

Sex-biased Patterns of Saprophagous Calypttratae (Diptera) Collected With Different Baits of Animal Origin

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ABSTRACT The aim of the present study was to explore the response behavior of males and females of different families of Calypttratae (Diptera) to two different baits (rotten liver and feces) as separate attractants. We describe the sex bias toward these baits for species of Calliphoridae, Muscidae, and Sarcophagidae and compare the general patterns of this sex bias according to the family or trophic guild. In total, 15 species of Sarcophagidae, 10 species of Muscidae, and 9 species of Calliphoridae were analyzed. A female-biased pattern was observed for most calliphorids and for all muscids, whereas a male-biased pattern was more frequent among sarcophagids. The female captures on each kind of bait were assessed as a potential indicator of potential breeding substrates of the species. Three different trophic guilds and their pattern of sex bias were compared. The results obtained allow hypothesizing on the biological traits of saprophagous species, especially on their potential breeding substrates, and assessing proper sampling methods.

KEY WORDS Sex bias, calypttratae, calliphoridae, sarcophagidae, muscidae

In most species with separate sexes, males and females are produced in balanced numbers, regardless of the mechanism of sex determination (Hardy 2002). However, female-biased sex ratios have been frequently recorded for many species of arthropods. In these cases, most female-biased sex ratios recorded are known only from field collections and samplings of adults, which are possibly subjected to a number of inherent collection biases. The adult sex ratio is influenced by the primary sex ratio, and also by sex differences in time of emergence, age at maturation, reproductive life span, survival of males and females, mating behavior, differential resource use and migration patterns (Hardy 2002). Ephemeral and patchy resources of decaying organic matter, such as carrion or dung, are usually exploited by several groups of Diptera soon after these resources become available. Such Diptera species can be divided into two basic groups according to their life history strategies. One group includes large size powerful dispersers with lower fecundity and slower larval development, which usually deposit living larvae and act as early colonizers, whereas the other includes smaller species that lay large masses of eggs and usually reach resources relatively later (Beaver 1977, Hanski and Kuusela 1980, Spivak et al. 1991). Hence, the use of organic matter is widely used to attract adult Diptera, and thus implies biases, as it occurs with many other insects.

Calyptrate flies have frequently been associated with humans and domestic animals and several worldwide species are attracted by substrates with higher concentrations of animal protein, as feces or carrion (Greenberg 1973, Graczyk et al. 2005). In such instances, the calyptrate species involved in organic matter decomposition have been largely studied by their role as mechanic vectors of pathogens (Graczyk et al. 2001), as facultative agents of myiasis (Hall and Wall 1995), and as forensic indicators (Smith 1986, Byrd and Caster 2001). Thus, knowledge of the ecology of these species is important not only for basic entomology but also for other fields of science and their applications. Based on the richness and abundance known for some groups (Brown et al. 2010), three main families of saprophagous calyptrate flies were targeted in this study: Calliphoridae, Muscidae and Sarcophagidae.

The adults of Calypttratae are very mobile organisms, which makes them difficult to sample at randomly selected sites. Therefore, there are many suitable methods and baited trap models to catch these flies (Smith 1986, Hall 1995, Erzinçlioglu 1996). One of the main effects involved in the use of baited traps is that their attractiveness can be different for males and females of different species (Muirhead-Thomson 1968). This implies a sex bias that strongly affects a sampling program, and may vary among species. There are large subsets of saprophagous species in which females use necrotic tissues or feces as protein sources for egg maturation as well as for egg- or larva-laying substrate (Avancini and Linhares 1988). Thus, female bias is expected during samplings for several species, but few

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studies have been conducted to identify how the type of bait affects the catches of the species. The need to deal with this gap in knowledge is important in applied entomology (e.g., successful application of control methods), basic taxonomy (e.g., sampling methods that ensure the catches of both sexes of a species), and ecological problems (e.g., estimates of the population density not based on the female proportion of the population).

