Seasonal pattern of avian Plasmodium-infected mosquitoes and implications for parasite transmission in central Panama

ARTICLE *in* PARASITOLOGY RESEARCH · AUGUST 2013

Impact Factor: 2.1 · DOI: 10.1007/s00436-013-3562-5 · Source: PubMed

2 AUTHORS:

Jose R Loaiza
Instituto de Investigaciones Cientificas y Se...
21 PUBLICATIONS **183** CITATIONS
SEE PROFILE

Matthew J. Miller
Villanova University
28 PUBLICATIONS **243** CITATIONS
SEE PROFILE

Parasitol Res
DOI 10.1007/s00436-013-3562-5

ORIGINAL PAPER

Seasonal pattern of avian *Plasmodium*-infected mosquitoes and implications for parasite transmission in central Panama

Jose R. Loaiza · Matthew J. Miller

Received: 10 May 2013 / Accepted: 25 July 2013

Abstract *Aedeomyia squamipennis* and *Culex* (*Melanoconion*) *ocossa*, two ubiquitous Neotropical mosquito species, are likely involved in the transmission of several bird pathogens in Gamboa, central Panama. However, knowledge on their eco-epidemiological profiles is still incomplete. Our goal in this study was to investigate temporal trends of vector density and their relationship with avian plasmodia prevalence. This information is central to identifying the risk posed by each vector species to the avian community locally. We found that *A. squamipennis* maintains stable population size across climatic seasons and thus maybe a more efficient vector of avian malaria than *C. ocossa*. In contrast, *C. ocossa*, which undergoes considerable population expansion in the rainy season and contraction in the dry season, is likely only an important avian malaria vector during part of the year. This is consistent with the larger number of parasite isolations and *Plasmodium* cyt *b* lineages recovered from *A. squamipennis* than from *C. ocossa* and might be explained by marked differences in their seasonality and host-feeding preferences. More *Plasmodium* PCR testing in mosquito communities from other areas of Panama might reveal additional vectors of avian plasmodia.

J. R. Loaiza (✉)
Centro de Biodiversidad y Descubrimiento de Drogas, Instituto de Investigaciones Científicas y Servicios de Alta Tecnología, Edificio 219, Clayton, PO 0843-01103, Ciudad del Saber, Panama
e-mail: jloaiza@indicasat.org.pa

J. R. Loaiza
Programa Centroamericano de Maestría en Entomología, Vicerrectoría de Investigación y Postgrado, Universidad de Panamá, Panama, Panama

M. J. Miller
Smithsonian Tropical Research Institute, Apartado Postal 0843-03092, Panama, Panama

Published online: 24 August 2013

Introduction

Malaria not only is the most devastating parasitic illness of mankind but also affects a wide range of other vertebrates, including reptiles and birds. As in humans, avian malaria is caused by apicomplexan parasites of the genus *Plasmodium* (Haemosporina: Plasmodiidae) (Levine 1988). Nonetheless, contrary to human malaria, the few observations on vectors suggest that mosquitoes of the subfamily Culicinae rather than Anophelinae transmit avian plasmodia (Huff 1965; Lourenço-de-Oliveira and De Castro 1991; Valkiūnas 2005; Njabo et al. 2010, 2011). One of the major obstacles to understanding the epidemiology of avian malaria in an endemic area is the lack of knowledge about parasite–vector interactions (Gager et al. 2008; Njabo et al. 2010; Carlson et al. 2011; Glaizot et al. 2012). For instance, information about seasonal trends of vector density and its relationship with *Plasmodium* species composition and prevalence could help to better understand risk and transmission dynamics locally (Kim et al. 2009; Sehgal 2010; Loiseau et al. 2010; Kim and Tsuda 2010). However, data about host demography and infection rates, and vector population dynamics, are rarely examined jointly, perhaps due to a shortage of interdisciplinary research among entomologists, ornithologists, and wildlife disease ecologists (Braga et al. 2011; Synek et al. 2012). As a result, the approach used to study avian malaria epidemiology has been traditionally incomplete, with only a few recent exceptions (Gager et al. 2008; Ishtiaq et al. 2008; Loiseau et al. 2010).

Entomological research in tropical areas has established a correlation between mosquito abundance and rainfall levels (Read and Adames 1980; Wolda and Galindo 1981). Some studies have attributed seasonal changes in vector density, female biting rates, and community structure to annual rainfall fluctuation and resultant breeding site shifts (Jones et al. 2004; Franklin and Whelan 2009). However, this trend might vary for areas where breeding sites are present year round (Service 2008). More generally, studies on human malaria have

depicted an apparent association between the number of clinical cases and *Plasmodium* infection rates in vector populations, which could be in part due to a higher human–vector contact during periods of peak mosquito abundance (Galardo et al. 2007). This assumption could equally pertain to the avian malaria system, but information on the temporal distribution of avian *Plasmodium* lineages in mosquitoes is still poorly described (Kim et al. 2009; Kim and Tsuda 2010; Carlson et al. 2011).

