Sex-based population structure of ectoparasites from Neotropical bats

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The structure and composition of populations may be molded by multiple evolutionary and ecological mechanisms, with natural selection affecting sex ratios as well as the distributions of each sex throughout the environment. To address sex-based aspects of population structure, I evaluated sex ratios, co-occurrence of the sexes, correlations of abundance of the sexes, and dispersion of individuals of each sex for each of 34 host–ectoparasite associations from Paraguayan bats. Of the 34 host–ectoparasite associations, 23 exhibited positive co-occurrence, 27 exhibited positive correlation of abundances, three exhibited male sex bias, one exhibited female sex bias, 27 had clumped distributions of males, and 26 had clumped distributions of females. No associations exhibited negative co-occurrence, negative correlation of abundance, or hyperdispersed males or females. There was no evidence for sexual segregation, sex-based niche partitioning, or intrasexual selection in any host–ectoparasite association. Previously proposed mechanisms (e.g. pre-partum sex bias, local mate competition, or mortality from host grooming) fail to explain observed patterns of sex bias. For ectoparasites of hosts that occupy permanent roost sites, sex-specific behaviour related to reproduction may make females more susceptible to off-host predation, and less likely to be present in samples from bats captured away from the roost. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **107**, 56–66.

ADDITIONAL KEYWORDS: aggregation of individuals – local mate competition – local resource competition – sex allocation – sex bias – sexual selection.

INTRODUCTION

The structure and composition of animal populations may be molded by multiple evolutionary and ecological mechanisms, with natural selection affecting sex ratios of populations as well as the distributions of sexes throughout the environment (Crow, 1986; Frank, 1990). Sex allocation theory makes specific predictions about how sex ratios should be selected for in order to optimize the contribution of individuals to subsequent generations (Fisher, 1958; Charnov, 1982; Crow, 1986). The default sex ratio for diploid parents of sexually reproducing species is 1 : 1, based on the assumption that the genotype of the parents determines the sex ratio of the progeny (Bull & Charnov, 1988), and because each sex will contribute equally to the genetic composition of the subsequent

generation (Fisher, 1958). Fisher's model has additional assumptions (e.g. biparentalism, Mendelian segregation of alleles, and random mating in an infinite population), and the violation of any assumption can result in unequal investment in the sexes and biased sex ratios (Charnov, 1982; Bull & Charnov, 1988). In general, empirical data support Fisher's model when assumptions are met, but there are many cases in which a violation of assumptions results in consistent deviations from a 1 : 1 ratio. For example, the assumption of random mating is violated by many species, and may give rise to male- or female-biased populations. The local mate competition hypothesis (Hamilton, 1967) explains the selection for more female progeny in fig wasps, in which mating takes place between offspring prior to the dispersal of just the females. In this scenario, few males can inseminate many females, and each female is a potential foundress of a future colony; therefore, group fitness

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favours an excess of females. In contrast, if only males disperse, competition for local resources among the non-dispersing females may result in greater fitness of male offspring, with selection leading to an excess of males (Clark, 1978).

In addition to affecting sex ratios, selection may mold patterns of distribution of the sexes in space, which may indicate which mechanisms drive selection and the allocation of resources to the sexes. If local resource competition is important, individuals of the same sex may become hyperdispersed to maximize fitness. Alternatively, if resources are distributed patchily, clumped distributions may emerge, with intense competition for local patches of resources. In addition, selection could favour distinct niches for each sex, which could reduce competition among siblings and enhance overall fitness. Such sex-specific niches have been documented for many taxa, including plants (Eppley, 2006), insects (Rusterholz & Erhardt, 2000; Joron, 2005), fish (Reimchen & Nosil, 2004), reptiles (Shine, 1986), birds (Bearhop *et al*., 2006), and mammals (Safi, König & Kerth, 2007).

HOSTS AND PARASITES AS MODEL SYSTEMS

Host–ectoparasite associations are useful model systems to study population structure. Hosts are well-defined sampling units: each host individual harbours a sample population; common host species provide replicate samples; and species-rich host taxa provide opportunities to determine if patterns are general or idiosyncratic. Host characteristics such as body size, sex, social system, and nesting/roosting ecology can affect ectoparasite population structure or diversity (e.g. Patterson, Dick & Dittmar, 2007, 2008; Presley & Willig, 2008; Presley, 2011). Consequently, host–parasite systems are ideal for investigations of sex-based aspects of population structure.

A study of sex ratios for 112 host–ectoparasite associations of streblid bat flies from Venezuelan bats found 36 associations that exhibited male sex bias (Dick & Patterson, 2008). Traditional explanations for sex bias (i.e. sampling bias, pre-partum sex bias, differential longevity, or dispersal capacity) were rejected in favour of sex-specific mortality risk from host grooming, with the hypothesis that larger females were not as successful avoiding host grooming as smaller male flies. In this study, I expand on this recent work with a more comprehensive analytical approach to evaluate aspects of population structure related to sex for five families of bat ectoparasite that differ in life-history traits. This approach provides a more rigorous evaluation of hypotheses (e.g. local mate competition and local resource competition) and potential mechanisms associated with patterns of sex bias in populations, as well as improved

understanding about the generality of patterns among taxa of ectoparasite or host. For 34 host–ectoparasite associations, analyses evaluate: (1) the existence of male or female sex bias; (2) if male and female ectoparasites exhibit similar patterns of distribution (co-occurrence or correlations of abundance) among hosts; and (3) if each sex is dispersed in a clumped, random, or even fashion among hosts.

