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Effects of age, diet, female density, and the host resource on egg load in *Anastrepha ludens* and *Anastrepha obliqua* (Diptera: Tephritidae)

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Abstract

Oocyte counts, used as a measure of egg load, were compared among three different age groups (15, 30 and 45 days) of two polyphagous species of tephritid fruit flies, *Anastrepha ludens* and *Anastrepha obliqua*, which were exposed to varying conditions of diet (sucrose vs sucrose and protein), availability of oviposition substrate (present vs absent), adult female density (1, 2 and 4 females/cage), and semiochemical context (presence vs absence of male pheromones and fruit volatiles). In both species, oocyte counts were higher in older females and for females fed sucrose and protein than for females fed sucrose only. The presence of artificial oviposition substrates influenced oocyte counts in *A. obliqua*, but not in *A. ludens*. Female density influenced oocyte counts in both species. Females maintained in groups had higher egg loads than isolated females. Finally, preliminary evidence suggests that semiochemical context influenced oocyte counts. Counts were highest for females in a room containing both fruit volatiles and male pheromone, lowest for females in a room containing neither volatiles nor pheromone, and intermediate for females in rooms containing either volatiles or pheromone but not both. Our results suggest that egg load is influenced by environmental factors in different ways in these two species. Egg load in *A. obliqua*, a species whose host fruits are highly ephemeral, is responsive to access to the host resource. By contrast, in *A. ludens*, a species infesting less ephemeral fruit, female density and age played a more important role than host stimuli. The role of ovarian maturation and oviposition in mediating these effects, as well as implications for mass rearing and pest management, are discussed.

Keywords: *Anastrepha*; Tephritidae; Oogenesis; Nutrition; Egg load; Social facilitation

1. Introduction

Oviposition in phytophagous and parasitic insects is a dynamic process in which individual females respond to variation in both host quality and availability in functional ways (Papaj and Rausher, 1983; Mangel and Roitberg, 1989; Godfray, 1994). A female's egg load (defined here as the number of mature oocytes in the ovaries) is regarded as an important determinant of ovipositional dynamics [rev. Minkenberg et al. (1992); though see Papaj (2000) for exceptions]. A number of behavioral traits are influenced by egg load, including the persistence with which females forage for oviposition sites, the probability that a host is accepted once found, and even the size of a female's clutch.

There is increasing evidence that, in many insects, the processes of ovarian maturation that contribute most immediately to egg load are dynamic as well, also responding to variation in host quality and availability in functional ways (rev. Papaj, 2000). For example, low host availability or quality appears to reduce egg production in various insect species (Fletcher et al., 1978;

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Fitt, 1986; Kostál, 1993; Hopkins and Ekbom, 1996). Sometimes effects of the host on ovarian maturation are due to nutrients obtained by feeding on the host itself. In other instances, maturation is cued by oviposition, such that whatever affects oviposition, in relation to host quality and availability, in turn affects ovarian maturation (Fletcher and Kapatos, 1983). In still other instances, the host appears to have direct sensory effects on maturation that are independent of feeding or oviposition on the host (Alonso-Pimentel et al., 1998; Lachmann and Papaj, 2001).

Aside from the host resource, a variety of other factors modify ovarian maturation. For example, a lack of protein in the diet delays development in many insects, including tephritid fly species (Hagen, 1953; Cangussu and Zucoloto, 1992; Blay and Yuval, 1997; Wheeler, 1996; Jácome et al., 1999). Similarly, mating status and social context influence the number of eggs that a female tephritid matures and eventually oviposits. In female *Bactrocera tryoni* (Frogg.), for example, rate of ovarian maturation increased when males were present and when the numbers of females per cage increased (Pritchard, 1970). The presence of males has been reported to increase oviposition rates, as well as total fecundity, in *Rhagoletis pomonella* (Opp and Prokopy, 1986).

This study had two basic aims. The first was to determine the extent to which effects of adult diet, host stimuli, and female density interact to influence ovarian development in *Anastrepha* flies. While numerous studies of insects have examined these factors singly in insects (Fletcher et al., 1978; Fletcher and Kapatos, 1983; Prokopy et al., 1995; Jácome et al., 1999), analyses of interactions among factors are rare (rev. Papaj, 2000). Yet recent work in our laboratory suggested to us that biotic and abiotic factors commonly interact to regulate fly physiology and behavior in a variety of important ways. For example, temperature, light intensity and food quality can modulate the time of day during which a female oviposits (Aluja et al., 1997) or can influence mating behavior (Aluja et al., 2000a).

A second and related aim of this study was to compare effects of these factors on ovarian development in two closely-related species, which differ in oviposition strategies. The Mexican fruit fly, *Anastrepha ludens* Loew, and the West Indian fruit fly, *Anastrepha obliqua* Macquart belong to the same taxonomic group (the *fraterculus* group) within the family Tephritidae (Norrbom et al., 2000). Both species are polyphagous, their larvae feeding on fruits of both economic and non-economic families of plants (Norrbom and Kim, 1988; Aluja, 1994; Aluja et al., 2000b). Although *A. ludens* has a longer life span, lifetime fecundity is similar for both species (Liedo et al., 1992). Ovarian development in both species is similar to that reported for other *Anastrepha* species. Under laboratory conditions, ovaries in each species reach full size when females are 14–16 days old (Martínez et al., 1995; Rámirez-Cruz et al., 1996; Bressan, 1996).

These similarities notwithstanding, the two species differ in important ways. They differ, for example, in clutch size. *Anastrepha ludens* lays eggs in batches (up to 40 eggs), whereas *A. obliqua* invariably lays a single egg per oviposition bout (Aluja et al., 2000a). In addition, the two species use different hosts that differ markedly in life history. The native host fruits of *A. obliqua* (mainly in the family Anacardiaceae) are small and numerous, synchronous in maturation and therefore highly ephemeral. *Spondias mombin* (L.), for example, can produce between 2500 and 20 000 fruits on a single tree over a relatively short period of time (Sivinski et al., 1997; FDF, personal observation). The host fruits of *A. ludens* in nature [mainly in the family Rutaceae (e.g. *Sargentia gregii* S. Wats. and *Casimiroa edulis* Llave and Lex)] (Aluja et al., 2000a) are, by contrast, less numerous and less synchronous in maturation and consequently much less ephemeral. Based on these differences in host predictability, we anticipated that the two species would differ in the manner in which environmental factors influenced egg load. In particular, we predicted that *A. obliqua* would be more sensitive to the presence of host stimuli than *A. ludens*.

2. Materials and methods

2.1. Insects

The flies used in this experiment emerged from pupae that were collected in the field from infested fruit. *A. ludens* was collected from *C. paradisi* Macfad. and *A. obliqua* from *Spondias purpurea* (L.). To prevent dehydration, pupae were covered with a 3 cm layer of moist vermiculite (~60% humidity).

2.2. Experimental factors

We manipulated a suite of environmental variables potentially affecting ovarian development in the two *Anastrepha* species:

1. diet;
2. presence of oviposition substrate;
3. female density;
4. male pheromone; and
5. host fruit volatiles (Fig. 1).