The town of Gamboa, located in central Panama, has been recognized as a rich environment for mosquito development (Heinemann and Belkin 1978; Christensen et al. 1996). In this place, the Chagres River empties in the main pathway of the Panama Canal and creates a massive artificial pond that contains a flourishing community of aquatic and semiaquatic plants (Galindo and Adames 1973). *Aedeomyia squamipennis* and *Culex* (*Melanoconion*) *ocossa* are two ubiquitous mosquito species present in Gamboa. The former is an established vector of several viruses and parasites of birds (Gabaldon et al. 1977a, b, 1981; Calisher et al. 1981; Dutary et al. 1989), whereas the latter has been hypothesized as a vector of enzootic and epizootic strains of Venezuelan equine viral encephalitis (VEEV) in humans (Galindo and Grayson 1971; Galindo et al. 1983; Turell et al. 1999). The bionomic profiles of these vectors have been studied in Panama before (Galindo and Adames 1972; Galindo 1972; Calisher et al. 1981; Dutary et al. 1989), but data regarding their temporal changes in density are still incomplete (Galindo and Adames 1973; Gabaldon and Ulloa 1980; Galindo et al. 1983). More recently, *A. squamipennis* and *C. ocossa* were found to be infected with nine mitochondrial DNA lineages of avian *Plasmodium* in Gamboa. Interestingly, each mosquito species carries unique phylogenetic lineages of *Plasmodium*, as determined by sequences of the cytochrome *b* gene, which are likely to be reproductively isolated parasite species. Yet, two of these vector-specific cyt *b* lineages overlapped in the Clay-colored robin, *Turdus grayi* (Gager et al. 2008). The authors of that paper suggest that this finding indicates tight parasite–vector coevolutionary relationships and the existence of two discrete epidemiological cycles of avian malaria in central Panama, perhaps determined by ecological or behavioral differences between *A. squamipennis* and *C. ocossa* (e.g., density, feeding preferences, vertical stratification, biting times, and the seasonal aspect of all these factors). Nevertheless, more recent evidence on parasite–vector interactions render low support to the coevolution view and propose various degrees of vector specialization for avian haemosporidians (Ishtiaq et al. 2008; Kim and Tsuda 2010, Kimura et al. 2010; Glaizot et al. 2012). Although results from Gager et al. (2008) improved our understanding about avian plasmodia epidemiology regionally, they do not provide insights as to the transmission risk posed by each vector species to the bird community locally. Our aims herein were (1) to describe the temporal trends of vector density in Gamboa, a place where breeding sites are likely to be present year round and (2) to examine temporal distribution of *Plasmodium* cyt *b* lineages in *A. squamipennis* and *C. ocossa*. This information will provide insights on the transmission dynamics of avian plasmodia in central Panama.

Materials and methods

Study site and data analysis

This research was conducted in Gamboa located approximately 25 km northwest of Panama City (Fig. 1). Two climatic periods take place in Gamboa: a dry season that extends from January through April (average rainfall 260 mm) and a rainy season that covers the rest of the year (average rainfall 2,700 mm). The area is flat and swampy and is surrounded by tropical humid–dry transitional forest; January is the driest month, and October is the wettest (Dutary et al. 1989). We conducted larval sampling at margins of the Chagres River and also in forest ponds using standard dipping techniques. Larvae were collected, after ten initial dips to determine positivity, using 50 dips per breeding site. At least ten breeding sites from each habitat type (river margin and forest pond) were checked every sampling period, and the same sites were revisited during subsequent field trips. Samples were sorted in the laboratory to the subfamily level and grouped according to instars in different plastic containers. This procedure was carried out twice a month during four consecutive months in the rainy and dry season, correspondingly. We used Fisher's exact test of independence in 2×2 tables to compare the proportion of collected fourth-instar larvae by habitat type and climatic season (wet and dry) for *A. squamipennis* and *C. ocossa* separately. Mosquito species identity was confirmed by male genitalia (reared specimens from larval collections) and larval skin preparations following the protocols listed by Thomas Gaffigan and James Pecor, available at http://www.wrbu.org/techniques.html. *C.* (*Melanoconion*) *ocossa* (formerly *Culex Mel. aikenii*) was the only taxa detected from the *Culex ocossa* species complex (Pecor et al. 1992). Female mosquitoes were pooled by species (20 specimens per pool generally collected from a single trap during the same night) and tested for the presence of avian *Plasmodium* cyt *b* lineages. More details about adult mosquito sampling, DNA extraction, PCR amplification, and sequencing procedures as well as partial results were reported in Gager et al. (2008), but data about temporal changes of vector density along with the seasonal distribution of *Plasmodium* cyt *b* lineages in mosquitoes are presented and discussed herein. Spearman's rank–order correlation was used to test for the existence of positive or negative monotonic relationships between adult mosquito density and the monthly values of