MATERIAL AND METHODS

PARAGUAYAN BATS AND THEIR ECTOPARASITES

Bats and their ectoparasites were collected from 28 sites distributed throughout Paraguay (Presley & Willig, 2008). All aspects of protocols for collection and processing were designed to minimize the likelihood of contamination (i.e. the assignment of ectoparasites to the wrong host individual). Research involving live animals conformed to guidelines of the American Society of Mammalogists (Sikes, Gannon & Animal Care and Use Committee of the American Society of Mammalogists, 2011), and was approved by the Animal Care and Use Committee of Texas Tech University. Details about collection, identification, and deposition of specimens are available elsewhere (Willig *et al*., 2000; Presley, 2004; Presley & Willig, 2008).

Fifty-four species of bat are known from Paraguay (López-González, 2005), representing six families and a diverse suite of foraging guilds. Frugivorous phyllostomids dominate the assemblages of eastern Paraguay, which is humid, physiographically diverse, and dominated by tall, humid forests. In contrast, insectivorous molossids dominate assemblages in the flooded and xeric environs of western Paraguay (Willig *et al*., 2000; López-González, 2004). Large areas adjacent to the Paraguay River are flooded, and support palm savannahs or marshlands, whereas western Paraguay is dominated by low xerophytic thorn-scrub. Vespertilionids occur throughout the country, but are never the predominant taxon of bat (López-González, 2004).

Paraguayan bats are infested by a diverse fauna of ectoparasitic arthropods, including bat flies (Nycteribiidae and Streblidae), bat bugs (Polyctenidae), fleas (Ischnopsyllidae), ticks (Argasidae and Ixodidae), and mites (Chirodiscidae, Macronyssidae, Myobiidae, Spinturnicidae, and Trombiculidae). Host grooming can be a primary cause of mortality for permanent ectoparasitic insects (Overal, 1980; Marshall, 1982), and presumably for acarids as well. If hosts are ineffective at grooming because of poor health or deformities, ectoparasite populations can increase quickly (Marshall, 1982). Adult stages of each insect family and of spinturnicid mites are

sexually dimorphic and easily discernable, allowing for the evaluation of sex-based population dynamics of infracommunities. Only adults were included in analyses.

In South America, most bat flies and bat bugs are associated with three families of host: Phyllostomidae, Vespertilionidae, and Molossidae (Presley, 2004; Dick & Gettinger, 2005; Presley & Willig, 2008). Streblids infest phyllostomids, noctilionids, and molossids. Nycteribiids are restricted to vespertilionids, and Paraguayan polyctenids occur only on molossids (Marshall, 1982; Presley, 2004). Streblids and polyctenids are highly host-specific (Presley, 2004; Dick & Gettinger, 2005; Dick, 2007; Dick & Patterson, 2007), and are usually associated with a single primary host species. In contrast, nycteribiids in Paraguay commonly occur on multiple host species of the same genus (Graciolli, Dick & Gettinger, 2006). South American spinturnicid mites are primarily associated with phyllostomids and vespertilionids (Presley, 2004), and differ in their host specificity.

ANALYTICAL APPROACH

The majority of non-primary bat–streblid associations result from disturbance transfers or contamination during sampling (Dick, 2007), and do not reflect natural host–parasite relationships. To eliminate effects of contamination, I defined primary host–ectoparasite relationships based on infestation parameters to establish a standardized threshold to identify relationships that were likely to be naturally occurring and appropriate for analysis. Ectoparasites that occurred on $\geq 5\%$ of the individuals of a host species, and with a mean abundance per inspected host individual ≥ 1.0, or with a specificity index ≥ 0.90, were considered to form primary relationships (Presley, 2011). Specificity is the proportion of individuals of an ectoparasite species that occur on a particular host species, and is calculated separately for each host–parasite association, and sums to 1.00 for each species of parasite for an entire host assemblage. These data are part of a collection of bats and ectoparasites that included 2909 bats, representing 44 species (López-González, 2005), which yielded 17 505 ectoparasites, representing 104 species, 39 genera, and 11 families (Presley, 2004); calculations of specificity were based on this collection. To ensure adequate sample sizes and statistical power, all analyses were restricted to primary associations in which ectoparasites were collected from ten or more host individuals.

