FEEDING HISTORY, AGE AND SEX INFLUENCE THE RESPONSE OF FOUR ECONOMICALLY IMPORTANT *ANASTREPHA* SPECIES (DIPTERA: TEPHRITIDAE) TO HUMAN URINE AND HYDROLYZED PROTEIN

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ABSTRACT. We studied the effect of dietary history and age on the response of *Anastrepha ludens, A. obliqua, A. serpentina* and *A. striata* (Diptera: Tephritidae) females and males to two types of food baits: human urine and hydrolyzed protein, both at the fresh and 5 day old stages. Baits were offered simultaneously to flies with water as the control treatment. Diets offered (along with water) to adults at the moment of emergence were either 1) sucrose, 2) a mixture of sucrose and protein, 3) sucrose plus bird feces, 4) an open fruit, or 5) water alone. Adults evaluated were either sexually immature (unmated, 2-3 day old) or mature (presumably mated, 16-20 day old). For each of the four species, adult responses to the baits varied as a function of age, sex and feeding history. Responses were always greater for sexually mature individuals (primarily females) when compared to sexually immature individuals. Feeding regime influenced the response of sexually immature males and females of all four species. The response of sexually immature individuals toward the baits was both weak (particularly when adults were fed on either open fruit or a mixture of sucrose and protein) and non-selective. Sexually mature protein-fed *A. ludens* and *A. striata* males and females exhibited a weak response to protein baits. Open fruit elicited a strong response to the baits in sexually mature *A. ludens* and *A. serpentina* females. Fresh and 5-day old urine baits were attractive to sexually mature *A. ludens* females, regardless of adult feeding history. Human urine was particularly attractive to sucrose-fed, *A. striata* females, and hydrolyzed protein was more attractive than urine to *A. striata* females that had previously fed on a combination of sucrose and bird feces. We conclude that adult responses toward different baits strongly depend on sex, adult diet, and reproductive state, and that such responses vary substantially among species. These findings have important practical implications with respect to fruit fly monitoring and control. KEY WORDS: *Anastrepha*, Diptera, Tephritidae, feeding history, physiological state, food baits, human urine, hydrolyzed protein.

RESUMEN. Estudiamos el efecto de la dieta y la edad de los adultos en la respuesta de machos y hembras de *Anastrepha ludens, A. obliqua, A. serpentina* y *A. striata* (Diptera: Tephritidae) hacia dos tipos de cebos alimenticios: orina humana y proteína hidrolizada. Ambos compuestos fueron evaluados en estado fresco y 5 días de añejamiento. Los cebos fueron ofrecidos simultáneamente a las moscas con agua como tratamiento control. Las dietas fueron ofrecidas a los adultos desde el momento de la emergencia y consistieron en uno de los siguientes tipos de alimento: 1) sacarosa, 2) una mezcla de sacarosa y proteína, 3) sacarosa y heces de pájaro, 4) un fruto abierto, o 5) únicamente agua. Las moscas tuvieron además acceso a agua *ad libitum*. Los

adultos fueron evaluados 2-3 días después de la emergencia (*i.e.*, sexualmente inmaduros, no apareados), o a los 16-20 días de edad (sexualmente maduros, presumiblemente apareados). En cada una de las especies evaluadas la respuesta de los adultos hacia los cebos alimenticios varió de acuerdo a la edad, el sexo y la dieta previa ingerida. Adultos sexualmente maduros (principalmente hembras) respondieron en mayor intensidad a los cebos en comparación con individuos sexualmente inmaduros. El tipo de alimento ingerido influenció significativamente la respuesta de machos y hembras de las cuatro especies evaluadas. La respuesta de adultos sexualmente inmaduros generalmente fue tenue (principalmente cuando los adultos se mantuvieron con sacarosa y proteína) y no fue selectiva. Hembras de *A. ludens* y *A. striata* sexualmente maduras y mantenidas con sacarosa y proteína respondieron de manera tenue a los cebos conteniendo proteínas. Una dieta a base de frutos propiciaron una respuesta marcada hacia los cebos en hembras de *A. ludens* y *A. serpentina* sexualmente maduras. Ambos tipos de orina fueron atractivos principalmente en el caso de hembras de *A. ludens* sexualmente maduras, independientemente del tipo de alimento ingerido. Este mismo tipo de cebo fue muy atractivo para hembras de *A. striata* sexualmente maduras y alimentadas de sacarosa, mientras que la proteína hidrolizada fue atractiva para hembras de esta misma especie mantenidas con sacarosa y heces de pájaro. Concluimos que el tipo de respuesta de adultos de las especies evaluadas dependieron de la edad, de la dieta previa ingerida y el sexo de los adultos, y que el nivel de respuesta hacia los cebos varió de manera importante entre especies. Nuestros resultados tienen importantes implicaciones prácticas en el contexto del monitoreo y control de moscas de la fruta del género *Anastrepha*.

PALABRAS CLAVE: *Anastrepha*, Diptera, Tephritidae, dieta previa, estado fisiológico, cebos alimenticios, orina humana, proteína hidrolizada.

