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A COMPARISON OF KENYAN *BIOMPHALARIA PFEIFFERI* AND *B. SUDANICA* AS VECTORS FOR *SCHISTOSOMA MANSONI*, INCLUDING A DISCUSSION OF THE NEED TO BETTER UNDERSTAND THE EFFECTS OF SNAIL BREEDING SYSTEMS ON TRANSMISSION

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ABSTRACT: In Kenya, schistosomes infect an estimated 6 million people with >30 million people at risk of infection. We compared compatibility with, and ability to support and perpetuate, *Schistosoma mansoni* of *Biomphalaria pfeifferi* and *Biomphalaria sudanica*, 2 prominent freshwater snail species involved in schistosomiasis transmission in Kenya. Field-derived *B. pfeifferi* (from a stream in Mwea, central Kenya) and *B. sudanica* (from Nawa, Lake Victoria, in western Kenya) were exposed to *S. mansoni* miracidia isolated from fecal samples of naturally infected humans from Mwea or Nawa. Juvenile (<6 mm shell diameter), young adult (6–9 mm), and adult snails (>9 mm) were each exposed to a single miracidium. *Schistosoma mansoni* developed faster and consistently had higher infection rates (39.6–80.7%) in *B. pfeifferi* than in *B. sudanica* (2.4–21.5%), regardless of the source of *S. mansoni* or the size of the snails used. *Schistosoma mansoni* from Nawa produced higher infection rates in both *B. pfeifferi* and *B. sudanica* than did *S. mansoni* from Mwea. Mean daily cercariae production was greater for *B. pfeifferi* exposed to sympatric than allopatric *S. mansoni* (583–1,686 vs. 392–1,232), and mean daily cercariae production among *B. sudanica* were consistently low (50–590) with no significant differences between sympatric or allopatric combinations. Both non-miracidia–exposed and miracidia-exposed *B. pfeifferi* had higher mortality rates than for *B. sudanica,* but mean survival time of shedding snails (9.3–13.7 wk) did not differ significantly between the 2 species. A small proportion (1.5%) of the cercariae shedding *B. pfeifferi* survived up to 40 wk post-exposure. *Biomphalaria pfeifferi* was more likely to become infected and to shed more cercariae than *B. sudanica,* suggesting that the risk per individual snail of perpetuating transmission in Kenyan streams or lacustrine habitats may differ considerably. High infection rates exhibited by the preferential self-fertilizing *B. pfeifferi* relative to the out-crossing *B. sudanica* point to the need to investigate further the role of host breeding systems in influencing transmission of schistosomiasis by snail hosts.

Vector-borne diseases including malaria, dengue, Zika virus, and trypanosomiasis continue to pose major challenges to public health (Smith et al., 1998; Greenwood and Mutabingwa, 2002; San Martín et al., 2010). Similarly, snail-transmitted infections also remain problematic in the developing world, and although snails are not vectors in a more conventional sense in that they do not bite their hosts to perpetuate transmission, they play an indispensable role in transmission and are considered to be vectors by the WHO (2016).

With a few exceptions, digeneans (digenetic trematodes or flukes) use snails as first intermediate hosts, enjoying a remarkably productive period of asexual reproduction within snails that culminates with the production of cercariae that may continue for months and in some cases over a year (Mutuku et al., 2014). The prolonged production and release of numerous cercariae into the environment gives the life cycles of human-infecting schistosomes considerable stability, thereby challenging control efforts. Given the vast populations of snails that occupy many natural transmission sites, control of snail-transmitted diseases is a formidable challenge. When schistosomiasis control has been most successful is when snail control has been implemented (Lelo et al., 2014; Njenga et al., 2014; Sokolow et al., 2016), highlighting the importance of knowing more about the biology of the snail hosts and their interactions with snail-transmitted parasites of human and veterinary concern.

The competence of snails to serve as hosts for schistosomes is influenced by several different factors including, but not limited to, infection prevalence as measured by the proportion of schistosome-exposed snails that actually produce and release (shed) cercariae, the length of time required to complete sporocyst development for the first release of cercariae following exposure to infection (the pre-patent period), the longevity of infected snails, duration of actual shedding of the schistosome-exposed snails, and daily output of cercariae from infected snails (Ibikounlé et al., 2012). It is also important to appreciate that schistosome snail hosts exist in complex environmental settings that can influence their capacity to support transmission. They must simultaneously cope with exposure to potential infection with several other species of digenetic trematodes, which may even be more common than schistosomes (Loker et al., 1981; Mohammed et al., 2016) and that also have the potential to cause castration, thereby strongly affecting fitness of the snails. Moreover, infection with other trematode species may alter susceptibility to infection with schistosomes (Spatz et al., 2012). Finally, the suitability of snail environments often varies dramatically with season (Charbonnel et al., 2005), which is

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anticipated to influence the snail's breeding system (for instance, selfing vs. out-crossing), which in turn might influence their competence in resisting infection by parasites (Howard and Lively, 1994; Gibson et al., 2016).

In Africa, transmission of intestinal schistosomiasis caused by *Schistosoma mansoni* is enabled by 12 species of *Biomphalaria* with *Biomphalaria pfeifferi* and *Biomphalaria sudanica* being the most prominent intermediate hosts in Kenya (Loker et al., 1993; Brown, 1994). *Biomphalaria pfeifferi* is widely distributed in tributaries feeding Lake Victoria, canals in the Mwea irrigation scheme in central Kenya, small impoundments, and both seasonal and perennial streams throughout the country, except in the tropical lowland belt along coastal Kenya. *Biomphalaria sudanica* is mainly found along the shores of Lake Victoria and other larger water bodies like Lake Jipe (Loker et al., 1993; Brown, 1994).