Four analytical approaches were used to evaluate patterns of male and female ectoparasite occurrence on individuals of primary hosts. First, patterns of co-occurrence were evaluated using presence–absence data. Second, correlations of abundances were used to evaluate aggregation or segregation of the sexes. Third, sex ratios were evaluated to determine if they significantly differed from 1 : 1. Fourth, analysis of dispersion of individuals determined if individuals of each sex were dispersed in a uniform, random, or clumped fashion. Analyses were conducted separately for each of 34 host–ectoparasite associations, representing 26 species, including three spinturnicids, four polyctenids, three nycteribiids, 15 streblids, and one ischnopsyllid. Associations involved 17 host species, including two noctilionids, seven phyllostomids, three vespertilionids, and five molossids (Presley, 2012). All analyses were conducted with an α level of 0.05.

Co-occurrence

Patterns of co-occurrence between males and females were evaluated using a null model approach in ECOSIM 7.0 (Gotelli & Entsminger, 2001), and presence–absence matrices, in which each row represented the sex of ectoparasite, and each column represented an individual host. The C-score metric (Stone & Roberts, 1990), which measures the average number of chequerboard units, was chosen because it is less sensitive to noise in the data, and has better interpretability than other metrics (Gotelli, 2000). If species co-occur more often than expected by chance, the C-score is less than expected by chance (i.e. a low chequerboard score).

A null model with fixed row totals and equiprobable columns was chosen a priori, and used in all analyses of co-occurrence. This combination of constraints and test statistic offers the best combination of desirable type-I and type-II error rates (Gotelli, 2000), and biological realism. More specifically, abundance and incidence may be sex-specific characteristics for some ectoparasites of bats (Fritz, 1983; Dick & Patterson, 2008), and each host individual should be capable of supporting both sexes of ectoparasite. Host individuals from which no parasites were collected represent 'empty' observations. Based on the extensive literature on Neotropical bat ectoparasites, there is no reason to expect that any particular individual of a primary host is an unsuitable host. Consequently, it is more likely that bats without ectoparasites remain free of parasites by chance, or by the choice or preference of the ectoparasites, than that they represent unsuitable habitats per se. Omitting empty hosts can lead to inflated type-II error rates by artificially restricting the host individuals available for ectoparasites to infest, creating a smaller null space than that represented by the data for host–parasite associations. Empty hosts were included via the empty sites option in ECOSIM 7.0, to ensure that the construction of null communities was consistent with our current biological understanding of bats and their ectoparasites.

Each observed C-score was compared with a distribution of C-scores calculated from 10 000 matrices generated based on the outlined null model criteria. To allow for the possibilities of segregation or aggregation of the sexes, co-occurrence analyses were conducted as two-tailed tests. The sexes were considered to be aggregated if the probability of a C-score from a randomly generated matrix being less than or equal to the observed C-score was ≤ 0.025 ($\alpha/2$), and were considered to be segregated if the probability of a C-score from a randomly generated matrix being greater than or equal to the observed C-score was ≤ 0.025. Null distributions may not be normal, requiring separate P values for each tail of the distribution (Gotelli & Entsminger, 2001). C-scores from randomly generated matrices that equal observed values are included in the calculation of both P values. Consequently, the sum of P values for a single analysis will be > 1.0 when such ties exist. To make the values of the C-score comparable, the standardized effect size (SES) was calculated using the mean and standard deviation of the distribution of C-scores. In general, SES values < -2 or > 2 represent significant results, depending on how closely the distribution of null values approximates a normal distribution.

Correlations of abundances

The strengths of interactions between males and females may not be sufficiently strong to manifest in analyses of presence–absence data, but may be reflected by in-concert changes in abundance. Because the focal aspect of correlations of abundance between males and females is that the relationship is monotonic, and not that it is linear, and also to avoid the unnecessary assumption that data had a bivariate normal distribution, Spearman rank correlations were chosen over Pearson product moment correlations.

Sex ratios

A Wilcoxon matched-pairs signed-ranks test (Siegel, 1956) was used to evaluate biases in sex ratios for each of the 34 host–ectoparasite associations. This test uses the direction and magnitude of differences in paired values, giving more weight to pairs that have a large difference than to pairs that have a small difference. Wilcoxon tests were conducted as two-tailed tests to detect male or female biases in sex ratios using the Wilcoxon option for related samples in SPSS 15.0.0 for Windows.

Dispersion of individuals among hosts

The coefficient of dispersion (CoD = variance/mean) was used to estimate the distribution of each sex among host individuals. A CoD of ~1.0 indicates a random distribution of individuals, whereas values < 1.0 indicate uniform distributions, and values > 1.0 indicate clumped distributions (Brower, Zar & von Ende, 1990). Significant deviations from random dispersion were evaluated via a χ^2 test, where the quotient of the sum of squares and the mean have a χ^2 distribution with $N - 1$ degrees of freedom (N = number of hosts).

Considerable debate surrounds the use of methods to maintain type-I experiment-wise error rates at predetermined α levels for suites of analyses (Hurlbert & Lombardi, 2003; Roback & Askins, 2005). Methods that attempt to maintain the experiment-wise error rate at an a priori level are overly conservative (e.g. Bonferroni sequential adjustment), and the application of such methods must inflate type-II error rates in order to reduce type-I error rates. For exploratory analyses such as those conducted herein, corrections for multiple tests are not recommended (Roback & Askins, 2005). I was more concerned about the consequences of ignoring results with ecological implications and with general patterns among all host–ectoparasite associations than about the potential for individual type-I errors, which are generally a minor concern for most ecological studies (Hurlbert & Lombardi, 2003). Therefore, I present exact P values for all analyses and interpret results without any correction for multiple tests.

