most common occlusal syndrome among American youths (1); in monkeys on hard diets the palate broadens as it gets higher.
18. Principal components analysis shows higher covariance in animals on hard diets (eigenvalue one is 4.65 and 4.21 for monkeys on soft diets) and less dispersion away from that common growth vector (eigenvalue two is 1.11 and 1.34, respectively).
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Associative Learning in Egglaying Site Selection by Apple Maggot Flies

Abstract. *Evidence is presented demonstrating that associative learning during oviposition in* Crataegus *or apple hosts can significantly influence the propensity of apple maggot flies to accept or reject these hosts in future encounters. The data suggest that within resource patches of a given host type there may be an enhancement of foraging efficiency.*

Insects of several orders undergo adaptive changes in behavior as a result of experience (that is, learning in its broadest sense) (*1*). For example, previous experience plays an important role in oviposition site preference pattern of certain hymenopterous parasitoids (*2*). The female, after having found and parasitized a few individuals of a given host type, learns to associate particular physical or chemical cues with that type, measurably increasing subsequent preference for it. Until now, the only strong evidence for associative learning in oviposition site selection in nonparasitoid insects is in *Drosophila* (*3*). Studies on butterflies (*4*) have been suggestive but inconclusive. In this report on oviposition of the apple maggot fly, *Rhagoletis pomonella*, into host *Crataegus* and apple fruit, we present data showing associative learning in egglaying site selection in a herbivorous insect.

Table 1. Acceptance of fruit for oviposition by *R. pomonella*. Flies tested in the laboratory were mature but had not been previously exposed to fruit. Flies tested in the field experiment were in an apple orchard that was at least 1 km from other fruit hosts of *R. pomonella*.

Test type	Fruit type	N	Percentage accepting fruit	P*
		Laboratory experiments		
Two-choice	*C. mollis*	48	94	
	Apple	56	46	≤ .001
One-choice	*C. mollis*	50	90	
	Apple	50	36	≤ .001
		Field experiment		
One-choice	*C. mollis*	50	0	
	Apple	50	62	≤ .001

*P values refer to comparisons between first and second items in each experiment.

Table 2. Acceptance of a fifth fruit for oviposition by trained (+) and untrained (−) flies. A fly was considered trained if it attempted oviposition in each of the first four fruits offered, and untrained if it did not attempt oviposition in at least one of the first four fruits offered.

First four fruits offered	Fly trained	Fifth fruit offered	N	Percentage accepting fifth fruit	P*
		Experiment 1			
Apple	+	*C. mollis*	29	52	
C. mollis	+	*C. mollis*	47	91	≤ .001
C. mollis	−	*C. mollis*	29	83	≤ .025
		Experiment 2			
C. mollis	+	Apple	49	20	
Apple	+	Apple	26	92	≤ .001
Apple	−	Apple	49	49	≤ .01
		Experiment 3†			
Apple	+‡	*C. viridis*	15	6	
C. viridis	+	*C. viridis*	17	94	≤ .001

*P values refer to comparisons with first item in each experiment. †All flies had trained on *C. viridis* and rejected apple when offered it as a fifth fruit 3 days prior to training under this protocol. ‡Nine of 24 flies failed to train on apple; 7 of 24 failed to train on *C. viridis*.

Flies used in laboratory assays emerged from puparia formed by larvae that infested apples collected from Orchard Hill trees in Amherst. At the time these assays were begun, the flies were mature but had not been previously exposed to fruit (hence, "naive"). In the two-choice test, a randomly selected fly was caged with one apple and one *Crataegus mollis* fruit for as long as fruit visits continued. Each fly was removed 5 minutes after the last fruit visit. In other laboratory tests, each randomly selected fly was offered a single fruit and permitted to remain there until it either accepted the fruit (attempted oviposition) or rejected it (left without attempting oviposition). If the fly neither accepted nor rejected the fruit within 10 minutes, these data were excluded from the analysis.

Each fly assayed in field tests had just finished ovipositing in a Red Delicious apple on an Orchard Hill tree and was offered, under the same protocol as in laboratory tests, a single fruit for acceptance or rejection.

In the two-choice test in the laboratory (Table 1) flies demonstrated a significant ovipositional preference for *C. mollis* over apple (*5*). Similarly, in a one-choice test in the laboratory (Table 1) flies exhibited a significantly greater propensity to attempt egglaying in *C. mollis* than in apple. In contrast, in a one-choice test in nature (in which we offered fruits from the same batches and on the same days as offered in the laboratory), not one of 50 flies observed on apple trees attempted oviposition in *C. mollis* (Table 1) (*6*).