The behavioral responses of adult fruit flies (Diptera: Tephritidae) to food-based lures and traps are influenced primarily by the type of food they ingest as adults and their physiological state (*e.g.*, hunger or reproductive state) (Robacker, 1991; Landolt and Davis-Hernández, 1993; Robacker *et al.*, 1996; Robacker, 1998). For instance, response of *Anastrepha ludens* (Loew, 1873) and *Ceratitis capitata* (Wiedemann, 1824) to food baits may vary according to the availability of host fruits, food resources and orchard ecological and climatic characteristics (Robacker, 1991; Heath *et al.*, 1997; Epsky *et al.*, 1999). Food sources for adult flies in nature include extrafloral secretions, fruit juices, honeydews, bird feces and bacteria (Hagen, 1958; Neilson and Wood, 1966; Nishida, 1980; Hendrichs and Hendrichs, 1990; Aluja and Birke, 1993; Aluja, 1994; Aluja *et al.*, 2000). This is relevant since the type of food ingested by adult flies not only affects their response to food-based lures, but can also have a profound influence on the quality and quantity of sexual pheromone produced (Landolt and Sivinski, 1992; Epsky and Heath, 1993), female fecundity and life expectancy (Jácome *et al.*, 1999), male mating competitiveness (Aluja *et al.*, 2001a), and female ovary development (Aluja *et al.*, 2001b).

The physiological state of adult flies influences their responsiveness to food-based lures in several ways (Rull and Prokopy, 2000). This has been studied in detail in recent years with emphasis on the effect of hunger and age on attraction of adult *Anastrepha* flies to protein-based baits. Work on *A. ludens* (Robacker, 1991; Robacker and García, 1993; Robacker *et al.*, 1996; Robacker, 1998; 1999) and *A. suspensa* (Loew) (Sharp and Chambers, 1983, Landolt and Davis-Hernández, 1993; Nigg *et al.*, 1995) stands out in this respect. For example, it has been shown that protein deprivation in *A. ludens* adults elicits subsequent responses toward protein baits (Robacker, 1991), odors released by bacteria (Robacker and García, 1993), and ammonia-based attractants (Robacker *et al.*, 1996; Robacker, 1998; Robacker *et al.*, 2000). Further, levels of protein consumption by *C. capitata*, *A. ludens*, *A. suspensa* and *A. serpentina* (Wiedemann, 1830) females are associated with their reproductive state, which is in turn related to ovarian development (Sharp and Chambers, 1983; Robacker, 1991; Landolt and Davis-Hernández, 1993; Aluja *et al.*, 2001b). The latter explains, in part, why females are more attracted than males to protein baits (Houston, 1981; Hendrichs *et al.*, 1991; Robacker, 1991; Landolt and Davis-Hernández, 1993).

Here we aimed to determine how dietary history and age (an indicator of reproductive state) of adults of four economically important species of *Anastrepha* (*A. ludens*, *A. obliqua*, (Macquart, 1835) *A. serpentina* and *A. striata* Schiner) influence male and female responses toward fresh and five-day old human urine and hydrolyzed protein in the laboratory. For three of these species (*i.e.*, *A. obliqua*, *A. serpentina* and *A. striata*), factors influencing adult responses to food baits have barely, or never, been studied before. We chose human urine because it is a cost-free, readily available, naturally occurring substance that has proven attractive to fruit flies in commercial fruit orchards (Hedström, 1988; Piñero *et al.*, 2003). Given its potential as a low-tech fruit fly attractant (Aluja, 1999), we wanted to learn more about the factors that influence fly responses to this lure. For comparative purposes we chose hydrolyzed protein, the most common commercially available bait used throughout the world. We worked with four of the seven economically important *Anastrepha* species (Aluja, 1994) in an attempt to compare responses among fly species. There is mounting evidence that not all these species respond with equal intensity to a single food bait or bait formulation (Aluja, 1999; Aluja *et al.*, 1989; 2001b) but potential causes for such differences have not been formally evaluated.

MATERIAL AND METHODS

This study was conducted at the Instituto de Ecología, in Xalapa, Veracruz, Mexico, situated at 19°30' north latitude and 96°55' west longitude, and an altitude of 1,300 m above sea level. Laboratory conditions were $25 \pm 1°C$, 60-70% relative humidity, and a photoperiod of 12:12 h (light/darkness).

Source of adult flies. All flies used in the experiments were obtained from wild populations. *Anastrepha ludens* were obtained from infested grapefruit (*Citrus paradisi* Macfadden, 1830)

and oranges (*Citrus sinensis* (L.) Osbek, 1765) (Rutaceae). Adult *A. serpentina* originated from infested mamey (*Calocarpum mammosum* (L.) Pierre, 1904) (Sapotaceae). *Anastrepha obliqua* adults were obtained from yellow plum (*Spondias purpurea* L., 1762) and jobo (*S. mombin* L., 1753) (Anacardiaceae) and *A. striata* adults stemmed from infested guavas (*Psidium guajava* L., 1753) (Myrtaceae). All field-collected fruit was transported to the laboratory, weighed and placed in plastic washbowls. Pupae were then collected and held in plastic containers (500 ml) with vermiculite until adult emergence.

Handling of adult flies. Within 24 hours after emergence, flies of a particular species were separated into cohorts of 30 individuals and placed in Plexiglas cages (30 x 30 x 30 cm; see Jácome *et al.*, 1999 for further details). Once flies were separated into cohorts, they were provided with water and one of the food sources described below (under feeding history). For each fly species, adults were further separated into two cohorts according to age: 2-3 days (sexually immature, unmated flies) and 16-20 days (sexually mature, presumably mated flies). It was at these two ages that the responses of individuals to the food baits (see below) were evaluated. We further note that the 16-20 day old class did not contain any individual of the 2-3 day old class that had been previously used for experimentation (*i.e.*, there were no repeated measures on any fly). All flies were maintained in the laboratory until observations began.

Feeding history. In the 2-3 day-old age group the following diets were offered to flies before exposing them to the human urine and hydrolyzed protein baits (see below under food baits): 1) sucrose and protein (S&P), 2) sucrose (SUC), 3) sucrose and bird feces (S&F), 4) open fruit (OF), and 5) water. Each food type was placed in a separate Petri dish (10-cm diameter) and introduced into a cage containing males and females

immediately after eclosion (only one food source plus water per cage). For the 16-20 day-old individuals, the same diets mentioned above were used with the exception of water because they did not survive for more than 5 days under this feeding regime.