RESULTS

Streblids were the most abundant (2469 individuals) and species-rich (27 species) ectoparasitic insect on Paraguayan bats, with polyctenids (ten species, 561 individuals), nycteribiids (five species, 87 individuals), and ischnopsyllids (three species, 32 individuals) being considerably less common and diverse. Spinturnicids were the second most abundant and species-rich (ten species, 1763 individuals) family of acarids, after macronyssids (22 species, 9766 individuals).

Significant aggregation (positive co-occurrence) of males and females occurred in 23 of the 34 associations (Table 1). No association had indications of segregation of the sexes, with all associations having the negative SES scores that indicate a positive association. Males and females of 27 associations exhibited significant positive correlations of abundances (Table 1).

Three associations exhibited male sex bias and one exhibited female sex bias (Table 1). Males (27 associations) and females (26 associations) exhibited clumped distributions in the majority of cases, with the remaining associations having random distributions (Table 2). Five species (*Periglischrus iheringi*,

Table 1. Analyses of patterns of co-occurrence, correlations of abundance, and sex ratios for male and female ectoparasites from 34 host–ectoparasite associations

Ectoparasite family / Ectoparasite species	Host species	Number of hosts	Co-occurrence			Correlations		Sex ratio analysis		
			SES	Aggregation P value	Segregation P value	ρ	P value	% males	Z	P value
Spinturnicidae										
Periglischrus iheringi	Artibeus fimbriatus	79	−2.21	**0.010**	0.997	0.408	**<0.001**	0.51	0.263	0.792
Periglischrus iheringi	Artibeus jamaicensis	42	−2.09	**0.013**	0.998	0.388	**0.011**	0.62	1.223	0.221
Periglischrus iheringi	Artibeus lituratus	351	−5.65	**<0.001**	1.000	0.411	**<0.001**	0.49	0.902	0.367
Periglischrus iheringi	Platyrrhinus lineatus	90	−2.40	**0.007**	0.999	0.379	**<0.001**	0.49	0.982	0.326
Periglischrus ojastii	Sturnira lilium	404	−4.33	**<0.001**	1.000	0.257	**<0.001**	0.45	0.836	0.403
Spinturnix americanus	Myotis albescens	87	−1.69	0.142	0.982	0.207	0.055	0.63	1.387	0.166
Spinturnix americanus	Myotis nigricans	128	−1.13	0.327	0.953	0.106	0.232	0.45	0.443	0.658
Nycteribiidae										
Basilia bequaerti	Eptesicus furinalis	69	−3.12	**0.004**	0.999	0.464	**<0.001**	0.63	1.387	0.166
Basilia carteri	Myotis albescens	87	−2.43	**0.012**	0.998	0.288	**0.007**	0.52	0.330	0.741
Basilia carteri	Myotis nigricans	128	−5.16	**<0.001**	1.000	0.639	**<0.001**	0.46	0.290	0.771
Basilia speiseri	Myotis nigricans	128	−3.67	**0.002**	1.000	0.387	**<0.001**	0.56	0.714	0.475
Polyctenidae										
Hesperoctenes fumarius	Molossus molossus	228	−3.28	**0.002**	0.999	0.246	**<0.001**	0.47	0.190	0.849
Hesperoctenes longiceps	Eumops patagonicus	526	−1.87	0.068	0.980	0.084	0.053	0.39	1.897	0.058
Hesperoctenes parvulus	Molossops temminckii	160	−3.56	**0.006**	0.999	0.326	**<0.001**	0.48	0.302	0.763
Hesperoctenes sp.	Eumops glaucinus	56	−3.08	**<0.001**	1.000	0.586	**<0.001**	0.39	0.694	0.487
Streblidae										
Aspidoptera falcata	Sturnira lilium	404	−5.16	**<0.001**	1.000	0.338	**<0.001**	0.45	1.571	0.116
Aspidoptera phyllostomatis	Artibeus fimbriatus	79	−1.41	0.195	0.966	0.192	0.090	0.32	1.807	0.071
Megistopoda aranea	Artibeus fimbriatus	79	−0.11	0.577	0.623	−0.020	0.858	0.61	1.613	0.107
Megistopoda aranea	Artibeus jamaicensis	42	−1.73	0.077	0.988	0.380	**0.013**	0.61	1.254	0.210
Megistopoda proxima	Sturnira lilium	404	−4.47	**<0.001**	1.000	0.279	**<0.001**	0.60	3.993	**<0.001**
Metelasmus pseudopterus	Artibeus fimbriatus	79	−1.27	0.227	0.956	0.144	0.206	0.47	0.277	0.782
Noctiliostrebla aitkeni	Noctilio leporinus	28	−2.45	**<0.001**	1.000	0.835	**<0.001**	0.54	1.145	0.252
Noctiliostrebla maai	Noctilio albescens	68	−3.48	**<0.001**	1.000	0.663	**<0.001**	0.55	1.745	0.081
Noctiliostrebla maai	Noctilio leporinus	28	−2.01	**0.015**	0.998	0.830	**<0.001**	0.54	0.740	0.459
Paradychiria fusca	Noctilio albescens	68	−2.40	**0.011**	0.999	0.688	**<0.001**	0.46	0.813	0.416
Paradychiria parvula	Artibeus lituratus	351	−6.66	**<0.001**	1.000	0.470	**<0.001**	0.53	0.755	0.450
Paratrichobius longicrus	Carollia perspicillata	75	−0.22	0.628	0.799	0.009	0.936	0.80	2.366	**0.018**
Strebla guajiro	Desmodus rotundus	51	−3.42	**<0.001**	1.000	0.777	**<0.001**	0.55	1.035	0.301
Strebla weidemanni	Carollia perspicillata	75	−2.46	**0.005**	0.999	0.380	**<0.001**	0.56	1.095	0.274
Trichobius joblingi	Eumops patagonicus	526	−3.16	0.031	0.998	0.145	**<0.001**	0.62	1.251	0.211
Trichobius jubatus	Molossus molossus	228	−2.61	0.052	0.995	0.180	**0.006**	0.57	0.688	0.491
Trichobius jubatus	Molossus rufus	100	−2.85	**0.008**	0.999	0.336	**<0.001**	0.41	1.538	0.124
Trichobius parasiticus	Desmodus rotundus	51	−3.64	**<0.001**	1.000	0.959	**<0.001**	0.62	2.426	**0.015**
Ischnopsyllidae										
Myodopsylla wolffsohni	Myotis albescens	87	−1.88	0.112	0.989	0.231	**0.032**	0.25	2.294	**0.022**