There are at least two possible explanations for the difference between these laboratory and field results. (i) The ovipositing flies on apple trees were individuals that had a propensity—either genetic or based on larval induction—to oviposit in apples or (ii) the ovipositing flies on apple trees had learned to accept apples and reject other potential hosts such as *C. mollis*. We believe the former explanation to be unlikely on the basis that (i) the flies observed in the laboratory originated as larvae the previous year from the same trees on which the flies observed in the field were found ovipositing; (ii) in laboratory assays, fewer than 3 percent of naive *R. pomonella* flies from different wild populations originating as larvae from apple exhibited a greater propensity to attempt oviposition in apples than in *C. mollis* (*7*); (iii) Orchard Hill trees bore abundant fruit and were isolated by 1 km or more from other hosts of *R. pomonella* flies, rendering substantial fly emigration (*8*) or immigration (*9*) unlikely; and (iv) preliminary evidence suggests lack of influence of induction during the larval stage of *R. pomonella* on the host acceptance pattern of ovipositing adults (*7*).

To substantiate that associative learning can play a significant role in the oviposition behavior of apple maggot flies, we conducted laboratory tests in which naive flies were trained experimentally. Training consisted of four successive ovipositions (2 minutes apart) in fruit of the same type. Two minutes afterward, the fly was offered a fifth fruit of a different or the same type. Flies trained on apple significantly rejected *C. mollis* as a fifth fruit, compared with flies trained on *C. mollis* or compared with randomly selected untrained flies offered a succession of five *C. mollis* fruits (experiment 1 in Table 2). Likewise, flies trained on *C. mollis* significantly rejected apple as a fifth fruit compared with flies trained on apple or compared with randomly selected untrained flies offered a succession of five apples (experiment 2 in Table 2).

Our final test was aimed at determining if a female could, through training on one fruit type, learn to reject a second type and then, after several days without fruit, be trained on the second type to reject the first. We initially offered flies *C. viridis* fruit (our supply of *C. mollis* had been exhausted) and selected for training on *C. viridis* those flies that attempted oviposition in it initially. Forty-eight flies that were trained on *C. viridis* and that rejected apple when it was offered as a fifth fruit 2 minutes afterward were maintained in cages without fruit for 3 days. We then attempted to retrain 24 of these flies on apple and 24 once again on *C. viridis*. A majority in each group became retrained. Those retrained on apple significantly rejected *C. viridis* as a fifth fruit, compared with those retrained on *C. viridis* (experiment 3 in Table 2).

The results of our laboratory tests, and other tests (not shown), demonstrate that, as a consequence of previous oviposition experience on apple or *Crataegus* hosts, apple maggot flies can learn to accept or reject these hosts in future encounters. We believe that such associative learning accounts to a large extent for our finding, in field experiments, of total rejection of *Crataegus* fruit offered to the flies ovipositing on apple trees. We do not know whether apple maggot flies are capable of forming a true search image for a particular host type, as is the case for blue jays foraging for insect prey (*10*). Nor do we know (i) whether all flies from all populations can learn equally well; (ii) how strong the host stimulus needs to be and how frequently it must be experienced to elicit learning; (iii) for how long a time memory of learning is retained under different degrees of prior experience; (iv) whether the stimulus that elicits learning is principally physical or chemical, or both; (v) to what extent learning influences apple maggot fly foraging behavior in nature under a variety of conditions (*6, 8*); and (vi) whether additional learning occurs through adult experience prior to maturity.

As postulated by Rausher (*11*), there may be at least three possible selective advantages of learning in oviposition site selection by a herbivorous insect. First, it may permit a female to concentrate its search effort on the host species of greatest local abundance (presuming the most abundant species to be the one encountered first), thereby decreasing the probability that the female will waste energy searching for a rarer host. Second, it may reduce dispersal, and hence mortality associated with dispersal, particularly when hosts are rather patchy in distribution. Third, it may enhance fitness in instances where survival is greater under successive generations of larval development on the same host rather than on different hosts, provided the first host encountered is likely to be the species on which the female grew as a larva. Present knowledge is insufficient to determine which of these advantages might accrue to *R. pomonella*, although we feel justified in proposing that associative learning enhances fly foraging efficiency within resource patches of a given host type.

Our evidence, along with that suggestive of associative learning in certain butterflies (*4*), leads us to predict that this phenomenon may be more widespread among insect herbivores than is realized. The overall behavioral similarity between apple maggot and the Mediterranean fruit fly, *Ceratitis capitata* (*12*) hints that learning might play a role in oviposition site selection in that species as well. In these as in other agriculturally important insects, associative learning, if it occurs, could bear strongly on the effectiveness of current and future pest management practices (*7*). Learning could also be a factor in the hypothesized sympatric speciational process in apple maggot flies (*13*), although present knowledge of both learning and speciational processes is too meager to justify speculation about the possible nature of the relation. Finally, associative learning in oviposition site selection in the apple maggot fly represents one more manifestation of the influence of previous experience (*14*) on the behavior of this insect.

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