Diets were prepared as follows: "S&P" corresponded to 15 g of Baker crystallized sucrose (T. J. Baker, Mallinckrodt Baker, S. A. de C. V., Xalostoc, México), 5 g of powdered protein (Yeast Hydrolysate Enzymatic, ICN Biomedicals, Aurora, Ohio, U. S. A.), and water. "SUC" consisted of 20 g of dry sucrose crystals. "S&F" was prepared by mixing 10 g of sucrose and 10 g of bird feces (obtained from *Turdus grayi* Bonaparte, 1838, locally known as "Primavera"). "OF" consisted of a slice of one of the preferred hosts for each fly species (*C. paradisi* for *A. ludens*, *Mangifera indica* L. (1753) for *A. obliqua*, *Manilkara zapota* (L.) P. Royen (1753) for *A. serpentina*, and *P. guajava* for *A. striata*). In the case of the diet "water", we used distilled water.

Food baits. The food baits offered to the adult flies during the observations were 1) fresh human urine, 2) 5-day old human urine, 3) fresh hydro-lyzed protein (Captor Plus®, Agroquímica Tridente, S. A. de C. V. México, D. F.), 4) hydrolyzed protein aged for five days, and 5) water as a control. Both urine and protein were used undiluted. The donor of human urine was a 26-year old male individual, with no kidney damage or any metabolic disorder (JP). His diet excluded coffee, alcohol, vitamin supplements, food condiments, soft drinks and cigarettes. This diet was started 15 days prior to the initiation of experiments and maintained throughout the study. pH values of 20 random samples of both types of human urine (fresh and 5-day old) were recorded using a pH meter (Cole-Parmer Model 59000-20, Chicago, Ill., U. S. A.). The chemical composition of both fresh and 5-day old human urine was characterized in a medical laboratory (Laboratorio Hernández-Blázquez, Coatepec, Veracruz, México). Results of this analysis (Table 1) indicated that urea and ammonia contents (the two compounds critical for the study) fell within the normal ranges for a healthy individual (normal ranges of urea and ammonia are 20-30 g/100 ml and 0.5-0.9 g/100 ml, respectively; Bell *et al.*, 1961; Anonymous, 1981).

Table 1

Chemical composition of a sample of both fresh and 5-day old human urine obtained from a single donor (male, 26 year-old), used in this study. Analysis was performed in a medical laboratory (Laboratorio Hernández-Blázquez, Coatepec, Veracruz, México). Mean pH values of 20 random samples of both types of human urine are also shown.

Compound	Fresh urine	5 d. old urine
Urea	18.8 g/100 ml	16 g/100 ml
Ammonia	0.044 g/100 ml	0.037 g/100 ml
Uric acid	0.222 g/100 ml	0.202 g/100 ml
Creatinine	0.001 g/100 ml	0.00081 g/100 ml
Sodium (Na^+)	105 mEq/l	96 mEq/l
Potassium (K^+)	3.1 mEq/l	2.5 mEq/l
Calcium (Ca^+)	84 mEq/l	75 mEq/l
pH	Mean= 6.9, S= 0.83 Range: 5.8-8.9 N= 20	Mean= 7.4, S= 1.3 Range: 5.4-9.9 N= 20

Experimental Protocol. To avoid odor mixture, experimental trials were carried out in a different laboratory from where flies were maintained. Three days before observations began, every fly was individually marked on the thorax with a distinctive spot of acrylic paint (Vinci de México, S. A. de C. V., México, D. F.). For the observations, we placed ten marked males and ten marked females of a single species, with a predetermined dietary history and age, in a Plexiglas cage (30 x 30 x 30 cm). Flies did not have access to food and water 12 hours before observations began. Upon initiating the observation period, five round, white plastic lids (2 cm of diameter) were simultaneously introduced into each experimental cage. Each lid contained a piece of cotton impregnated with 2 ml of one of the five food baits to be tested (all 4 baits and water were thus represented in every trial).

We recorded the number of flies feeding on each one of the food sources, as well as the sequence of each feeding event, over a period of seven hours (9:00-16:00) for every cage. We observed up to a maximum of four cages simultaneously in any given day. The relative position of each one of the lids (*i.e.*, baits) was randomly changed every hour in every cage. For each *Anastrepha* species, observations were replicated ten times for each combination of age and feeding history. Overall, we observed 7,200 individually marked flies (1,800 per species).

Statistical analyses. For all analyses, the dependent variable used was the number of adults that responded to food baits (*i.e.*, were seen feeding on one of the plastic lids containing the baits impregnated in cotton). Separate analyses were carried out for the 2-3 day old adults and 16-20 day old adults after exploratory analyses indicated that there were marked differences between age groups in their response to the food baits. To determine possible differences in the global responses (*i.e.*, without considering dietary history)

between sexes, Hotelling T^2 multivariate tests were carried out for each species, and at each age. We ran two-way Multivariate Analyses of Variance (MANOVA) to determine, at each age, possible differences in the global responses of males and females to the food baits according to the fly species (Factor 1), and previous diet (Factor 2). As all multivariate tests performed were significant for the interaction fly species x previous diet, we then proceeded to separately examine the effect of previous diet on responses by each species. Adult preferences for specific food baits were based only on numerical (not statistical) comparisons because the response to a particular food bait is not independent of the response to the other types of food baits offered within the same arena (Peterson and Renaud, 1989). All statistical analyses were performed using Statistica® (StatSoft, 1999). To perform the statistical analyses we considered only the first visit made by each adult to any food bait. We deemed this critical in light of the fact that there could be a learning effect if a fly had previously fed on the same or on different baits. Despite this precaution, data corresponding to subsequent visits (*i.e.*, repeated visits to particular baits) were also subjected to analyses, but because of the similar patterns to those found for the first visits such results are not presented here.

