adult wasp can eliminate potential fitness benefits of learning. Second, because of seasonal changes in fruit availability, the fruit types used were guava and kumquat. A preliminary experiment revealed no preference for either fruit by experimentally naïve wasps (58% of 24 wasps chose kumquat and 42% chose guava). Here the fruit items containing host eggs presented to a wasp were either a single guava with four host egg clutches, or two kumquats, each with two host egg clutches. We used two kumquats because of their smaller sizes, which cannot support the development of four clutches of host eggs.

At the end of each session, we marked all fruit items that contained host eggs; at the end of each day, we placed all fruits from the same wasp on top of a small, plastic container with a screen lid, which was positioned inside a larger plastic container with a thin layer of dry sand at the bottom and a screen cover. Liquid from the fruits was drained from the small container when necessary on subsequent days. A month after the behavioral part of the experiment, we removed all pupae and larvae from the sand and decomposed fruit and
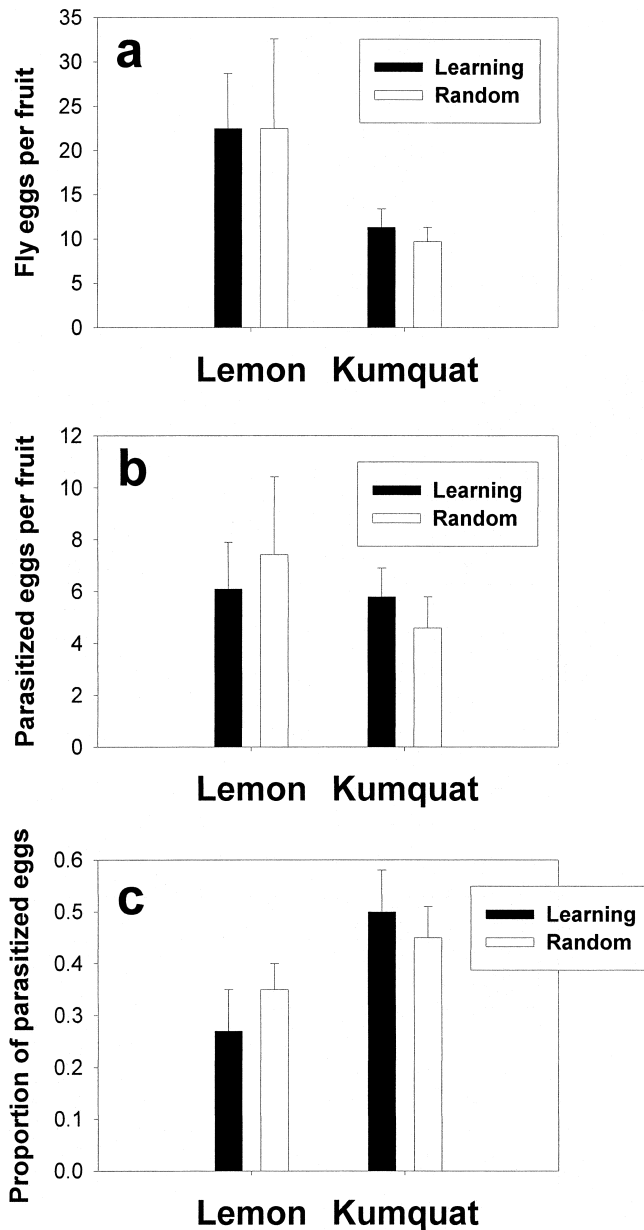
Figure 2
(a) The average (+SE) number of fly eggs per fruit encountered by wasps of each treatment group, (b) the mean number of eggs parasitized per fruit, and (c) the average proportion of eggs parasitized per fruit.

Figure 3
The percentage of learning wasps that chose fruit containing fly eggs in each of the six sessions of experiment 2 and 3. Sessions 1–3 were on day 1 and sessions 4–6 were on day 2. The expected random choice was 50%.

did not forget overnight, as indicated by the high score at the first session on day 2 (session 4). The overall scores of individual wasps ranged between 2/6 to 6/6, or 33–100% correct choices, with an overall mean of 85 ± 6% (mean ± SE).

Fitness. The number of wasp offspring was significantly higher for the learning than for random group ($F_{1, 15}$ = 5.2, $p < .05$; Figure 4b). The effect of fruit type was nonsignificant ($p > .4$).

