Short communication

Trophic interactions between parasitoids and necrophagous flies in Central Argentina

Ana P. Sereno [a], Adriana Salvo [a,b], Moira Battán-Horenstein [a,c,*]

[a] Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba, Av. Velez Sarsfiel 299, Córdoba, Argentina
[b] Centro de Investigaciones Entomológicas de Córdoba, IMBIV/CONICET, Argentina
[c] Instituto de Diversidad y Ecología Animal (CONICET-UNC), Facultad de Ciencias Exactas, Físicas y Naturales, UNC, Argentina

ABSTRACT

Larvae of necrophagous flies in the families Calliphoridae, Sarcophagidae and Muscidae are the main exploiters of decaying organic matter. Knowledge of insect species associated with each stage of decay can be used to estimate the time since death in the crime scene. Dipteran larvae are attacked by a rich community of parasitoids, including species of Braconidae, Ichneumonidae and Pteromalidae (Hymenoptera: Parasitica). This study examined the parasitic complex associated with flies of forensic and sanitary importance in the city of Córdoba (Argentina). Sampling was conducted at two sites with different urbanization levels from December 2012 to March 2013; parasitoids were collected using fly traps baited with beef liver. Rates of parasitism and of parasitized pupae were estimated and species composition was analyzed for both communities. Sarcophagidae was the most abundant family, represented by two species, followed by Calliphoridae. *Nasonia vitripennis* Ashmead (Hymenoptera) was the most abundant species and was collected from a wider variety of hosts. To the best of our knowledge, this is the first study providing accurate information about trophic interactions between calyptrate dipteran species and their hymenopteran parasitoids in central Argentina.

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1. Introduction

Carrion and dung are nutritionally rich but discrete and ephemeral resources used by several groups of organisms, with insects being those of highest species richness and abundance (Hanski, 1991). Among the commonly named "carrion flies", the dipteran families Calliphoridae, Muscidae, Sarcophagidae and Fanniidae are considered important decomposers because their immature stages use carrion as a food source for development. The temporal sequence of species arrival (faunal succession) is predictable under given environmental conditions and is closely linked to the carrion decay process (Anderson and Van Laerhoven, 1996). Knowledge about the insect species associated with each of the different stages of decay as well as about the temperature-dependent development of these species can be used to estimate the postmortem interval (PMI) or time since death (Anderson and Van Laerhoven, 1996). In the decomposing remains, a large number of dipteran larvae and pupae attract a rich community of parasitoids, including species belonging to the families Braconidae, Ichneumonidae, Pteromalidae, Figitidae, Eulophidae, Chalcididae, and Diapriidae (Hymenoptera Parasitica). Accurate information concerning taxonomical and bioecological aspects of host–parasitoid relationships in these communities is relevant for two reasons. Firstly, given that parasitoids have a longer development than their hosts, the knowledge of their trophic relationships, within the context of insect succession patterns, may contribute to a better estimation of PMI (Voss et al., 2009); secondly, parasitoid species have an effect on populations of some dipteran species of medical and veterinary importance, since they kill their host. Indeed, hymenopteran parasitoids have been largely employed as biological control of pest flies (Geden and Hogsette, 2006), which can be mechanical vectors of pathogenic bacteria, enteropathogens, protozoan parasites, viruses (Wanaratana et al., 2011) and may produce myiasis to humans and other vertebrates (Linhares, 1981).

The population dynamics and community organization of host–parasitoid systems have been extensively studied in the context of crop pest management, and very infrequently in other types of systems involving carrion or dung (Voss et al., 2009). In Argentina, studies on blow flies and their parasitoid community are scarce (Salvo and Battán-Horenstein, 2008; Battán Horenstein and

Salvo, 2012), and information concerning trophic relationships is particularly lacking. The aim of this study was to accurately identify interactions between parasitoid species and host fly species of forensic and sanitary importance, and to assess the strength of those interactions. Our results may contribute to the forensic entomology field, by increasing the accuracy in determining the PMI, as well as to the biological control field, by providing valuable information for the regulation of synantropic flies.