2.2.1. Factor 1

The effect of diet on egg load was assessed at two levels, well-fed (ad libitum dry sucrose and protein in 3:1 proportions) and stressed (dry sucrose only, in limited quantities). Females receiving only sucrose were

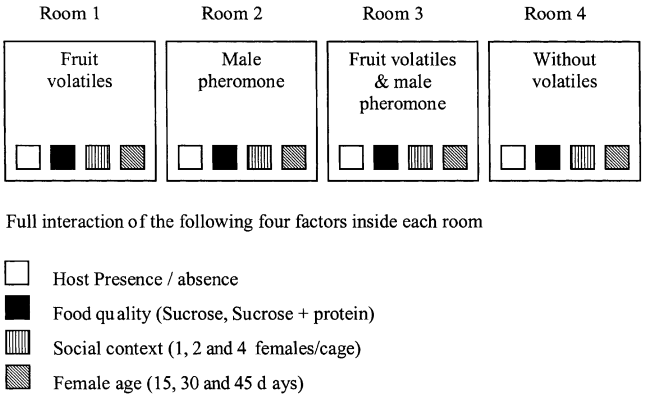

Fig. 1. Experimental design showing the four rooms with the semiochemical treatments and the full interaction of the other four factors evaluated (host presence, female flies age, food quality and social context). Room dimensions (height, length, width) were: 3.0; 2.4 (4.2 in rooms 2 and 3); and 2.1 m.

additionally starved for three days between feeding opportunities. This procedure did not result in large differences in mortality for either species, while ensuring a substantial difference between treatments in level of food quality.

2.2.2. Factor 2

The influence of an ovipositional substrate was tested using artificial hosts similar to those described by Boller (1968). These oviposition devices consisted of 2.5 cm diameter spheres of agar (Bacteriological Agar, Sigma Chemical Co. St. Louis, USA) wrapped in Parafilm (American National Can™ Neenah, WI, USA). Female flies can readily penetrate the parafilm and agar with their ovipositors and lay eggs in the agar. Agar was colored green with Colorante Alimenticio McCormick Verde (Herdez, Mexico). Eggs were not counted owing to the large numbers of agar spheres used in the study. Two full time technicians would have been needed to count the hundreds of eggs oviposited into the spheres every day. Instead, we counted the number of mature oocytes and used this number as a measure of egg load, in females grouped by treatment combination and age class (15, 30, or 45 days post-emergence).

2.2.3. Factor 3

To evaluate the effect of female density (i.e., number of females per cage) on ovarian maturation, we maintained females in 500 ml containers (cages) at densities of 1, 2 or 4. The containers were made from white plastic and had the following dimensions: 8 cm height, 11.4 cm top diameter, and 9 cm bottom diameter. We cut a 10-cm hole in all container lids and covered it with organdi cloth to permit ventilation and penetration of volatiles [sex pheromones and fruit odors (factors 4 and 5 described below)] into the cages. In those containers in which agar spheres were present, we varied the number of spheres in direct proportion to the number of females. This design generated a confound between artificial fruit number and female density. However, the design also ensured that any stimulating effect of the presence of other females was not offset by competition among females for access to oviposition substrate. Since we were particularly interested in detecting any stimulating effects of female density in the presence of an oviposition substrate, we chose to minimize any effects of competition in this way.

2.2.4. Factor 4

To evaluate the effect of male sex pheromone on egg load, cages containing 30 males of the same species as the females being tested were placed in each corner of a given experimental room containing cages of test females (for room dimensions see Fig. 1 legend). Cages with males were 30×30×30 cm in size and had a Plexiglas frame and floor. To permit aeration, four cage walls were covered with Teflon-fiber screens. The front of the cage was covered with nylon stocking.

2.2.5. Factor 5

To evaluate the effect of volatile compounds emanating from fruits on egg load, 5 kg of mature fruit were placed in the four corners of a given experimental room containing cages of test females (for room dimensions see Fig. 1 legend). The mango cultivar Tommy Atkins was offered to *A. obliqua* and the grapefruit cultivar Marsh to *A. ludens*.

2.3. Experimental design

This study employed a multi-factorial experimental design in which diet, oviposition substrate, female density, and each kind of odor stimuli were manipulated independently of one another. Each of the first three factors was represented in each of four rooms, maintained under the same environmental conditions [mean±SEM; T=26±1°C, RH 59–72% (68.3±0.6) and light 90±10 fc]. Owing to the challenges of generating and distributing odor stimuli, the fourth and fifth factors (male pheromone and host fruit volatiles) were manipulated on a room-by-room basis (Fig. 1).

Given the large number of other treatment combinations that we intended to evaluate, it was impossible to obtain enough rooms of the required size to replicate pheromone/fruit volatiles combinations within the same period of time. Instead, we intended to employ a randomized block design in which pheromone/volatiles treatments were replicated over time, with the various pheromone/volatiles treatments systematically reassigned to rooms over successive replications. Unfortunately, problems with fruit availability prevented us from replicating the pheromone/volatiles treatments in this way. As a result, the pheromone and fruit volatiles treat-

ments were, by the experiment's end, confounded with room identity. In our data analysis, we consequently inspected for trends among pheromone/volatiles combinations in each species, but refrained from drawing firm conclusions about the impact of male pheromone and fruit volatiles on egg load. Rather than consider sex pheromone or fruit volatiles as treatments under these circumstances, we treated odor combination/room as a blocking factor (Fig. 1) and analyzed the data accordingly.

2.4. Experimental protocol

Pupae were divided into four groups of equal size and placed in plastic containers. Groups of pupae were placed in one of four rooms, each characterized by a different pheromone/volatiles combination (Fig. 1). This procedure ensured that flies were exposed to odor stimuli well before emerging as adults. As soon as adults emerged, males and females were separated. Females were placed in 500 ml plastic containers (cages) and randomly assigned to a treatment regime. Adults were not removed from their emergence rooms until after days 15, 30 or 45.

At one of three ages, 15, 30, or 45 days post-emergence, any and all surviving females in a cage of a given treatment combination were isolated and frozen at −18°C. Later, we dissected females and counted number of mature oocytes as a measure of egg load. Prior to dissection, individuals were fixed in a 1% formaldehyde and phosphate buffer solution (14.2 g monobasic phosphate and 13.8 g dibasic phosphate in 2000 ml water). A single female from a given cage was then dissected, oocytes separated from ovarian tissue under a dissecting microscope, and the number of mature oocytes counted. In all, at least six females from each treatment group were dissected (a total of 2437 dissections over the entire study).

2.5. Statistical analysis

As discussed above, we treated room/odor combination as a blocking factor in the statistical analysis, and compared the effects of diet, social context, host presence, and age at sexual maturation on the response variable, egg load, using a four-way analysis of variance (ANOVA), nested within blocks (Crawley, 1993). Data were analyzed using Statistica software for Windows (Statistica, 1998. Statsoft Inc. version 5.1, Tulsa, OK).

3. Results

For *A. ludens*, a randomized-blocks four-way ANOVA incorporating age, composition of diet, host presence, and female density, as well as all possible interactions among these factors accounted for 37.4% of the overall variance in number of mature oocytes (i.e., egg load). For *A. obliqua*, an identical randomized-blocks four-way ANOVA accounted for 50.9% of the overall variance in number of mature oocytes.

3.1. General pattern over female age

In both *A. ludens* and *A. obliqua*, oocyte counts varied with female age, with counts lowest at Day 15 and highest at Day 30 (Fig. 2A; *A. ludens*; $F_{2,1340}$=5.53, $p<0.005$; *A. obliqua*; $F_{2,1017}$=4.07, $p<0.02$).

3.2. Effect of diet

By far, the single most important variable accounting for variation in oocyte count in both species was composition of diet (Fig. 2B). In both species, oocyte counts for females provided with an ad libitum diet of protein and sugar were much higher than counts for females provided limited access to sugar only (*A. ludens*, $F_{1,1340}$=684.32, $p<0.0001$; *A. obliqua*, $F_{1,1017}$=896.29, $p<0.0001$). For both species, there was a significant two-way interaction between age and diet (Fig. 3; *A. ludens*, $F_{2,1340}$=10.99, $p<0.0001$; *A. obliqua*, $F_{2,1017}$=4.24, $p<0.02$). In *A. ludens*, well-fed females had higher oocyte counts at Days 30 and 45 than on Day 15, whereas food-stressed females had slightly higher counts on Day 15 than on Days 30 and 45. In *A. obliqua*, well-fed females had distinctly higher oocyte counts at Days 30 than on Days 15 and 40, whereas food-stressed females harbored virtually no oocytes at all, regardless of age.

