Persistent hotspots of S*chistosoma mansoni* transmission in the Kenyan waters of Lake Victoria: a search for snail-related answers beneath the waves

Snails and hotspot transmission of *S. mansoni* in Lake Victoria

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Abstract

Background

Schistosoma mansoni transmission in Lake Victoria is often intense and hard to interrupt with praziquantel (PZQ) treatment alone. Following termination of a five-year annual treatment campaign, we sought to reveal underlying explanations by investigating snail-related aspects of transmission in six PZQ non-responsive villages that lie within a transmission hotspot located along the west-facing Kenyan shore of the lake, and in four PZQ-responding villages facing the more protected waters of Winam Gulf.

Methodology/Principal Findings

We examined on four occasions over a two-year period the relative abundance and *S. mansoni* prevalence for *Biomphalaria sudanica* along the lake shore and for *B. choanomphala* mostly from deeper waters, and acquisition of *S. mansoni* worms by sentinel mice, at 20 sampling sites (two per village). *Biomphalaria sudanica* was found at all sites and abundance did not differ significantly between hotspot and responding villages. By contrast, *B. choanomphala* was found at all six hotspot villages and only one responding village. Overall, more *B. sudanica* (83/10,249, 0.81%) were positive for *S. mansoni* than *B. choanomphala* (3/2,120, 0.14%). *S. mansoni* prevalence among *B. sudanica* in hotspot villages (60/5,346, 1.12%) was higher than in responding villages (23/4,903, 0.47%), and sentinel mice worm recoveries were also higher in hotspot villages, though variability among sampling times and locations precluded statistical significance in either case. The ratio of predatory echinostome to schistosome infections in snails was significantly higher at responding than hotspot sites.

Conclusions/Significance

All ten villages offer considerable opportunities for transmission because of abundant *Biomphalaria* populations. Hot spot villages had higher prevalence of snails positive for *S. mansoni* suggesting transmission trends higher than in responding villages. Although recovery of *B. choanomphala* including individuals infected with *S. mansoni* was relatively low, these snails offer a different, potentially underestimated, deeper water avenue for transmission largely unavailable in the responding villages.

Author summary

Schistosomiasis is a widespread disease of poverty that has been difficult to control despite mass drug administration (MDA) campaigns throughout affected regions. After treatment, people become rapidly reinfected because there is a reservoir of schistosome parasites in freshwater snail vectors. Our study investigated the role of snails in transmission of *S. mansoni* at 10 villages near Lake Victoria that were part of a recently-concluded MDA campaign. The villages were categorized by the campaign as “responding” if prevalence was substantially reduced over five years, or as “hotspots of transmission” if prevalence diminished only slightly or increased despite MDA. We measured relative abundance and *S. mansoni* prevalence in local snail populations, and infection rates of sentinel mice, at four sampling times over 13 months. Opportunities for transmission were high at all 10 villages, though hotspot villages yielded a higher prevalence of infected snails and higher worm recoveries from sentinel mice. Results were suggestive of higher transmission in hotspot villages though variability among locations and times precluded statistical significance. Hotspot sites also differed from responding sites in that the deep-water vector, *B. choanomphala*, was primarily present only at hotspots, and echinostomes (trematodes that prey on *S. mansoni* sporocysts in snails), were relatively fewer.

Introduction

One of the most prevalent and persistent of the world's neglected tropical diseases is human schistosomiasis, caused primarily by three members of the digenetic trematode genus *Schistosoma*, *S. mansoni* and *S. japonicum* being responsible for intestinal schistosomiasis and *S. haematobium* for urogenital schistosomiasis [1]. There is currently considerable momentum to bring schistosomiasis under control and to proceed to elimination efforts [2] that would eventually nullify its under-appreciated impact on public health [3, 4]. An essential weapon in the elimination of schistosomiasis is the drug praziquantel (PZQ) which has been used extensively in a variety of control programs that have had considerable impact in reducing prevalence and intensity of infection [5]. There is also a growing appreciation for the need for integrated control programs taking into account sanitation, provision of safe water, education, and acknowledging the fundamental role played by freshwater snails as vectors of the disease [6, 7]. Although snails are not vectors in a conventional sense, much of the success of schistosome parasites is owed to their molluscan hosts which support the prolific production of human-infective cercariae, and that often exist in huge populations across immense freshwater habitats such as Lake Victoria. Here we focus our attention on the relationship of *Biomphalaria* snails to PZQ-based control efforts for *S. mansoni* in and around the portion of Lake Victoria in Kenya.