As part of a series of studies exploring the role of Kenyan *Biomphalaria* snails in the transmission of *S. mansoni*, we first examined the compatibility of field-derived *B. pfeifferi* to *S. mansoni* miracidia obtained from infected school children (Mutuku et al., 2014). We were particularly interested in learning if *S. mansoni* exhibits a greater degree of compatibility with its local *B. pfeifferi* snail populations than it does with other *B. pfeifferi* populations further removed geographically. Both sympatric and allopatric combinations of parasites and snails exhibited high compatibility (approximately 50% at a dose of 1 miracidium per snail), with an increase in infection and mortality rates as the miracidial dose was increased. Approximately 3% of *B. pfeifferi* from Asao, western Kenya, exposed to a low dose of sympatric miracidia (1 or 5) continued to shed cercariae for as long as 58 wk post-exposure (PE). We were also interested in comparing *B. pfeifferi* and *B. sudanica* with respect to their role in transmission of *S. mansoni*. Using a polymerase chain reaction (PCR) assay to detect *S. mansoni* in snails, we established that during 24 days of pre-patent development following exposure to a single *S. mansoni* miracidium, 48.3% of *B. pfeifferi* harbored successfully developing parasites as compared to only 23.5% for *B. sudanica*. At 40 days PE, by which time it was expected that any successful infection should have culminated in cercariae production, only 14.7% of *B. sudanica* had either shed cercariae or harbored viable parasites, whereas the comparable figure for *B. pfeifferi* was 47.6% (Lu et al., 2016). These results are suggestive that *B. pfeifferi* offers a more conducive environment for schistosome development than *B. sudanica* during the pre-patent period.

In contrast to the study of Mutuku et al. (2014), which dealt exclusively with *B. pfeifferi*, one aim of the current study was to examine the relative compatibility of field-derived Kenyan *B. pfeifferi* and *B. sudanica* to *S. mansoni*. Additionally, we were interested in learning if *B. pfeifferi* was more susceptible to infection with *S. mansoni* derived from a sympatric locality, one in which its transmission likely depended exclusively on *B. pfeifferi,* and if this snail species would be equally susceptible to *S. mansoni* taken from an allopatric area where *B. sudanica* routinely transmitted the parasite. Conversely, is *B. sudanica* more compatible with *S. mansoni* derived from the same location where it is routinely transmitted by *B. sudanica*? We were also interested in documenting other parameters associated with vectorial competence including length of pre-patent period, the number of cercariae produced daily, and the duration of shedding of cercariae by infected snails.

MATERIALS AND METHODS

Parasite and snail sources

Schistosoma mansoni eggs were obtained from pooled fecal samples from 5 school children aged 6–12 yr from Mukuo village, Mwea, Kirinyaga County, central Kenya (GPS coordinates 00°40′54″S, 037°20′36″E, altitude 1,098 m), or from Nawa village, Kisumu County, western Kenya (GPS coordinates 00°06′12″S, 034°42′75″E, altitude 1,272 m). Schistosome eggs were concentrated and hatched, and miracidia used to infect snails as described by Mutuku et al. (2014). Snails were collected from the field from Mukou stream, in Mwea, central Kenya, and *B. sudanica* were collected from Nawa beach, on the shores of Lake Victoria, in Nawa village, Kisumu, and identifications were confirmed as *B. sudanica* or *B. pfeifferi* based on known geographical and habitat preferences (Loker et al., 1993; Brown, 1994; Dejong et al., 2001, 2003; Steinauer et al., 2009) and conchological characters (Brown, 1994). The 2 species are distinct genetically and were not found coexisting in the habitats we examined. *Biomphalaria* snails collected were isolated and screened for digenean infection, and any snail found to be shedding cercariae of any type was discarded. Prior to exposure to *S. mansoni*, all non-shedders were maintained for an additional 4 wk in aquaria, both to adapt the snails to laboratory conditions and to permit re-screening to determine if they were still negative for digenean infections (Mutuku et al., 2014).

Experimental design

A reciprocal cross-infection experiment was conducted whereby *B. pfeifferi* snails from Mwea and *B. sudanica* from Nawa were exposed to *S. mansoni* miracidia from either Mwea or Nawa. Snails to be exposed to *S. mansoni* were categorized into 3 groups depending on size (shell diameter)/age: (1) juveniles <6 mm shell diameter; (2) young adults 6–9 mm; and (3) adults >9 mm (a total of 6 sympatric and 6 allopatric combinations). For each of the 12 possible combinations, 100 pre-screened snails found not to be shedding any digenetic trematodes were exposed to 1 *S. mansoni* miracidium each. For each of the 3 snail size categories from the 2 locations, a group of 100 snails was not exposed to the parasite and served as unexposed controls. A total of 1,800 snails and 1,200 miracidia were used for this experiment. Starting at 1 wk PE, the snails were examined once a week, for any snails shedding *S. mansoni* cercariae, for over a period of at least 24 wk, or until the snails died using the procedure described below. Snails were counted and screened individually for evidence of shedding schistosome or any other cercariae, and the number of surviving snails recorded. For snails that were found to be shedding, the total number of cercariae they produced for 2 hr between 1000 hr and 1200 hr was determined as described below.

Determination of cercariae output from infected snails

Each snail was placed in an individual well of a 24-well plastic culture plate, each well containing 1 ml of aged de-chlorinated water. The plate was placed in indirect sunlight for 2 hr between 1000 hr and 1200 hr. Individual wells were then examined under a dissecting microscope for presence of cercariae. For the snails that had shed cercariae, the contents of the well were mixed gently using a micropipette, and an aliquot of 50 µl was then obtained and placed in a gridded Petri dish. Two drops of Lugol's iodine

were then added to stain and immobilize the cercariae, which were then counted with the aid of a dissecting microscope and a tally counter. The number of cercariae counted was multiplied by 20 to obtain the total number of cercariae that were produced by the snail during the 2 hr period. This procedure was used for all the shedding snails at 6, 10, and 14 wk PE.

Ethical considerations

Approval for this study was obtained from the KEMRI Scientific and Ethics Review Unit (SERU) and was referenced SERU SSC No. 2373 and from the University of New Mexico (UNM) Institutional Review Board and referenced 18115. Children were selected for enrollment into the study because they are the most vulnerable to schistosomiasis, contribute significantly to environment contamination and parasite transmission, are easily accessible from their schools, and are regularly offered treatment. Recruitment of human study subjects and their participation and care was done as described previously (Mutuku et al., 2014). Consent to participate in the study was obtained from parents or guardians. The information and data obtained from the study participants were stored securely within KEMRI on password-protected computers. This study was conducted with the approvals of the National Commission for Science, Technology and Innovation (NACOSTI), Permit NACOSTI/P/16/9609/12754, and the National Environment Management Authority (NEMA), Permit NEMA/AGR/46/2014.

Statistical analyses

Data analysis was conducted using IBM SPSS version 21.0 statistical software and Microsoft Excel. Descriptive statistics such as proportions were used to summarize categorical variables, while measures of central tendency such as mean, standard error, and range were used to summarize continuous variables. Odds ratio (OR) and 95% confidence interval (CI) were used to estimate the strength of association between outcome and exposure variables. A P value less than 0.05 was considered statistically significant.