3.3. Effect of artificial host models

The two species differed in the effect of artificial host models on oocyte counts (Fig. 4A). In *A. obliqua*, counts were generally lower in the presence of host models than in their absence (Fig. 4A; $F_{1,1017}$=7.83, $p<0.006$); in *A. ludens*, the pattern was reversed, counts being higher in the presence of host models than in their absence. However, the differences for *A. ludens* were not significant (Fig. 4A; $F_{1,1340}$=2.26, $p>0.1$).

The effect of host model on oocyte counts in *A. obliqua* depended on age (Fig. 5; $F_{2,1017}$=7.86, $p<0.0005$). As females aged in the presence of a host model, oocyte counts declined progressively. As females aged in the absence of a host model, by contrast, oocyte counts increased.

The effect of host model on oocyte counts in *A. obliqua* also depended on diet ($F_{1,1017}$=7.90, p=0.005). As noted above, food-stressed females carried virtually no eggs, regardless of the presence of hosts; oocyte counts for ad libitum-fed females, by contrast, were

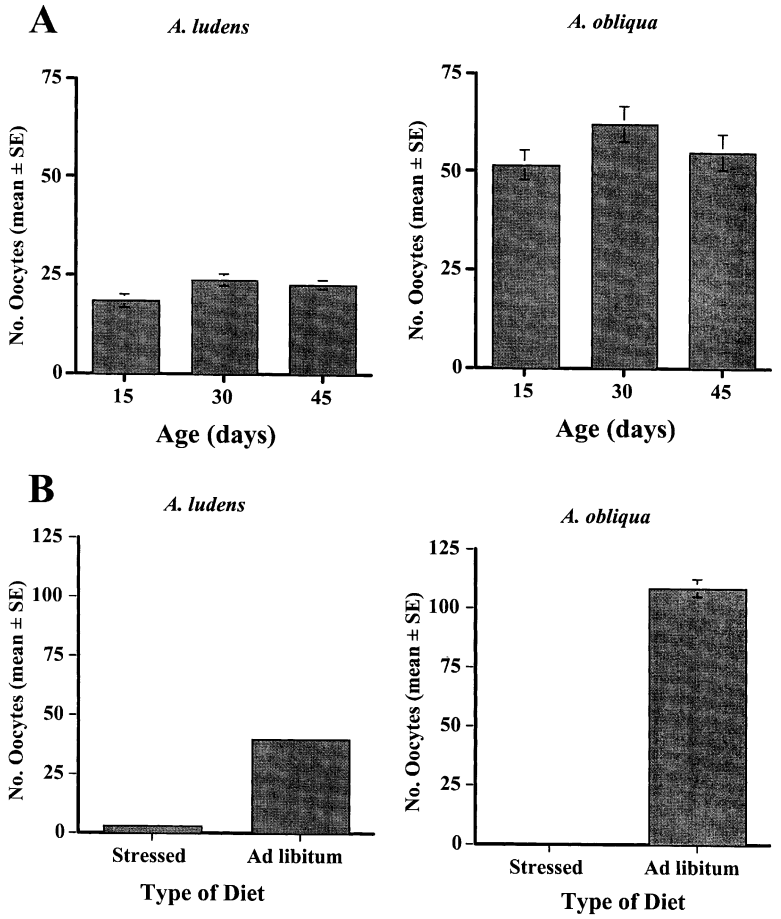

Fig. 2. Mean number of oocytes produced by *A. ludens* and *A. obliqua* females (A) at three ages and, (B) under two dietary quality conditions.

lower in the presence of host models than in their absence.

Finally, in *A. obliqua*, there was a significant three-way interaction involving age, diet and host ($F_{2,1017}$=7.28, p<0.001) and a significant three-way interaction involving age, host and density ($F_{2,1017}$=7.16, p<0.001).

3.4. Effect of female density

In both species, females maintained in groups of two and four per cage had higher oocyte counts than females maintained in isolation (Fig. 4B; *A. ludens*, $F_{2,1340}$=10.32, p<0.0001; *A. obliqua*, $F_{2,1017}$=7.90, p<0.0005). For both species, there was a significant interaction between female density and diet (Fig. 6; *A. ludens*, $F_{2,1340}$=3.50, p<0.05; *A. obliqua*, $F_{2,1017}$=8.01, p<0.0005). However, the form of the interaction differed between the species. In *A. obliqua*, the effect of female density was evident only in well-fed females, because food-stressed females produced virtually no eggs at any density. In *A. ludens*, food-stressed females produced small numbers of eggs and, in fact, the effect of female density was proportionally greater for food-stressed females than for well-fed ones.

In *A. ludens*, female density also interacted with age (Fig. 7A; $F_{2,1340}$=2.55, p<0.05). At Day 15, oocyte counts increased progressively with female density, being maximal at densities of four females. At Days 30 and 45, by contrast, oocyte counts were maximal at densities of two females. In *A. ludens*, female density also interacted with host model presence in a significant way (Fig. 7B; $F_{2,1340}$=3.87, p<0.03). When hosts were present, oocyte counts increased over female density to a maximum value at the highest female density. When hosts were absent, by contrast, oocyte counts were maximal at the intermediate density of two females. Finally, this pattern was most clear-cut for ad libitum-fed females, generating a significant three-way interaction, involving diet, host and female density ($F_{2,1340}$=3.17, p<0.05).

3.5. Effect of blocking factor

For each species, oocyte counts varied from room to room, and in the same way; however, the block effect

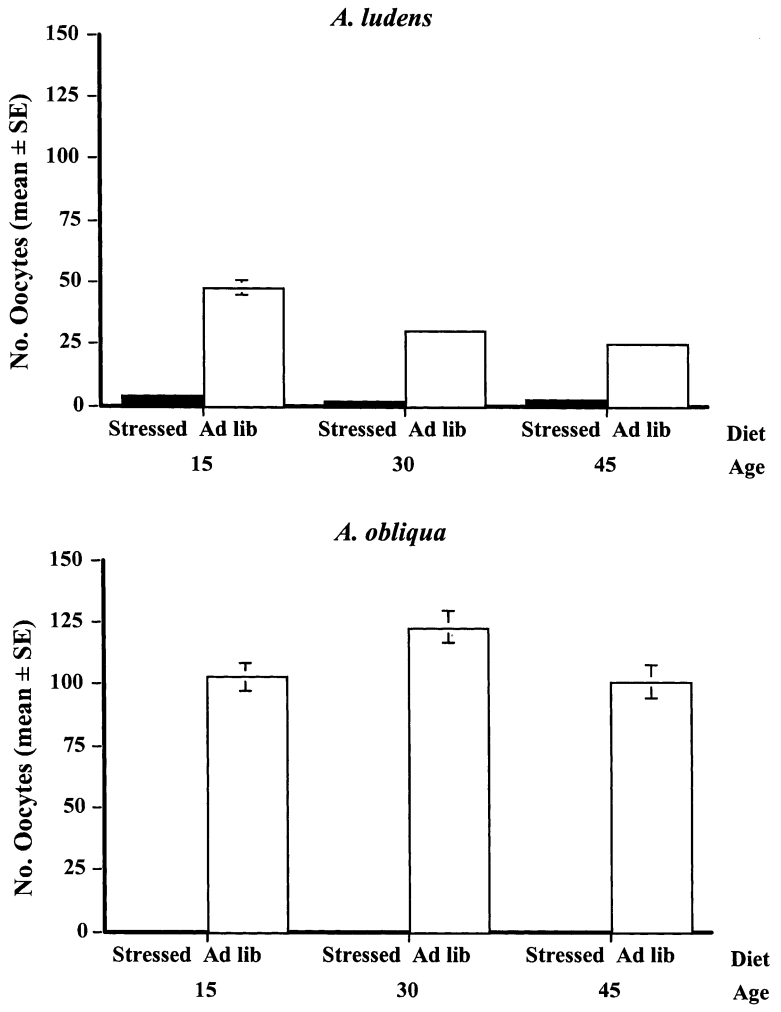

Fig. 3. Mean number of oocytes produced by *A. ludens* and *A. obliqua* females of three ages, under two dietary quality conditions.