RESULTS

For each species, male and female response to food baits increased dramatically from age 2-3 to age 16-20, and the extent of such response varied strongly depending on the previous diet ingested and the fly species. Accordingly, results are presented separately for each age group. Table 2 shows male and female global responses to food baits as a function of all factors evaluated in this study fly species, diet, and age.

Table 2

Percentage of 2 to 3- and 16 to 20-day old males and females of four economically *Anastrepha* species that responded to a bait (values were pooled over all four baits tested), according to fly species and feeding history. Diets: S&P= sucrose and protein, SUC= sucrose alone, S&F= sucrose and bird feces, OF= open fruit (preferred host for each species), and water.

Species	Diet	Males 2-3 d	Males 16-20 d	% Increase in Response between Age Classes[1]	Females 2-3 d	Females 16-20 d	% Increase in Response between Age Classes[1]
A. ludens	S&P	16	25	56.3	30	38	26.7
	SUC	25	28	12.0	19	50	163.2
	S&F	19	25	31.6	24	53	120.8
	OF	9	20	122.2	7	58	728.6
	Water	49	----	----[2]	62	----	----[2]
A. obliqua	S&P	24	26	8.3	19	38	100.0
	SUC	14	42	200.0	26	52	100.0
	S&F	23	45	95.7	19	74	289.5
	OF	26	33	26.9	37	59	59.5
	Water	43	----	----[2]	51	----	----[2]
A. serpentina	S&P	17	17	0.0	24	31	29.2
	SUC	22	35	59.1	28	56	100.0
	S&F	27	32	18.5	37	47	27.0
	OF	4	66	1550.0	18	79	338.9
	Water	56	----	----[2]	62	----	----[2]
A. striata	S&P	33	58	75.8	38	54	42.1
	SUC	38	70	84.2	40	71	77.5
	S&F	29	55	89.7	33	61	84.8
	OF	56	56	0.0	65	81	24.6
	Water	37	----	----[2]	50	----	----[2]

[1] Increase in response to baits when comparing newly emerged individuals (2-3 days of age) and sexually mature individuals (16-20 days of age).

[2] This diet was not offered to 16-20 day old individuals since adults fed only on water do not survive for more than 5 days (Jácome *et al.*, 1999).

Sexually immature (2-3 day old) flies. For each of the fly species observed, no differences in the global response (*i.e.*, number of adults that responded to baits regardless of feeding history) between males and females were detected at this age (Hotelling T^2 test; df 5, 94; $P > 0.05$ in all cases). Conversely, we determined important differences in adult responses (*i.e.*, pooling males and females) to the food baits according to previous diet and fly species evaluated (MANOVA Rao's R= 1.31; df 120, 1341; P= 0.018 for the interaction between "diet" and "species"). When adult responses were examined separately for each species we found that the response of *A. ludens*, *A. obliqua* and *A. serpentina* adults toward baits was influenced by the previous diet (MANOVA Rao's R= 2.00, P= 0.002, Rao's R= 1.55, P= 0.03, and Rao's R= 1.91, P= 0.003, respecti-
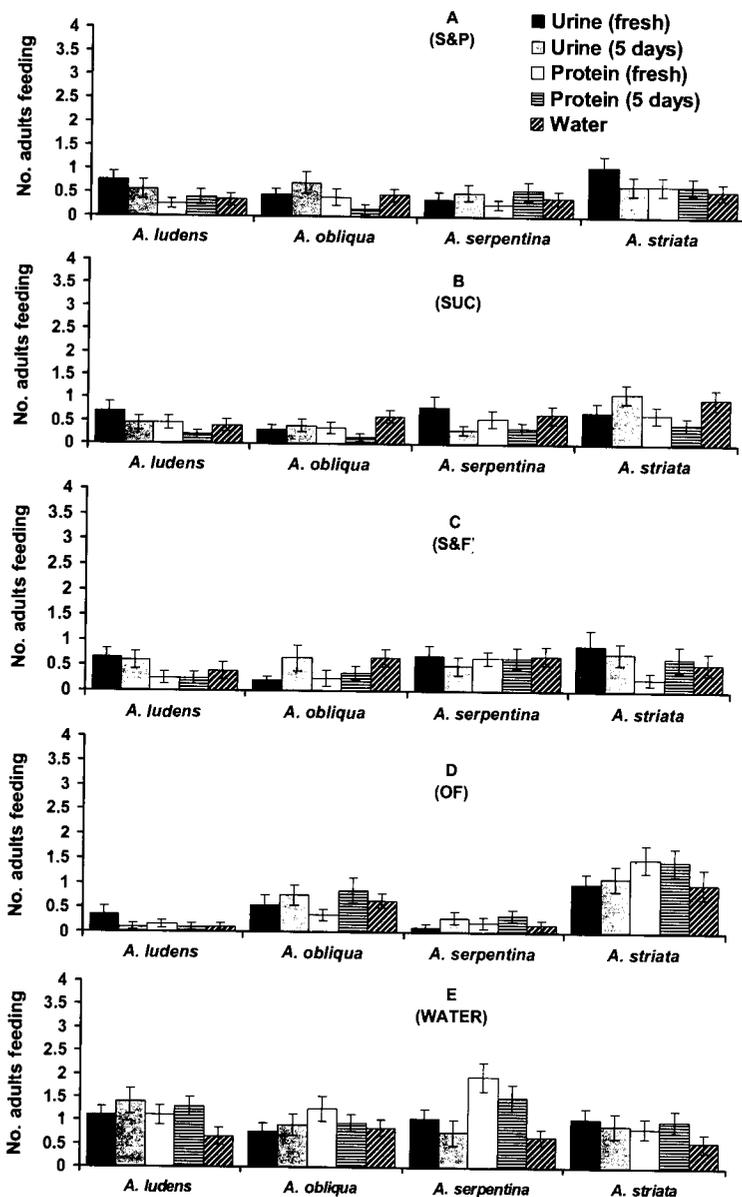
FIGURE 1. Number (mean ± SE) of sexually immature (2-3 day old) *Anastrepha ludens*, *A. obliqua*, *A. serpentina*, and *A. striata* adults that responded to different food baits, according to their adult feeding history: (A) sucrose and protein (S&P), (B) sucrose (SUC), (C) sucrose and bird feces (S&F), (D) open fruit (OF), and (E) water alone. This figure shows adult responses regardless of sex since sexually immature males and females responded similarly to baits (Hotelling T^2 test; df= 5, 94; $P > 0.05$ for each of the four species studied).

