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Mating Behavior and Basic Biology of *Haywardina cuculi* (Diptera: Tephritidae), a Poorly Known Species Exhibiting High Variability in Copulation Duration

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Abstract The natural history and mating behavior of a species of tephritid fruit fly in the poorly studied genus *Haywardina* are described for the first time. *Haywardina cuculi* Hendel larvae were recovered over four field seasons from infested fruit of *Vassobia breviflora* (Sendtn.) Hunz, which constitutes a new host plant record for this species. Recovered pupae emerged as adults over 183 days on average, suggesting that most individuals became dormant. Adult flies engaged in sexual activity as soon as two days after emergence, were highly promiscuous, and displayed large variability in copulation duration. As for most tephritid species in the subtribe Carpomyini, *H. cuculi* exhibited a resource defense mating system. Fly activity peaked around noon. Copulation could last from 9 min to 17 h, with most copulations beginning in the afternoon and lasting until the following day. We discuss the potential significance of copulation duration variability in light of mate guarding and sperm competition hypothesis and outline future research to understand the evolution of life history and these behavioral strategies.

Dr. Juan Rull, Dr. Solana Abraham, and Dr. Pablo Schlisermann contributed equally to this work.

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Keywords Tephritidae · carpomyini · mate guarding · sperm competition · copulation duration

Introduction

Estimates of global insect diversity range from 5 to 30 million species (May 1988; Stork et al. 2015), yet less than two million of these have been described (Costello et al. 2012). Further, our knowledge on insect ecology, evolution and behavior rests on detailed studies of only a handful of thoroughly known species and would greatly benefit from a wider perspective. The basis of a sound understanding of the ecology of an organism and evolution of behavioral patterns begins with studies on its natural history (Dayton 2003), a field of study that has been neglected since the nineteenth century.

In the case of tephritid fruit flies, a diverse group of phytophagous insects composed of more than 4000 species (Norrbon 2004), knowledge on mating systems and behavioral ecology rests heavily on scrutiny of a few species of economic importance (Sivinski 1999). In the case of the Carpomyini tribe, a group composed of 123 species arranged in 12 genera (Smith and Bush 1999; Norrbom 2004), most knowledge stems from studies on the species of economic importance in the genus *Rhagoletis* and, in particular the apple maggot fly, *Rhagoletis pomonella* (Walsh) (Boller and Prokopy 1976; Prokopy and Papaj 2000).

As far as we know, the mating system of all Carpomyini can be categorized as male resource defense, where males await for females seeking host fruit for oviposition, defend such egg laying/mating arenas from intruding males, and copulate without performing any sort of courtship (Boller and Prokopy 1976; Prokopy and Papaj 2000). Based on studies performed on *R. pomonella*, and a few other species in the genus, both males and females are highly promiscuous, and the last male to copulate fertilizes most of the eggs laid by the female (Opp and Prokopy 2000). Such a condition may favor the evolution of mate guarding among competing males (Alonso-Pimentel and Papaj 1996; Opp et al. 1996). With the exception of walnut infesting *Rhagoletis* in the *suavis* species group, female *Rhagoletis* (no species in other genera within Carpomyini have been studied) lay a single egg per fruit, their offspring are the subject of strong intraspecific competition, and competing females deposit a host marking pheromone that deters other females from selecting occupied fruit (Prokopy and Papaj 2000; Nufio and Papaj 2001). Most species in the subtribe are monophagous, stenophagous, or oligophagous, and host specialization results in the evolution of diapause regimes tightly linked to host fruiting phenology (Smith and Bush 1999).

Within Carpomyini, the genus *Haywardina* is composed of only four species, two occurring in Argentina (*H. cuculi* Hendel and *H. obscura* Norrbom), one in Peru (*H. cuculiformis* Aczél), and one in Peru and Ecuador (*H. bimaculata* Norrbom) (Norrbom 1994). Definitive host records are available for only one species in the genus, and known hosts belong in the Solanaceae. *Haywardina cuculi* has been reared from *Solanum tchoneuron* Lillo and observed in large numbers (including copulating pairs) on *S. argentinum* Bitter & Lillo. Other host plant records for *Haywardina* are questionable (Norrbom 1994). Here, we were able to rear *H. cuculi* from fruit of *Vassobia breviflora* (Sendtn.) Hunz., which represents a new host plant record.

Vassobia breviflora, is a pioneer shrub or small tree up to nearly 15 m in height, native to southern Brazil, Bolivia, Paraguay, Uruguay, and northern and central Argentina (Masetevach and Barboza 2013). It grows on forest edges between 200 and 2000 m (Palacio et al. 2016). Fruits are depressed-globose berries, orange to red when ripe, and 7–9 mm in diameter (Masetevach and Barboza 2013; Bernacki et al. 2015). The fruiting phenology shows the largest fruit crop between November and February, but individual plants may bear fruit throughout the year across its distributional area (Bernacki 2014; Bernacki et al. 2015; Palacio et al. 2015). Moreover, the geographic distribution of *V. breviflora* largely encompasses the known geographic distribution of *Haywardina*. Therefore, this plant-insect association constitutes a good opportunity to generate knowledge on the biology of these fruit flies.