was significant only for *A. obliqua* ($F_{3,1017}$=3.69, p<0.02). Oocyte counts in both species were highest in room 3, in the presence of fruit volatiles and male pheromone, lowest in room 4, in the absence of both fruit volatiles and male pheromone, and intermediate in rooms containing either fruit volatiles or male pheromone but not both (Fig. 8). An obvious interpretation consistent with these results, at least in *A. obliqua*, is that fruit volatiles and male pheromone independently facilitate egg production and that their facilitating effects are additive or perhaps synergistic in nature. However, because volatile/pheromone combinations could not be replicated, owing to limitations on space and fruit availability, we cannot discount the alternative interpretation that unknown differences among experimental rooms, differences having nothing to do with male pheromone or fruit volatiles, generated these patterns.

3.6. Summary of species patterns

A summary of tests of significance for the various main effects and interactions is presented in Table 1. For both species, diet, age and female density had similarly positive effects on oocyte counts. For both species, diet had a greater impact on oocyte counts than any other factor. Not surprisingly, in both species, effects of age and of female density on oocyte counts in both species depended significantly on diet (Age×Diet and Diet×Density effects, Table 1).

The two species differed chiefly with respect to the effect of an artificial host model on oocyte counts; in *A. ludens*, there was no effect whereas, in *A. obliqua*, oocyte counts were lower in the presence of a host model than in its absence. In *A. obliqua*, the strong influence of host models on oocyte counts was associated with significant two-way interactions involving age and diet (Age×Host and Diet×Host effects, Table 1). These interactions were not significant for *A. ludens*.

For both species, significant three-way interactions among factors were relatively rare, and the four-way interaction among factors was not significant (Table 1).

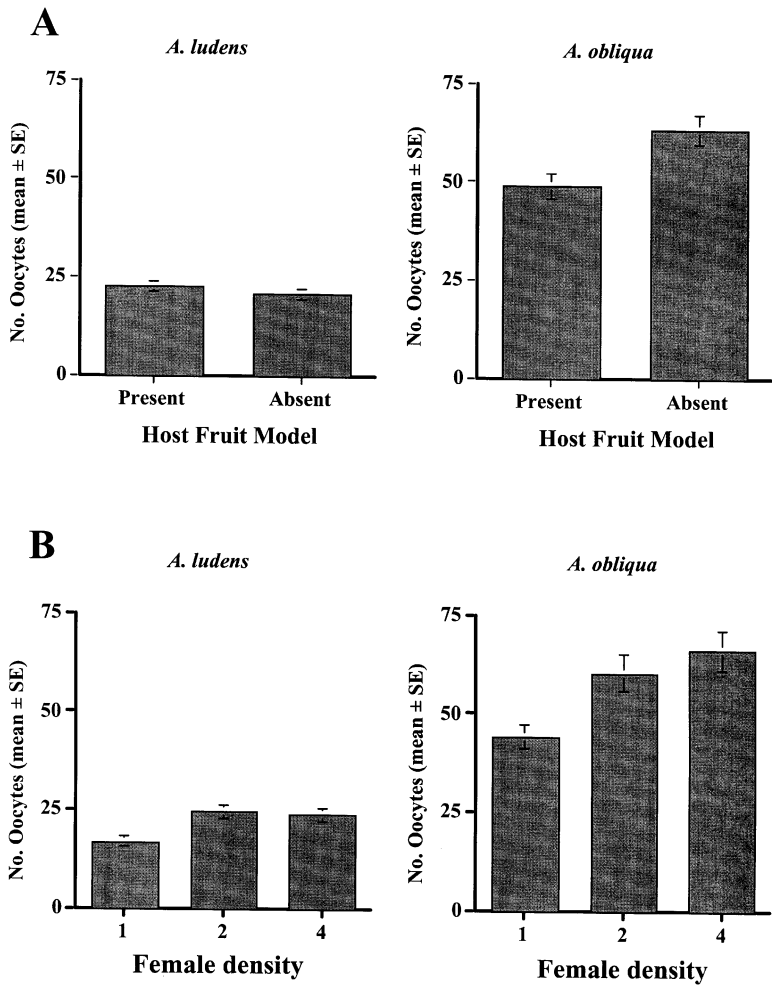

Fig. 4. Mean number of oocytes produced by *A. ludens* and *A. obliqua* females (A) with and without the stimulus of an artificial host and (B) at three female density (females/cage) conditions.

4. Discussion

4.1. Diet as a dominant factor in ovarian maturation

Adult nutrition is undoubtedly the most important factor regulating ovarian maturation in most insects, including fruit flies (Fletcher, 1989; Wheeler, 1996). Fletcher (1989) considered adult diet even more influential than temperature in that regard. Jácome et al. (1999), working with *A. serpentina* Wiedemann, found that both the quality and availability of adult food profoundly affect individual fitness. Thus, it is not surprising that in our study, where both quality and quantity were varied simultaneously, diet had a dramatic effect on egg load in both *Anastrepha* species studied. So pervasive was the effect of diet in our study that it interacted strongly with other factors in each species. Diet interacted with effects of age in both species (Fig. 6), such that well-fed females showed a clear pattern of increased egg load with age, whereas stressed females did not. The effect of female density on oocyte counts in each species similarly depended on diet, being evident only in well-fed females.

Our observation that adult protein intake is key to egg production is consistent with other results for higher Diptera (rev. Wheeler, 1996). The link between protein and ovarian maturation is a function of juvenile hormone (JH) and ecdysteroid production. The interactions of these hormones are complex and not completely understood. In *Drosophila*, starvation affects the rate of transcription of yolk-peptide genes, an effect not due directly to either JH or ecdysteroids (Bownes and Blair, 1986).

4.2. Effects of female density and 'social facilitation'

Given the importance of diet in ovarian development, increases in female density could conceivably generate competition for essential nutrients and thereby reduce rates of ovarian maturation. In our study, food was alternately so superabundant or so limited that competition among females was unlikely to influence maturation. In fact, in both study species, egg loads of females held