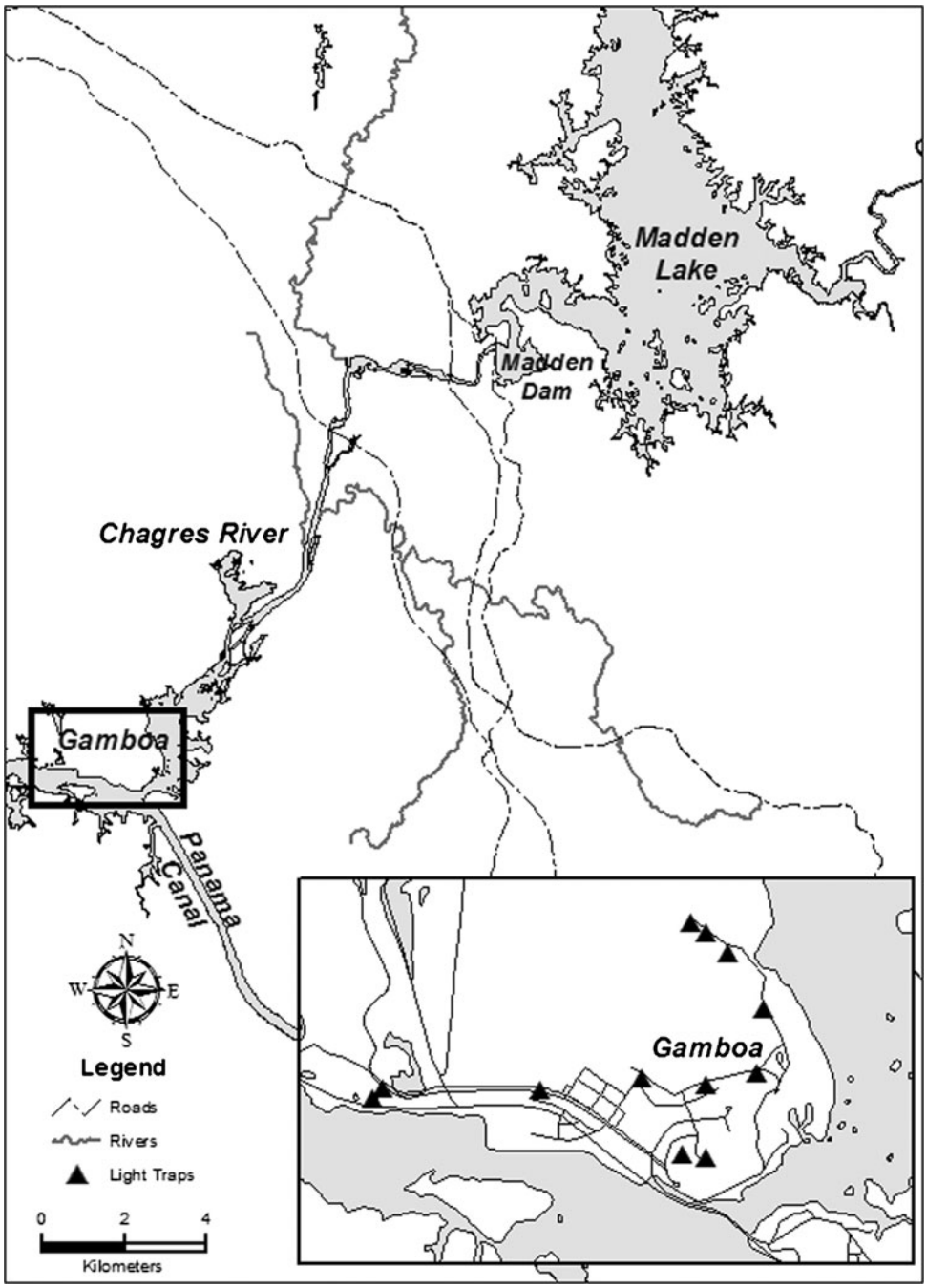

Fig. 1 Map showing the locations of 12 CDC-baited light traps in the secondary forest of Gamboa (*triangles*), central Panama

accumulated rainfall in Gamboa; here, we only included the most common species of mosquitoes (Table 1).

The minimum infection rate (MIR=number of PCR-positive pools/number of collected mosquitoes×1,000) was estimated and plotted by month for both *A. squamipennis* and *C. ocossa*. This is a more conservative approach to compare *Plasmodium* infection rates in mosquitoes, because it assumes that only one individual of the pooled sample is infected; however, this method can underestimate transmission risk when infection rates are high (Bustamante and Lord 2010). Furthermore, to avoid overinterpretation of the data (e.g., making inferences about *Plasmodium* cyt *b* lineages that might not complete their developmental cycle in the vector), MIR estimates were carried out using only the two most common *Plasmodium* cyt *b* lineages isolated from *A. squamipennis* and *C. ocossa* (Gager et al. 2008). The software package R version 2.14.1 was used, and $P<0.05$ was employed as a cutoff for statistical analysis.

Table 1 Spearman's rank correlation coefficient (rho) and *P* values for monotonic relationships between mosquito density and the values of monthly accumulated rainfall (in millimeters) in Gamboa, Panama

Mosquito species	Spearman's rank correlation coefficient (rho) and *P* values		
A. squamipennis	rho=0.25	*S*=850.2506	*P*=0.349
A. albimanus	rho=0.32	*S*=460.6772	*P*=0.223
A. triannulatus	rho=0.26	*S*=860.2653	*P*=0.321
C. nigripalpus	**rho=0.81**	***S*=127.2806**	***P*=0.0001**
C. erraticus	**rho=0.81**	***S*=129.1894**	***P*=0.0001**
C. ocossa	**rho=0.98**	***S*=8.0236**	***P*=0.0001**
M. indubitans	rho=−0.22	*S*=527.776	*P*=0.404
C. nigricans	**rho=−0.47**	***S*=1,001.473**	***P*=0.051**
M. dyari	**rho=−0.51**	***S*=1,029.772**	***P*=0.041**
M. titillans	**rho=−0.49**	***S*=1,017.745**	***P*=0.047**

Negative and positive significant relationships are shown in bold

Results

Three patterns of temporal variation in adult mosquito density were observed: some species such as *Culex nigripalpus*, *Culex erraticus*, and *C. ocossa* showed significant positive monotonic relationships with the monthly values of accumulated rainfall in Gamboa and were more abundant during the wet season, increasing in numbers between September and December (Table 1; Figs. 2a and 3a). In contrast, other species like *Coquillettidia nigricans*, *Mansonia dyari*, and *Mansonia titillans* showed significant negative monotonic relationships with rainfall levels and were more abundant in March toward the end of the dry season (Table 1; Fig. 2b). Finally, a few species, including *A. squamipennis*, displayed more or less constant abundance, being collected in regular numbers in both seasons and depicting no relationship with rainfall (Table 1; Figs. 2c and 3b). We found no significant association between breeding site type and climatic season for the larval proportion of *A. squamipennis* (Fisher's exact test, $df=1$, $P<0.10$) (Fig. 2d). However, we did recover a highly significant effect (Fisher's exact test, $df=1$, $P<0.0018$) for *C. ocossa*; larval density was higher in forest ponds during the rainy season and higher in river margins during the dry season (Fig. 2d).

Twenty of the 435 pools screened were positive for *Plasmodium* parasite, and a greater percentage of positive pools were recovered from *A. squamipennis* than from *C. ocossa* (11/39=28.2 % vs 9/76=11.8 %, in that order). Furthermore, nine *Plasmodium* cyt *b* lineages were obtained from the PCR screening of 8,700 individuals representing 11 mosquito species by Gager et al. (2008). Six *Plasmodium* cyt *b* lineages were exclusively obtained from *A. squamipennis* while three were only isolated from *C. ocossa*, with PAN6 and PAN2 being the most common species, respectively (Table 2). Furthermore, 14 positive pools (70 %) and 6 cyt *b* lineages were recovered between November and December, when a second major peak of rainfall occurs in Gamboa. In contrast, six positive pools (30 %) and three cyt *b* lineages were detected during the dry season (Table 2). The values of MIR for *A. squamipennis* remained above 5.0 across seasons, but MIR values for *C. ocossa* decreased from 8.0 in the rainy season to 0 during the dry season (Fig. 3c). Finally, PAN1 and PAN7 were detected twice during the study, PAN1 in November and February and PAN7 in February and March, in that order. Other *Plasmodium* cyt *b* lineages were detected only once during the rainy season (Table 2).