Although the physiological reproductive state of flies (i.e., their ovarian development or mated or unmated condition) influences fly behavior (Stoffolano et al. 1990; Stoffolano et al. 1995a,b), observations on general patterns of response to different baits can provide the first insight into the biological traits of the species. This could be especially useful for highly diversified and poorly known communities of saprophagous Calyptratae, as it occurs in temperate areas of the Neotropics. To explore the response of adult Calyptratae to different baits, we performed comparative studies using rotten liver and feces as attractants. The aim of this work is to describe the sex bias toward two different bait types occurring for the species of three families of Calyptratae flies (Calliphoridae, Muscidae, and Sarcophagidae) on the different baits, and to describe and compare general patterns of sex bias for the Calyptratae according to the family or trophic guild. Also, the female captures obtained on each kind of bait were assessed as an indicator of potential breeding substrates of the species. The results may offer a valuable insight into patterns of resource use, and will allow hypothesizing on the biological traits of saprophagous species, their potential breeding substrates, and assessing proper sampling methods.

Material and Methods

Study Site and Trapping Methodology. The study sites were located in Almirante Brown district, 30 km south Buenos Aires city, Argentina (Fig. 1). This district covers 129.33 km² with a population of >0.5 million people. The climate is temperate and

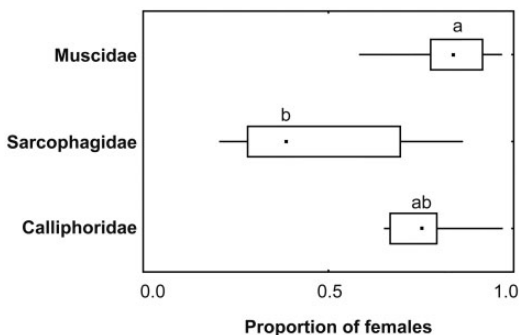


Fig. 1. Differences in sex ratio (proportion of females) between Calliphoridae, Muscidae, and Sarcophagidae obtained in Almirante Brown, Buenos Aires, Argentina. Different letters indicate significant differences ($P < 0.05$), as determined by the Kruskal–Wallis test.

humid, with a mean annual precipitation of ~1,000 mm.

To obtain representative samples of the fly species inhabiting the selected area (e.g., synanthropic and nonsynanthropic species), three sites placed under different degrees of human intervention along an urbanization gradient including urban (34° 50'15.02" S, 58° 23'52.75" W), suburban (34°49' 36.90" S, 58° 24'16.56" W), and rural (34° 51'29.38" S, 58° 23'17.75" W) sites, respectively, were selected for this work. The samples were taken from May 2005 to April 2007 at monthly intervals, when daily conditions were appropriate (sunny and dry weather). Each month, flies were trapped on attractive baits with a hand net. The two types of baits used consisted of 200 g of rotten cow liver (pieces of liver were placed into glass vials for 5 d stored in an isolated cool room, but without temperature control) and 200 g of fresh dog feces (hereafter, liver and feces, respectively). To avoid biases in samples between sun-loving or shade-loving species, both bait types were simultaneously placed in two points: under the shade of trees (shady condition) and in open pasture or garden (sunny condition), totaling four baits per site. The minimum spacing distance for the baits at each site was 30 m. Captured flies were killed in situ by placing them in glass vials with carbon tetrachloride and then stored in paper containers. All the fly specimens collected were sexed and identified to species level. Taxonomic identifications were performed using specialized literature of local fauna: Calliphoridae (Marilyn and Schnack 2002, Olea and Marilyn 2013), Muscidae (Patitucci et al. 2013), and Sarcophagidae (Mulieri et al. 2010). Voucher specimens are housed in the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina.