DISCUSSION

In the controlled test for associative learning, wasps showed a good ability to associate hosts with fruit type after experiencing each fruit type for 15 min (Figure 1), consistent with previous results from parasitoid wasps and other insects (e.g., Dukas, 1998; Lewis and Takasu, 1990; Tully, 1996; Turlings et al., 1993). Here we extended such previous findings by evaluating whether these short-term effects of learning on choice are replicated when wasps are allowed to engage in host-finding behavior over a long time, and whether such long-term use of learning translates into a fitness advantage compared to the null model of random choice with long search duration.

Wasps that used learning for choosing host substrate consistently chose the fruit type they had experienced with host eggs throughout the 2-day experiment (Figure 3). This indicates that various interfering activities and long time intervals do not hinder the expression of learning typically revealed in short-term studies. Moreover, the long-term use of learning translated into significantly higher fitness than the null model of random choice (Figure 4). This was the case when fitness was measured either as the number of eggs laid (experiment 2) or as the number of adult offspring (experiment 3).

In experiments 2 and 3, learning wasps that chose a fruit with no host eggs and random wasps assigned to a fruit with no host eggs spent the whole time period with that fruit. Effectively, this aspect of the protocol implied a long search duration. In the field, such search consists of time spent on a fruit searching for host eggs and time spent flying to fruit and choosing another fruit to land on. Our laboratory simulation did not include these two separate components. In the field, the proportion of time spent searching in relation to the time spent egg laying depends on factors such as fruit and fly-egg densities, the spatial distributions of these resources, a wasp's ability to fly long distances, and weather conditions. Under

placed the pupae in small, plastic cups with a thin layer of sand at the bottom and a plastic lid with a few small holes. A month afterward, we counted all adult wasps. We replicated the experiment three times, the first replicate including two learning and two random wasps and the other two replicates having four wasps in each treatment. Hence, for each treatment group, we had a total of 10 wasps, 5 experiencing guava and 5 experiencing kumquat with host eggs.

Results
Behavioral observations. During the initial test, 8 out of the 10 wasps of the learning treatment chose the type of fruit that had contained eggs during the preceding sampling. Subsequent choices were 80–90% correct over the six sessions spanning over 2 days (Figure 3). As in experiment 2, the wasps
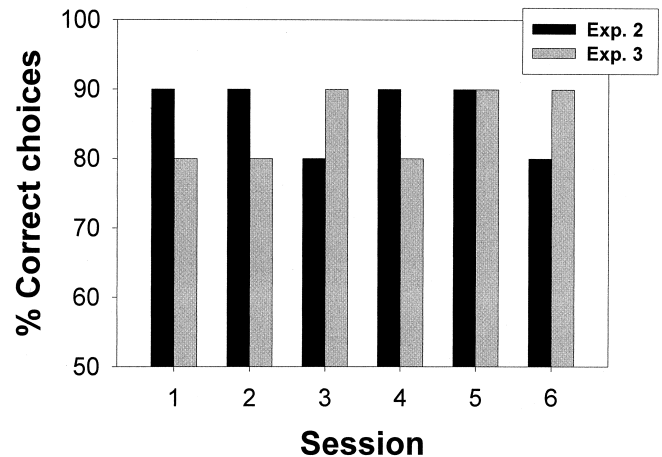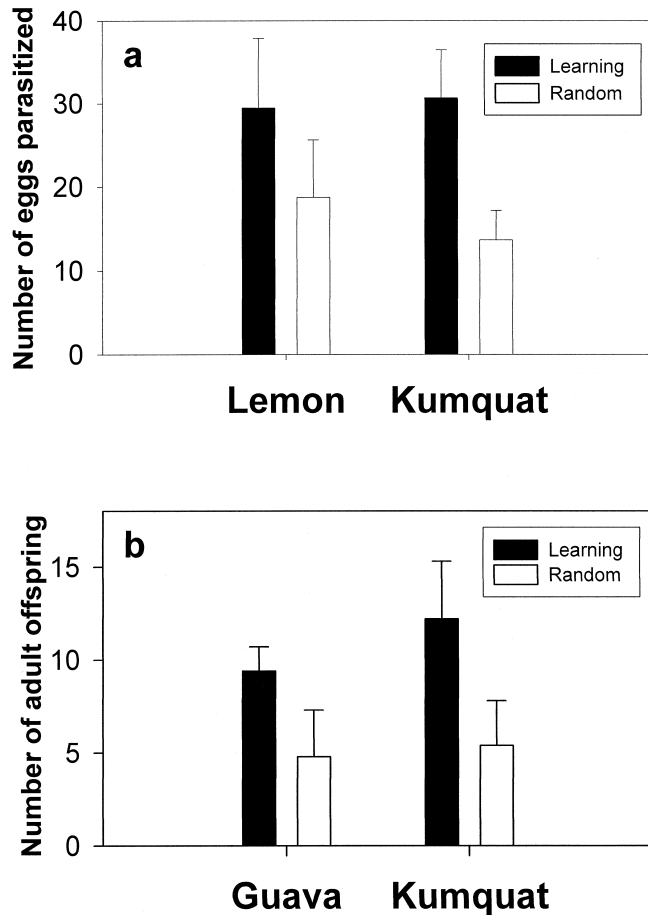
Figure 4
(a) The average number of host eggs parasitized by wasps in experiment 2, and (b) the average number of adult offspring of wasps in experiment 3.