Discussion

Seasonal trends of vector density in Gamboa

In most tropical areas, mosquito populations are expected to oscillate cyclically as precipitation fluctuates, because the number of available breeding sites is a function of rainfall (Read and Adames 1980; Wolda and Galindo 1981; Franklin and Whelan 2009). However, a few tropical sites maintain suitable larval habitats year round (Galindo et al. 1983; Service 2008). In Gamboa, the Chagres River is a complex hydrologic system that allows for the establishment of a great variety of aquatic plants. These plants grow along the margins and serve as ideal habitats for mosquito development, varying temporarily in quantity due to stream discharge conditions (Fig. 1). Therefore, rainfall fluctuation around Gamboa modifies water levels and flow in this river and influences the availability of mosquito habitats in two ways. First, during the rainy season, water level is high and currents are strong; thus, margins get overflooded, and plant development is disrupted. However, at the same time, numerous temporary water pools are created inside the adjacent forest (Galindo and Adames 1973). In contrast, during the dry season, temporary water pools disappear as the forest floor dries out, but in turn, the Chagres River offers better conditions for the development of aquatic vegetation (Aguila 1987). As a consequence, mosquito species associated with aquatic vegetation in Gamboa should increase in density during the dry season, while those exploiting temporary forest floor water pools should increase in numbers during the rainy season (Galindo et al. 1983; Lopez and Lozovei 1995; Jones et al. 2004). Our results support this view, to some extent, because population densities for *C. nigricans*, *M. dyari*, and *M. titillans* increased considerably between January and March when *Pistia stratiotes*, *Eichchornia crassipes*, and other floating plant species are most common in Gamboa (Galindo and Adames 1973; Aguila 1987). Immature stages of these taxa have morphological adaptations to take oxygen directly from plant roots. Hence, their populations increase significantly along with plant development because larvae can avoid predation more effectively. Nevertheless, Galindo (1972) reported a tight association between immature stages of *C. ocossa* and *P.*

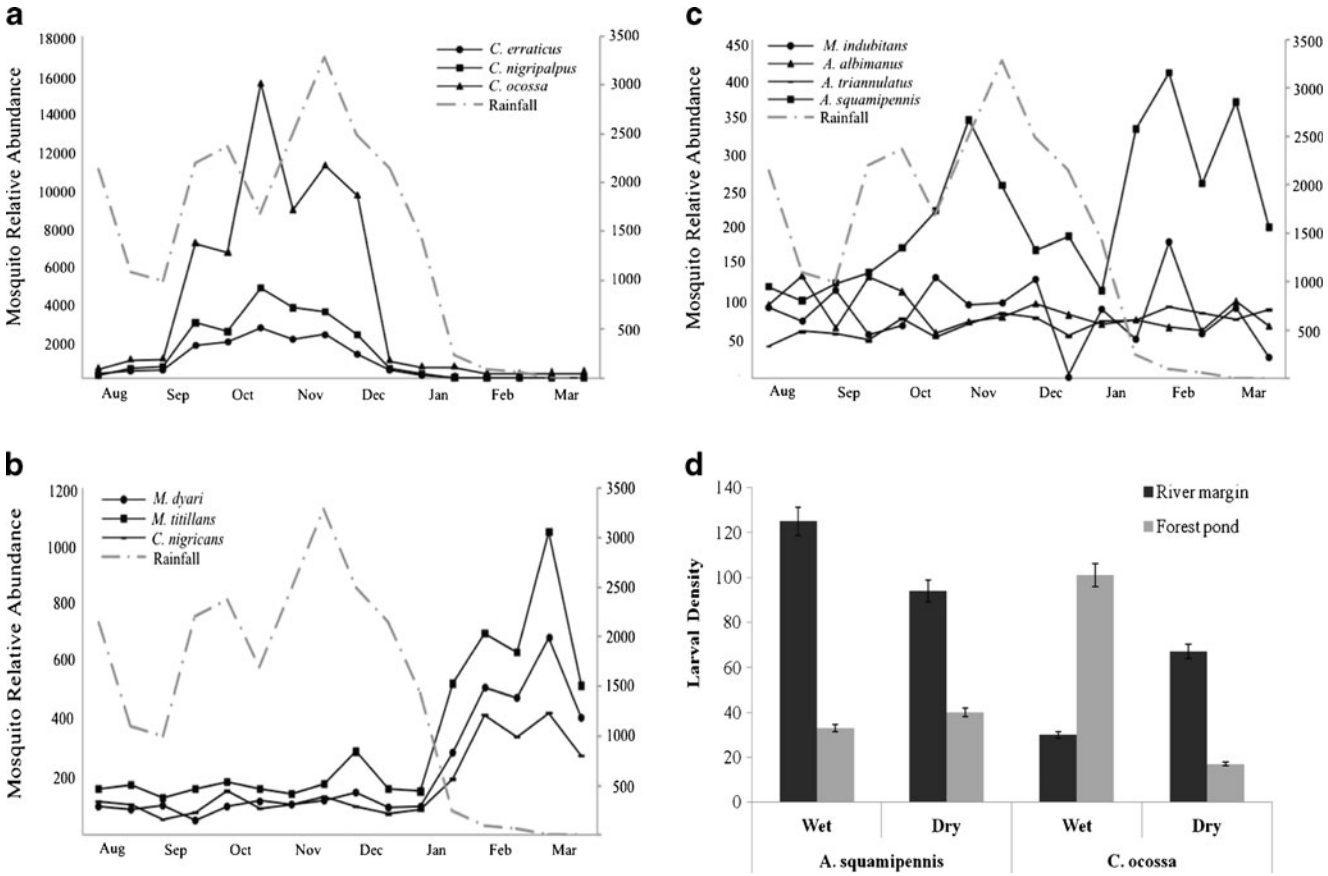

Fig. 2 Temporal changes in mosquito density and rainfall levels. **a** *C. erraticus*, *C. nigripalpus*, and *C. ocossa*. **b** *M. dyari*, *M. titillans*, and *C. nigricans*. **c** *A. squamipennis*, *Mansonia indubitans*, *Anopheles albimanus*, and *Anopheles triannulatus*. **d** Proportion of collected four-instar larvae of *A. squamipennis* and *C. ocossa* by breeding site type and climatic season