2. Materials and methods

2.1. Trapping of flies and parasitoids

The study was conducted in the city of Cordoba, central Argentina (31° 200′ S, 64° 100′ W, elevation 440 m a. s. l.). The climate is temperate, mesothermal, with average temperatures ranging from 11° C in winter to 24° C in summer; average annual precipitation is 800 mm and rainfalls prevail from December to March (Jarsún et al., 2003). Trapping was performed at two study sites, which were characterized by the number of buildings per hectare from the analysis of satellite images (Google Earth). One of the sites, hereinafter referred to as "urban", was located in a densely built neighbourhood (approximately 22 buildings per hectare), with a dominance of backyards and small or no front yards; this site included park areas (approximately 1 ha) and commercial buildings. The other site, herein after referred to as "suburban", was located at a low-density built-up neighbourhood (approximately eight buildings per hectare), with bigger home gardens, higher number of trees but fewer areas dedicated to recreational activities than the urban site. Sampling traps (adapted from Hwang and Turner, 2005) for carrion flies were baited with 400 g of cow liver and placed at each site on four dates in summer (from December 2012 to March 2013), during the period of highest activity of carrion flies. Female flies, attracted by the smell of decay, enter the trap to lay their eggs or larvae in the bait. The traps were remained exposed to attack by parasitoids at the sites for 10 days, the average time for fly larvae to reach the pupal stage. After this period, pupae were removed manually and individually placed in emergence containers and kept in the laboratory until emergence of adult flies or parasitoids. Pupal and larval stages of carrion flies and all emerged adult insects were identified on the basis of their morphology using taxonomic keys (Noyes 1980; Gibson et al., 1997; Masner and García, 2002; Whitworth, 2006) and comparison with reference material belonging to scientific collection to Dr. A. Salvo.

2.2. Data analysis

The following variables were estimated for host and parasitoid communities: number of species, number of individuals (abundance) and species composition. Parasitism rates were estimated as the number of parasitoids emerged from pupae divided by the number of collected pupae multiplied by 100. This variable indicates the successful emergence of parasitoids and the consequent number of adult parasitoids effectively available to affect the next generation of flies. Parasitized pupae from which no adults emerged were dissected and the percentage of parasitized pupae estimated.

3. Results

3.1. Community composition

A total of 2.092 puparia of Diptera were collected during the experiments and a total of 1.119 Hymenoptera parasitoids were obtained from fly pupae. Table 1 shows the abundances of the Diptera and parasitoids collected from each site. The flies were represented by the families Calliphoridae, Sarcophagidae, Muscidae and Fanniidae. The most abundant family was Sarcophagidae, represented by the species Sarcophaga (Liopypia) argyrostoma (Robineau-Desvoidy) and S. (L) crassipalpis Macquart. These species were collected only from the urban site. Calliphoridae, represented by species of the genera Lucilia, Chrysomya and Cochliomyia, was the second most abundant family (Table 1). Chrysomya albiceps, collected mainly in December, was the most abundant species, whereas species of the genus Lucilia (L. sericata Meigen, L. eximia Robineau-Desvoidy and L. cuprina Wiedemann), were less abundant, but were captured from both sites on the four sampling dates. Cochliomyia macellaria (F.) was the least frequent species, being found only at the suburban site. Muscidae was represented by Musca domestica L. and Muscina stabulans (Fallén); the former was the most abundant species at the suburban site, whereas the latter represented less than 1% of individuals reared at the urban site. The genus Fannia (Fanniidae) was collected from both study sites, but represented less than 1% of the collected Diptera individuals.

Four species of hymenopteran parasitoids emerged from collected pupae in different frequencies and on different dates (Table 1). At both sites, Nasonia vitripennis (Pteromalidae) was the most abundant species, followed by Tachinaephagus zaelandicus Ashmead (Encyrtidae). Nasonia vitripennis emerged from the pupae collected during December 2012 and January and March 2013, but was not recorded in February. Emergence of T. zaelandicus increased gradually from January to March. Parasitoids of the genus Trichopria (Diapriidae) and of the species Brachymeria podagrica Fabricious (Chalcididae) showed a low frequency, the former being collected only from the suburban site and the latter, from the urban site, mostly during February.