Sex Bias. Only dominant species (≥ 30 individuals) were taken into account for statistical analyses. To compare behavior of sex bias at the family level, we considered the observation of each species as independent observations. The estimator used for this purpose was P_f : total proportion of females. Normal distribution and homoscedasticity of data were not obtained for female proportions; hence, we applied comparisons using nonparametric analyses. The comparisons between baits for each family were assessed by means of the nonparametric Wilcoxon test for dependent samples (Zar 1996). The comparisons between the three families were assessed by means of the nonparametric Kruskal–Wallis analysis of variance (ANOVA) for independent samples, followed by a posteriori comparisons using Tukey's procedure (Zar 1996). In this case, the value considered for the analysis was the total proportion of females (by pooling the captures made in both baits) for each species.

To describe the pattern of each species, a nonparametric chi-square test (Zar 1996) was used to describe the overall trend in sex bias. The captures on both baits were pooled, and the species were classified into three categories: females-biased or male-biased species, if there were statistical differences from the expected sex ratios of 1:1, and not biased species, if

there were no statistical differences from the expected sex ratios of 1:1.

Contingency tables were used to examine the relationship between the two categorical variables: sex and type of bait. We applied the chi-square test for each species to test whether the sex ratio obtained was independent of the type of bait. In cases when the sample analyzed was small and did not fit with the conditions necessary for the application of the chi-square test (if $n \leq 300$), then the Fisher exact test was applied (Freeman and Halton 1951). In all cases, the analysis was performed on the data set, providing that observed frequencies were different than 0 or that an expected frequency was higher than 1.

Potential Breeding Substrates and Trophic Groups. Among Calyptratae, females strongly select the sites and the proper substrates to deposit their offspring (Archer and Elgar 2003). Accordingly, to evaluate the preference for breeding substrates of species, only the female captures were taken into account. Frequencies of captures of females on baits were compared and statistical differences from an expected ratio of 1:1 were assessed by using a nonparametric chi-square test (Zar 1996). The species were classified as Coprophagous (C) or Necrophagous (N) in relation to their association to a given kind of bait, or as Generalistic (G) if no significant association was found. Additionally, the sex ratio between the three groups was compared by means of the nonparametric Kruskal–Wallis ANOVA for independent samples.

Results

During the two years sampled, 34,433 individuals of saprophagous Calyptratae were collected at the three sites studied (21,858 Calliphoridae, 9,320 Sarcophagidae, and 3,255 Muscidae). In total, 15 species for Sarcophagidae, 10 species for Muscidae, and 9 species for Calliphoridae were represented in samples with >30 specimens.

Comparison of the Patterns of sex Bias Among Calyptratae Families (Total Flies Captured). The patterns of sex ratios were compared among families. The sex ratios obtained revealed differences among the three families (Kruskal–Wallis $H = 12.35$; $P = 0.002$). A strong female-biased sex ratio was obtained for Calliphoridae and Muscidae, whereas and higher proportions of males were detected for Sarcophagidae, although differences were significant only between Sarcophagidae and Muscidae in pairwise comparisons (Fig. 1).

Effect of the Bait Type on the sex Ratio at Family Level. Regarding the patterns of sex bias at the family level, the effects of the bait type on the sex ratio showed no significant differences for Calliphoridae (Pf feces: 0.68 ± 0.25 ; Pf liver: 0.75 ± 0.17 ; Wilcoxon pair test $T = 12$; $P = 0.213$) and Sarcophagidae (Pf feces: 0.45 ± 0.23 ; Pf liver: 0.50 ± 0.21 ; Wilcoxon pair test $T = 27$; $P = 0.06$). The effect of the bait type on the sex ratio was significant for Muscidae, and this effect was due to the higher proportion of females

obtained on liver (Pf feces: 0.79 ± 0.12 ; Pf liver: 0.86 ± 0.14 ; Wilcoxon pair test $T = 9$; $P = 0.02$).