RESULTS

Duration of pre-patent period for *S. mansoni* in snails

The pre-patent period for *S. mansoni* was found to be shorter in *B. pfeifferi* than in *B. sudanica* (Fig. 1). For all *S. mansoni* and *B. pfeifferi* combinations, some snails were shedding cercariae by 4 wk PE, with sympatric combinations having higher proportions of early shedders (3–5.8%) compared to allopatric combinations (1.4–1.5% shedders). Allopatric combinations showed higher proportions of snails beginning shedding at 6 wk PE. For *B. sudanica*, except for young adults exposed to sympatric *S. mansoni*, none of the other groups had shed by 4 wk PE, and only a small percentage (1.2–3.4%) shed by 5 wk PE. Even for juvenile *B. sudanica*, it took up to 6 wk for shedding to commence. There was no obvious overall tendency for younger snails to shed earlier than older snails.

Prevalence of *S. mansoni* infection in snails

With respect to infection prevalence as measured by shedding of cercariae, snails in each exposure combination attained their peak prevalence of infection at 6 wk PE, with an overall 30.6% prevalence among the 755 surviving exposed snails. *Biomphalaria sudanica* were significantly less likely to develop cercariae-producing infections (48 of 414 surviving snails or 8.6%) than *B. pfeifferi* (183 of 341 surviving snails or 53.7%), regardless of the size of snails used or the source of *S. mansoni* (Fig. 1; Suppl. Tables S1, S2). For *B. sudanica* of all 3 size/age groups, the sympatric *B. sudanica*–transmitted Nawa *S. mansoni* isolate produced higher prevalence of infection than the allopatric *B. pfeifferi*–transmitted *S. mansoni* isolate from Mwea, but the differences were not significant. The opposite was true for *B. pfeifferi* where the allopatric Nawa isolate of *S. mansoni* achieved higher prevalence levels than the sympatric Mwea isolate (significant for juvenile snails). In other words, for both snail species, and for all 3 size/age classes, the Nawa-derived *S. mansoni* isolate always produced more patent infections than the Mwea-derived *S. mansoni* isolate. The overall percentage of infection achieved among all snails exposed to Nawa *S. mansoni* (145 of 369 or 39.3%) was significantly higher ($P = 0.0078$) than for *S. mansoni* derived from Mwea (86 of 386 snails or 22.3%) (Fig. 1; Table S2). Overall, relative to the juvenile snail prevalence level of 32.7%, infection prevalence of young adult and adult snails were not significantly different (27.7%, OR = 0.79 [95% CI = 0.55–1.13]; $P = 0.1938$), and (31.8%, OR = 0.96 [95% CI = 0.64–1.42]; $P = 0.8213$), respectively (Table S1).

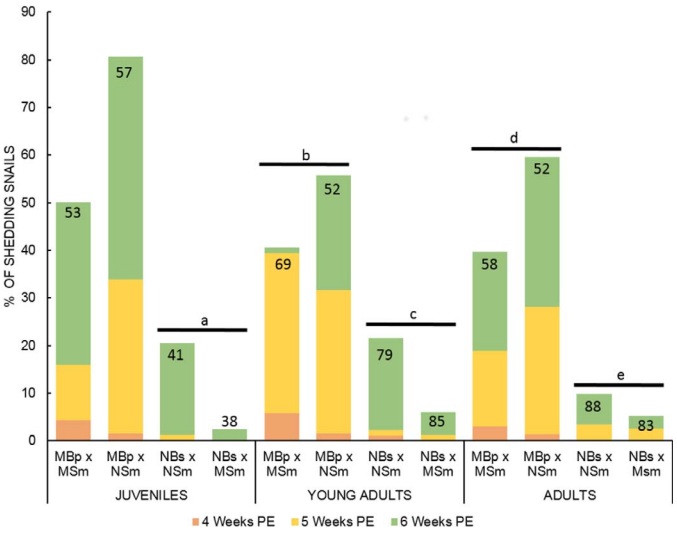

FIGURE 1. Percentage of snails in the different age categories shedding cercariae of *Schistosoma mansoni* (*Sm*) from Nawa or Mwea at 4, 5, 6 wk post-exposure (PE) to single schistosome miracidium. NBs represents *Biomphalaria sudanica* Nawa; MBp, *Biomphalaria pfeifferi* Mwea; MSm, *S. mansoni* Mwea; NSm, *S. mansoni* Nawa. The number of snails alive in each group at 6 wk PE is indicated within each bar. As determined at 6 wk PE, the groups united by a horizontal bar with a common letter were not significantly different from one another with respect to percentage of snails infected. Other comparisons among groups are discussed in the text. Color version available online.

Snail mortality by 10 wk post-exposure to *S. mansoni*

Except for the adult snails, mortality was higher for *B. pfeifferi* than *B. sudanica,* regardless of infection status (Fig. 2; Table II). Overall, relative to juvenile exposed snails, mortality among the unexposed control snails for both *B. sudanica* and *B. pfeifferi* was

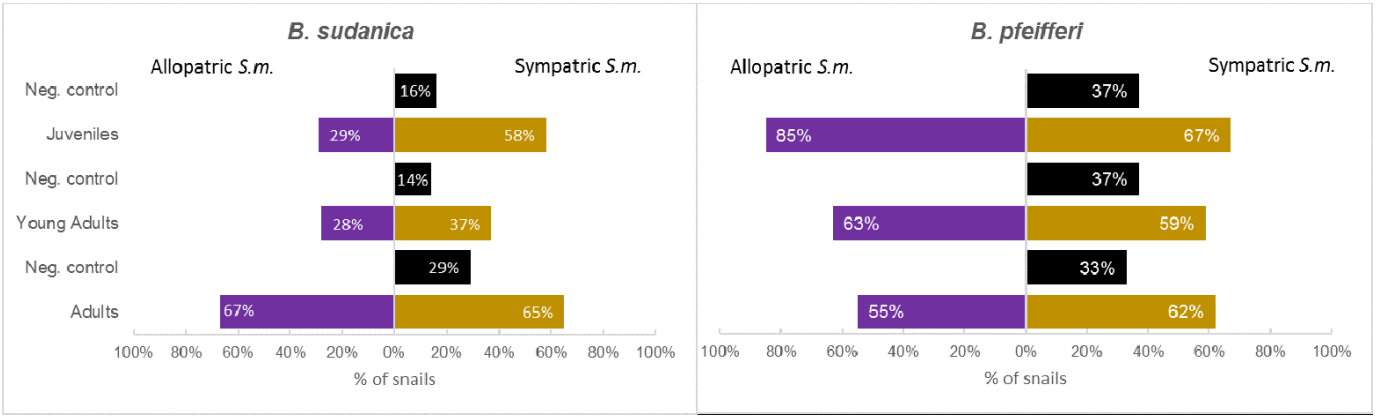

FIGURE 2. Mortality rates for different snail-parasite combinations, at 10 wk post-exposure. Black bars represent mortality rate for unexposed control groups for each of the corresponding snail categories. Snail mortality is expressed as a percentage of the initial number of snails in a combination. Neg. control = Negative (Unexposed control), *S.m.* = *Schistosoma mansoni*. Color version available online.