stratiotes in the Chagres River and anticipated low survival rates for *C. ocossa* in the absence of this plant species (Galindo and Adames 1972). Our findings differ in that *C. ocossa* peaked in density between September and December when less sunlight, smaller amounts of organic matter, and strong currents would have precluded the growth of *P. stratiotes* in the Chagres River (Aguila 1987). There are two potential explanations for this outcome. Firstly, *C. ocossa* could breed in association with other plant species, some of which might thrive during the rainy season in the Chagres River (Galindo and Adames 1973). Secondly, *C. ocossa* might exploit semipermanent water pools that formed inside the forest, particularly those that harbor small clumps of *P. stratiotes* (Galindo et al. 1983). Noticeably, results from Fisher's exact test using larval collections of this species, different habitat types, and climatic seasons give more support to this latter hypothesis (Fig. 2d). Alternatively, periodic flushing in the Chagres River by heavy rain might dislodge *P. stratiotes* clumps from banks upstream, which may float downstream and concentrate around Gamboa, thus increasing breeding habitat for *C. ocossa*, and also give rise to novel populations that remain attached to the leaves of *Pistia* and move along with them (Galindo 1972; Galindo and Adames 1973). This dispersal mechanism could be further facilitated by water flushing from several Chagres tributaries (e.g., Chilibre and Cabuya) or by periodic water spills from the Madden Dam (Fig. 1).

On the other hand, larvae and pupae of *A. squamipennis* have been collected in numerous plant species in the Chagres River. Among these, *Jussiaea natans*, *Salvinia rotundifolia*, *Hydrilla verticillatta*, *E. crassipes*, *Heteranthera reniforme*, *P. stratiotes*, and *Ludwigia helmintorhiza* are more abundant during either the dry or rainy season, but may also be present across seasons (Galindo and Adames 1973, Aguila 1987). Accordingly, although the density of *C. ocossa* in Gamboa appears to be regulated by the annual rainfall cycle triggering water discharge changes in the Chagres River and water pool formation in the forest, regular numbers in *A. squamipennis* seem more related with a greater diversity of emergent, submerged, and floating plant species that provide optimal developmental sites for this species year round (Gabaldon et al. 1981; Lopez and Lozovei 1995). Results from Fisher's exact test using larval collections of *A. squamipennis*, different habitat types, and climatic seasons support these observations and propose a broader breeding niche for this species than for *C. ocossa*.

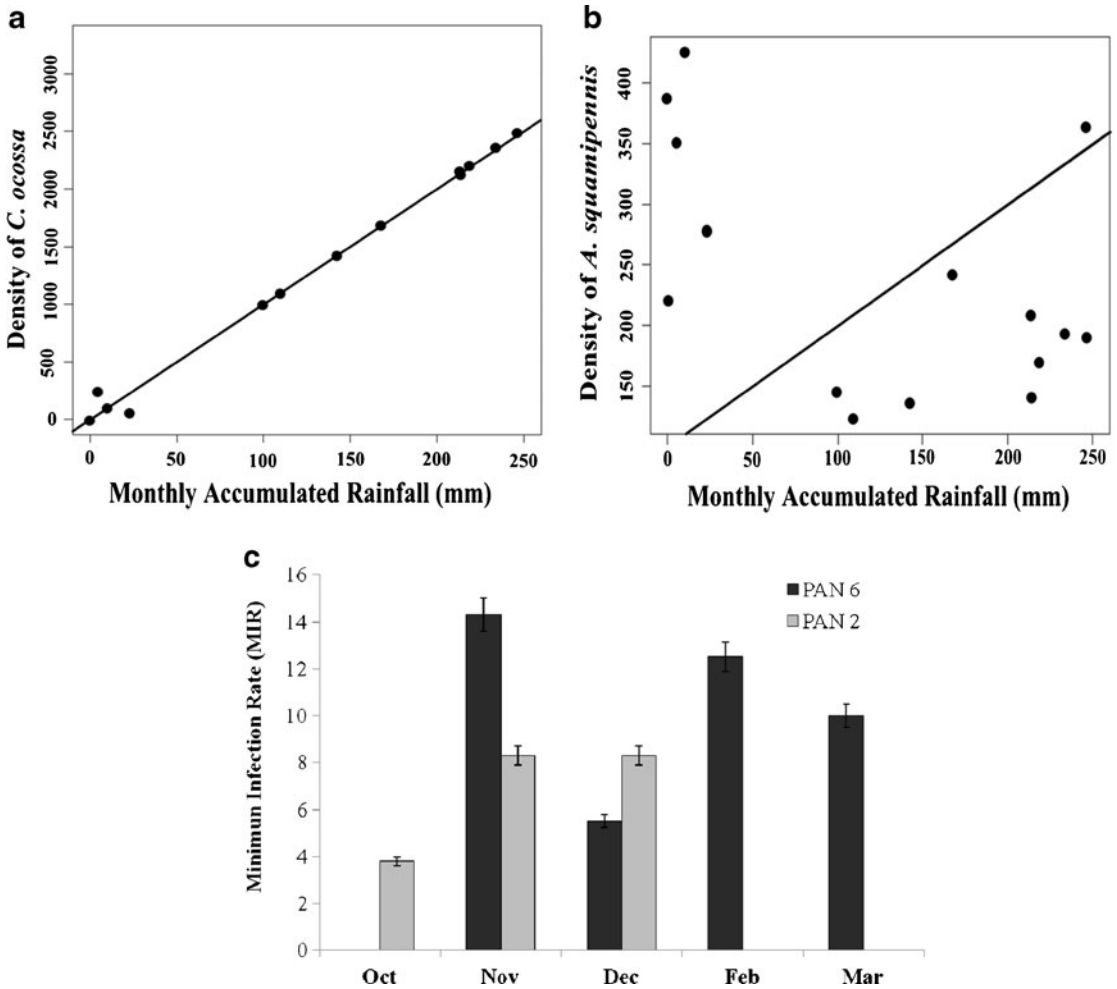

Fig. 3 Comparison of the relationships between vector density (**a**=*C. ocossa*; **b**=*A. squamipennis*) and the values of monthly accumulated rainfall (in inches) in Gamboa. Spearman's rank correlation coefficient (*rho*) and *P* values for *A. squamipennis* and *C. ocossa* are shown in Table 1. **c** MIR values for PAN6 and PAN2 cyt *b* lineages, respectively. *MIR*=-number of positive pool/number of mosquitoes collected×1,000. September–October (wet season), January–March (dry season)