Overall sex Bias of Calyptratae Species (Total Flies Captured on Liver + Bait). In close relation to the previous results, a significant female-biased pattern was observed for all muscids and most calliphorids. Among Calliphoridae, the sole exception to the female-biased pattern was *Sarconesia chlorogaster* Wiedemann, which exhibited a male-biased trend. Within Sarcophagidae, five species were female-biased: *Oxysarcodexia bicolor* Lopes, *Oxysarcodexia paulistanensis* (Mattos), *Ravinia sueta* (Wulp), *Tricharaea* (*Sarcophaga*) *occidua* (F.), and *Tricharaea* (*Tricharaea*) *brevicornis* (Wiedemann), while the rest showed a male-biased pattern of sex ratio (Table 1).

Sex Ratio of Species and Bait Type. Thirty species were analyzed to test whether the pattern of sex ratio obtained was independent of the type of bait used. A large proportion of species (21 species) showed independence between the proportional capture of sexes and the type of bait. Exceptions were found for three species of Calliphoridae [*Lucilia chuvia* (Walker), *Lucilia sericata* (Meigen), and *Calliphora vicina* Robineau-Desvoidy] and two of Muscidae (*Muscina stabulans* Fallén and *Psilochaeta pamptiana* Shannon & Del Ponte; Table 1). In such cases, the female bias was not inverted, but differences in the proportional abundance of sexes were detected, as a result of a more balanced pattern observed on feces. Within Sarcophagidae, *Helicobia aurescens* Townsend and *Oxysarcodexia terminalis* (Wiedemann) showed overall male-biased patterns with significant differences in the proportional abundance of sexes between baits and balanced numbers on liver. A similar trend was found in the female-biased sarcophagid *O. paulistanensis*. In the case of *T. (S.) occidua* (Table 1), this species was also a female-biased species but more balanced on feces.

Female Captures as an Indicator of Potential Breeding Substrates. The frequency of females captured on liver was significantly higher than that of females captured on feces for every calliphorid species, suggesting a unique Necrophagous behavior among the species of this family. In contrast, Sarcophagidae contain both generalist and coprophagous species. Within Muscidae, the females of three species [*Musca domestica* L., *Ophyra aenescens* (Wiedemann), *Ophyra chalcogaster* (Wiedemann)] showed Necrophagous behavior, while the remaining seven species acted as Coprophagous (Table 1).

Two species of Sarcophagidae, *Udamopyga percita* Lopes and *Sarcophaga* (*Lipoptilocnema*) *lanei* Townsend, were not considered for this analysis due to their low female numbers (Table 1), and one species of Muscidae (*Op. aenescens*) was regarded as necrophagous because the females were exclusively captured on liver.

Sex bias of Calyptratae Functional Groups According to Their Potential Breeding Substrate. Taking into account the preference of females of the species for a given bait, the comparisons between the three different functional groups of flies showed no differences in sex bias (Kruskal–Wallis $H = 4.51$; $P = 0.105$). Generalist flies (i.e., species

Table 1. Total frequencies of females, overall trend of sex ratio (female or male biased) and number of individuals of both sexes obtained on feces and liver for Calypttratae species in Almirante Brown, Buenos Aires, Argentina