some natural settings, which we simulated here, search duration may be relatively long. In other words, our experiments represented one extreme within a wide range of possibilities, the one which would maximize our chances of finding positive effects of learning on fitness. This is legitimate because, first, we have documented the existence of feasible conditions where learning positively affects fitness, and second, we verified that other aspects of parasitoid behavior and physiology allow for learning to positively affect fitness.

The random wasps encountered fruit with host eggs at a lower frequency than the learning wasps (Figure 3). Hypothetically, the random wasps could compensate by laying more eggs than learning wasps once on a fruit with host eggs. Alternatively, if experience on the fruit strongly affects parasitization rate once a wasp is on a fruit with host eggs, the random wasp could lay fewer eggs on a host-infested fruit. However, neither possibility is supported by the data, which shows no between-treatment difference in the number or proportion of eggs parasitized per fruit (Figure 2b,c).

In experiment 2, we examined actual availability of host eggs and patterns of parasitism by individual wasps (Figure 2). Because we could only estimate the number of egg clutches flies actually laid in fruit, we discovered only at the end of the experiment the unintended outcome of a larger average number of eggs in lemon than kumquat (Figure 2a). This difference, however, did not translate into a larger number of parasitized eggs in lemon than in kumquat (Figure 2b), perhaps because wasps were limited by the rate of finding and para-

sitizing eggs once on a fruit with host eggs. Alternatively, wasps may have used a danger-spreading strategy of limiting the number of eggs laid per single fruit.

Although it seems obvious that learning should have positive effects on fitness, evidence in support of that assertion in any species is rare. The only relevant studies we are familiar with are two rather restrictive experiments documenting that, in a single test following training, learning was associated with increased reproductive success of male fruit flies and fish (Gailey et al., 1985; Hollis et al., 1997). In addition, Dukas and Bernays (2000) have recently documented that learning increased the growth rate of grasshoppers in an experiment that lasted over 12 days of the last nymphal stage. The scarcity of published data may indicate researchers' failures to document positive fitness consequences of learning because negative results typically remain unpublished. Indeed, one of us (R.D.) failed twice to obtain positive results in experiments similar to the ones described below. In both sets of experiments, fruit flies (*D. melanogaster*) and the parasitoid wasp *Diachasmimorpha tryoni* showed weak tendencies to use experience in the more realistic settings required for the fitness test, even though both species show significant associative learning under less realistic, highly controlled conditions used in typical learning tests (Davis, 1996; Dukas, 1999; Tully, 1996). In both experiments, individuals appeared to rely mostly on innate biases: attraction to yeast odors and conspecifics by fruit flies and attraction to decomposed fruit by the parasitoids (Dukas R, unpublished data). These failures suggest that at least in some insects and in some situations, fitness consequences of learning may be small and thus difficult to quantify empirically even in the experimental settings where learning should be advantageous.

When will learning enhance fitness?

Our results allow us to evaluate the conditions under which learning can have a positive effect on fitness. First, the environment must consist of patterns that can enhance performance if learned (e.g., Dukas, 1998; Stephens, 1993). In our protocol, we used the realistic scenario of host eggs confined to one out of two available fruit types. Although we chose the simplest possible protocol, one can readily imagine more realistic situations, including a larger number of fruit types and some spatial and temporal variance. For example, instead of the zero–one protocol, host egg distribution may be a random variable with mean close to zero in one fruit type and close to one in the other, with the means changing slightly over time. It is known from experiments with bumble bees that such variance decreases learning rate (Dukas and Real, 1993), but we do not know the relation between variance and learning in parasitoid wasps; neither do we know the threshold of variance that would eliminate positive effects of learning on fitness compared to random choice. Moreover, we know little about the magnitude of spatial and temporal variance in the field, although this is critical for evaluating the relative importance of learning in nature. Further studies evaluating natural variation in host distribution and the effect of variance on learning are much needed. Meanwhile, our results may be taken as the extreme case documented under no variance, which informs us about the maximum possible positive fitness effect of learning.