Here, in an effort to expand the existing basis of knowledge on the biology and behavior of an unstudied genus of tephritid fruit flies, we report data on host plant affiliation, distribution, infestation levels (host use), life history, parasitism levels and performed behavioral observations on adults of *H. cuculi*, a species in the subtribe Carpomyini. We hypothesized that *H. cuculi* would tend to exhibit a univoltine life-history and resource defense mating system.

Materials and Methods

Infestation Levels

Fruit collections were carried out from 2009 to 2013 during the main fruiting period of *V. breviflora* (November, December and January). Fruit was recovered on and under *V. breviflora* tree canopies at an ecotone between two phytogeographical regions (Yungas and Chaco Serrano) in northwestern Argentina, in three provinces: Salta, Tucumán, and Catamarca (Coordinates of collection sites can be viewed in Table 1). A total of 101 *V. breviflora* trees were surveyed. Sample size varied according to relative availability of fruits per tree throughout collecting month, year, and location.

For every sample, collected fruits were weighed, counted and placed in perforated plastic baskets over moist sterilized sand to recover pupae. Plastic trays were kept under shelter at ambient environmental conditions for a month. For each sample, a subset of 10 fruit was individually weighed and its soluble sugar content measured in brix degrees with a hand held refractometer.

Sand was observed at 7-d intervals, and the time from collection of infested fruit to fly pupation was recorded. In order to estimate infestation levels, the indices of larvae per kg of fruit and percent infested fruit were calculated. Recovered pupae were counted and kept in 200 ml plastic cups with sand and a voile cover until emergence of adults for taxonomic identification. Plastic cups were kept in an environmentally controlled chamber at 25 ± 1 °C, $65 \pm 10\%$ RH until adult eclosion. The time elapsed from pupation to eclosion of adults was recorded. Percent parasitism was estimated as the total number of parasitoids over the total number of fly pupae obtained from fruit samples.

To estimate adult and parasitoid emergence and the time elapsed from pupation to adult emergence 563 *H. cuculi* pupae from El Cadillal, El Cajón, El Siambón, Hualinchay and Villa Padre Monti (Table 1) were monitored.

Table 1 Collection dates, sites, number of fruit, total and individual fruit weight, sugar content, total number of recovered pupae, and infestation levels by *Haywardina cuculi* on *Vassobia breviflora*

Collection date	Locality (Province)	Fruit (Plants)	Kg of fruit (g) (Mean ± SE)	Brix (mean ± SE)	Pupae N°	Larvae per kg of fruit IL, (% infested fruit)
11/2011	San Andrés (Salta) 23° 04' 39.6" S; 64° 49' 03.6" W	278 (2)	0.06 ± 0.01	n/d	0	0.00 (0)
	Isla de Cañas (Salta) 22° 58' 48.7" S; 64° 33' 04.8" W	117 (1)	0.01 ± 0.01	n/d	0	0.00 (0)
12/2010	San Ignacio (Tucumán) 23° 04' 19.6" S; 64° 33' 27.5" W	607 (4)	0.13 ± 0.01	n/d	0	0.00 (0)
12/2012	El Cadillal (Tucumán) 26° 36' 51.6" S; 65° 11' 27.2" W	2843 (32)	0.49 ± 0.01	15.92 ± 0.53	452	927.94 (15.89%)
01/2013	El Cajón (Tucumán) 26° 31' 59.63" S; 64° 52' 00.02" W	1667 (3)	0.22 ± 0.00	n/d	137	626.43 (8.21%)
	El Siabomb (Tucumán) 26° 43' 12.95" S; 65° 47' 13.22" W	145 (1)	0.02 ± 0.00	11.90 ± 0.77	19	1032.61 (13.10%)
	Escaba (Tucumán) 27° 40' 03.2" S; 65° 48' 10.6" W	2498 (10)	0.78 ± 0.02	19.67 ± 2.12	157	201.28 (6.28%)
	Hualinchay (Tucumán) 26° 18' 02.33" S; 65° 37' 04.70" W	9340 (30)	1.68 ± 0.02	14.2 ± 0.68	65	40.98 (0.7%)
	Padre Monti (Tucumán) 26° 28' 59.88" S; 64° 58' 00" W	340 (5)	0.03 ± 0.00	n/d	29	892.31 (8.52%)
	Río Grande (Tucumán) 26° 46' 03.3" S and 65° 28' 21.7" W	457 (3)	0.05 ± 0.02	11.98 ± 0.50	12	239.04 (2.62%)

Table 1 (continued)

Collection date	Locality (Province)	Fruit (Plants)	Kg of fruit fruit	Fruit individual weight (g) (Mean ± SE)	Brix (mean ± SE)	Pupae N°	Larvae per kg of fruit II, (% infested fruit)
12/2009	El Durazno (Catamarca) 28° 05' 55.3" S; 65° 36' 01.4" W	2346 (6)	1.27	0.11 ± 0.01	17 ± 1.00	0	0.00 (0)
	Higuerilla (Catamarca) 27° 48' 57.4" S; 65° 46' 54.2" W	995 (4)	0.21	0.36 ± 0.01	s/d	5	0.02 (0.05%)

In order to perform behavioural observations, adult *H. cuculi* recovered from fruit collections were exposed to cherry tomatoes. Pupal recovery from cherry tomatoes was limited, yet *V. breviflora* fruit was no longer available when rearing was attempted. Exposed fruit was handled as described above to recover pupae, and mating behaviour of newly emerged adults was observed and recorded continuously until the first deaths were noted for a cohort. Due to the lack of knowledge on duration of dormancy and environmental cues used for its regulation, we were unable to synchronize eclosion of adults in large numbers, and performed behavioural observations on small cohorts as the opportunity arose.