Africa harbors 90% of the global burden of *S. mansoni* [8]. Across the continent, 12 species of snails in the genus *Biomphlaria* perpetuate transmission of this parasite. In Kenya, there are multiple vectors that appear to be adapted to distinctive habitats: 1) *B. pfeifferi*, a stream inhabitant whose distribution includes tributaries feeding Lake Victoria, canals in the Mwea irrigation scheme in central Kenya, and in small impoundments and both seasonal and perennial streams throughout the country, except in the tropical lowland belt along the coast; 2) *B. sudanica*, mainly found along shores of Lake Victoria and its surrounding swamps, and in Lake Jipe; and 3) *B. choanomphala*, a deeper water inhabitant of Lake Victoria [9, 10]. The latter two taxa are likely members of the same species [11, 12], and although they are

frequently referred to as ecophenotypes or ecomorphs, their distinctive conchological differences persist upon lab culture (Mutuku, personal observations). Which of the two formal names, *sudanica* or *choanomphala,* deserves precedence requires further study and molecular confirmation using specimens from the type localities (see discussion in [11]).

Prevalence of intestinal schistosomiasis in Kenya is highest (> 50%) in the Mwea irrigation scheme, central Kenya and in the Lake Victoria basin in western Kenya. Recent studies assessing the prevalence of *S. mansoni* infection in school children in the lake basin [13, 14] reported overall prevalence of 60.5% and 69%, respectively, with island areas having 2-fold higher prevalence rates than mainland villages. Schools closer to the lake had higher prevalence rates than those further from the lake. This would seem to be associated with more frequent contact with lake water containing infected snails, rendering such children more vulnerable to infection or re-infections after treatment.

Several chemotherapy-based initiatives have been undertaken to control schistosomiasis in this region. Among them, the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) project implemented school-based and village-wide annual mass drug administration (MDA) in varying treatment levels for 5 consecutive years (2011-2015) for 150 Kenyan villages situated on or near the eastern shore of Lake Victoria [15]. In schools that reported significant declines in prevalence, the initial prevalence ranged from 52-81%, and following treatment declined to 5.5-19%, and were termed “responding” sites (Table 1). However, in other schools, PZQ treatment resulted in smaller reductions in prevalence, starting from 60-91% before to 39-83% prevalence following treatment, with one school actually experiencing an increase in prevalence over the course of the study [15]. These sites were referred to as “non-responding” sites and generally fell within a persistent hotspot of infection identified by [15].

In the current study, building upon the results of Wiegand et al. [15], we investigated the hypothesis that the hotspot villages, relative to the responding villages, are adjacent to habitats containing more snails and more infected snails, resulting in greater numbers of infectious cercariae in the environment. These factors would contribute to persistently high prevalence in the face of control operations. During the two years immediately following the cessation of the SCORE-sponsored MDA project, on four separate occasions, we surveyed *Biomphalaria* populations and their schistosome infection rates adjacent to 10 villages with different degrees of responsiveness to treatment. We examined both shore-associated *B. sudanica* and *B. choanomphala* dredged from deeper waters. *B. pfeifferi* was not found in the habitats sampled. Additionally, we concurrently monitored the presence of viable *S. mansoni* cercariae by placing sentinel mice at water contact sites associated with each village and later perfused the mice for evidence of infection. Below we describe the results of looking beneath the waves to ascertain possible snail-related influences on the pattern of persistent hotspots noted for *S. mansoni* along the Kenyan shore of Lake Victoria.

Materials and methods

Study sites

The study involved ten villages along the shores of Lake Victoria, western Kenya. The villages had been subjected to annual school based mass drug administration (MDA) for 5 consecutive years. Six of the villages (Minya, Agok, Migiro, Miyandhe, Kanyibok, Usenge,) had persistent high prevalence of *S. mansoni* (designated hotspot villages) despite the MDA while four (Kotieno, Seka Dok, St. Douglas Weta, Mumbo) recorded a significant drop in infection prevalence (responding villages). In each village, two shoreline habitats were identified where there was evidence of human-water contact activities, and these were established as our

sampling sites. Each of the 20 sampling sites was visited at four times (April 2016, August 2016, January 2017 and May 2017).

Fig 1: Map showing position of study villages along the shore of Lake Victoria, Kenya. The map was constructed using QGIS version 2.18.22 and 3.2.1, the basemap layer was added from the standard terrain tiles provided from http://maps.stamen.com through the OpenLayers function. The tiles are © Stamen Design, under a creative commons attribution (CC BY 3.0) license. The data for the locations were obtained manually using cellular phone GPS functionality according to the WGS1984 mercator projection.