A second condition needed for learning to have a positive effect on fitness is that an animal must possess sufficiently robust sensory, learning, and memory abilities that allow it to make clear associations between a stimulus and an environmental state and remember that association until the next times that association is relevant. That is, it is feasible that an individual can learn a certain association and use it immedi-

ately but not after getting involved in other tasks. For example, it may be that after spending a couple hours laying eggs on one fruit, a parasitoid would just move to the next closest fruit item, paying no attention to the fact that this fruit type had not contained host eggs in earlier sampling. In our experiment, such a hypothetical situation could result in learning wasps choosing a correct fruit type immediately after the short sampling periods in session 1, but choosing randomly at the start of session 2 and subsequent sessions, a pattern that we did not observe.

A third condition required for learning to positively affect fitness is that learning must result in significant time savings that can be used directly or indirectly for additional reproduction. For example, learning can allow an individual to restrict host search to a subset of the available substrates (e.g., Papaj and Vet, 1990), but random search may be as successful if it takes a relatively short time to visit and abandon empty substrates. In our experiments, a wasp was allowed a single choice per session, and this implied a relatively long search duration for the wasps (of either treatment) that chose a fruit devoid of host eggs. In the field, such search has the two components of searching for fruit and searching for host eggs once on a fruit. One can imagine cases where search is shorter than simulated in our experiments, and hence the positive fitness contribution of learning is lower than we documented.

Similarly, if the rate of egg laying or the total number of eggs laid is a limiting factor (e.g., Heimpel and Rosenheim, 1998), time savings may not translate into increased egg laying. For example, *B. arisanus* is proovigenic, meaning that egg load at maturity may be close to the maximum number of eggs a wasp can lay (Flanders, 1950; Jervis and Kidd, 1986). Therefore, it is possible that under natural settings, advantages of learning may be masked due to egg limitation if nonlearners merely have somewhat lower egg laying rate but they lay a similar number of eggs over a longer period.

Finally, mortality due to predation or abiotic factors can either diminish or magnify the fitness effect of learning. If mortality rate is high and equally affects learners and nonlearners, it may be more difficult to detect the fitness benefit of learning in the field. Alternatively, if mortality rate is higher during flying between substrates and searching for hosts than during egg laying, the fitness benefits of learning may be more substantial if learners spend less time searching. It is difficult to include predation in an experiment such as ours, but it would be useful to evaluate the relative mortality risk while flying between substrates, searching for hosts on a substrate, and during probing and egg laying. Such knowledge will help us evaluate the degree to which learning can increase fitness in natural settings.

In our experiments, the random treatment consisted of wasps capable of learning but prevented from expressing it. One could argue that these wasps paid the physiological cost of possessing a learning machinery (see DeWitt et al., 1998) and that it would be more appropriate to compare learning individuals to ones that do not possess learning ability. Although this is correct, it is perhaps technically unfeasible. Moreover, although learning probably incurs a physiological cost, perhaps the ecological cost of learning is typically the dominant one. The ecological cost consists of the time devoted to sampling the environment and using inferior alternatives. It may also include errors and continuous sampling throughout life, which may be associated with heightened predation risk as well. Ultimately, it will probably be possible to evaluate the physiological and ecological costs of learning by identifying and studying closely related species that are either capable or incapable of learning. Preliminary studies in mollusks and parasitoid wasps indicate that such between-species

variation might indeed exist (Potting et al., 1997; Wright et al., 1996).

To further understand the evolution and ecology of learning abilities, we must document how and to what extent learning affects animal fitness in nature. Conducting a sufficiently controlled experiment on fitness consequences of learning under fully natural settings is difficult. Here we provided a foundation for such line of research by documenting that learning can have a positive effect on a parasitoid's fitness under simulated laboratory settings. In the near future, we intend to extend our approach to natural conditions. Simultaneously, we plan to quantify the ecological parameters that would determine the potential for learning to have positive effects on an insect's fitness in the field.

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