	Bait preference (females)	Chi-square	Overall trend	Sex ratio				Chi-square (contingency table)
				Feces		Liver		
				♂	♀	♂	♀	
CALLIPHORIDAE								
<i>Chrysomya albiceps</i> (Wiedeman, 1819)	N	P < 0.001	female biased	0	4	198	6076	–
<i>Chrysomya megacephala</i> (Fabricius, 1794)	N	P < 0.001	female biased	0	5	46	168	–
<i>Lucilia sericata</i> (Meigen, 1826)	N	P < 0.001	female biased	160	237	469	2167	P < 0.001
<i>Lucilia cluvia</i> (Walker, 1849)	N	P < 0.001	female biased	1125	1513	1343	5979	P < 0.001
<i>Lucilia cuprina</i> (Wiedemann, 1830)	N	P < 0.001	female biased	10	17	60	176	<i>P</i> = 0.249
<i>Calliphora lopesi</i> Mello, 1962	N	P = 0.008	female biased	6	7	8	21	<i>P</i> = 0.298
<i>Calliphora vicina</i> Robineau-Desvoidy, 1830	N	P < 0.001	female biased	127	112	473	1013	P < 0.001
<i>Sarconesia chlorogaster</i> (Wiedemann, 1830)	N	P < 0.001	male biased	28	14	106	56	<i>P</i> = 0.881
<i>Cochliomyia macellaria</i> (Fabricius, 1775)	N	P < 0.002	female biased	1	17	14	102	<i>P</i> = 0.692
SARCOPHAGIDAE								
<i>Ravinia sueta</i> (Wulp, 1895)	C	P < 0.001	female biased	56	140	19	30	<i>P</i> = 0.166
<i>Oxysarcodexia culmiforceps</i> Dodge, 1966	G	<i>P</i> = 0.383	male biased	59	46	61	38	<i>P</i> = 0.431
<i>Oxysarcodexia paulistanensis</i> (Mattos, 1919)	C	P < 0.001	female biased	449	1421	458	980	P < 0.001
<i>Oxysarcodexia terminalis</i> (Wiedemann, 1830)	C	P = 0.004	male biased	193	65	64	36	P = 0.042
<i>Oxysarcodexia bicolor</i> Lopes, 1946	G	<i>P</i> = 0.106	female biased	3	20	2	11	<i>P</i> = 0.999
<i>Oxysarcodexia varia</i> (Walker, 1836)	G	<i>P</i> = 0.062	male biased	230	74	161	53	<i>P</i> = 0.912
<i>Udamomyia percita</i> Lopes, 1938	?	–	male biased	41	7	24	10	<i>P</i> = 0.103
<i>Tricharaea (Sarcophaga) occidua</i> (Fabricius, 1794)	C	P < 0.001	female biased	815	1476	339	946	P < 0.001
<i>Tricharaea (Tricharaea) brevicornis</i> (Wiedemann, 1830)	G	<i>P</i> = 0.622	female biased	6	20	1	17	<i>P</i> = 0.211
<i>Helicobia aurescens</i> (Townsend, 1927)	G	<i>P</i> = 0.229	male biased	112	52	74	65	P = 0.007
<i>Microcerella muehni</i> (Blanchard, 1939)	G	<i>P</i> = 0.635	male biased	87	53	79	58	<i>P</i> = 0.477
<i>Sarcophaga (Bercaea) africa</i> (Wiedemann, 1824)	G	<i>P</i> = 0.086	male biased	25	12	36	22	<i>P</i> = 0.447
<i>Sarcophaga (Liopygia) crassipalpis</i> Macquart, 1839	C	P = 0.027	male biased	38	18	7	7	<i>P</i> = 0.212
<i>Sarcophaga (Lipoptilocnema) lanei</i> Townsend, 1934	?	–	male biased	31	10	8	4	<i>P</i> = 0.711
<i>Sarcophaga (Lipoptilocnema) koehleri</i> (Blanchard, 1939)	C	P = 0.008	male biased	66	35	34	16	<i>P</i> = 0.537
MUSCIDAE								
<i>Gymnodia</i> spp.	C	P < 0.001	female biased	9	103	1	23	<i>P</i> = 0.999
<i>Musca domestica</i> Linnaeus, 1758	N	P < 0.001	female biased	14	164	53	566	<i>P</i> = 0.768
<i>Muscina stabulans</i> (Fallén, 1817)	C	P < 0.001	female biased	33	120	23	182	P = 0.007
<i>Mydaea plaumanni</i> Snyder, 1941	C	P < 0.001	female biased	52	206	4	35	<i>P</i> = 0.188
<i>Myospila obscura</i> (Shannon & Del Ponte, 1926)	C	P < 0.001	female biased	19	35	8	12	<i>P</i> = 0.788
<i>Neurotrixa felsina</i> (Walker, 1849)	C	P < 0.001	female biased	296	396	58	103	<i>P</i> = 0.118
<i>Ophyra aeneascens</i> (Wiedemann, 1830)	N	–	female biased	0	0	24	116	–
<i>Ophyra chalcogaster</i> (Wiedemann, 1824)	N	P = 0.042	female biased	1	9	0	20	–
<i>Psilocheata chalybea</i> (Wiedemann, 1830)	C	P < 0.001	female biased	20	114	3	49	<i>P</i> = 0.089
<i>Psilocheata pampliana</i> (Shannon & Del Ponte, 1926)	C	P < 0.001	female biased	76	218	10	80	P = 0.004