Behavioral Observations

On the day of emergence, adults were placed in plastic 15 × 15 × 15 cm transparent cages with ad libitum access to water and food. Flies were fed a diet consisting of sugar (57.9%) (Ledesma S.A., Jujuy, Argentina), hydrolyzed yeast (14.5%) (Yeast Hydrolyzed Enzymatic, MP Biomedicals®), hydrolyzed corn (27.3%) (Gluten Meal, ARCOR®, Tucumán, Argentina), and vitamin E (0.3%) (Parafarm®, Buenos Aires, Argentina) (w/w) (Jaldo et al. 2001).

For behavioral and mating observations, four cohorts of newly hatched adults (0–2 days after adult emergence) were maintained at 26 ± 2 °C, light was provided by a window and artificial lights switched on at 7:00 and off at 20:00 h. Cohorts were examined and observed daily until the death of at least one fly. Cohort 1 was composed of three females and two males (1.5:1 sex ratio) and observed for 71 consecutive days. Cohort 2 was composed of five females and six males (0.88:1 sex ratio) and observed for 27 consecutive days. Cohort 3 was composed of three females and two males (1.5:1 sex ratio) and observed for 21 consecutive days. Cohort 4 was composed of four females and three males (1.33:1 sex ratio) and observed for 18 consecutive days. In total, the reproductive behavior of 15 females and 13 males was analyzed.

Observations consisted of periodical scanning of cages (c.a. every 15 min) and recording of behavioral activities. Scanning encompassed a daily time period from 6:00 am in the morning until 22:00 pm in the evening. Several random observations were made at irregular intervals from 22:00 to 6:00 a.m. to verify the general lack of nocturnal activity of flies.

Description of General Precopulatory, Copulatory, and General Behavioral Patterns

Based on notes taken during observations, some typical pre-mating and mating sequences and some general behavioral patterns were described.

Daily Activity and Mating Patterns and Duration of Copulation

General behavioral activities were categorized and recorded as follows: i) Inactivity (where all the flies in a cage were observed standing motionless); ii) Reduced activity (where one or more flies were observed walking or grooming); iii) Activity (when at least one fly was observed in flight, signaling, or egg laying). In the case of mating activity, mating events were recorded when there was at least one pair *in copula*. Due to

the small size of the flies, and limited number of individuals, it was difficult to mark the flies with paint or a small piece of paper, as is regularly done with other fly species (Rull et al. 2013). We were therefore unable to record individual mating rate or mating success. Estimates of mating frequency for each sex are based on the total number of recorded copulations for the total number of observed individuals independently of individual mating success.

Two distinct copulation durations were observed and classified as follows: “short” copulations, which started and ended in the course of the same day, and “long” copulations”, where the pair remained *in copula* overnight and separated the following day.

Statistical Analysis

Copulation duration (from the time of the beginning of the copulation to its end) and mating latency (from time the lights were turned on until the beginning of copulation in a given day) were compared with non-parametric Mann-Whitney U tests between short and long copulations.

The observed frequency of activity, reduced activity, inactivity and the total number of copulations among two hour periods from 6:00 A.M. to 22:00 P.M. were compared to a uniform distribution of activity and a uniform distribution of copulations (respectively) across two-hour periods by means of observed vs. expected X^2 tests.

To analyze factors affecting copulation duration (the time in minutes from the beginning of copulation to its end) a model was built using STATISTICA software v.7® (Statsoft Inc. Tulsa, Oklahoma), with, cohort (1 to 4), fly age (in days), and mating latency (from the time the lights were turned on until the beginning of copulation in a given day) as factors. A backwards stepwise regression was run with the final model only retaining those factors that significantly explained variability in copulation duration (dependent variable).

Results

Infestation Levels

A total of 4.96 kg of *V. breviflora* fruit were collected (21,633 fruits) over four years at 12 localities in three Northwestern provinces of Argentina yielding overall 880 *H. cuculi* pupae (Table 1). Infestation levels varied from 0.05% to 15.89%, with a single larva developing per fruit. Sugar content of infested fruit ranged from 11.90 ± 0.77 to 19.67 ± 2.12 °Bx.