vely [df 40, 138 in all cases]). In these three species, when adults were fed only water, they responded more to baits than when they were fed any other diet (Table 2). Figure 1 shows adult responses (*i.e.*, males and females combined) toward each of the food baits offered according to previous diet and fly species. Although formal statistical comparisons among food baits were not performed to determine bait preferences (see statistical analyses section), in general, responses can be considered as weak and non-selective. This was especially true for *A. ludens* and *A. serpentina* adults that were fed OF (Fig. 1 D). Strongest responses were observed for *A. serpentina* to protein baits after previous exposure to water alone (Fig. 1 E).

Responses of 2-3 day old male and female *A. striata* were not influenced by the previous diet offered (MANOVA Rao's R= 0.76, df 20, 136; P= 0.76), although the greatest response occurred when flies (both males and females) were fed OF (Table 2). In general, adults of this fly species responded more to food baits (also in a non-selective manner), than adults of the other species.

Sexually mature (16-20 day old) flies. As mentioned before, responses to food baits increased substantially at this age when compared to 2-3 day old individuals (see Table 2). In some cases (*e.g.*, *A. ludens* females fed on OF), such increase in responsiveness was very striking (~700%). In addition, responses of sexually mature (*i.e.*, 16-20 day old) adults toward food baits were greatly influenced by both the previous diet offered to adults and by fly species.

Sexually mature *A. ludens*, *A. obliqua* and *A. serpentina* adults exhibited, gender-dependent responses to food baits (Figs. 2 and 3). In general, females showed significantly greater and more selective responses to the baits than did conspecific males (Hotelling T^2 test F= 6.64, P< 0.01; F= 5.07, P< 0.01; and F= 2.46, P= 0.04, respectively [df 5, 74 in all cases]). In the case of

A. striata males and females exhibited similar responses to the baits (Hotelling T^2 test: F= 0.88; df 5, 74; P> 0.05).

The extent of the responses to food baits of sexually mature *A. ludens*, *A. obliqua*, and *A. striata* males was not influenced significantly by dietary history (MANOVA Rao's R= 0.34, P= 0.99, Rao's R= 1.31, P= 0.21, and Rao's R= 1.23, P= 0.26, respectively [df 15, 88 in all cases]). However, in *A. striata* the response toward the food baits was more selective than in the other two species. Specifically, we found that SUC-fed males responded more to fresh human urine than to any other bait, but S&F-fed males preferred fresh hydrolyzed protein (Fig. 2 A-D). Dietary history had a large effect on male *A. serpentina* responses to baits (MANOVA Rao's R= 3.01, df 15, 88; P< 0.001). For this fly species, OF elicited an increase in response of 16-20 day old males when compared to 2-3 day old conspecifics (Table 2), in all food baits (Fig. 2 D). Overall, the weakest responses were generally found in S&P-fed males, particularly in the case of *A. serpentina* (Table 2), in which no apparent preferences for any particular bait were observed (Fig. 2 A).