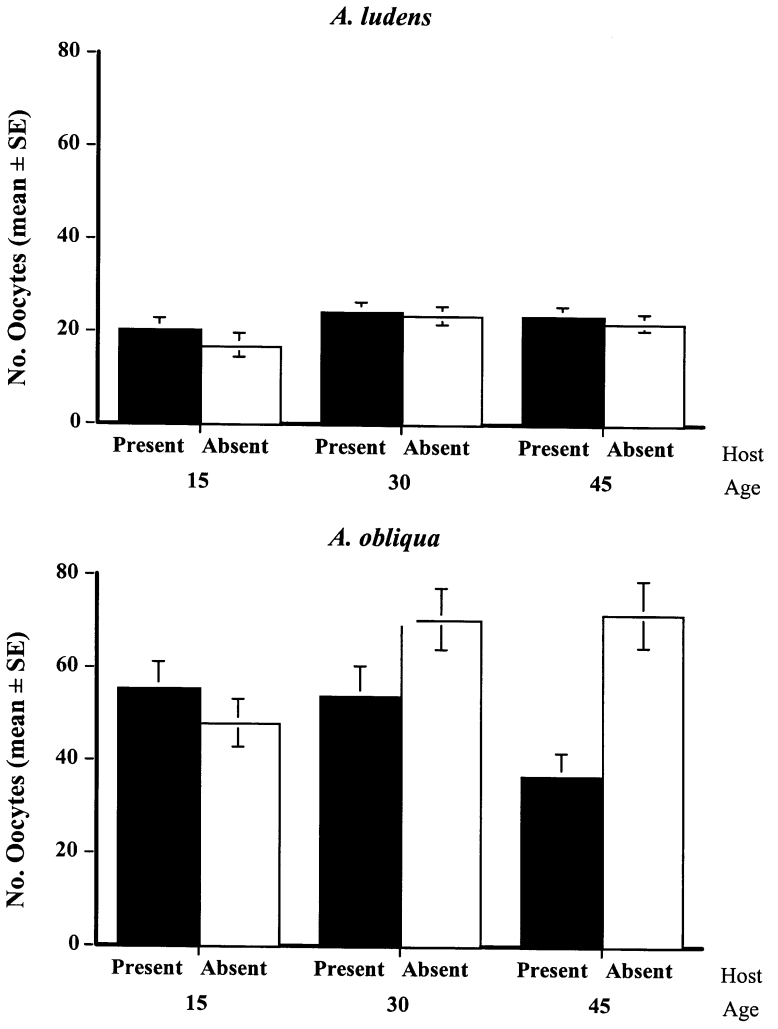

Fig. 5. Mean number of oocytes produced by *A. ludens* and *A. obliqua* females of three ages with and without the stimulus of an artificial host.

with other females were significantly greater than egg loads in isolated females (Fig. 3). This result is consistent with results of Pritchard (1970) in which *B. tryoni* females produced larger ovaries when caged in groups than when caged singly.

These results are particularly interesting in light of reports of 'social facilitation' in which oviposition is facilitated in the presence of other ovipositing females (Prokopy and Bush, 1973; Prokopy and Reynolds, 1998). In our study, the facilitating effect of female density on egg load did not require access by females to an oviposition substrate (cf Fig. 6B). We can conclude from this result that socially-mediated facilitation in ovarian maturation is not mediated by socially-mediated facilitation in oviposition. However, based on these results and on the known effects of egg load on oviposition behavior (rev. Minkenberg et al., 1992), it is reasonable to propose the converse possibility, namely that a facilitating effect of female density on oviposition may be based in part on a facilitating effect of female density on ovarian maturation.

The effect of female density on egg production may be indirect, involving, for example, an increase in protein intake by females, which in turn boosts egg production or an increase in oviposition, which triggers maturation of new eggs. Alternatively, the effect may be a direct effect, independent of protein intake. In either case, the underlying physiological mechanism is not known. It may be similar to that of cockroaches (*Blattella germanica*), where grouping releases the brain from inhibition, inducing secretion of JH and subsequently egg development (Gadot et al., 1989). Similar patterns have been observed in social insects (Wheeler, 1996).

4.3. Host deprivation and egg load

The two *Anastrepha* species studied here each responded to deprivation of an oviposition substrate over a 30–45 day period by accumulating mature oocytes (Fig. 4). Such a pattern may relate to the polyphagous habit of these species. The polyphagous species *B.*

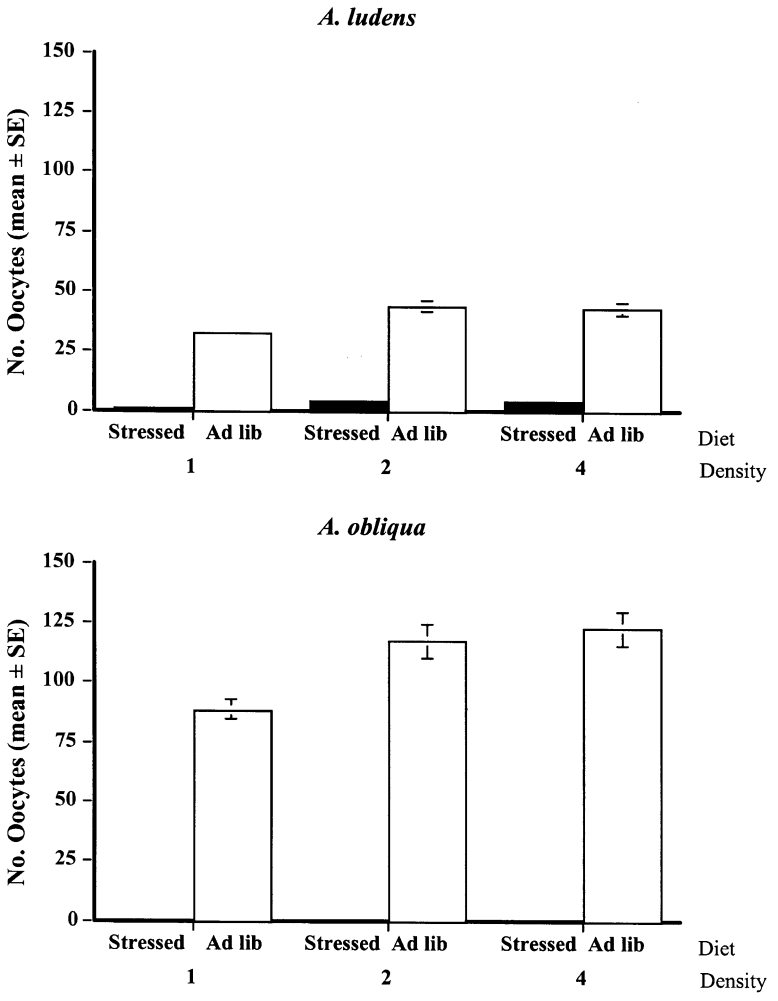

Fig. 6. Mean number of oocytes produced by *A. ludens* and *A. obliqua* females under three crowding conditions (female density), and two dietary quality conditions.

tryoni, for example, accumulates eggs when deprived of hosts, whereas egg load in the specialist species *Blattella cacuminatus*, *Blattella cucumis* and *Blattella jarvisi* does not increase under host deprivation (Fitt, 1986). Fitt suggested that the association is causal in nature, specifically that the pattern of physiological control of oocyte maturation dictates diet breadth in herbivores. He argued that specialist species stop maturing oocytes when deprived of a favored host and remain choosy; generalists accumulate eggs when deprived of a favored host and gradually become less selective as a consequence.

At odds with Fitt's perspective is a pattern observed in *Delia* fly species. In both the generalist *Delia platura* and the specialist *Delia antiqua*, rates of egg maturation appear to decrease in response to host deprivation (Weston and Miller, 1987; Weston et al., 1992). However, the mechanisms are apparently different in the two species. In *D. antiqua*, egg maturation is tied closely to egg deposition. Egg maturation slows under host deprivation simply because egg deposition decreases. In *D. platura*, by contrast, egg maturation appears to decrease under host deprivation even before unlaid eggs accumulate. As a consequence, the generalist fly may well be less likely (not more likely as Fitt predicted) than the specialist fly to accumulate eggs under host deprivation and, in so doing, relax its selectivity. Whether or not selectivity in *D. platura* changes under host deprivation has evidently not been assessed.

Fitt's arguments also imply an obligatorily negative association between egg load and selectivity, an association, which has been documented in many, though not all, insects (Minkenberg et al., 1992; Papaj, 2000). Whether or not egg load is negatively correlated with selectivity in *Anastrepha* flies is not known at present. Based on Fitt's arguments and our present results, we would predict such a correlation.