3.2. Host-parasitoid interactions

Hosts of parasitoid species were two on average (± 0.7; n = 4 parasitoid species) and flies were attacked by two species on average (± 0.57; n = 4 fly species). Fig. 1 shows the interactions between dipteran hosts and hymenopteran parasitoids at the study sites. At the urban site, N. vitripennis caused the highest parasitism percentage, reaching 14% of dipteran pupae collected in January (Table 2). At the suburban site, the highest rates of parasitism were found in February, with T. zaelandicus being the most common species (22% of parasitized pupae), followed by Trichopria sp., with 15% (Table 2). Larvae of Fannia sp., C. macellaria and M. stabulans developed in very low numbers and were not attacked by parasitoids (Fig. 1)

4. Discussion

The results obtained in this study allowed us to describe the species composition of both Dipteran hosts and parasitoid communities as well as to establish the trophic interactions between species. The host community was mainly dominated by Sarcophagidae and Calliphoridae, two families of high forensic importance, including important decomposer species, whose larvae use organic substrates such as carcasses and dung as food source. The species of these families are commonly used in forensic contexts, are very frequent in the carrion succession (Battán Horenstein and Salvo, 2012) and can be vectors of different pathogens that affect human health (Linhares, 1981).

Nasonia vitripennis was the most abundant species within the parasitoid community at both sites. The high abundance of adults captured in traps may be related to the gregarious nature of this species. Our data suggest a preference of N. vitripennis for urban environments, which is in agreement with, who recorded this species more frequent in densely populated areas than in populations of low density in Nova Iguacu, Brazil. The second most

Table 1
Diptera species and hymenopteran parasitoids collected from two sites in Córdoba City.

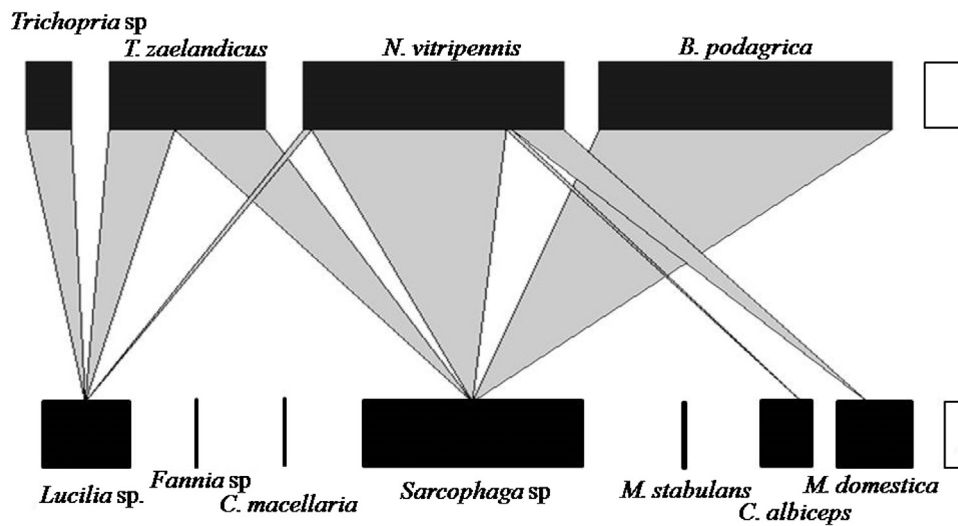| Diptera		Urban				Suburban			
Family	Genus/species	D	J	F	M	D	J	F	M
Sarcophagidae	*Sarcophaga* (L.) spp.	183	281	299	294	–	–	–	–
Muscidae	*M. domestica*	–	–	4	2	42	180	–	187
	M. stabulans	–	–	–	11	–	–	–	–
Fanniidae	*Fannia* spp.	–	–	1	–	–	–	–	2
Calliphoridae	*Lucilia* spp.	15	19	–	–	–	122	84	–
	C. albiceps	102	–	–	–	257	1	–	–
	C. macellaria	–	–	–	–	–	–	6	–
Total		**300**	**300**	**304**	**307**	**299**	**303**	**90**	**189**
Hymenoptera									
Pteromalidae	*N. vitripennis*	27	307	–	74	170	–	–	–
Encyrtidae	*T. zaelandicus*	–	–	–	298	–	7	121	–
Diapriidae	*Trichopria* spp.	–	–	–	–	–	–	16	–
Chalcididae	*B. podagrica*	6	57	36	–	–	–	–	–
Total		**33**	**364**	**36**	**372**	**170**	**7**	**137**	**–**