The overall trend (females or male biased species) are assigned by mean of statistical differences from the expected sex ratios of 1:1 ($P < 0.01$). Chi-square of contingency tables referred to examine the relationship between the sex and type of bait (significant results in bold). Chi-square test referred to bait preference of females indicates differences from an expected bait ratio 1:1 (significant results in bold).

which females are equally attracted to both baits) consisted only of sarcophagid species and, as it occurs within this family, the group exhibited higher variability in the sex ratio than coprophagous and necrophagous flies (Table 1; Fig. 2).

Discussion

Field collections of Diptera using baits do not provide definitive evidence about the ecological context in which sex ratios are expressed because we know little about the mating behavior and population structure of most species of this order (Hardy 2002). However, while important aspects of the biology, including the mating dynamics and structure of natural populations, have remained largely unstudied, the observations on the sex ratio of the species occurring in relation to patchy ephemeral resources may be suggestive and provide a first understanding on several of these issues.

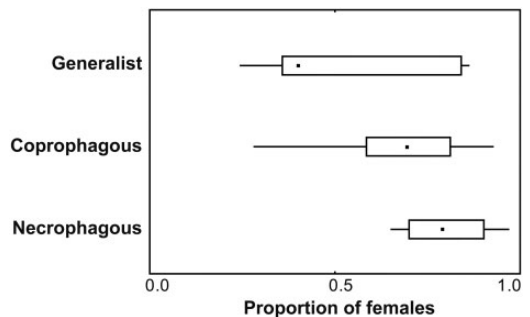


Fig. 2. Differences in sex ratio (proportion of females) between functional groups (coprophagous, necrophagous, or generalist) obtained in Almirante Brown, Buenos Aires, Argentina.

Unbiased sex ratios at hatching seem to be the rule among Calypttraeae, as documented for Calliphoridae, Muscidae, and Sarcophagidae (Bonatto 1996, De Arriba and Costamagna 2006, Barros-Souza et al. 2012, Khan et al. 2012). Consequently, the wide spectrum of unbalanced adult sex ratios obtained for Calypttraeae in association with decaying organic matter is the result of ecological traits of the species. The mating system of the species and the differences in the protein requirements of the sexes to reach sexual maturity may play an important role in the observed sex ratios of the species studied here.

The females of Calypttraeae are often anautogenous, requiring protein sources to develop their first batch of eggs or become sexually receptive, which suggests that mating in the field might usually occur at or near a protein source (Kitching 1977, Cook 1994). In the case of some blowflies, males have been observed downwind from an odor source where they intercept and attempt to mate with females (Cook 1994). Blowflies usually spend short periods in copula (a few minutes) even after numerous, successive matings (Thomas 1991, Cook 1994). In contrast, sarcophagids copulate for considerable periods of time, and mating take place for a few consecutive hours (Thomas 1950). Males of Sarcophagidae are extremely aggressive, frequently harass females (irrespective of whether they belong to their own species or genus), and outnumber females directly on the oviposition substrates. This behavior may reflect that males are subjected to strong mating competition, and behaviors ensuring precedence to reach mating are strongly selected (Wilkinson and Johns 2005). Both mating strategies and their associated behavioral patterns may underlie differences in the sex ratio observed for Calliphoridae and Sarcophagidae in relation to the substrates used for this study.