Adult Ecllosion and Parasitism

Out of 563 *H. cuculi* pupae, 57.19% emerged on average 183.60 ± 8.98 days after pupation. With respect to 322 *H. cuculi* adults, 143 were female and 179 were male. Associated with this host plant-herbivorous system, an undescribed parasitoid in the genus *Utetes* (Hymenoptera: Braconidae) was found. From the total of 563 *H. cuculi* pupae, 39 parasitoids were recovered (8 ♀ and 31 ♂) (Table 2). All identified plants

Table 2 Time elapsed in days from pupation to adult (flies and parasitoids) emergence and percent of parasitism for *Haywardina cuculi* pupae collected in six locations of Northwestern Argentina

Location	Time to adult emergence (Mean \pm SE) in days	N° Pupae	N° of adults	N° of adult parasitoids	% parasitoidism
Rio Grande	(224 \pm 38.1)	32	13	0	0
El Cajón	(246 \pm 17.2)	137	88	19	13.9
Padre Monti	(173.2 \pm 28.3)	68	39	2	2.9
El Siambom	(113 \pm 11.1)	19	4	1	5.3
El Cadillal	(170.4 \pm 15.3)	234	138	16	6.8
Hualinchay	(156.2 \pm 12.6)	73	40	1	1.4

and insects were deposited in the Fundación Miguel Lillo permanent collection as voucher specimens.

Behavioral Descriptions

As recorded for other species in the subtribe Carpomyini, no behavior related to pheromone emission, calling, or wing fanning was observed among *H. cuculi* males. When facing another individual, independently of its sex, flies engaged in wing waving, usually semaphoring, without much further escalation. Males occupying the same fruit were occasionally observed performing very brief ritualistic boxing matches. Males seeking to mate jumped on females and attempted to force copulations without courtship. Females could resist mountings by shaking and flying away and occasionally dropping to the cage floor and rolling on the ground. Several copulations began on fruit guarded by males and visited by females for egg laying, but many others began on the cage walls, ceiling or floor. After mounting and intromission, females tended to hold their wings outward at a 90 degree angle from their body. This mating posture was generally abandoned a few minutes after initiation of copulation and females held their wings at rest during the rest of copulation.

Diel Activity and Mating Patterns

Flies in the dark, whether alone or in copula, were invariably observed to be inactive. There was a mid-morning activity peak between 10:00 and 12:00 with activity declining gradually towards dusk ($X^2 = 763.3$; d.f. = 7; $P < 0.001$). A similar pattern was observed for reduced activity ($X^2 = 456.8$; d.f. = 7; $P < 0.001$), whereas inactive flies were observed at higher frequencies during early morning 6:00–8:00 and late afternoon-night 18:00–20:00; 20:00–22:00 ($X^2 = 733.8$; d.f. = 7; $P < 0.001$), (Fig. 1). Flies were generally observed inactive overnight, with pairs engaged in long copulations also remaining motionless. The peak of mating activity occurred slightly after the peak of general activity, with most copulations beginning between 12:00 and 14:00 ($X^2 = 76$; d.f. = 7; $P < 0.001$), but, mating could begin at any time during daylight (Fig. 2).

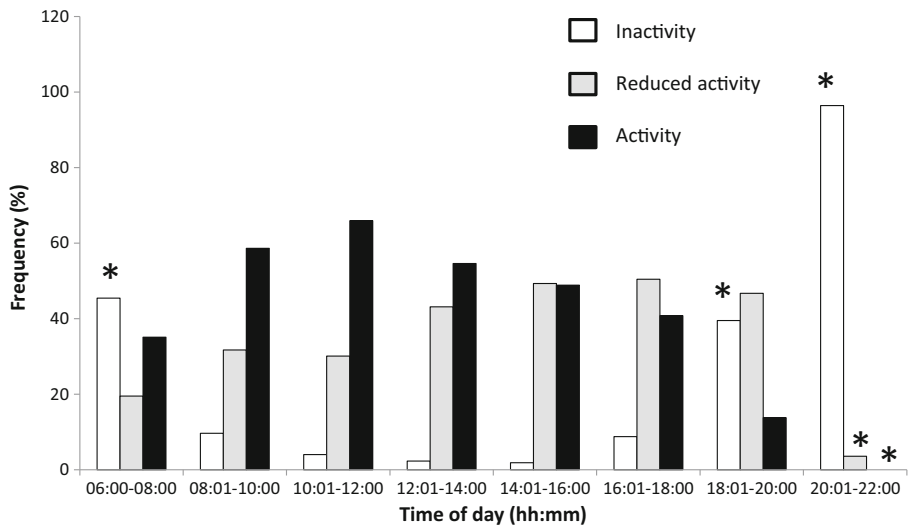


Fig. 1 Diel activity patterns of four cohorts of caged *Haywardina cuculi* adults observed over 21–71 day periods

Duration of Copulations

In total, 166 short copulations and 55 long copulations were recorded (short copulations started and ended in the course of the same day, while for long copulations pairs remained *in copula* overnight and separated the following day).

A backwards stepwise regression revealed that copulation duration was explained by mating latency ($R^2 = 0.21$; $F_{1217} = 58.57$; $P < 0.01$). Cohort and fly age were removed from the model. In general, copulations that initiated early in the day were of shorter duration than those that initiated late in the day, which usually continued overnight and separated the following day (Figs. 3 and 4).