Vector contribution to avian plasmodia transmission in Gamboa

The role of *A. squamipennis* as a vector of avian *Plasmodium* was first recognized by Gabaldon et al. (1977a), who detected mosquito infections after the mosquitoes fed on both passeriform and nonpasseriform birds. The same author acknowledged a marked preference of *A. squamipennis* for avian blood as compared to other vertebrate classes (Gabaldon et al. 1977b). This strong ornithophilic feeding preference was also substantiated by Christensen et al. (1996) in central Panama, who detected *A. squamipennis* feeding primarily on birds (86.6 %) and to a much lesser degree on amphibians/reptiles (7.3 %) and mammals (6.1 %). In contrast to *A. squamipennis*, *C. ocossa* has been shown to feed more readily on mammals than on birds and also regularly takes blood from amphibians and reptiles (Galindo 1972; Tempelis and Galindo 1975; Christensen et al. 1996). Therefore, steady numbers across seasons due to a broader breeding niche and strong ornithophilic feeding preference suggest that *A. squamipennis* transmits avian *Plasmodium* regularly to the bird community in Gamboa, whereas low population density during the dry season and a more opportunistic feeding behavior are likely to interrupt transmission by *C. ocossa* due to lower bird–vector contact. These assumptions are consistent with the finding of more frequent parasite isolations and a greater diversity of *Plasmodium* cyt *b* lineages recovered from *A. squamipennis* than from *C. ocossa*, despite higher number of mosquitoes analyzed for the latter species (Gager et al. 2008).

Results from Gager et al. (2008) suggest the existence of two distinct epidemiological cycles of avian malaria as a consequence of *Plasmodium* cyt *b* lineage assemblages not sharing the same vector species. In this particular case, it is possible that PAN6 infects passerine birds year round due to steady populations of *A. squamipennis* in Gamboa, whereas PAN2 might only appear seasonally in sampled birds due to higher bird–vector contact when populations of *C. ocossa* increase significantly (Fig. 3c). The epidemiological partition suggested by Gager et al. (2008) might also be shaped by dissimilar feeding patterns

Table 2 Temporal associations between avian *Plasmodium* cyt *b* lineages, mosquito vectors, and host species in Gamboa, Panama

Vector species	*Plasmodium* cyt *b* lineages	Isolation time	Host species in Gamboa
A. squamipennis	PAN1 (EU600217)		*Thraupis episcopus*
		November (1)	*Sporophila americana*
		February (1)	*Cynanerpes cyaneus*
	PAN4 (EU600220)	November (1)	–
	PAN5 (EU600221)	November (1)	*Thraupis episcopus*
			Sporophila americana
			Cynanerpes cyaneus
	PAN6 (EU600222)	November (1)	–
		December (1)	–
		February (2)	*Turdus grayi*
		March (1)	
	PAN8 (EU600224)	December (1)	–
	PAN9 (EU600225)	December (1)	–
C. ocossa	PAN2 (EU600218)	October (1)	–
		November (2)	–
		December (2)	
	PAN3 (EU600219)	October (2)	*Turdus grayi*
	PAN7 (EU600223)	February (1)	–
		March (1)	–

GenBank accession codes and the number of isolations for the *Plasmodium* cyt *b* lineages reported in Gager et al. (2008) are shown inside brackets in the second and third columns, respectively

in these mosquito species because *A. squamipennis* is acrodendrophilic (Galindo and Adames 1973), which explains its low adult density in Gamboa where traps were set at the ground level, while *C. ocossa* feeds preferentially at the ground level (Jones et al. 2004). This view further suggests that higher *Plasmodium* lineage diversity and infection rate should be uncovered with more pools of *A. squamipennis* from the forest canopy, and it might also explain the lack of plateau on the cyt *b* lineage recovery chart from Gager et al. (2008). Overall, these outcomes also agree with the results of previous investigations on human arboviral disease transmission in central Panama where isolations of Gamboa serogroup viruses from *A. squamipennis* occur year round, but cases of VEEV normally appear during the rainy season, when *C. ocossa* expands its range considerably (Galindo 1972; Galindo and Adames 1973; Galindo et al. 1983; Dutary et al. 1989). The fact that more positive pools and cyt *b* lineages were detected during the rainy season advocate for a higher transmission rate at this point of the year, but this finding could simply be a sampling artifact as fewer mosquito pools were collected during the dry season (Gager et al. 2008). We note that PAN7 was only isolated from *C. ocossa* in February and March when vector density was low; whether this is a consequence of bird migration patterns (Neotropical–Nearctic migratory passerines arrive in Panama in September and depart northward beginning in March), seasonal changes in host-feeding preferences by *C. ocossa*, or higher bird susceptibility to mosquito bites during nestling times (resident passerines begin nesting in March) (e.g., Valkiūnas 2005; Sehgal 2010; Kim and Tsuda 2010; Bonneaud et al. 2009) remains to be determined.