Saprophagous Calypttraeae are a complex guild of flying insects including several functional groups taking into account their response to substrates such as animal protein source. The results of this study highlight the wide range of sex-ratio patterns displayed by saprophagous Calypttraeae in response to different bait stimuli. A diversified response was observed not only between species but also at the family level, a fact that seems to agree with the highly diversified ecological traits observed for the Calypttraeae as a whole (Wiegmann et al. 2011). We found differences in the global pattern of sex ratio both at the family level and at the species level within each family.

Few works have compared the sex ratios at the family level, and this is the first study comparing the sex ratio of several species belonging to different families of Calypttraeae in the Neotropics. The previous works have analyzed separately the sex ratio for Calliphoridae (Mendes and Linhares 1993a), Muscidae (Mendes and Linhares 1993b), and Sarcophagidae (Mendes and Linhares 1993c). In comparison to our results, these experiments showed similar trends (female bias) for Calliphoridae and Muscidae, but not for Sarcophagidae. Similarly, our results are different from those obtained by Martín-Vega and Baz (2013) in an Old World sarcosaprophagous community, where

female-biased samples were clearly observed for Calliphoridae, Muscidae, and Sarcophagidae. The observed differences for the sex ratio obtained for Sarcophagidae can depend on two main possible causes. One explanation may be the inherent effect of the trapping methodology on the fly behavior (e.g., distinct degree of fly attractiveness or avoidance to the trapping method used). Males of some sarcophagid species may be reluctant to penetrate into the trapping devices, as those used by Martín-Vega and Baz (2013) and Mendes and Linhares (1993c). Instead, for the present work, flies feeding or resting on the baits or landing on the surroundings were directly captured when the baits were netted, thus increasing the capture of males in the sample. Another main factor might be the inherent differences in the behavior of the species in each community. We consider that both factors might contribute to the general differences in sex ratio observed among both assemblages. In this regard, it is noted that species that appeared in both experiments [*C. vicina*, *Chrysomya albiceps* (Wiedemann), *M. domestica*, and *M. stabulans*] shared the same behavior (female biased) associated with the substrate composed of rotten animal tissue.

The capture of females of the species not only seems to describe the feeding preferences of females, but also allows hypothesizing about potential breeding substrates of the species. Considering the Calliphoridae as a test case because of their previously well-documented carrion feeding behavior of their immature individuals (Hanski 1987, Vargas and Wood 2010), all calliphorid species showed significantly higher frequencies of females on liver. This result indicates that the observations on the behavior of the adult females can act as good indicators of the breeding behavior of the species. On the other hand, the sarcophagids *U. percita* and *S. (L.) lanei* showed the lowest female captures, suggesting a quite different breeding substrate preference than vertebrate carcasses or feces. In the case of *U. percita*, the species has been previously recorded breeding on land snails (Lopes 1969) and is most probably specialized to exploit dead mollusks (Lopes 1940), thus avoiding substrates such as feces or rotten vertebrate tissues.

The results obtained highlight that the feces attracted significantly more females of Muscidae and Sarcophagidae than the liver. The preference observed for the muscid females belonging to the genus *Neurotrixa* and for sarcophagid females belonging to the genera *Oxysarcodexia* and *Tricharaea* may indicate the coprophagous habits of their larvae. Previous studies indicate the emergence of species of these genera from different kinds of feces (D'Almeida 1986, 1994; Hernandez 1989; Mendes and Linhares 2002), and support our findings.

On the other hand, larvae of species belonging to *Gymnodia* (= *Brontaea*) and *Ravinia* have been described as predatory larvae on feces (Hernandez 1992, Mendes and Linhares 2002). A similar predatory behavior is displayed by the third-instar larvae of the genera *Ophyra* and *Chrysomya* in carrion communities (Skidmore 1985, Faria and Godoy 2001). Taking into

account the inherent complexity of larval behavior, our results were able to detect the coprophilous or necrophilous habits for all mentioned species, but hypotheses for individual species must be proposed to indicate the preferred laying-substrate, but not the true feeding guild to which their larvae belong (i.e., true coprophagous or necrophagous larvae, obligate, or facultative predatory larvae). These findings are useful in the context of poorly known taxa, as it occurs with rich families as Muscidae and Sarcophagidae in South America. For example, the behavior of the New World species belonging to *Psilochaeta*, *Myospila*, or *Mydaea* in relation to different kinds of substrates was never observed, and results here obtained can properly drive and put into a context the future researches regarding the life cycle or larval behavior of these groups.