Analyses of co-occurrence were based on standardized effect size (SES) for the C-score metric in ECOSIM (Gotelli & Entsminger, 2001). Spearman rank (ρ) correlations were used to evaluate patterns of abundance of male and female ectoparasites. The ratio of male to female ectoparasites was evaluated via a non-parametric Wilcoxon test. Analyses were conducted for all host species for which adult ectoparasites were recorded from at least ten host individuals, and analyses were restricted to primary host–parasite associations. All analyses included empty hosts (i.e. host individuals that were inspected for ectoparasites but not infested). Significant results ($P \leq 0.05$) are set in bold.

Table 2. Analysis of the dispersion of individuals conducted separately for female and male ectoparasites from 34 host–ectoparasite associations

Ectoparasite family / Ectoparasite species	Host species	Number of hosts	Females		Males	
			CoD	*P* value	CoD	*P* value
Spinturnicidae						
Periglischrus iheringi	*Artibeus fimbriatus*	79	1.928	**< 0.001**	1.542	**0.002**
Periglischrus iheringi	*Artibeus jamaicensis*	42	1.176	0.204	2.847	**< 0.001**
Periglischrus iheringi	*Artibeus lituratus*	351	3.523	**< 0.001**	1.700	**< 0.001**
Periglischrus iheringi	*Platyrrhinus lineatus*	90	7.775	**< 0.001**	1.935	**< 0.001**
Periglischrus ojastii	*Sturnira lilium*	404	2.677	**< 0.001**	1.772	**< 0.001**
Spinturnix americanus	*Myotis albescens*	87	1.219	0.082	1.378	**0.012**
Spinturnix americanus	*Myotis nigricans*	128	1.838	**< 0.001**	1.385	**0.003**
Nycteribiidae						
Basilia bequaerti	*Eptesicus furinalis*	69	1.202	0.123	1.176	0.151
Basilia carteri	*Myotis albescens*	87	1.487	**0.002**	1.124	0.203
Basilia carteri	*Myotis nigricans*	128	2.662	**< 0.001**	1.764	**< 0.001**
Basilia speiseri	*Myotis nigricans*	128	1.288	**0.016**	1.906	**< 0.001**
Polyctenidae						
Hesperoctenes fumarius	*Molossus molossus*	228	1.905	**< 0.001**	0.881	0.901
Hesperoctenes longiceps	*Eumops patagonicus*	526	1.519	**< 0.001**	1.060	0.163
Hesperoctenes parvulus	*Molossops temminckii*	160	1.486	**< 0.001**	1.547	**< 0.001**
Hesperoctenes sp.	*Eumops glaucinus*	56	26.924	**< 0.001**	6.025	**< 0.001**
Streblidae						
Aspidoptera falcata	*Sturnira lilium*	404	1.899	**< 0.001**	1.496	**< 0.001**
Aspidoptera phyllostomatis	*Artibeus fimbriatus*	79	1.158	0.161	0.936	0.639
Megistopoda aranea	*Artibeus fimbriatus*	79	1.069	0.318	0.956	0.590
Megistopoda aranea	*Artibeus jamaicensis*	42	2.439	**< 0.001**	2.164	**< 0.001**
Megistopoda proxima	*Sturnira lilium*	404	1.328	**< 0.001**	1.443	**< 0.001**
Metelasmus pseudopterus	*Artibeus fimbriatus*	79	0.897	0.729	0.910	0.700
Noctiliostrebla aitkeni	*Noctilio leporinus*	28	4.313	**< 0.001**	4.557	**< 0.001**
Noctiliostrebla maai	*Noctilio albescens*	68	2.140	**< 0.001**	3.055	**< 0.001**
Paradychiria fusca	*Noctilio leporinus*	28	6.649	**< 0.001**	8.691	**< 0.001**
Paradychiria parvula	*Noctilio albescens*	68	7.443	**< 0.001**	3.620	**< 0.001**
Paratrichobius longicrus	*Artibeus lituratus*	351	1.469	**< 0.001**	1.380	**< 0.001**
Strebla guajiro	*Carollia perspicillata*	75	0.946	0.610	1.655	**< 0.001**
Strebla weidemanni	*Desmodus rotundus*	51	4.540	**< 0.001**	7.722	**< 0.001**
Trichobius joblingi	*Carollia perspicillata*	75	1.287	**0.049**	1.428	**0.009**
Trichobius jubatus	*Eumops patagonicus*	526	1.163	**0.006**	1.413	**< 0.001**
Trichobius jubatus	*Molossus molossus*	228	0.965	0.636	1.454	**< 0.001**
Trichobius jubatus	*Molossus rufus*	100	1.313	**0.020**	1.433	**0.003**
Trichobius parasiticus	*Desmodus rotundus*	51	7.176	**< 0.001**	13.015	**< 0.001**
Ischnopsyllidae						
Myodopsylla wolffsohni	*Myotis albescens*	87	1.814	**< 0.001**	1.279	**0.042**