significantly lower at 20.0%, (OR = 0.16 [95% CI = 0.12–0.23]; $P < 0.0001$) and 36.0%, (OR = 0.37 [95% CI = 0.27–0.51]; $P < 0.0001$), respectively. For the miracidium-exposed snails, relative to juvenile snails, young adult snails were 41% less likely to die; however, there was no significant increase in mortality for the adult snails when all the snails were considered together 62.20% (OR = 1.11 [95% CI = 0.84–1.48]; $P < 0.4686$).

Survival of snails with *S. mansoni* cercariae infections

For snails shedding *S. mansoni* cercariae (Table I), except for the adult snails, *B. sudanica* had higher mean survival time compared with *B. pfeifferi*, with *B. sudanica* exposed to sympatric *S. mansoni* achieving slightly longer mean survival times (9.5–12.2 wk) than with allopatric *S. mansoni* (9.5–11.0 wk). However, whereas no infected *B. sudanica* survived past 29 wk PE, a small percentage (1.5%) of young and adult *B. pfeifferi* exposed to sympatric *S. mansoni* survived for up to 40 wk PE. By comparison, the longest recorded survival of unexposed *B. sudanica* and *B. pfeifferi* was 43 and 49 wk, respectively.

TABLE I. Mean survival time for *S. mansoni* cercariae-shedding snails.

Snail-parasite combination*	Mean survival times (wk)	Standard error	Confidence interval (95%)	
			Lower	Upper
Juveniles				
Mwea Bp × Mwea Sm	10.9	0.6	9.7	12.1
Mwea Bp × Nawa Sm	9.3	0.4	8.6	10.1
Nawa Bs × Nawa Sm	11.8	0.9	10.0	13.6
Nawa Bs × Mwea Sm	10.5	1.5	7.5	13.5
Young adults				
Mwea Bp × Mwea Sm	10.1	0.8	8.5	11.8
Mwea Bp × Nawa Sm	10.6	0.8	9.0	12.1
Nawa Bs × Nawa Sm	12.2	0.5	11.1	13.2
Nawa Bs × Mwea Sm	11.0	0.4	10.1	11.9
Adults				
Mwea Bp × Mwea Sm	13.7	1.3	11.1	16.3
Mwea Bp × Nawa Sm	11.7	0.4	10.8	12.5
Nawa Bs × Nawa Sm	9.5	0.9	7.8	11.2
Nawa Bs × Mwea Sm	9.5	2.5	4.5	14.5

* Bp = *B. pfeifferi*, Bs = *B. sudanica*, Sm = *S. mansoni*.

Cercariae production

Overall, infected *B. pfeifferi* produced more cercariae than *B. sudanica*, with almost all the mean counts for the former being higher than the latter species (Fig. 3; Table II). For *B. pfeifferi*, sympatric combinations usually had a higher mean cercariae production (583 [95% CI 404–762] to 1,686 [95% CI 886–2,486]) than allopatric combinations (392 [95% CI 255–529] to 1,232 [95% CI 936–1,528]). There was no obvious trend for smaller snails to produce fewer cercariae than bigger/older snails, but the highest counts did come from young adult or adult snails. There was no obvious overall tendency for cercariae production to either increase or decrease over 3 successive periods of observation spread over an interval of 56 days. The highest number of cercariae produced at a single observation time was 4,460 by an adult *B. pfeifferi* exposed to sympatric *S. mansoni*.

For *B. sudanica*, generally more cercariae were produced by snails with sympatric than allopatric *S. mansoni* infections (161 [95% CI 103–218] to 360 [95% CI 279–441]), but again this was not always the case. In these monomiracidial infections, no *B. sudanica* snails produced more than 1,000 cercariae during the observation period, though this was common with infected *B. pfeifferi* with sympatric *S. mansoni*.

DISCUSSION

Following exposure to *S. mansoni*, for *B. pfeifferi* relative to *B. sudanica*, we found the pre-patent period tended to be shorter, the prevalence of infection as measured by shedding significantly higher, the mortality at 10 wk higher, average survivorship of infected snails marginally shorter, and the average daily production of cercariae significantly higher. In general, the size/age of the snails exposed (<6, 6–9, and >9 mm shell diameter) did not strongly affect most parameters studied for either snail species.

In our hands, following exposure to a single miracidium, Kenyan field isolates of *B. pfeifferi* consistently attained a prevalence of infection of more than 40%. We have now noted

TABLE II. Analysis of mean cercariae production within each snail-parasite combination at 6, 10, and 14 wk post-exposure (PE).