The importance of protein in the female adult diet of calyptrate flies has been well-documented (Stoffolano et al. 1990, Stoffolano et al. 1995a). While carbohydrates seem to cover the high caloric requirements in males for mobility, sources with animal protein content are needed by females for their ovariole maturity and as oviposition or larviposition substrates. This aspect is better known in the case of Calliphoridae. While both stages of calliphorid females, mated gravid and unmated previtellogenic, are attracted to carcasses either as oviposition media or as protein source for sexual maturity, respectively, previtellogenic females may be attracted to feces only as protein sources (Stoffolano et al. 1990). Previous studies have demonstrated that the effect on calliphorid males seems to be less drastic, and that males obtain enough protein from poor substrates like feces to reach sexual development in shorter feeding periods of times than females (Stoffolano et al. 1995b). Males of blowflies are relatively less frequent on carcasses or on rotten tissues than females and several previous works have shown that this kind of response is very common among calliphorids (MacLeod and Donnelly 1957, Woodburn and Vogt 1982). In the Neotropics, the conspicuous exceptions are the blowflies belonging to the subfamily Toxotarsinae, in which captures are either balanced (Mulieri et al. 2006) or male-biased (Mariluis et al. 2008). In this work, the single toxotarsine fly displaying the previously described male-biased behavior was *S. chlorogaster*. One possible explanation is that males and females of *S. chlorogaster* appear to meet on oviposition sites to mate, displaying a sarcophagid-like mating system.

Competition plays a major role in dung and carrion insect communities and there are several ecological, behavioral, and physiological adaptations for an efficient resource use. A rapid start of resource use associated with viviparity is made possible by several calyptrate groups that are successful as colonizers of extremely ephemeral resources such as feces, or as opportunistic generalist species (Denno and Cothran 1975, Hanski 1987). Thus, many species and clades of viviparous Sarcophagidae and Muscidae have been recorded as coprophagous or associated with dung (Meier et al. 1999). Our study supports the association of viviparity with these kinds of life history strategies. The group of generalist species consisted only of

Sarcophagidae, a family in which viviparity appears to be a ground plan. Coprophagous species also recorded a relatively high proportion of viviparous species and consisted of a mixed assemblage with several Sarcophagidae among them. In contrast, the necrophagous group consisted exclusively of oviparous species belonging to Calliphoridae and Muscidae.

Also, competition for resources affects behavior and, consequently, could modify the niche breadth of the species. For this study, we considered each species as independent observations, and such condition was probably reached (or almost reached) during the coldest period of the year, because the lower richness and abundance of flies promote a reduction in species interactions. However, during summer, sex ratio responses could be partly modified as a consequence of increased interactions among species. Consequently, the sex ratio expressed by any species may vary from place to place in relation to the characteristics of the coexisting species or community composition.

Finally, the placement of baits in the field to obtain specimens of flies for taxonomic purposes is a practice commonly used by entomologists. From the point of view of taxonomist researchers, the differential response of sexes to different baits is a major question in samples targeted to taxonomic collection. We conclude that the liver is unsuitable for the collection of large numbers of males of certain species of Calliphoridae and Muscidae, in which the capture of a single male simply by chance requires high sampling efforts (e.g., *Cochliomyia macellaria* F., *Gymnodia* spp., or *Ophyra* spp.). In contrast, the use of baits such as feces or rotten tissues to obtain saprophagous species of Sarcophagidae appears to be a proper method in terms of cost-effectiveness for a family whose systematics is widely based on male morphology.

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