Coefficient of dispersion (CoD) and *P* values from associated χ^2 tests to evaluate whether male or female ectoparasites are: hyperdispersed (CoD < 1, with a significant χ^2 test); clumped (CoD > 1, with a significant χ^2 test); or randomly distributed on host individuals. Significant results ($P \leq 0.05$) are set in bold.

Spinturnix americanus, *Basilia carteri*, *Megistopoda aranea*, and *Trichobius jubatus*) of ectoparasite had primary associations with more than one host species. In general, results for these ectoparasites were similar, regardless of host species, with *M. aranea* exhibiting the greatest level of host-specific population structure, differing in sex ratio and dispersion of males and females (Tables 1 and 2).

DISCUSSION

POSITIVE CO-OCCURRENCE AND ABUNDANCE CORRELATIONS

Sexual segregation is common in species that do not require the constant presence of both sexes for breeding or rearing young (Ruckstuhl & Neuhaus, 2005). Such segregation reduces intraspecific competition by broadening the overall niche space of the species, and increasing resources available to pregnant or nursing females, resulting in enhanced fitness. Sexual segregation is associated with niche partitioning or sexual size dimorphism in many plants, invertebrates, and vertebrates (e.g. Shine, 1986; Rusterholz & Erhardt, 2000; Reimchen & Nosil, 2004; Joron, 2005; Bearhop *et al.*, 2006; Eppley, 2006; Safi *et al.*, 2007). Females of some families of bat ectoparasites can produce several offspring from a single copulation: female streblids have spermatheca, but it is not known for how long they store sperm (Marshall, 1981). Reproductive rates for most bat ectoparasites are low compared with arthropods in general, including for the Streblidae (one offspring per 10 days; Overal, 1980), Nycteribiidae (one offspring per 9 days), Polyctenidae (observations suggest that they reproduce at slower rates than dipterans), and Spinturnicidae (egg and larval stages occur entirely within the female for one offspring at a time; Rudnick, 1960). Consequently, the persistent cohabitation of males and females may not be required. Moreover, bats are capable of grooming all body parts to kill ectoparasites (Marshall, 1982; Fritz, 1983). This suggests that the number and sizes of spaces on a host that are safe from grooming activity are limiting, which has the potential to increase intraspecific competition, including between the sexes.

If competition is an important driver affecting the distribution of sexes among hosts, sexes should co-occur less often than expected by chance, and abundances should be negatively correlated on host individuals. For bat ectoparasites, sexes from a majority of associations exhibited positive co-occurrence (68%) or positive correlations of abundance (79%), with none having negative co-occurrence or negative correlations of abundance (Table 1). These patterns were not restricted to particular families of ectoparasite, as > 60% and > 70% of the associations from each family exhibited positive co-occurrence or positive correlations of abundance, respectively.

Within primary host populations, ectoparasites may preferentially infest particular host individuals. Adult females of most Neotropical bat species are found in harems (many females and one male) or maternity colonies (only females), and most adult males are solitary. Consequently, selection forces associated with opportunities for host transfer (the dispersal of offspring and outbreeding) and resource abundance (the number of available hosts) favour the infestation of female bats over male bats, leading to greater abundances on female bats (Patterson *et al.*, 2008; Presley & Willig, 2008; Presley, 2011). Indeed, given the choice between an adult female bat and an adult male bat, spinturnicid mites preferentially select adult females (Christie *et al.*, 2007). Combined with prior analyses (Presley & Willig, 2008; Presley, 2011), this suggests that conspecific ectoparasites preferentially inhabit the same hosts based on sex-based host characteristics, and that intraspecific competition does not structure ectoparasite populations. Rather, selection based on host characteristics is likely to mold the patterns of distribution and abundance of ectoparasites among host individuals.