Snail size	Snail species	Snail source	Parasite source	Number of shedding snails			Mean cercariae output for 2 hr (95% CI)			*P* value
				6 wk PE	10 wk PE	14 wk PE	6 wk PE	10 wk PE	14 wk PE	
Juveniles	*B. sudanica*	Nawa	Nawa	17	7	4	258 (146–370)	451 (274–628)	176 (27–325)	0.0627
	B. sudanica	Nawa	Mwea	2	1	0	150 (51–249)	250		0.2207
	B. pfeifferi	Mwea	Mwea	25	10	5	984 (789–1,179)	583 (404–762)	624 (454–794)	0.015
	B. pfeifferi	Mwea	Nawa	46	10	2	1,032 (862–1,201)	392 (255–529)	568 (306–829)	0.0009
Young adults	*B. sudanica*	Nawa	Nawa	17	11	1	161 (103–218)	250 (173–328)	60	0.0261
	B. sudanica	Nawa	Mwea	5	3	0	310 (48–572)	590 (243–937)		0.1797
	B. pfeifferi	Mwea	Mwea	28	14	5	1,051 (814–1,288)	760 (489–1,031)	1,686 (886–2,486)	0.0425
	B. pfeifferi	Mwea	Nawa	29	9	8	607 (442–772)	513 (288–739)	880 (660–1,100)	0.1033
Adults	*B. sudanica*	Nawa	Nawa	4	2	0	227 (81–374)	360 (279–441)		0.1649
	B. sudanica	Nawa	Mwea	2	1	0	120 (40–200)	50		0.2207
	B. pfeifferi	Mwea	Mwea	23	15	10	851 (628–1,073)	859 (613–1,104)	1,476 (782–2,170)	0.0736
	B. pfeifferi	Mwea	Nawa	30	23	1	1,232 (936–1,528)	535 (379–691)	585	0.0016

this to be the case whether *S. mansoni* is derived from locations sympatric to the *B. pfeifferi* isolate (both for the Asao stream in west Kenya and now observed twice for *B. pfeifferi* from canals in the Mwea rice scheme in central Kenya) or from allopatric locations (Mutuku et al., 2014). High susceptibility of *B. pfeifferi* to allopatric *S. mansoni* isolates has been shown to be the case regardless of whether the *S. mansoni* isolates come from regions in which *B. pfeifferi* is the usual host or from regions where *B. sudanica* is the normal host. For instance, 80% of juvenile *B. pfeifferi* from Mwea in central Kenya became infected following exposure to a single miracidium of *S. mansoni* from Nawa, Lake Victoria, approximately 300 km to the west. Our results are in agreement with most but not all previous studies (Frandsen, 1979; Southgate et al., 2000; Ibikounlé et al., 2012; Adriko et al., 2013; Lu et al., 2016) in documenting high levels of compatibility of *S. mansoni* with both sympatric and allopatric *B. pfeifferi*, including those in which snail and schistosome originated from different continents (Frandsen, 1979; Ibikounlé et al., 2012).

In contrast to Adriko et al. (2013) and in agreement with Frandsen (1979), our results consistently show lower levels of success for *S. mansoni* in *B. sudanica*, in either sympatric or allopatric combinations. The Nawa isolate of *S. mansoni*, which was recovered from individuals from the shores of Lake Victoria, where their infections most probably originated from *B. sudanica*, never infected more than 25% of sympatric *B. sudanica*, even though this same isolate proved to be very compatible with allopatric *B. pfeifferi* (80% prevalence). Comparing the responses to *S. mansoni* of lab-reared *B. sudanica* and field-derived *B. pfeifferi* using a combination of an *S. mansoni*–specific PCR-based detection assay, dissection, and shedding methods (Lu et al., 2016), it was noted that more *B. pfeifferi* (54.5%) than *B. sudanica* (38.9%) were positive for *S. mansoni* at 1–4 days PE. This suggested that penetration of miracidia was somewhat higher for *S. mansoni* in *B. pfeifferi*. By 8–24 days PE, the proportion of dissection-positive/PCR positive snails was over 2 times higher in *B. pfeifferi* than in *B. sudanica*. By 40 days PE, the proportion of all snails that was unequivocally positive for *S. mansoni* was 3.2 times higher, and the proportion of all snails that was shedding was over 12 times higher than for *B. sudanica*. *Schistosoma mansoni* also developed faster in *B. pfeifferi* than in *B. sudanica* (Lu et al., 2016).

Also of considerable relevance to understanding the capacity of snails to transmit schistosomiasis is their longevity, especially their duration of shedding. We observed that *B. pfeifferi* of all age

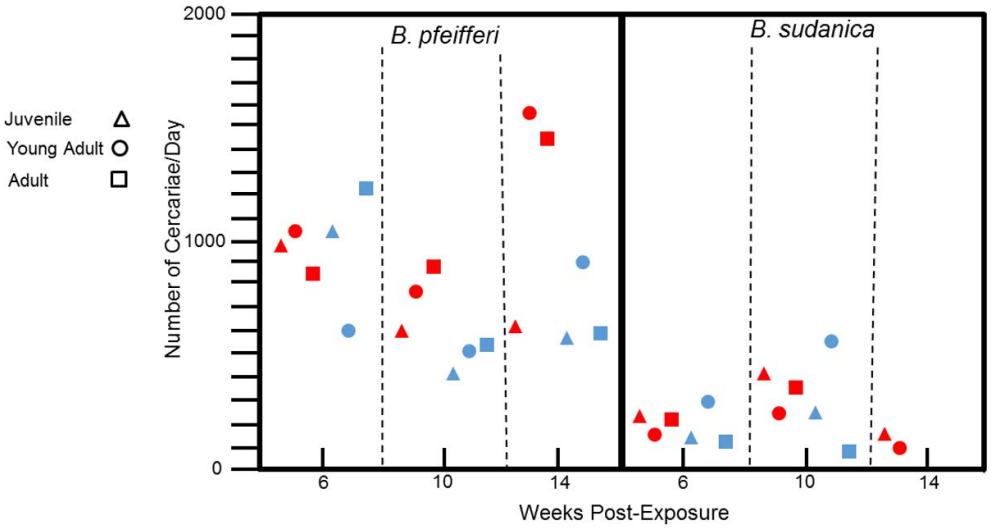

FIGURE 3. Mean cercariae produced by *Biomphalaria pfeifferi* and *Biomphalaria sudanica* at 6, 10, and 14 wk post-exposure. Sympatric combinations are represented in red and allopatric combinations in blue. Color version available online.

groups, both unexposed controls and exposed snails, suffered high mortality by 10 wk PE, generally higher than seen for *B. sudanica*. Exposure combinations with high prevalence of infection had particularly high mortality by 10 wk PE, so this provides some explanation for the high mortality observed. Another factor we cannot exclude is that the *B. pfeifferi* used were transported from central to western Kenya and may have suffered both from transportation and different environmental conditions at Kisian. We have also noted that some *B. pfeifferi* isolates adapt better to laboratory conditions than others. In spite of the high mortality at 10 wk PE, some *B. pfeifferi* nonetheless survived and continued to shed for up to 40 wk PE. In contrast, none of the exposed *B. sudanica* survived past 29 wk PE. As previously noted (Mutuku et al., 2014), some individual field-derived *B. pfeifferi* when experimentally exposed to *S. mansoni* as young adults can support production of *S. mansoni* cercariae for over a year.