In the case of sexually mature females, responses to food baits as influenced by adult diet were highly variable and as a consequence, no clear pattern across species emerged (Fig. 3). For example, dietary history influenced responses by *A. obliqua* and *A. serpentina* females to the food baits in a different manner (MANOVA Rao's R= 3.61, P< 0.001; Rao's R= 2.70, P= 0.002, respectively, [df 15, 88 in both cases]). While in *A. obliqua* the greatest overall response was observed when adult diet was S&P (three of four baits, Fig. 3 A) or SUC (two of four baits, Fig. 3 B), in *A. serpentina* the greatest overall response was observed when females were fed OF (two of four baits, Fig. 3 D). Importantly, for any particular *Anastrepha* species, responses to specific baits
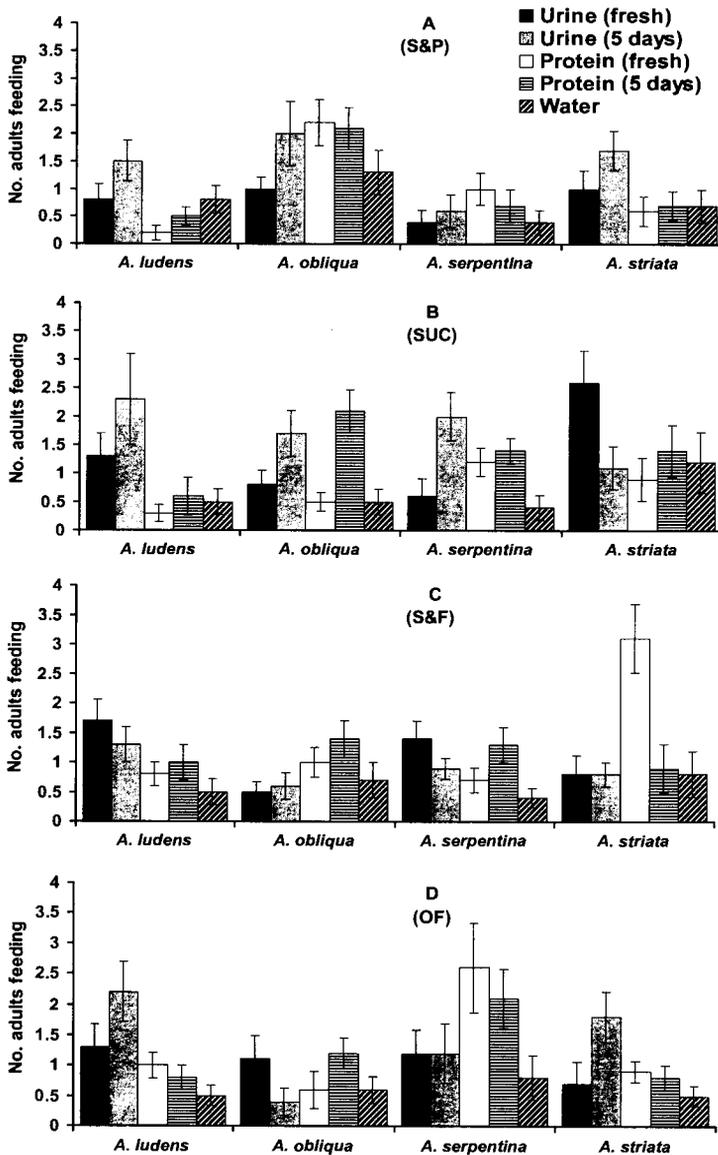
FIGURE 2. Number (mean ± SE) of sexually mature (16-20 day old) *Anastrepha ludens*, *A. obliqua*, *A. serpentina*, and *A. striata* males that responded to different food baits, according to their adult feeding history: (A) sucrose and protein (S&P), (B) sucrose (SUC), (C) sucrose and bird feces (S&F), and (D) open fruit (OF).

were not consistent when considering all previous adult nutritional histories. For example, in the case of *A. serpentina* the greatest response to fresh urine was observed under S&F (Fig. 3 A) and to fresh protein under OF (Fig. 3 D). In the case of *A. obliqua* there was a strong response to fresh and 5-day old urine under S&F (Fig. 3 A) and a weak one under OF (Fig. 3 D). In the case of *A. ludens* response to human urine (fresh and 5-day old) was consistently the highest under all adult diets (Fig. 3 A-D) but in *A. striata* this was not the case. In the latter species, the highest response was observed to fresh urine under SUC (Fig. 3B) and to fresh protein under S&F (Fig. 3C).

DISCUSSION

We determined important both inter- and intraspecific differences in the response of adult flies toward human urine and hydrolyzed protein, which were strongly dependent on feeding history and age (a rough indicator of reproductive state). In all four species, adult responses to food baits were higher and more selective for sexually mature adults than for sexually immature individuals. Responses of sexually immature individuals to the food baits were gender-independent in all four species but this was not the case with sexually mature individuals. Based on the above, several themes emerge for discussion: (1) differences among species in response to the same baits, (2) age- and sex-dependent responses to baits that were not uniform across all species, (3) attenuated or increased response to baits as influenced by type of diet ingested by adults, and (4) overall performance of human urine in comparison to hydrolyzed protein.

1. Differences among species in response to same baits. As mentioned in the introduction, not all economically important *Anastrepha* spe-cies appear to respond with equal intensity to a particular bait or bait formulation (Aluja, 1999;

Aluja *et al.,* 1989; 2001b). In this study we tested this hypothesis by using a standardized protocol that enabled us to establish comparisons among species. Such supposition was further confirmed by us while working on the responses of wild flies to human urine, chicken feces and hydrolyzed protein in guava, mango, grapefruit and sapodilla orchards (Piñero *et al.,* 2003; M. Aluja and J. Piñero, unpub. data). In the field we found that, while for *A. fraterculus* females human urine proved very attractive, *A. ludens* responded significantly more strongly to hydrolyzed protein. However, here we noticed that *A. ludens* responded more to human urine than to hydrolyzed protein regardless of the feeding history.