Fig. 1. Food web diagram depicting the interactions observed between parasitoid and carrion fly species in Córdoba, Argentina. Each species at the top (parasitoids) and bottom (flies) levels is represented by a rectangle, whose size is proportional to the total number of emerged adults. The width of the lines linking species indicates interaction strengths (number of parasitoids obtained on each fly host). Size of empty rectangles at right of top and bottom levels are equivalent to 100 parasitoids and 100 flies respectively.

Table 2
Number of Diptera collected (N), number of parasitized pupae (n) and percentage parasitism (% P) at two study sites.

Urban		D (N = 300)		J (N = 300)		F (N = 304)		M (N = 307)	
Parasitoid	Host	n	% P	n	% P	n	% P	n	% P
N.vitripennis	*Sarcophaga* (L.) spp.	1	0,33	43	14,33	–	–	6	1,95
	Lucilia spp.	–	–	3	1	–	–	–	–
B. podagrica	*Sarcophaga* (L.) spp.	3	1	17	5,67	34	11,18	–	–
T. zaelandicus	*Sarcophaga* (L.) spp.	–	–	–	–	–	–	24	7,82
Suburban		D (N = 299)		J (N = 303)		F (N = 90)		M (N = 189)	
Parasitoid	Host	n	% P	n	% P	n	% P	n	% P
N. vitripennis	*C. albiceps*	11	3,68	–	–	–	–	–	–
	M. domestica	2	0,67	–	–	–	–	–	–
T. zaelandicus	*Lucilia* spp.	–	–	4	1,32	20	22,23	–	–
Trichopria sp.	*Lucilia* spp.	–	–	–	–	14	15,56	–	–

abundant species was *T. zaelandicus*, which is a gregarious parasitoid that attacks the third instars of Diptera, including urban flies (Ferreira de Almeida et al., 2002).

Regarding the host-parasitoid associations, *N. vitripennis* was the most abundant species, showing the broadest host range, followed by *T. zealandicus*. *Nasonia vitripennis* was mainly associated with *Sarcophaga* sp., attacking other species of the families Calliphoridae and Muscidae in the absence of *Sarcophaga* sp., in accordance with the already mentioned generalist feeding habits of this species (Marchiori, 2004). Several authors have suggested the high potential of this species as a biological control agent of Diptera pests (Marchiori, 2004). Carvalho et al. (2005) mentioned

T. zealandicus as the most important parasitoid of *C. megacephala* species (Calliphoridae); however, in our study, specimens of this species emerged from pupae of *Lucilia* and *Sarcophaga* (L). Marchiori (2004) found *B. podagrica* to be associated with Sarcophagidae, Calliphoridae and Muscidae pupae; in this study it was observed emerging only from pupae of *Sarcophaga*. In this work *Sarcophaga* sp. had the largest number of parasitized pupae, although it was collected in less abundance than other parasitoids; our finding is in agreement with observations made by Marchiori (2001), who attributed this phenomenon to the solitary behavior of the species.

This study describes for the first time the trophic relationships between Diptera hosts and their hymenopteran parasitoids in Córdoba city, Argentina, and provides the basis for future studies. Accurate knowledge of host-parasitoid interactions may be very useful in establishing PMI estimates in cases when traditional fly indicator species have completed their development cycle. Parasitoids exhibiting a wide host range and high rates of parasitism in the field are likely to be valuable forensic indicators, as these species are likely to be found in and collected from crime scenes (Voss et al., 2009). Both *N. vitripennis* and *T. zealandicus* have a great potential as postmortem interval indicators, since both species can parasitize several flies of forensic importance. In addition, the data presented here contribute to the development of biological control plans of these flies of medical and veterinary importance.

Acknowledgements

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