With respect to the number of cercariae released per day, on average *B. pfeifferi* produced more cercariae than *B. sudanica*. For individual *B. pfeifferi*, >2,000 cercariae were often recovered from a 2-hr shedding period. By contrast, none of the experimentally exposed *B. sudanica* were observed to shed more than 900 cercariae in a comparable interval, in agreement with results by Frandsen (1979). Low infection and cercariae production levels help to explain the difficulties we have experienced in the past in trying to maintain the *S. mansoni* life cycle in the laboratory in *B. sudanica*. In contrast to our results, a study utilizing Ugandan *Biomphalaria* snail isolates demonstrated that *B. sudanica* produced more cercariae than *B. pfeifferi* though the difference was not significant (Adriko et al., 2013).

The results of our study are also of relevance to evolutionary biologists interested in the Red Queen hypothesis, particularly the topic of how sexual reproduction in host populations influences susceptibility to coevolving parasites. Studies of other digenean-snail combinations have shown that high prevalence of digenean infection favors higher proportions of sexually reproducing snails. This is because the parasites are able to adapt readily to clonal asexual hosts and achieve high levels of sterilizing infection among them, whereas host individuals produced by sexual crosses may express rare traits conferring higher resistance to infection (Howard and Lively, 1994; Vergara et al., 2014; Gibson et al., 2016). With respect to the present study, we note as have several others that *B. pfeifferi* is highly susceptible to *S. mansoni* and in nature is often infected with several digenetic trematodes (Loker et al., 1981; Mohammed et al., 2016). A number of studies have documented that although *B. pfeifferi* is capable of cross-fertilization, it is a strong preferential self-fertilizer (Jarne and Theron, 2001; Charbonnel et al., 2005; Campbell et al., 2010). The latter mode of reproduction generally leads to an excess of homozygosity and a loss of genetic diversity, and *B. pfeifferi* populations consist of a series of relatively demarcated lineages separated from one another by strong preferential selfing (Jarne and Theron, 2001). These are all characteristics that might be expected to favor high levels of parasitism. We lack a general understanding in *S. mansoni* transmission foci for how many such *B. pfeifferi* lineages exist, how spatially distinct or temporally stable they are, and how much they may vary if at all with respect to susceptibility to *S. mansoni* or to the many other species of digenetic trematodes with which they must contend. These other digenean species can be as common or more so than *S. mansoni* and also impose fitness costs as they also can cause castration (Esch and Fernandez, 1994; Lafferty and Kuris, 2009). It will be interesting to learn if under heavy pressure from parasitism including *S. mansoni* and other abundant digeneans like amphistomes (Laidemitt et al., 2016) whether *B. pfeifferi* populations increase out-crossing rates such that they might then fare better in a co-evolutionary arms race with outcrossing parasites (King et al., 2011; Koskella et al., 2011; Singh et al., 2015). Alternatively, perhaps some genotypes of *B. pfeifferi* are more resistant to digeneans than others, and selective pressure from digenean parasitism may favor increased frequency of resistant genotypes. Although the impermanent nature of the stream habitats often colonized by *B. pfeifferi* may prevent stable parasite populations from building such that the presumed advantages in rapid colonizing ability favored by self-fertilization might predominate over advantages in resistance to parasites resulting from sex, our studies suggest high levels of parasitism can persist for years in streams that do not necessarily flood or dry on an annual basis (M. R. Laidemitt, pers. comm.). Further investigation of the interactions between digenean parasitism (including *S. mansoni*) and both outcrossing rates and population composition studies for *B. pfeifferi* are clearly warranted, including efforts to determine if out-crossed progeny enjoy greater resistance to digenean infection.

Biomphalaria sudanica and *B. choanomphala* also deserve consideration in a broader evolutionary context. *Biomphalaria choanomphala* has been characterized as an out-crosser (Standley et al., 2014). In the context used by the authors, this designation was meant to apply to both *B. choanomphala* and *B. sudanica* given that other studies suggested the 2 regularly undergo genetic exchange (the 2 taxa represent distinctive ecophenotypes) and *B. choanomphala* is the name with taxonomic priority (Standley et al., 2011). Here we use the name *B. sudanica* rather than *B. choanomphala* to apply to the shore-inhabiting form, because the latter is generally considered to be a deep-water snail. In any case, the exact nature of the genetic exchange between the 2 named taxa deserves further study for additional populations. Also, assuming that the lakeshore-inhabiting form *B. sudanica* is an out-crosser, it is of interest and consistent with theory that its experimental susceptibility to infection with either sympatric or allopatric *S. mansoni* is lower than seen with *B. pfeifferi*. Furthermore, overall prevalence levels with *S. mansoni* and other digeneans appear to be lower in *B. sudanica* than in *B. pfeifferi* in natural habitats monitored for over 2 yr (M. R. Laidemitt, pers. comm.) though extraneous environmental factors might play an important role in dictating such infection levels as well. Any gain in resistance achieved by *B. sudanica* by out-crossing might be expected to affect not just *S. mansoni*, but as many as 16 additional digenean species, many transmitted by shoreline-inhabiting birds, that commonly infect this snail in nature as well. Although experimentation with additional isolates is needed, the low infection levels we retrieve with *B. sudanica* following experimental exposure to *S. mansoni* are suggestive of the presence in *B. sudanica* of resistance traits that may prove useful with respect to developing new control efforts based on introductions of resistant snails into natural populations of schistosome-susceptible snails. Last, the relationships between *B. choanomphala* and digenean infection deserve much more scrutiny. This taxon is typically but not always recovered from deeper lake water. Deepwater habitats have been considered as coevolutionary "cold spots" as compared to shallower shoreline habitats frequented by avian definitive

hosts (Howard and Lively, 1994; King et al., 2009). For *B. choanomphala*, does its preferred habitat provide a refugium from digenean infection? Although it is clear that *B. choanomphala* can be infected by *S. mansoni* in deeper water, the extent to which it is exposed to this and other digenean species may be considerably diminished relative to *B. sudanica*. If so, then does this taxon when in deep water revert to more frequent self-fertilization, possibly abandoning the costs of maintaining resistance to digenean infection given their lower exposure rates (Sheldon and Verhulst, 1996)?