Recent studies detected the same *Plasmodium* cyt *b* lineages in congeneric mosquito species and also in mosquito species from different genera and proposed different levels of parasite–vector specialization (Ishtiaq et al. 2008, 2010; Ejiri et al. 2009; Kim and Tsuda 2010, Kimura et al. 2010; Glaizot et al. 2012). However, parasite transmission by PCR-positive mosquito species was not confirmed, and therefore, these findings have to be interpreted cautiously. Additional searches for *Plasmodium*-infected mosquitoes will have to be undertaken to rule out the possibilities that PAN6 and PAN2 co-occur in *A. squamipennis* and *C. ocossa* and also that other unidentified mosquito vectors (Njabo et al. 2010; Carlson et al. 2011; Glaizot et al. 2012), or even nonmosquito species (e.g., *Culicoides*), could participate as vectors of avian *Plasmodium* transmission in the Panama Canal region (Santiago-Alarcon et al. 2012). Moreover, because whole mosquito bodies were used in this study for *Plasmodium* screening instead of heads and thoraxes, research about vector competence is still needed to verify transmission capabilities of these parasites by their corresponding vectors. As both *A. squamipennis* and *C. ocossa* are active throughout the night, studies on bird nocturnal ecology and vector blood preferences across avian species should further clarify their contributions to the transmission of specific *Plasmodium* cyt *b* lineages in Gamboa. Finally, broader bird sampling and coordinated research by ornithologists and entomologists will be necessary to advance our understanding of avian malaria epidemiology and wildlife

disease ecology. Substantial work on the basic ecology of avian *Plasmodium* transmission remains, especially in the Neotropics.

Acknowledgments We thank Andrea Gager, formerly from Princeton University, for the opportunity to collaborate on her Ph.D. thesis project; Eldredge Bermingham; and Oris I. Sanjur from the Smithsonian Tropical Research Institute (STRI) for logistical support and academic guidance. We acknowledge the help of Sara Veronica Pinzon, Jose R. Rovira, and Jorge Morales in collecting mosquito larvae in Gamboa. Additionally, we thank James Pecor from the Walter Reed Biosystematics Units (WRBU), U.S.A., and Luis Guillermo Chaverry from the National Institute of Biodiversity of Costa Rica (INBio) for assisting with species identification in the subgenus Melanoconion of *Culex*. Milton Solano produced the map on Fig. 1, Eyda Gomez offered logistic support throughout the study, and Marilyn Scott, from McGill University, critically commented on an earlier version of this work. Funding was provided by INDICASAT AIP, STRI, and the National Secretariat for Science, Technology, and Innovation of Panama (SENACYT)–Research Investigator Award (SNI) granted to JRL.

References

Aguila Y (1987) Efecto inmediato de dos herbicidas sobre las larvas de *Mansonia* y *Coquillettidia* (Diptera: Culicidae) al ser aplicados a cinco malezas acuáticas. Tesis de maestría. Universidad de Panamá. 64 páginas

Bonneaud C, Sepil I, Milá B, Buermann W, Pollinger J, Sehgal RNM, Valkiūnas G, Iezhova TA, Saatchi S, Smith TB (2009) The prevalence of avian *Plasmodium* is higher in undisturbed tropical forests of Cameroon. J Trop Ecol 25:439–447

Braga EM, Silveira P, Belo NO, Valkiūnas G (2011) Recent advances in the study of avian malaria: an overview with an emphasis on the distribution of *Plasmodium* spp. in Brazil. Mem Inst Oswaldo Cruz 106:3–1

Bustamante DM, Lord CC (2010) Sources of error in the estimation of mosquito infection rates used to assess risk of Arbovirus transmission. Am J Trop Med Hyg 82:1172–1184

Calisher CH, John SL, Gustavo J, Thomas PM, Ernesto GV, Marta SS, Werner LJ (1981) Viruses isolated from *Aedeomyia squamipennis* mosquitoes collected in Panama, Ecuador and Argentina establishment of the Gamboa serogroup. Am J Trop Med Hyg 30:219–223

Carlson J, Martinez J, Cornel AJ, Loiseau C, Sehgal RNM (2011) Implications of *Plasmodium* parasite infected mosquitoes on an insular avifauna: the case of Socorro Island, Mexico. J Vector Ecol 36:213–220

Christensen HA, De Vasquez AM, Boreham MM (1996) Host-feeding patterns of mosquitoes (Diptera: Culicidae) from Central Panama. Am J Trop Med Hyg 55:202–208

Dutary BE, Petersen JL, Peralta PH, Tesh RB (1989) Transovarial transmission mosquito, *Aedeomyia squamipennis*. Am J Trop Med Hyg 40:108–113

Ejiri H, Sato Y, Sawai R, Sasaki E, Matsumoto R, Ueda M, Higa Y, Tsuda Y, Omori S, Murata K, Yukawa M (2009) Prevalence of avian malaria parasite in mosquitoes collected at a zoological garden in Japan. Parasitol Res 105:629–633

Franklin DC, Whelan PI (2009) Tropical mosquito assemblages demonstrate 'textbook' annual cycles. PLoS ONE 12:e8296

Gabaldon A, Ulloa G (1980) Holoendemicity of malaria: an avian model. Trans R Soc Trop Med Hyg 74:501–507

Gabaldon A, Ulloa G, Godoy N, Marquez E, Pulido J (1977a) *Aedeomyia squamipennis* (Diptera: Culicidae) vector natural de malaria aviaria en Venezuela. Bol Dir Malariol San Amb 17:9–13

Gabaldon A, Ulloa G, Pulido J, Sutil E (1977b) Especies de la Familia Culicidae que presentan ornitofilia en Venezuela. Bol Dir Malariol San Amb 17:3–8

Gabaldon A, Ulloa G, Pulido J (1981) Distribución geográfica, ecología y etología de *Aedeomyia squamipennis*, importante vector natural de malaria aviaria en Venezuela. Bol Dir Malariol San Amb 21:103–113

Gager AB, Loaiza JR, Dearborn CD, Bermingham E (2008) Do mosquitoes filter the access of *Plasmodium* cytochrome *b* lineages to an avian host? Mol Ecol 17:2552–2561

Galardo AK, Arruda M, D'Almeida CAA, Wirtz R, Lounibos LP, Zimmerman RH (2007) Malaria vector incrimination in three rural riverine villages in the Brazilian Amazon. Am J Trop Med Hyg 76:461–469

Galindo P (1972) Endemic vectors of Venezuelan encephalitis. PAHO Sc Public 243:249–253