4.4. The role of access to oviposition substrate on egg load

The similarity in ovarian response to host deprivation notwithstanding, our species differed in terms of how an

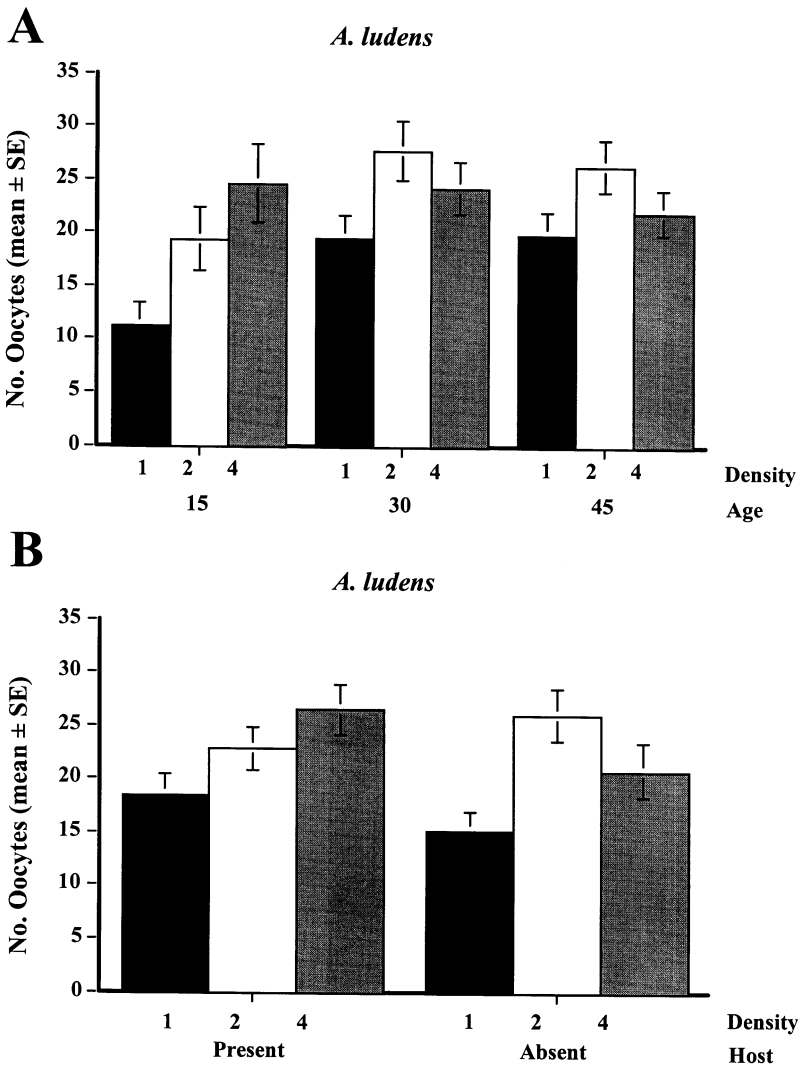

Fig. 7. Mean number of oocytes produced by *A. ludens* females under (A) three crowding conditions (female density) at three ages and (B) three crowding conditions (female density) at three ages with and without the stimulus of an artificial host.

artificial host model affected egg load. In *A. obliqua*, egg load was consistently lower in the presence of host models than in their absence. In *A. ludens*, by contrast, host models were not associated with lower egg loads; in fact, the trend was in the opposite direction.

Whether these differences reflect differences in ovarian maturation or oviposition behavior is difficult to determine. Since eggs laid into host models were not counted, it is not possible to assess the absolute impact of host models on ovarian maturation versus oviposition in either species. Nevertheless, it is reasonable to infer, based on our data, that our species differed in how the rate of ovarian maturation was balanced against the rate of oviposition. In *A. obliqua*, the rate of oviposition appeared to outpace the rate of maturation, such that egg loads declined over time in the presence of the host model. In *A. ludens*, by contrast, these rates did not differ in this way; in fact, oocyte counts tended to be higher in the presence of a host model, suggesting that the rate at which eggs were matured may have exceeded the rate at which those eggs were laid.

This apparent species difference in the balance of ovarian maturation and oviposition is somewhat surprising in light of the fact that *A. ludens* lays its eggs in batches, whereas *A. obliqua* lays its eggs singly. Intuitively, one might expect the batch-layer to be the more likely to run short of eggs in the presence of hosts. Clearly, this did not happen. Rather, the species difference is consistent with a known difference between the species in their response to variation in host density. Whereas *A. ludens* females lay a relatively constant number of eggs over time, *A. obliqua* females adjust oviposition activity according to host density (F. Díaz-Fleischer and M. Aluja, unpublished data). As hosts become available, oviposition activity in *A. obliqua* increases. The data presented here suggest that, at least under experimental conditions, oviposition by *A. obliqua* in the presence of host models may be ‘ramped up’ more than

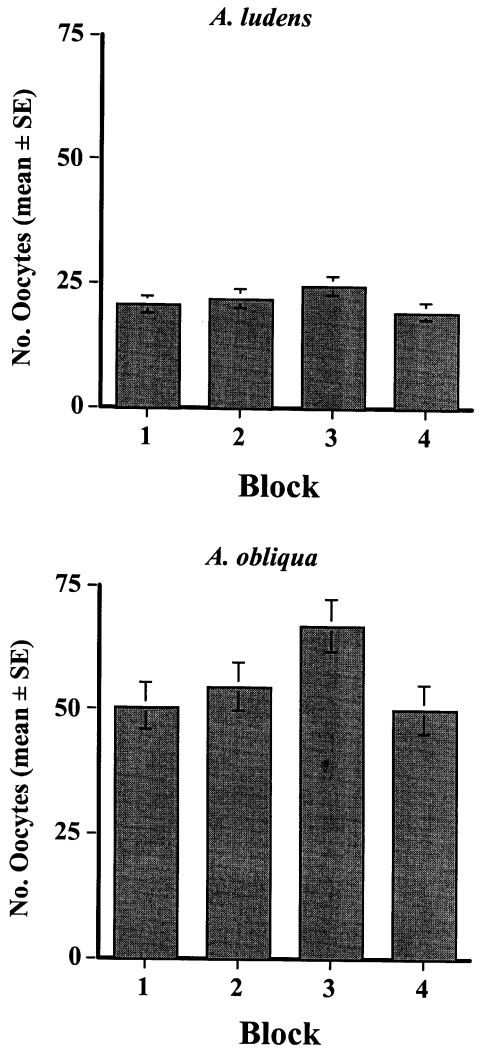

Fig. 8. Mean number of oocytes produced by *A. ludens* and *A. obliqua* females in each one of the four blocks (semiochemical context): 1, presence of fruit volatiles; 2, male pheromones; 3, fruit volatiles and male pheromones; and 4, without semiochemical stimulants.

oogenesis, leading to the observed decrease in oocyte counts.

We suspect that the apparent species difference in the balance between oviposition and oogenesis relates to a difference in the phenologies of each species' hosts. The hosts of *A. obliqua* generally mature more synchronously than those of *A. ludens* and thus the availability of *A. obliqua*'s hosts are more highly ephemeral. We postulate that oviposition in *A. obliqua* is designed to respond rapidly to a sudden increase in host availability, to the extent that an especially rapid boost in egg deposition is traded off against the risk of running short of eggs. Possibly this rapid ovipositional response trades off against other factors affecting fitness, such as dispersal ability or long-term fecundity.