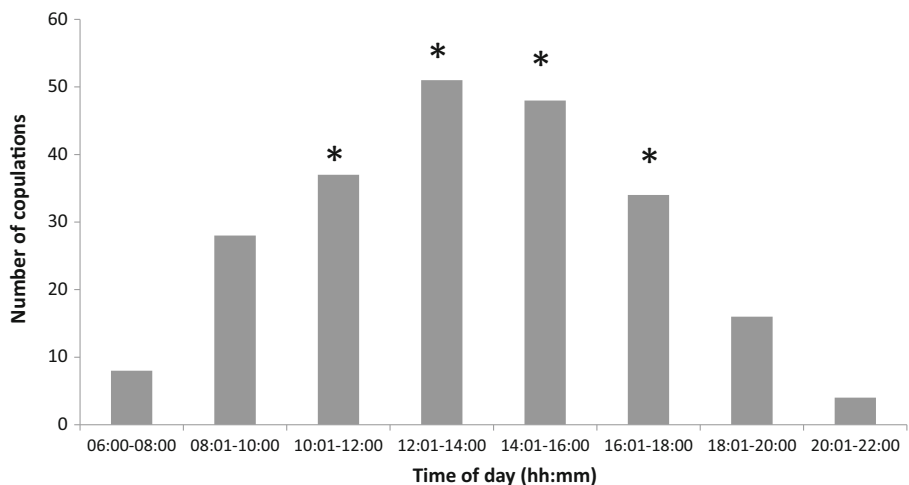


Fig. 2 Number of copulations recorded over two-hour periods for four cohorts of caged *Haywardina cuculi* adults observed over 21–71 day periods

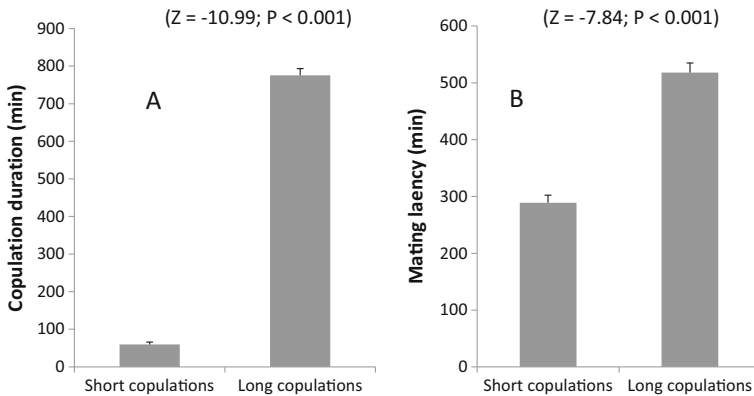


Fig. 3 Copulation duration (a) and latency to mating (b) of four cohorts of caged *Haywardina cuculi* adults observed over 21–71 day periods

Copulation duration and mating latency differed between short and long copulations. Short copulations were significantly shorter than long copulations (Mann-Whitney, $Z = -10.99; P < 0.001$). Short copulations began earlier in the day than long copulations (Mann-Whitney, $Z = -7.84; P < 0.001$) (Fig. 3).

Mating Rate

Overall, the total number of observed copulations was 221. Accordingly, each female ($N = 13$) mated an average of 0.87 times per week, while each male ($N = 15$) did so on an average of 0.74 times per week. Such figures do not take into account differential mating success.

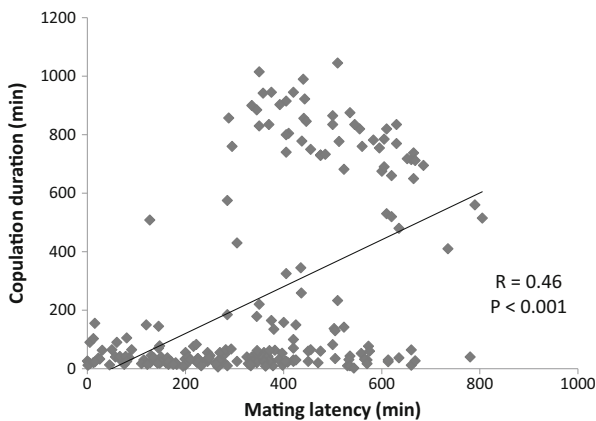


Fig. 4 Copulation duration and mating latency of all recorded copulations ($N = 221$) of four cohorts of caged *Haywardina cuculi* adults observed over 21–71 day periods

Discussion

Haywardina cuculi was found infesting fruit of *Vassobia breviflora* in riparian habitats at the foothills of the Yungas in Northeastern Argentina, which represents a new host plant record for the species. However, infestation levels during four field seasons were low (0.05 to 15%), and should be compared to those exploiting other plants such as *Solanum tnchoneuron* and *S. argentinum* reported by Norrbom (1994) as hosts. A single larva developed per fruit, as is common among species in the subtribe Carpomyini (Averill and Prokopy 1987), perhaps owing to the fact that host fruits are small and very abundant. A large proportion of available fruit remained uninfested, suggesting either that gravid females are selective with respect to egg laying sites or occur at low densities. Infested fruit tended to have high concentrations of soluble sugars. Parasitism by an unidentified species of *Utetes* was 14%. Larvae developed within a two week period and pupated outside of fruit. Adults could emerge within a month, but most individuals took an average of 183 days, suggesting that a large proportion of the population became dormant. Adults began sexual activity within one or two days after emergence and were found to be highly promiscuous. Adult activity peaked around midday in laboratory conditions. The mating system can be classified as resource defense by males, which is typical of Carpomyini, and observations revealed an extraordinarily high variability in copulation duration, which may be tied to sperm competition among males. Copulations beginning late in the afternoon, after 4:00 pm, were usually longer in duration than those starting earlier in the day. Such findings open avenues for future interesting research testing hypotheses on mate guarding.