NO EVIDENCE FOR INTRASEXUAL SELECTION

Sexual selection (Andersson, 1994) may manifest as intersexual selection (competition in the attraction of mates) or intrasexual selection (competition via elimination of same-sex rivals, usually among males). In addition, competition among females may occur if they defend productive areas of limiting resources. Consequently, competition may occur within either sex, depending on social structure and resource distribution or abundance. Individual bats differ in their value to ectoparasites, with many ectoparasites preferentially inhabiting female hosts (Christie *et al.*, 2007; Patterson *et al.*, 2008; Presley & Willig, 2008). If there is competition among female ectoparasites for the highest value hosts or among males for access to females on those high-value hosts, females or males may be hyperdispersed among hosts as a result of competitive interactions. Alternatively, if host selection has a greater effect on fitness than intrasexual competition, individuals of each sex should have clumped distributions. Males (79%) and females (76%) exhibited clumped distributions (Table 2) for the majority of associations, indicating that host selection is more important than competition among individuals of the same sex. It is possible that intersexual selection occurs, with male ectoparasites competing to mate with females. Indeed, this seems likely as male abundances were highly correlated with female abundances in most associations (Table 1), indicating that males are tracking the same resources as females or that females are the resource being tracked (reproductive filter; Dick & Patterson, 2007).

SEX BIAS, HOST GROOMING, AND SEXUAL SIZE DIMORPHISM

Analysis of sex ratios for streblids from Venezuelan bats (Dick & Patterson, 2008) documented male sex

bias in nearly one-third (36 of 112) of host–ectoparasite associations, with only one having female bias (Dick & Patterson, 2008). In comparison, 16% (three out of 18) of streblid associations from Paraguay exhibited male bias, with one association approaching significance for male bias and one association approaching significance for female bias (Table 1). Multiple potential reasons for the differences in results between studies exist. Each study took steps to prevent contamination of samples (Dick & Patterson, 2008; Presley & Willig, 2008), making effects related to contamination or careless field collection methods unlikely. Sample sizes were generally larger for Venezuela than for Paraguay; however, insufficient power does not appear to be an underlying cause, as sample sizes in Paraguay were sufficient to detect non-random patterns in correlations of abundance, co-occurrence of sexes, and dispersion of individuals for the majority of host–ectoparasite systems (Tables 1 and 2). Nonetheless, it is true that with larger sample sizes smaller differences in sex ratio will be considered statistically significant. Eight host–ectoparasite associations with male bias in Venezuela were also evaluated in Paraguay: three of these were the only Paraguayan associations to exhibit significant male bias, and four of the remaining associations had more males than females. This suggests that sex bias may be geographically invariant for particular host–ectoparasite associations, and that geographic variation in overall patterns may result from changes in host assemblage composition, and not from changes in ectoparasite responses.

The typical explanations proffered for biased sex ratios, such as pre-partum bias, sampling effects, differential longevity or dispersal, and local mate competition, could not explain sex bias in Venezuelan streblids (Dick & Patterson, 2008). To summarize, data on streblids at eclosure are consistent with a 1 : 1 pre-partum sex ratio (but see Dittmar et al., 2011), thereby effectively rejecting that mechanism. Male streblids are more active than females (Marshall, 1981), making them more likely to quickly abandon captured hosts and to be under-represented in collections. Females of ectoparasitic insects have significantly greater longevity than males (Marshall, 1981 and sources therein; Dick & Patterson, 2008 and sources therein), which is in opposition to male sex bias. Wing development of streblid species varies from fully functional wings to winglessness, and wing functionality may affect dispersal ability. No correlation existed between wing development and sex bias, indicating that differential dispersal of the sexes is unlikely to explain patterns of male bias. Because the local mate competition hypothesis predicts female sex bias rather than male sex bias, it is refuted by the male bias of streblids. Rather, differential risk of

mortality from host grooming, considered a primary source of streblid mortality (Marshall, 1982), was proposed to explain the male sex bias of streblids (Dick & Patterson, 2008).

Streblids exhibit three distinct adaptation syndromes associated with particular niche spaces on the host body (i.e. on the fur, in the fur, and on the patagia; Dick, 2005). Male bias was not associated with ecomorphological groups, as 14, nine, and 13 associations that exhibited male bias in Venezuela (Dick & Patterson, 2008) are adapted to live on the patagia, on the fur, and in the fur, respectively. Each ecomorphological group was represented by one of the three male-biased associations from Paraguay. If grooming mortality is responsible for sex bias, it is not specific to streblids that live on particular parts of the host body.