Another observation of interest from our data is that *S. mansoni* from Nawa, where it is transmitted by *B. sudanica*, produced higher infection levels in both snail species than the *B. pfeifferi*–transmitted isolate of *S. mansoni* isolate from Mwea. This is consistent with the idea that ongoing coevolutionary interactions of *S. mansoni* with a sexually reproducing host confers on it properties of infectivity that guarantee it a higher likelihood of success when confronted with a selfing species like *B. pfeifferi*. Study of further reciprocal exposure experiments involving the same 2 snail species and isolates of *S. mansoni* derived from each would be of interest to further document this possibility. Also of interest would be to learn if and how the interactions between sexual vs. selfing snails might also influence trade-offs that might occur with respect to virulence in the definitive host (Davies et al., 2001).

In conclusion, we were interested in determining which of the two most prominent intermediate host snails for *S. mansoni* in Kenya is more efficient in transmission of the intestinal schistosomiasis parasite by measuring traits that affect transmission. At least some *B. sudanica* and *B. pfeifferi* could support full development of either allopatrically or sympatrically derived *S. mansoni* regardless of snail size/age, but *B. pfeifferi* were significantly more likely to become infected and had higher daily rates of cercariae production than *B. sudanica*. Even though *B. sudanica* seems less efficient in transmission of the parasite on a per snail basis, this species occurs in vast numbers in its natural habitat. Abundance may thus compensate for low compatibility such that *B. sudanica* can readily sustain transmission in communities living around the shores of the lake. The persistence of a proportion of long-term *B. pfeifferi* shedders, though it may seem insignificant, could nonetheless play a significant role in initiating reinfections in the face of sustained mass drug administration. Because of differences in the breeding systems of *B. pfeifferi* and *B. sudanica*, the interactions of these 2 host species with *S. mansoni* and other digeneans may prove to be instructive in understanding the importance of sex in resistance to parasites.

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LITERATURE CITED

Adriko, M., C. J. Standley, B. Tinkitina, G. Mwesigwa, T. K. Kristensen, J. R. Stothard, and N. B. Kabatereine. 2013. Compatibility of Ugandan *Schistosoma mansoni* isolates with *Biomphalaria* snail species from Lake Albert and Lake Victoria. Acta Tropica **128:** 303–308.

Brown, D. S. 1994. Freshwater snails of Africa and their medical importance, 2nd ed. CRC Press, Boca Raton, Florida, 608 p.

Campbell, G., L. R. Noble, D. Rollinson, V. R. Southgate, J. P. Webster, and C. S. Jones. 2010. Low genetic diversity in a snail intermediate host (*Biomphalaria pfeifferi* Krauss, 1848) and schistosomiasis transmission in the Senegal River Basin. Molecular Ecology **19:** 241–256.

Charbonnel, N., R. Rasatavonjizay, E. Sellin, P. Brémond, and P. Jarne. 2005. The influence of genetic factors and population dynamics on the mating system of the hermaphroditic freshwater snail *Biomphalaria pfeifferi*. Oikos **108:** 283–296.

Davies, C. M., J. P. Webster, and M. E. Woolhous. 2001. Trade-offs in the evolution of virulence in an indirectly transmitted macroparasite. Proceedings of the Royal Society of London: Biological Sciences **268:** 251–257.

DeJong, R. J., J. A. Morgan, W. L. Paraense, J. P. Pointier, M. Amarista, P. F. Ayeh-Kumi, A. Babiker, C. S. Barbosa, P. Brémond, A. Pedro Canese, et al. 2001. Evolutionary relationships and biogeography of *Biomphalaria* (Gastropoda: Planorbidae) with implications regarding its role as host of the human bloodfluke, *Schistosoma mansoni*. Molecular Ecology and Evolution **18:** 2225–2239.

Dejong, R. J., J. A. Morgan, W. D. Wilson, M. H. Al-Jaser, C. C. Appleton, G. Coulibaly, P. S. D'Andrea, M. J. Doenhoff, W. Haas, M. A. Idris, et al. 2003. Phylogeography of *Biomphalaria glabrata* and *B. pfeifferi*, important intermediate hosts of *Schistosoma mansoni* in the New and Old World tropics. Molecular Ecology **12:** 3041–3056.

Esch, G. W., and J. C. Fernandez. 1994. Snail-trematode interactions and parasite community dynamics in aquatic systems: A review. American Midland Naturalist **131:** 209–237.

Frandsen, F. 1979. Studies of the relationship between *Schistosoma* and their intermediate hosts. III. The genus *Biomphalaria* and *Schistosoma mansoni* from Egypt, Kenya, Sudan, Uganda, West Indies (St. Lucia) and Zaire (two different strains, Katanga and Kinshasa). Journal of Helminthology **53:** 321–348.

Gibson, A. K., J. Y. Xu, and C. M. Lively. 2016. Within-population covariation between sexual reproduction and susceptibility to local parasites. Evolution **70:** 2049–2060.

Greenwood, B., and T. Mutabingwa. 2002. Malaria in 2002. Nature **415:** 670–672.

Howard, R. S., and C. M. Lively. 1994. Parasitism, mutation accumulation and the maintenance of sex. Nature **367:** 554–557.

Ibikounlé, M. G., R. Mouahid, M. Nguéma, N. G. Sakiti, D. Kindé-Gasard, A. Massougbodji, and H. Moné. 2012. Life-history traits indicate local adaptation of the schistosome parasite, *Schistosoma mansoni*, to its snail host, *Biomphalaria pfeifferi*. Experimental Parasitology **132:** 501–507.

JARNE, P., AND A. THERON. 2001. Genetic structure in natural populations of flukes and snails: A practical approach and review. Parasitology **123:** 27–40.

KING, K. C., L. F. DELPH, J. JOKELA, AND C. M. LIVELY. 2009. The geographic mosaic of sex and the Red Queen. Current Biology **15:** 1438–1441.

KING, K. C., J. JOKELA, AND C. M. LIVELY. 2011. Parasites, sex, and clonal diversity in natural snail populations. Evolution **65:** 1474–1481.

KOSKELLA, B., D. VERGARA, AND, C. M. LIVELY. 2011. Experimental evolution of sexual host populations in response to parasites. Evolutionary Ecology Research **13:** 315–322.

LAFFERTY, K. D., AND A. M. KURIS. 2009. Parasitic castration: The evolution and ecology of body snatchers. Trends in Parasitology **25:** 564–572.