Dormancy is conceived as a state of suppressed development that evolved among animals to cope with long periods of adverse conditions (Košťál 2006). In the subtropics, both relatively low winter temperatures and precipitation fluctuations, influence phenological cycles. "Overwintering" mechanisms among animal species evolving in such environments is poorly understood, especially the complex cues that trigger and halt dormancy (Danks 2007). Specialized subtropical insects exhibit a great wealth of behavioral, demographic, and physiological mechanisms to bridge difficult periods between host availability (Danks 2002). Among tropical and subtropical tephritids specialized species may exhibit great adult longevity (Aluja et al. 2000), slow larval/pupal winter development (Thomas 1997; Papadopoulos et al. 1998), quiescence (Silverman and Goeden 1980) and diapause (Norrbom et al. 2014). In the case of *H. cuculi*, while adults may be relatively long lived, most individuals appear to enter a state of dormancy during the pupal stage, as evidenced by variable pupae-adult developmental periods. Dormancy has been reported for some subtropical species of hymenopteran parasitoids exploiting tephritids in the Yungas (Ovruski et al. 2016), yet nothing is known about the environmental cues that regulate this apparently facultative state. Host plant affiliation, phenology, and local environmental conditions appear to have selected for univoltine *H. cuculi*. As a consequence, reproductive behavior is seasonally concentrated around the fruiting period of *V. breviflora* during the warm and wet summer of North Western Argentina (December to February). It has been proposed for non-frugivorous tephritids, that species where adults seeking reproduction return to aggregate on their F1-generation host plant when it becomes suitable for egg laying,

can be classified as aggregative (Headrick and Goeden 1998), a life history strategy that tends to select for resource defense polygyny mating systems (Headrick and Goeden 1994). The frugivorous *H. cuculi* also appears to fall into the aggregative species classification.

Behavioral observations revealed that males and females of *H. cuculi* are polygamous and copulations are distributed throughout the day with a peak from 12:00 to 16:00 h. This temporal pattern could differ from that found in nature given that during the fruiting season of *V. breviflora*, midday maximal temperatures can frequently reach more than 35 °C (<http://www.accuweather.com/> 2015 El Cadillal). We observed two copulation types, one labeled as “short copulation”, which started early in a given day and ended in the same day. These copulations varied from as short as ten minutes to as long as 9 h. The other copulation type termed long copulation, typically started late during a given day, usually in the afternoon, and ended the following day usually before 10:00 am. These copulations varied from 6 h to as long as 17 h in duration. Additionally, although we were unable to record individual mating success, we observed that both males and females were highly promiscuous and mated multiply. High variability in duration of copulations is interesting and merits further investigation, several hypotheses could explain selection for these behavioral patterns (Wilkinson and Johns 2005). Some tephritid species have been found to guard females after copulation during egg laying (Dodson 1997), in some species egg laying activity can be concentrated during particular periods of the day (Brévault and Quilici 2000), in the case of *H. cuculi*, if egg laying in nature occurs early in the day, males initiating copulation during late afternoon, could remain *in copula* in an attempt to ensure that females fertilize their eggs the following day during oviposition. Such a pattern could explain the positive correlation found between mating latency and copula duration, but would require systematic field observations of egg-laying behavior and studies on sperm use patterns.

Prolonged copulation has been observed among several orders of insects (Thornhill and Alcock 1983; Alcock 1994; Andrés and Cordero Rivera 2000; García-González and Gomendio 2004). Among non-frugivorous Tephritidae, the majority of species studied are aggregative and display a variety of mating strategies, including resource defense polygyny, and paternal assurance strategies, such as mate guarding, male combat, and lengthy copulation durations (Headrick and Goeden 1994). Hypotheses proposed to explain the adaptive significance of prolonged copulation include the “sperm loading” hypothesis, which implies that sperm transfer increases with increased copulation duration (Dickinson 1986), conferring to the male an advantage over his competitors through the number of sperm stored by his mate. On the other hand, there is the “in-copula guarding” hypothesis, when males *in copula* act as a mating plug to prevent female remating before egg-laying, thus ensuring paternity (Alcock 1994). In the case of *H. cuculi* no hypothesis can be currently favored without further testing.

The operational sex ratio has been found to affect mating duration of some species of Tephritidae exhibiting resource defense mating systems (Alonso-Pimentel and Papaj, 1996) where copulations in male biased environments were found to be longer than in female biased environments, both in the laboratory and the field. In-copula guarding would be important among species where there is no female sexual inhibition after copulation, as for *H. cuculi*, and this could be a male mechanism to avoid sperm competition. Such male tactic assumes that copulation duration is at least in part under

male control, which seems to be true for some insects such as damselflies (Miller, 1987), but not for some species of true fruit flies (Perez-Staples et al. 2010; Abraham et al., 2014) and remains unknown for *H. cuculi*. On the other hand, prolonged copulations could serve both as a mate guarding strategy and as an aid in sperm transfer.