Such variations in responses by one or different *Anastrepha* species to the same baits, even when controlling for some physiological factors, make the task of finding a lure that will be equally effective for all economically important *Anastrepha* species even more difficult. Given that, for example, *A. ludens, A. obliqua, A. serpentina,* and *A. striata* exhibit such different mating behaviors and ovarian maturation schedules, which are strongly influenced by the sort of food ingested (Aluja *et al.,* 2000; 2001ab), it is very unlikely that they will respond equally to the same stimulants. Also, species like *A. striata* exhibit trophallaxis and this could explain why sexually immature adults of this fly species responded more to food baits than adults of the other species. If a male is to be competitive, it will have to pass to the female high quality liquids through trophallaxis (Aluja *et al.,* 1993). Therefore, feeding early on a protein-based food (bait in our case here) is critical. But the latter may not be as critical to, for example, *A. ludens* adults. Larvae of these species are known to feed on seeds and pulp (Aluja *et al.,* 2000). As a result, adults could possibly emerge with a substantial protein reserve and not be attracted to proteinaceous lures early on in their adult lives.
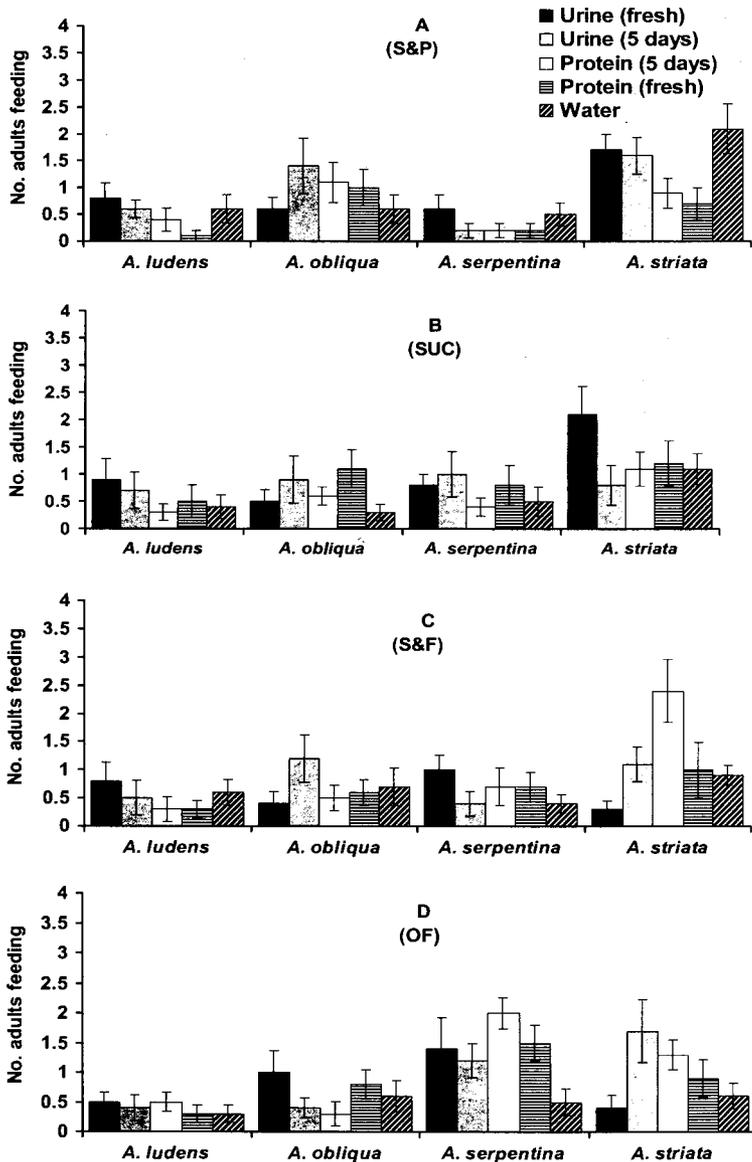
FIGURE 3. Number (mean ± SE) of sexually mature (16-20 day old) *Anastrepha ludens*, *A. obliqua*, *A. serpentina*, and *A. striata* females that responded to different food baits, according to their adult feeding history: (A) sucrose and protein (S&P), (B) sucrose (SUC), (C) sucrose and bird feces (S&F), and (D) open fruit (OF).

Furthermore, the ecological conditions prevalent in a mango, citrus, sapodilla or guava orchard are very different and will undoubtedly influence levels of trap and bait effectiveness (Celedonio-Hurtado, 1995; Aluja *et al.,* 1996). For example, the conditions in a mature mango orchard are moist and shaded, whereas in a guava orchard they usually are drier and sunnier. Therefore, a trap will not likely perform equally well under both ecological conditions. We therefore suggest that more studies be performed to understand the idiosyncrasies of each species, to learn more about their differences and similarities with respect to sexual pheromones, and their responses to fruit volatiles that share attractive elements. All this information will undoubtedly facilitate the development of more effective traps.

2. Age- and sex-dependent responses to baits that were not uniform across all species. Responses of sexually mature adults (16-20 day old) were substantially greater than those exhibited by very young, sexually immature individuals (2-3 day old). Similar associations between age and fly responses to food baits have also been reported by Nigg *et al.* (1995) for *A. suspensa* and by Prokopy *et al.* (1992) and Rull and Prokopy (2000) in *C. capitata* and *Rhagoletis pomonella* (Walsh, 1867), respectively. Our results suggest that during the first days after emergence, both males and females feed little and indiscriminately upon any carbohydrate-containing substances that allow them to survive. Later, as their reproductive systems begin to mature, they seek out protein sources (Robacker, 1991; Landolt and Davis-Hernández, 1993; Nigg *et al.,* 1995). Thus, it is not surprising that when sexually immature *A. ludens* and *A. obliqua* adults were maintained on water alone (*i.e.,* without any other food source) for 2-3 days, the response to food baits was greater than for adults fed on other diets, with no preference for any particular bait. However, *A. serpentina* adults offered water alone preferred

the hydrolyzed protein bait. This may be attributable to greater protein requirements of females of this fly species because they produce larger eggs as compared to *A. ludens*, *A. obliqua* and *A. striata* (Emmart, 1933; Norrbom, 1985; Carroll and Wharton, 1989; Selivon and Perondini, 1999).

Importantly, the response of sexually mature *A. ludens*, *A. obliqua*, and *A. serpentina* females was not only greater, but also was more selective than that of sexually mature males. Such differences probably are a consequence of a higher protein demand by females to guarantee the development of ovaries and the production of eggs. For each of the fly species studied here, the nature of the responses by males and females was also a function of the previous diet ingested (see next section for a detailed discussion of this).