LAIDEMITT, M. R., E. T. ZAWADZKI, S. V BRANT, M. W. MUTUKU, G. M. MKOJI, AND E. S. LOKER. 2016. Loads of trematodes: Discovering hidden diversity of paramphistomoids in Kenyan ruminants. Parasitology **144:** 131–147.

LELO, A. E., D. N. MBURU, G. N. MAGOMA, B. N. MUNGAI, J. H. KIHARA, I. N. MWANGI, G. M. MAINA, J. M. KINUTHIA, M. W. MUTUKU, E. S. LOKER, ET AL. 2014. No apparent reduction in schistosome burden or genetic diversity following four years of school-based mass drug administration in Mwea, central Kenya, a heavy transmission area. PLoS Neglected Tropical Diseases **8:** e3221. https://doi.org/10.1371/journal.ptnd.0003221.

LOKER, E. S., B. V. HOFKIN, G. M. MKOJI, B. N. MUNGAI, J. KIHARA, AND D. K. KOECH. 1993. Distributions of freshwater snails in southern Kenya with implications for the biological control of schistosomiasis and other snail-mediated parasites. Journal of Medicine and Applied Malacology **5:** 1–20.

LOKER, E. S., H. G. MOYO, AND S. L. GARDNER. 1981. Trematode–gastropod associations in nine non-lacustrine habitats in the Mwanza region of Tanzania. Parasitology **83:** 381–399.

LU, L., S. M. ZHANG, M. W. MUTUKU, G. M. MKOJI, AND E. S. LOKER. 2016. Relative compatibility of *Schistosoma mansoni* with *Biomphalaria sudanica* and *B. pfeifferi* from Kenya as assessed by PCR amplification of the *S. mansoni* ND5 gene in conjunction with traditional methods. Parasites & Vectors **9:** 166. https://doi.org/10.1186/s13071-016-1457-x.

MOHAMMED, N. A., H. MADSEN, AND A. A. AHMED. 2016. Types of trematodes infecting freshwater snails found in irrigation canals in the East Nile locality, Khartoum, Sudan. Infectious Diseases of Poverty **5:** 16. https://doi.org/10.1186/s40249-016-0108-y.

MUTUKU, M. W., C. K. DWENI, M. MWANGI, J. M. KINUTHIA, I. N. MWANGI, G. M. MAINA, L. E. AGOLA, S. M. ZHANG, R. MARANGA, E. S. LOKER, ET AL. 2014. Field-derived *Schistosoma mansoni* and *Biomphalaria pfeifferi* in Kenya: A compatible association characterized by lack of strong local adaptation, and presence of some snails able to persistently produce cercariae for over a year. Parasites & Vectors **7:** 533. https://doi.org/10.1186/s13071-014-0533-3.

NJENGA, S. M., F. M. MUTUNGI, C. N. WAMAE, M. T. MWANJE, K. KEVIN, K. K. NJIRU, AND M. J. BOCKARIE. 2014. Once a year school-based deworming with praziquantel and albendazole combination may not be adequate for control of urogenital schistosomiasis and hookworm infection in Matuga District, Kwale County, Kenya. Parasites & Vectors **7:** 74. https://doi.org/10.1186/1756-3305-7-74.

SAN MARTÍN, J. L., O. BRATHWAITE., B. ZAMBRANO, J. O. SOLÓRZANO, A. BOUCKENOOGHE, G. H. DAYAN, AND M. G. GUZMÁN. 2010. The epidemiology of dengue in the Americas over the last three decades: A worrisome reality. American Journal of Tropical Medicine and Hygiene **82:** 128–135.

SHELDON, B. C., AND S. VERHULST. 1996. Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology & Evolution **11:** 317–321.

SINGH, N. D., D. R. CRISCOE, AND S. SKOLFIELD. 2015. Fruit flies diversify their offspring in response to parasite infection. Science **349:** 747–750.

SMITH, D. H., J. PEPIN, AND A. H. STICH. 1998. Human African trypanosomiasis: An emerging public health crisis. British Medical Bulletin **54:** 341–355.

SOKOLOW, H. S, C. L. WOOD, I. J. JONES, S. J. SWARTZ, M. LOPEZ, M. H. HSIEH, K. D. LAFFERTY, A. M. KURIS, C. RICKARDS, AND G. A. DE LEO. 2016. Global assessment of schistosomiasis control over the past century shows targeting the snail intermediate host works best. PLOS Neglected Tropical Diseases **10:** e0004794. https://doi.org/10.1371/journal.pntd.0004794.

SOUTHGATE, V. R., L. A. TCHUENTÉ, A. THÉRON, J. JOURDANE, A. LY, C. MONCRIEFF, AND B. GRYSEELS. 2000. Compatibility of *Schistosoma mansoni* Cameroon and *Biomphalaria pfeifferi* Senegal. Parasitology **121:** 501–505.

SPATZ, L., S. M. CAPPA, AND M. O. DE NUNEZ. 2012. Susceptibility of wild populations of *Biomphalaria spp*. from neotropical South America to *Schistosoma mansoni* and interference of *Zygocotyle lunata*. Journal of Parasitology **98:** 1291–1295.

STANDLEY, C. J., S. L. GOODACRE, C. M. WADE, AND J. R. STOTHARD. 2014. The population genetic structure of *Biomphalaria choanomphala* in Lake Victoria, East Africa: Implications for schistosomiasis transmission. Parasites & Vectors **7:** 524. https://doi.org/10.1186/s13071-014-0524-4.

STANDLEY, C. J., C. M. WADE, AND J. R. STOTHARD. 2011. A fresh insight into the transmission of schistosomiasis: A misleading tale of *Biomphalaria* in Lake Victoria. PLoS ONE **6:** e26563. https://doi.org/10.1371/journal.pone.0026563.

STEINAUER, M. L., B. HANELT, L. E. AGOLA, G. M. MKOJI, AND E. S. LOKER. 2009. Genetic structure of *Schstosoma mansoni* in western Kenya: The effects of geography and host sharing. International Journal for Parasitology **39:**1353–1362.

VERGARA, D., J. JOKELA, AND C. M. LIVELY, 2014. Infection dynamics in coexisting sexual and asexual host populations: Support for the Red Queen hypothesis. American Naturalist **184:** 22–30.

WHO [WORLD HEALTH ORGANIZATION]. 2016. Vector-borne diseases. Available at: http://www.who.int/mediacentre/factsheets/fs387/en/. Accessed 24 September 2017.