The notion that *A. obliqua* places more of an evolutionary premium on a capacity for short-term bursts in

Table 1
Summary of tests of significance in a randomized-block four-way ANOVA's run for the *A. ludens* and *A. obliqua* experiments on egg load

Effect	*A. ludens*	*A. obliqua*
Block	NS[a]	*
Age	**	*
Diet	****	****
Host	NS	**
Density	****	***
Age×Diet	****	*
Age×Host	NS	***
Age×Density	*	NS
Diet×Host	NS	**
Diet×Density	*	***
Host×Density	*	NS
Age×Diet×Host	NS	**
Age×Diet×Density	NS	NS
Diet×Host×Density	*	NS
Age×Host×Density	(*)	NS
Age×Diet×Host×Density	NS	NS

[a] NS, No signficance.
* $p<0.05$.
** $p<0.005$.
*** $p<0.0005$.
**** $p<0.0001$.

oviposition in response to sudden host availability than does *A. ludens* is consistent with a difference between the two species in what might be termed a female's 'standing egg load'. Under a given set of conditions of diet and female density, and in the absence of oviposition substrate, egg load in *A. obliqua* always far exceeded that of *A. ludens* (cf. Figs 3A, 4, 5 and 7), despite the fact that the two species are relatively similar in size.

A relatively high egg load probably confers upon *A. obliqua* a relatively high capacity to respond to a sudden increase in host availability. In terms of standing egg load, *A. obliqua* is physiologically 'prepared' for the sudden onset of high host abundance. This preparedness to lay eggs presumably trades off against something else of fitness value, such as dispersal ability or long-term fecundity. If it did not, *A. ludens* would presumably have as high a standing egg load as does *A. obliqua*.

4.5. Interactions among effects

Our study contrasts with previous studies, which analyzed effects of various environmental factors on egg load, but which were not designed to assess interactions among those factors (e.g., Prokopy and Bush, 1973; Fletcher et al., 1978; Fletcher and Kapatos, 1983; Jácome et al., 1999). Clearly, factors affecting egg load in the two *Anastrepha* species interact in significant ways. The possibility for yet more interactions among factors is suggested by patterns relating to the presence

or absence of male pheromone and fruit volatiles (Fig. 7). For both species, oocyte counts were highest in the room containing both male pheromone and host fruit volatiles, lowest in the room containing neither pheromone nor volatiles, and intermediate in the rooms containing one or the other type of odor. This pattern resulted in a statistically significant block effect in the *A. obliqua* experiment (Table 1), where male pheromone and fruit volatiles appeared to interact in an additive fashion or perhaps even synergistically to increase egg load. Since logistical constraints prevented replication of the pheromone/volatile combinations, we cannot be certain that the block effect on egg load was due to 'semiochemical context' and not to idiosyncrasies of the particular rooms to which a given semiochemical context was assigned.

That sex pheromone should promote egg load is nevertheless consistent with reports in the literature for other insects that male presence influences ovarian development. In *Rhagoletis pomonella*, for example, the presence of males increased oviposition rates and total fecundity (Opp and Prokopy, 1986). In *Delia radicum*, onset of oviposition is delayed and oosorption initiated if females are not mated (Kostál, 1993). Frequently such effects of males are mediated through mating, including transfer of nutrients from the male to the female during copulation. In such cases, mating can influence ovarian development in the same way as diet in those cases in which males transfer proteins to females during copulation.

In *Drosophila silvestris*, though, acceleration in onset of vitellogenesis in the presence of males was not due to mating (Craddock and Boake, 1992) and may instead involve a direct effect of male courtship stimuli (Boake and Moore, 1996). To our knowledge, nobody has demonstrated an effect of male pheromone alone on ovarian development in insects, so that the present findings, though not conclusive, are certainly intriguing. Whether such a phenomenon has a different underlying mechanism than an effect of mating, which involves protein transfer, may depend on the extent to which male pheromone affects protein feeding. Protein intake was not measured in our study.

Surprisingly, an influence of host volatiles on ovarian development is known in only very few cases. In *D. antiqua* (Meigen), the lack of volatile host stimulants retarded the beginning of oviposition and delayed oviposition cycles (Weston and Miller, 1987; Weston et al., 1992). In the classic work by Hillyer and Thorsteinson (1969), allylisothyocyanate was found to promote ovarian development in *Plutella xylastella* (L.), though evidence suggested that it was perceived through contact rather than olfactorily. There is strikingly little direct evidence of an effect of host volatiles on ovarian development and so the present findings, though also not conclusive, are again intriguing.

4.6. Practical implications

The results of this study are of relevance to the control of these species, both of which are insect pests. In the case of *A. obliqua*, various environmental factors (including perhaps fruit volatiles) ought to be considered in programs designed for the mass-rearing and release of sterile individuals. For example, the release of volatile stimulants in rearing areas would allow egg production periods to be established at earlier ages. It would also yield more eggs, once female reproductive cycles have been synchronized. Another interesting application is the development of traps and baits using species-specific environmental stimuli. For example, a trap combining fruit volatiles and male sexual pheromones would probably facilitate the capture of gravid and/or virgin females. However, our results suggest that the advantage of using these volatiles in trap capture would, in theory at least, trade off against a possible disadvantage associated with facilitation of ovarian maturation. Finally, our results suggest that a common strategy for controlling both *Anastrepha* species is unfeasible. Indications to date suggest that the host stimuli influencing ovarian development are similar in kind to those influencing orientation behavior in a given insect species (Papaj, 2000). We therefore suspect that the two species will vary in their orientation response to a single type of bait or attractant (visual or olfactory) because their ovarian responses to environmental stimuli in this study varied. The question of differential responses of economically important *Anastrepha* species to standard bait formulation (e.g., protein hydrolysate) had been already addressed by one of us (M. Aluja) 11 years ago (Aluja et al., 1989) and based on our results here, should be addressed in detail in future studies on bait trap development.

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References

Alonso-Pimentel, H., Korer, J.B., Nufio, C., Papaj, D.R., 1998. Role of colour and shape stimuli in host-enhanced oogenesis in the walnut fly, *Rhagoletis juglandis*. Physiological Entomology 23, 97–104.

Aluja, M., 1994. Bionomics and management of *Anastrepha*. Annual Review of Entomology 39, 155–178.

Aluja, M., Jiménez, A., Piñero, J., Camino, M., Aldana, L., Valdés, M.E., Castrejón, V., Jácome, I., Dávila, A., Figueroa, R., 1997. Daily activity patterns and within-field distribution of papaya fruit flies (Diptera: Tephritidae) in Morelos and Veracruz, Mexico. Annals of the Entomological Society of America 86, 502–520.

Aluja, M., Cabrera, M., Guillén, J., Celedonio, H., Ayora, F., 1989. Behaviour of *Anastrepha ludens, A. obliqua* and *A. serpentina* (Diptera: Tephritidae) on a wild mango tree (*Mangifera indica*) harbouring three McPhail traps. Insect Science and its Application 10, 309–318.

Aluja, M., Piñero, J., Jácome, I., Díaz-Fleischer, F., Sivinski, J., 2000a. Behavior of flies of the genus *Anastrepha*. In: Aluja, M., Norrbom, A.L. (Eds.), Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, DelRay Beach, FL, pp. 375–408.

Aluja, M., Piñero, J., López, M., Ruíz, C., Zúñiga, A., Piedra, E., Díaz-Fleischer, F., Sivinski, J., 2000b. New host plant and distribution records in Mexico for *Anastrepha* spp., *Toxotrypana curvicauda* Gerstaecker, *Rhagoletis zoqui* Bush, *Rhagoletis* sp., and *Hexachaeta* sp. (Diptera: Tephritidae). Proceedings of the Entomological Society of Washington 102, 802–815.

Blay, S., Yuval, B., 1997. Nutritional correlates of reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae). Animal Behaviour 54, 59–66.

Boake, C.R.B., Moore, S., 1996. Male acceleration of ovarian development in *Drosophila silvestris* (Diptera, Drosophilidae): What is the stimulus? Journal of Insect Physiology 42, 649–655.