The effect of the age structure of populations on fly responses to proteinaceous baits was addressed by Houston (1981) and more recently by Epsky *et al.* (1993). We believe that based on the above-cited work and our results here, further studies on this topic are warranted. So far and with a few exceptions (*e.g.,* Nigg *et al.,* 1995), female flies captured in protein-baited traps or baits offered under laboratory conditions, were simply divided into sexually immature and mature individuals. Further, the underlying assumption has been that since females require protein for ovary development, they respond more strongly to protein baits than males (Aluja, 1994). But here we show that the age classes used in these types of studies should be further subdivided. Our results indicate that very young adults, respond indiscriminately to baits (*i.e.,* independent of type) and we attribute this to the fact that when newly emerged, adult flies seek out any food source in order to survive (discussed above). In this respect and in the case of *A. ludens*, it has been for example shown that carbohydrate consumption early in life is important for survival

(Robacker, 1991). It is only when ovary development begins or the rate of development increases, that protein ingestion appears to become critical. For example, in studies on ovary development of *A. serpentina* and *A. striata* (Martínez *et al.,* 1995, and Ramírez-Cruz *et al.,* 1996, respectively) it was shown that from date of emergence to day 5 and 3 (*A. serpentina* and *A. striata*, respectively) ovary development is not apparent. Some growth can be measured between ages 4 to 9 days and then an enhanced rate of development is observed between ages 10 and 14 days. The latter matches well our observations (*i.e.,* lack of response of 2-3 day-old flies to food-based lures). We therefore suggest that in future studies, fly cohorts be subdivided into the following age classes: 1-3, 4-10 and 10-15 days of age. The latter could render the process of result interpretation more accurate and could shed further light into the dynamics of fly responses to food baits.

3. Attenuated or increased response to baits as influenced by type of diet ingested by adults. A diet based on open fruits seems to produce contrasting responses of adults toward food baits depending on the fly species and the age. For instance, open fruit elicited the weakest response of *A. ludens* and *A. serpentina* sexually immature flies toward baits. In the case of *A. striata*, open fruit provoked immature flies to exhibit a greater response to the food baits than when adults were fed water alone. On the contrary, open fruit favored the highest responses in sexually mature *A. ludens* and *A. serpentina* females. A possible explanation for such contrasting results is that different species of fruits (used as larval or adult food) confer different nutrients to the adults. In the case of *A. striata*, it is possible that guava, the food source (and preferred host) offered to the adults contained phagostimulant components. An alternative explanation is that there are some nutrients that adults require,

which are not present in guava fruit and, as a consequence, adult *A. striata* responded strongly to the food baits offered that presumably contained those chemicals. Whatever the case may be, we believe that this represents the first evidence indicating that different species of fruit elicit strong or weak responses toward food baits depending on the fruit type, the fly species, and the age of the adult flies. These findings have significant practical implications for the development of protein- and plant-based attractants and thus deserve, in our opinion, further investigation.

In all four species, the ingestion of proteins elicited the weakest response of sexually mature females toward baits (see Table 2). Moreover, in some instances (*e.g., A. serpentina*) a diet based on protein also elicited a weak, non-selective response of both sexually mature males and females (see Figs. 2 and 3). These results, were to be expected since Robacker (1991) had already alluded to the fact that when *A. ludens* adults fed on sugars or proteins, the subsequent "hunger" for these nutrients was reduced.

Sexually mature SUC-fed (*i.e.,* protein deprived) *A. ludens, A. serpentina* and *A. striata* males and females did not seem to prefer proteins over human urine, a result that has at least two possible explanations: 1) Human urine emitted some volatiles (*e.g.,* ammonia) that were particularly attractive to SUC-fed adults (for example Robacker *et al.,* 2000 show NH_3 to be attractive to sugar fed but not sugar starved *A. ludens*), and which probably overrode the volatiles emitted by the hydrolyzed protein, and 2) Feeding only on carbohydrates blocked protein hunger. The last explanation, termed "junk food syndrome", was proposed by Jácome *et al.* (1999) who determined that adult *A. serpentina* preferred sucrose (*i.e.,* junk food), even when they had the choice of feeding on a high quality, protein-rich food. The impact of such an unusual preference for carbohydrates over proteins was a reduction in fe-

male fecundity and life expectancy (Jácome *et al.*, 1999). In the case of the stable fly (*Stomoxys calcitrans* L. 1758) (Diptera: Muscidae), the release of carbohydrates into the hemolymph apparently alters blood-seeking behavior by removing the hunger stimulus necessary to activate the search for blood meals (Jones *et al.*, 1992).

4. Overall performance of human urine in comparison to hydrolyzed protein. Combined results from this study, and the one by Piñero *et al.* (2003), suggest that human urine is indeed attractive to some species of *Anastrepha*, although such attractiveness is strongly influenced by factors such as age, gender and dietary history. As mentioned before, human urine was particularly attractive to sexually mature *A. ludens* females, regardless of feeding history. Despite the fact that formal statistical comparisons in adult responses among food baits could not be performed (see section of statistical methods for further details), human urine seemed to outperform hydrolyzed protein in the case of sexually mature *A. striata* females fed on S&P, SUC, or OF. Conversely, hydrolyzed protein was much more attractive than human urine to sexually mature *A. serpentina* females fed on open fruits, and to sexually mature *A. striata* males and females fed on S&F.

Concluding remarks. We conclude that responses of *Anastrepha* adults toward food baits depend strongly on the type of food ingested, reproductive state, and gender and that such responses vary considerably among species. We therefore believe, that before attempting to improve the effectiveness of food baits under field conditions, it is imperative to gain a better understanding of the effect that factors such as larval feeding behavior (*e.g.*, determining if they do so on seeds), adult oviposition, feeding and mating behavior, and ovarian maturation schedules (and what drives them), have on adult responses to baits. Equally important, is the determination of

the effect that the availability of host fruits within and outside the orchards (*e.g.*, wild hosts), orchard characteristics, and climatic conditions can exert on the response of adults to food-based baits.

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