Galindo P, Adames AJ (1972) Colonization of *Culex Melanoconion aikenii* (Aiken & Rowland, 1906) in Panama. Mosq News 32:196–200

Galindo P, Adames AJ (1973) Ecological profile of *Culex* (*Melanoconion*) *aikenii* (Diptera: Culicidae), vector of endemic Venezuelan encephalitis in Panama. Environ Entomol 2:81–86

Galindo P, Grayson MA (1971) *Culex* (*Melanoconion*) *aikenii*: natural vector in Panama of endemic Venezuelan encephalitis. Science 172:594–595

Galindo P, Adames AJ, Peralta PH, Johnson CM, Read R (1983) Impacto de la hidroeléctrica de Bayano en la transmisión de arbovirus. Rev Med Panama 8:89–134

Glaizot O, Fumagalli L, Iritano K, Lalubin F, Van Rooyen J, Christe P (2012) High prevalence and lineage diversity of avian malaria in wild populations of Great Tits (*Parus major*) and mosquitoes (*Culex pipiens*). PLoS ONE 7(4):e34964

Heinemann SJ, Belkin JN (1978) Collection records of the project "Mosquitoes of Middle America". Panama, including the Canal Zone. Mosq Syst 10:119–196

Huff CG (1965) Susceptibility of mosquitoes to avian malaria. Exp Parasitol 16:107–132

Ishtiaq F, Guillaumot L, Clegg SM, Phillimore AB, Black RA, Owens IP, Mundy NI, Sheldon BC (2008) Avian haematozoan parasites and their associations with mosquitoes across Southwest Pacific Islands. Mol Ecol 17:4545–4555

Ishtiaq F, Clegg SM, Philimore AB, Black RA, Owens IPF, Sheldon BC (2010) Biogeographical patterns of blood parasite lineage diversity in avian hosts from southern Melanesian islands. J Biogeogr 37:120–132

Jones JW, Turell MJ, Sardelis MR, Watts DM, Coleman RE, Fernandez R, Carbajal F, Pecor JE, Calampa C, Klein TA (2004) Seasonal distribution, biology, and human attraction patterns of culicine mosquitoes (Diptera: Culicidae) in a forest near Puerto Almendras, Iquitos, Peru. J Med Entomol 41:349–360

Kim KS, Tsuda Y (2010) Seasonal changes in the feeding pattern of *Culex pipiens pallens* govern the transmission dynamics of multiple lineages of avian malaria parasites in Japanese wild bird community. Mol Ecol 19:5545–5554

Kim KS, Tsuda Y, Sasaki T, Kobayashi M, Hirota Y (2009) Mosquito blood-meal analysis for avian malaria study in wild bird communities: laboratory verification and application to *Culex sasai* (Diptera: Culicidae) collected in Tokyo, Japan. Parasitol Res 105:1351–1357

Kimura M, Darbro JM, Harrington LC (2010) Avian malaria parasites share congeneric mosquito vectors. J Parasitol 96:144–151

Levine ND (1988) The protozoan phylum Apicomplexa. CRC, Boca Raton

Lourenço-de-Oliveira R, De Castro FA (1991) *Culex saltanensis* Dyar, 1928: natural vector of *Plasmodium juxtanucleare* in Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz 86:87–94

Loiseau C, Iezhova TA, Valkiūnas G, Chasar A, Hutchinson A, Buermann W, Smith T, Sehgal RN (2010) Spatial variation of haemosporidian parasite infection in African rainforest bird species. J Parasitol 96:21–29

Lopez J, Lozovei AL (1995) Ecologia do mosquito (Díptera: Culicidae) em criadouros naturais e artificiais de áreas rural do Norte do Estado do Paraná, Brasil, I – coleta ao longo do leito de Ribeirão. Rev Saúde Publica 29:183–197

Njabo KY, Cornel AJ, Sehgal RNM, Loiseau C, Buermann W, Harrigan RJ, Pollinger J, Valkiūnas G, Smith TB (2010) *Coquillettidia* (Culicidae: Diptera) mosquitoes are natural vectors of avian malaria in Africa. Mal J 8:193

Njabo KY, Cornel AJ, Bonneaud C, Toffelmier E, Sehgal RNM, Valkiūnas G, Russell AF, Smith TB (2011) Nonspecific patterns of vector, host and avian malaria parasite associations in a central African rainforest. Mol Ecol 20:1049–1061

Pecor JE, Mallampalli VL, Harbach RE, Peyton EL (1992) Catalog and illustrated review of the subgenus *Melanoconion* of *Culex*. Contr Entomol Inst 27:1–228

Read RG, Adames AJ (1980) Atmospheric stimulation of man-biting activity in tropical insects. Environ Entomol 9:677–680

Santiago-Alarcon D, Havelka P, Schaefer HM, Segelbacher G (2012) Bloodmeal analysis reveals avian *Plasmodium* infections and broad host preferences of *Culicoides* (Diptera: Ceratopogonidae) vectors. PLoS ONE 7(2):e31098

Sehgal RNM (2010) Deforestation and avian infectious diseases. J Exp Biol 213:955–960

Service M (2008) Medical entomology for students. Cambridge University Press, Cambridge, p 289

Synek P, Munclinger P, Albrecht T, Votypka J (2012) Avian haemosporidians in haematophagous insects in the Czech Republic. Paraitol Res. doi:10.1007/s00436-012-3204-3

Tempelis CH, Galindo P (1975) Host-feeding patterns of *Culex* (*Melanoconion*) and *Culex* (*Aedinus*) mosquitoes collected in Panama. J Med Entomol 12:205–209

Turell MJ, Barth J, Coleman RE (1999) Potential for Central American mosquitoes to transmit epizootic and enzootic of Venezuelan equine encephalitis virus. J Am Mosq Control Assoc 15:295–298

Valkiūnas G (2005) Avian malaria parasites and other haemosporidia. CRC Press, Boca Raton

Wolda H, Galindo P (1981) Population fluctuations of mosquitoes in the non-seasonal tropics. Ecol E 6:99–106