Boller, E.F., 1968. An artificial egging device for the European cherry fruit fly *Rhagoletis cerasi*. Journal of Economic Entomology 61, 850–852.

Bownes, M., Blair, M., 1986. The effects of a sugar diet and hormones on the expression of the *Drosophila* yolk-protein genes. Journal of Insect Physiology 33, 941–947.

Bressan, S., 1996. Desenvolvimento e potencial reproductivo das femeas de *Anastrepha obliqua* (Macquart, 1835) (Diptera, Tephritidae) em condicoes naturais. Revista Brasilera do Entomologia 40, 11–16.

Cangussu, J.A., Zucoloto, F.S., 1992. Nutritional value and selection of different diets by adult *Ceratitis capitata* flies (Diptera: Tephriitdae). Journal of Insect Physiology 38, 485–491.

Craddock, E.M., Boake, C.R.B., 1992. Onset of vitellogenesis in female *Drosophila silvestris* is accelerated in the presence of sexually mature males. Journal of Insect Physiology 38, 643–650.

Crawley, M.J., 1993. GLIM for Ecologists. Blackwell Scientific, Oxford.

Fitt, G.P., 1986. The influence of a shortage of host of the specificity of oviposition behaviour in species of *Dacus* (Diptera: Tephritidae). Physiological Entomology 11, 133–143.

Fletcher, B.S., 1989. Life history strategies of tephritid flies. In: Robinson, A.S., Hooper, G. (Eds.), Fruit Flies: Their Biology, Natural Enemies and Control. Elsevier, Amsterdam, pp. 195–208.

Fletcher, B.S., Kapatos, E., 1983. The influence of temperature, diet and olive fruits on the maturation rates of female olive flies at different times of the year. Entomologia Experimentalis et Applicata 33, 244–252.

Fletcher, B.S., Pappas, S., Kapatos, E., 1978. Changes in the ovaries of olive flies (*Dacus oleae* (Gmelin)) during the summer, and their relationship to temperature humidity and fruit availabilty. Ecological Entomology 3, 99–107.

Gadot, M., Burns, E., Schal, C., 1989. Juvenile hormone biosynthesis and oocyte development in adult female *Blattella germanica*: effects of grouping and mating. Archives of Insect Biochemistry and Physiology 11, 189–200.

Godfray, H.J.C., 1994. Parasitoids: Behavioral and Evolutionary Ecology. Monographs in Behavior and Ecology. Princeton University Press, Princeton, NJ.

Hagen, K.S., 1953. Influence of Adult Nutrition upon the Reproduction of Three Fruit Flies Species. Joint Legislative Committee on Agriculture and Livestock Problems. Third special Report on Oriental Fruit Fly. Senate of State of California, California, pp. 72–76.

Hillyer, R.J., Thorsteinson, A.J., 1969. The influence of the host plant or males on ovarian development or oviposition in diamondback moth *Plutella maculipennis* (Curt.). Canadian Journal of Zoology 47, 805–816.

Hopkins, R.J., Ekbom, B., 1996. Low stimuli reduce egg production in the pollen beetle *Meligethes aeneus*. Physiological Entomology 21, 118–122.

Jácome, I., Aluja, M., Liedo, P., 1999. Impact of adult diet on demographic and population parameters of the tropical fruit fly *Anastrepha serpentina* (Diptera: Tephritidae). Bulletin of Entomological Research 89, 165–175.

Kostál, V., 1993. Oogenesis and oviposition in the cabbage root fly, *Delia radicum* (Diptera: Anthomyiidae), influenced by food quality, mating status and host plant deprivation. European Journal of Entomology 90, 137–147.

Lachmann, A., Papaj, D.R. 2001. Effect of host stimuli on ovariole development in the walnut fly, *Rhagoletis juglandis* (Diptera, Tephritidae). Physiological Entomology (in press).

Liedo, P., Carey, J.R., Celedonio, H., Guillen, J., 1992. Size specific demography of three species of *Anastrepha* fruit flies. Entomologia Experimentalis et Applicata 63, 135–142.

Mangel, M., Roitberg, B.D., 1989. Dynamic information and host acceptance by a tephritid fruit fly. Ecological Entomology 14, 181–189.

Martínez, I., Hernández-Ortiz, V., Luna, R., 1995. Desarrollo y maduracion sexual en *Anastrepha serpentina* (Wiedemann) (Diptera: Tephritidae). Acta Zoologica Mexicana 65, 75–88.

Minkenberg, O.P.J.M., Tatar, M., Rosenheim, J.A., 1992. Egg load as a major source of variability in insect foraging and oviposition behaviour. Oikos 65, 134–142.

Norrbom, A.L., Kim, K.C., 1988. A list of the reported host plants of the species of *Anastrepha* (Diptera: Tephritidae). USDA–APHIS Miscellaneous Publication No. 81-52, p. 114.

Norrbom, A.L., Zucchi, R.A., Hernández-Ortiz, V., 2000. Phylogeny of the genera *Anastrepha* and *Toxotrypana* (Trypetinae: Toxotrypanini) based on morphology. In: Aluja, M., Norrbom, A.L. (Eds.), Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, DelRay Beach, FL, pp. 299–342.

Opp, S.B., Prokopy, R.J., 1986. Variation in laboratory oviposition by *Rhagoletis pomonella* (Diptera: Tephritidae) in relation to mating status. Annals of the Entomological Society of America 79, 705–710.

Papaj, D.R., 2000. Ovarian dynamics and host use. Annual Review of Entomology 45, 423–448.

Papaj, D.R., Rausher, M.D., 1983. Individual variation in host location by phytophagous insects. In: Ahmad, S. (Ed.), Herbivorous Insects: Host-seeking Behaviour and Mechanisms. Academic Press, New York, pp. 77–124.

Pritchard, G., 1970. The ecology of a natural population of Queensland fruit fly, *Dacus tryoni*. III. The maturation of females flies in relation to temperature. Australian Journal of Zoology 18, 77–89.

Prokopy, R.J., Bush, G., 1973. Oviposition by grouped and isolated apple maggot flies. Annals of the Entomological Society of America 66, 1197–1200.

Prokopy, R.J., Reynolds, A.H., 1998. Oviposition enhancement through socially facilitated behavior in *Rhagoletis pomonella* flies. Entomologia Experimentalis et Applicata 86, 281–286.

Prokopy, R.J., Cooley, S.S., Luna, I., Duan, J.J., 1995. Combined influence of protein hunger and egg load on the resource foraging behavior of *Rhagoletis pomonella* flies (Diptera; Tephritidae). European Journal of Entomolology 92, 655–666.

Rámirez-Cruz, A., Hernández-Ortiz, V., Martínez, I., 1996. Maduracion ovárica en la "mosca de la guayaba" *Anastrepha striata* Schiner (Diptera: Tephritidae). Acta Zoologica Mexicana 69, 105–116.

Sivinski, J., Aluja, M., López, M., 1997. Spatial and temporal distributions of parasitoids of Mexican *Anastrepha* species (Diptera: Tephritidae) within the canopies of fruit trees. Annals of the Entomological Society of America 90, 604–618.

Weston, P.A., Miller, J.R., 1987. Influence of ovipositional resource quality on fecundity of seedcorn fly (Diptera: Anthomyiidae). Environmental Entomology 16, 400–404.

Weston, P.A., Keller, J.E., Miller, J.R., 1992. Ovipositional stimulus deprivation and its effects on lifetime fecundity of *Delia antiqua* (Meigen) (Diptera: Anthomyiidae). Environmental Entomology 21, 560–565.

Wheeler, D., 1996. The role of nourishment in oogenesis. Annual Review of Entomology 41, 407–431.