Further research on the natural history of *H. cuculi* will contribute to understanding the evolution of overwintering mechanisms in subtropical insects and the evolution of mate guarding. In particular, establishing the fruiting phenology and infestation levels on other reported hosts such as *S. trichoneuron* and *S. argentinum* will allow to determine the seasonal duration of host availability, which coupled with laboratory experiments exploring the length of dormancy under different temperature/duration/humidity regimes, demographic studies examining longevity/fecundity, and physiological mechanisms underlying pupal diapause in subtropical parasitoids and their hosts, will contribute to the understanding of overwintering strategies evolving among subtropical insects. Behavioral observations of diel activity patterns in the field, with emphasis on mating and egg-laying, coupled with laboratory studies examining sperm precedence and comparing sperm transfer for different mating durations and conspecific densities will contribute to understanding the evolution of mate guarding among competing males.

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References

- Abraham S, Rull J, Mendoza M, Liendo MC, Devescovi F, Roriz AK, Kovaleski A, Segura DF, Vera MT (2014) Differences in sperm storage and remating propensity between adult females of two morphotypes of the *Anastrepha fraterculus* (Diptera: Tephritidae) cryptic species complex. *Bull Entomol Res* 104:376–382
- Alcock J (1994) Postinsemination associations between males and females in insects: the male guarding hypothesis. *Annu Rev Entomol* 39:1–21
- Alonso-Pimentel H, Papaj DR (1996) Operational sex ratio versus gender density as determinants of copulation duration in the walnut fly, *Rhagoletis juglandis* (Diptera: Tephritidae). *Behav Ecol Sociobiol* 39:171–180
- Aluja M, Piñero J, Jácome I, Díaz-Fleischer F, Sivinski J (2000) Behavior of flies in the genus *Anastrepha* (Trypetinae: Toxotrypanini). *Fruit flies (Tephritidae): Phylogeny and Evolution of Behavior*. CRC Press, Boca Raton, pp 375–408
- Andrés JA, Cordero Rivera A (2000) Copulation duration and fertilization success in damselfly: an example of female cryptic choice? *Anim Behav* 59:695–703
- Averill AL, Prokopy RJ (1987) Intraspecific competition in the tephritid fruit fly *Rhagoletis pomonella*. *Ecology* 68:878–886
- Bernacki FG (2014) Biología floral y frutal de *Vassobia breviflora* (Sedn.) Hunz. (Solanaceae) en el noroeste argentino [dissertation]. San Miguel de Tucumán: Universidad Nacional de Tucumán
- Bernacki FG, Albormoz P, Valoy M, Ordano M (2015) Anatomía de flor y fruto de *Vassobia breviflora* (Solanaceae) en el sur de las Yungas australes (Argentina). *Phyton-Revista Internacional de Botánica Experimental* 84:478–487
- Boller EF, Prokopy RJ (1976) Bionomics and management of *Rhagoletis*. *Annu Rev Entomol* 21:223–246
- Brévault T, Quilici S (2000) Diel patterns of reproductive activities in the tomato fruit fly, *Neoceratitis cyanescens*. *Physiol Entomol* 25:233–241