Female streblids are larger than males and spend a large portion of adulthood gravid with developing larvae, thereby enhancing sexual size dimorphism. The larger size is thought to make female flies more susceptible to grooming-related mortality than males. Nycteribiids are closely related to streblids and share many of the same life-history characteristics, including large females that spend most of adulthood gravid with developing larvae. Consequently, female nycteribiids should be subject to the same elevated grooming mortality risk that is proposed to cause sex bias in streblids; however, none of the Paraguayan nycteribiids exhibited male sex bias. Moreover, Marshall (1981) examined 16 associations involving nycteribiids: four exhibited female sex bias rather than male sex bias. It is possible that grooming mortality can account for the excess of males in streblids, but it is not clear that sexual size dimorphism or females being gravid most of their lives are the causes. Polyctenid populations generally have an excess of females (Marshall, 1981) or equal sex ratios (Table 1), but sexual size dimorphism is not as pronounced because of viviparity, with nymphal stages that feed on the host. Spinturnicid mites are sexually size dimorphic, with females typically more than twice the size of males (Herrin & Tipton, 1975), and locomote poorly when not on patagia, making them susceptible to pressures of host grooming. Nonetheless, spinturnicid sex ratios did not differ from 1 : 1. Because the differential grooming hypothesis relies mainly on sexual size dimorphism, size differences between species should also affect susceptibility to grooming, with both sexes of larger species being more susceptible to grooming than either sex of smaller species (Dittmar et al., 2011). Therefore, if size has an effect on grooming mortality, that effect would be more likely to structure assemblages of ectoparasites than to cause biased sex ratios. The preponderance of evidence suggests that differential grooming is unlikely

to be a primary factor affecting male sex bias in ectoparasites of bats.

Two viable alternative explanations for male sex bias in streblids are: (1) that females experience higher rates of off-host predation; or (2) the timing of larviposition by females leads to bias in samples collected from bats in flight. Phyllostomid bats, the primary hosts of Neotropical streblids, use a great variety of roost types that differ in permanence, and in the protection afforded to bats, including under green foliage, branches, or bark, termite nests, hollow logs, tree holes, buildings, tunnels, mines, and caves (Patterson *et al*., 2007). Populations of bats that use permanent and enclosed structures are larger, and exhibit greater roost fidelity than bats that roost in less permanent structures (Kunz & Lumsden, 2003). Consequently, remaining in the roost during times of bat activity represents less risk of disassociation from the host for ectoparasites in permanent roosts (e.g. caves or buildings) than for those in temporary roosts (e.g. foliage or bark). Off-host depredation rates on adult streblids in permanent roosts can be considerable, especially for ectoparasites that move a considerable distance from host roosting locations to pupal deposition fields (Dittmar *et al*., 2009, 2011). For cave-roosting bats, spiders and ants were documented to prey heavily on streblids at pupal deposition fields, or between the location of roosting bats and the deposition field (Dittmar *et al*., 2009, 2011). Because females travel to and from the deposition field to deposit each pupa, and males remain in the area of the hosts, females are at far greater risk from arthropod predators than are males (Dittmar *et al*., 2011). This mechanism would lead to male sex bias in the roost and on the host, but may be restricted to ectoparasites of hosts that roost in large permanent structures (e.g. caves or mines). The permanence of roosts increases the likelihood that newly emerged flies find a host, increasing reproductive success and leading to greater parasite loads on hosts in those types of roosts (ter Hofstede & Fenton, 2005; Patterson *et al*., 2007). However, this permanence also provides opportunities for arthropod predators to prey on the predictable and abundant resource represented by the ectoparasites: an occurrence that is far less likely to arise in small, ephemeral roosts in foliage or under loose bark.

In the absence of off-host predation, the timing of behaviour associated with reproduction may cause observations of male bias. Gravid females leave the host to larviposit shortly before hosts come out of their torpor and leave the roost to forage. The timing of larviposition could lead to male sex bias in samples taken from flying bats, whereas samples from the roost, taken while hosts are present, would be more reflective of the true sex ratio of the population (Dittmar *et al*., 2011). In permanent roost structures, streblids can be found in abundance in roosts, even after bats have left to forage (Dick & Patterson, 2006). If male bias on active bats is caused by a larger proportion of females remaining in the roost, then male bias should be more common on hosts that use permanent roosts. Therefore, female behaviours associated with reproduction, as well as the increased predation risk, may contribute to a male sex bias of streblids from hosts that use permanent roost structures.

Of the 39 host–streblid associations with male bias from Venezuela and Paraguay, 26 use permanent roost structures (e.g. buildings, bridges, tunnels, mines, or caves; Linares, 1998). This observation certainly does not test the hypotheses of depredation or larviposition that may cause male sex bias, but it does suggest that a relationship may exist between host roosting ecology and male bias in samples collected from bats in flight. Moreover, the fact that ectoparasites incapable of leaving the host body to avoid grooming (polyctenids and spinturnicids) do not exhibit male bias (Table 1; Marshall, 1981) is consistent with each of these hypotheses, but not with the differential grooming hypothesis. Consequently, host-specific roosting ecology may explain patterns of sex bias in ectoparasites that may result from a combination of sampling bias and differential off-host predation risk.

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