- Costello MJ, Wilson S, Houlding B (2012) Predicting total global species richness using rates of species description and estimates of taxonomic effort. *Syst Biol* 61:871–883
- Danks HV (2002) The range of insect dormancy responses. *Eur J Entomol* 99:127–142
- Danks HV (2007) The elements of seasonal adaptations in insects. *Can Entomol* 139:1–44
- Dayton PK (2003) The importance of the natural sciences to conservation. *Am Nat* 162:1–13
- Dickinson JL (1986) Prolonged mating in the milkweed leaf beetle *Labidomera clivicollis clivicollis* (Coleoptera: Chrysomelidae): a test of the “sperm-loading” hypothesis. *Behav Ecol Sociobiol* 18:331–338
- Dodson GN (1997) Resource defense mating system in antlered flies, *Phytalmia* spp. (Diptera: Tephritidae). *Ann Entomol Soc Am* 90:496–504
- García-González F, Gomendio M (2004) Adjustment of copula duration and ejaculate size according to the risk of sperm competition in the golden egg bug (*Phyllomorpha laciniata*). *Behav Ecol* 15:23–30
- Headrick DH, Goeden RD (1994) Reproductive behavior of California fruit flies and the classification and evolution of Tephritidae (Diptera) mating systems. *Stud Dipterol* 1:194–252
- Headrick DH, Goeden RD (1998) The biology of nonfrugivorous tephritid fruit flies. *Annu Rev Entomol* 43: 217–241
- Jaldo HE, Gramajo MC, Willink E (2001) Mass rearing of *Anastrepha fraterculus* (Diptera: Tephritidae): a preliminary strategy. *Fla Entomol* 84:716–718
- Košťál V (2006) Eco-physiological phases of insect diapause. *J Insect Physiol* 52:113–127
- Masetevach Becerra M, Barboza GE (2013) Vassobia. In: Zuloaga FO et al (eds) *Flora Fanerogámica Argentina: Programa ProFlora. Dicotyledoneae, Solanaceae*, Córdoba, Museo Botánico, IMBIV, pp 148–150
- May RM (1988) How many species are there on earth? *Science* 241:1441–1449
- Miller PL (1987) An examination of prolonged copulation of *Ischnura elegans* (Van der Linden) (Zygoptera: Coenagrionidae). *Odonatologica* 16:201–207
- Norrbom AL (1994) New species and phylogenetic analysis of *Cryptodacus*, *Haywardina*, and *Rhagoletotrypeta* (Diptera: Tephritidae). *Insecta Mundi* 288
- Norrbom AL (2004) Updates to biosystematic database of world Diptera for Tephritidae through 1999. *Diptera Data Dissemination Disk (CD-ROM)* 2
- Norrbom AL, Castillo-Meza AL, García-Chavez JE, Aluja M, Rull J (2014) A new species of *Anastrepha* (Diptera: Tephritidae) from *Euphorbia tehuacana* (Euphorbiaceae) in Mexico. *Zootaxa* 3780:567–576
- Nufio CR, Papaj DR (2001) Host marking behavior in phytophagous insects and parasitoids. *Entomol Exp Appl* 99:273–293
- Opp SB, Prokopy RJ (2000) Multiple mating and reproductive success of male and female apple maggot flies, *Rhagoletis pomonella* (Diptera: Tephritidae). *J Insect Behav* 13:901–914
- Opp SB, Spisak SA, Telang A, Hammond SS (1996) Comparative mating systems of two *Rhagoletis* species: the adaptive significance of mate guarding. *Fruit fly pests: a world assessment of their biology and management*. St. Lucie Press, Delray Beach, pp 43–49
- Ovruski SM, Schliserman P, Aluja M (2016) Occurrence of diapause in neotropical parasitoids attacking *Anastrepha fraterculus* (Diptera: Tephritidae) in a subtropical rainforest from Argentina. *Austr Entomol* 55:274–283
- Palacio FX, Valoy M, Bernacki FG, Sánchez MS, Núñez-Montellano MG, Varela O, Ordano M (2015) Bird fruit consumption results from the interaction between fruit-handling behaviour and fruit crop size. *Ethol Ecol Evol* 28:00–00. doi:10.1080/03949370.2015.1080195 [Published online: 29 Sep 2015]
- Papadopoulos NT, Katsoyannos BI, Carey JR (1998) Temporal changes in the composition of the overwintering larval population of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. *Ann Entomol Soc Am* 91:430–434
- Perez-Staples D, Weldon CW, Radhakrishnan P, Prenter J, Taylor PW (2010) Control of copula duration and sperm storage by female Queensland fruit flies. *J Insect Physiol* 56:1755–1762
- Prokopy RJ, Papaj DR (2000) Behavior of flies of the genera *Rhagoletis*, *Zonosemata*, and *Carpomya* (Trypetinae: Carpomyina). *Fruit flies (Tephritidae): Phylogeny and Evolution of Behavior*. CRC Press, Boca Raton, pp 219–252
- Rull J, Abraham S, Kovaleski A, Segura DF, Mendoza M, Liendo M, Vera MT (2013) Evolution of pre-zygotic and post-zygotic barriers to gene flow among three cryptic species within the *Anastrepha fraterculus* Complex. *Entomol Exp Appl* 148:213–222
- Silverman J, Goeden RD (1980) Life history of a fruit fly, *Procecidochares* sp., on the ragweed, *Ambrosia dumosa* (gray) Payne, in southern California (Diptera: Tephritidae). *Pan Pac Entomol* 56:283–288
- Sivinski J (1999) Breeding habits and sex in families closely related to the Tephritidae: opportunities for comparative studies of the evolution of fruit fly behavior. *Fruit flies (Tephritidae): Phylogeny and Evolution of Behavior*. CRC Press, Boca Raton, pp 23–37

- Smith JJ, Bush GL (1999) Phylogeny of the subtribe Carpomyina (Trypetinae), emphasizing relationships of the genus *Rhagoletis*. Fruit Flies (Tephritidae): *Phylogeny and Evolution of Behavior*. CRC Press, Boca Raton, pp 187–217
- Stork NE, McBroom J, Gely C, Hamilton AJ (2015) New approaches narrow global species estimates for beetles, insects, and terrestrial arthropods. *Proc Natl Acad Sci* 112:7519–7752
- Thomas DB (1997) Degree-day accumulations and seasonal duration of the pre-imaginal stages of the Mexican fruit fly (Diptera: Tephritidae). *Fla Entomol* 80:71–78
- Thornhill R, Alcock J (1983) The evolution of insect mating systems. Harvard University Press, Cambridge
- Wilkinson GS, Johns PM (2005) Sexual selection and the evolution of mating systems in flies. *The Biology of the Diptera*. Columbia University Press, New York, pp 312–339