Table 1: *S. mansoni* prevalence in schools within the study villages showing response to praziquantel treatment following five years of Mass Drug Administration. Data acquired from and shown with permission of the SCORE project

SCHOOL	Prevalence (%) Year 1 (2011)	Prevalence (%) Year 2 (2012)	Prevalence (%) Year 3 (2013)	Prevalence (%) Year 4 (2014)	Prevalence (%) Year 5 (2015)
Hotspot villages					
MINYA	68.6	77	75	74	82
AGOK	60.5	59.8	60.2	67	39
MIGIRO	69.4	76.7	49.2	72	48
MIYANDHE	78.6	70.5	71.4	73.5	54
KANYIBOK	91.8	93	83.7	84	83
USENGE	69.4	68	65	46.5	65
Responding villages					
KOTIENO	52.9	40.9	19	16	12
SEKA DOK	81.6	73.2	55.8	48	19
ST.DOUGLAS WETA	55.2	27.4	24	19	19
MUMBO	57.7	30	11.5	15.6	5.5

Snail Sampling

Two methods were used to collect snails from each of the 20 designated sampling sites: scooping from the shore or dredging by boat. From the shore, two experienced collectors scooped snails for 15 minutes using long-handled scoops (stainless steel sieve with a mesh size of 2 × 2 mm, supported on an iron frame). Snails were collected offshore by passing a dredge (0.75 m long and 0.4 m wide with attached sieve, 2 × 2 mm mesh size) along the bottom. At each sampling site a series of dredge hauls covering a combined length of 150 meters was made. Material collected was sorted and live biomphalarid snails were retrieved. All snails were taken to the laboratory in KEMRI, Center for Global Health Research, Kisumu where they were sorted into species based on shell morphology characteristics, using standard taxonomic identification keys [16], counted and screened.

Snail screening

Each snail was placed in an individual well of a 24-well plastic culture plate containing 1 ml of aged de-chlorinated tap water. The plate was placed in indirect sunlight for 2 hours between 10:00-13:00hr. Individual wells were then examined with the aid of a dissecting microscope for presence of cercariae. Using standard identification keys [17], cercariae were identified to basic taxonomic groups, and have been further identified with molecular methods as parts of other studies [18]. The snails were then maintained in the laboratory as described by Mutuku et al., [19] for four weeks and screened again to enable detection of snails that harbored pre-patent infections at the time of collection.

Sentinel mice exposures

Mice were exposed to lake water at each near shore sampling site in floating cages that ensured the mice were exposed to a depth of no more than 5 mm. The cages were made from a plastic container measuring 20 X 14 X 7 cm with the bottom of the container replaced with wire mesh with openings measuring 5 x 5 mm. Styrofoam blocks were attached to the plastic

container to insure the cage floated at the desired position. Lids of the containers were perforated with 5 mm diameter holes to allow air circulation. The abdomen of each mouse was shaved to facilitate cercariae penetration. For each of the shoreline sampling habitats, five cages each with 2 mice were tethered adjacent to locations where snails were collected (total of 20 mice per village per sampling time). Mice were in contact with the water for 3 hours (11:00 hr – 14:00 hr), an interval when shedding of cercariae by *S. mansoni*-infected snails is expected to be at its peak (Steinauer et al., 2008)**.** Mice from each sampling site were then marked with an identifying ear tag and maintained together until perfused as described by Tamarath et al. [20] at eight weeks post exposure. Recovered worms were counted and tabulated with respect to village, sampling site and sampling time.

Ethics statement

All experiments involving mice were approved by institutional animal care and use committee (IACUC) at the Kenya Medical Research Institute (Protocol KEMRI/ACUC/03.10.15). All protocols and practices for the handling and manipulation of mice were in accordance with the guidelines of the American Veterinary Medical Association (AVMA) for humane treatment of laboratory animals.

Results

Overview of *B. sudanica* and *B. choanomphala* recovered from the 10 villages

A total of 12,369 snails (10,249 *B. sudanica* and 2,120 *B. choanomphala*) were collected from the study sites during four collection periods (Table 2, Fig 2). No *B. pfeifferi* were found in the lake habitats sampled. Snails of *B. sudanica* were recovered along the shoreline from all 20 sampling locations while those identified as *B. choanomphala* were dredged from deeper water at all sampling locations in hotspot villages. *B. choanomphala* was also found in the scooping samples from shallow water habitats in two hotspot locations, Kanyibok and Usenge. At Usenge

Beach, the shallow water *B. choanomphala* persisted for at least five months with populations of *B. sudanica* found nearby. *B. choanomphala* was also occasionally recovered from the shore intermingled with *B. sudanica* in the Anyanga beach sampling site at Kanyibok. Mumbo was the only responding village from which *B. choanomphala* was found (from deep water habitats only). Of note is that Mumbo is located closer to the hotspot villages than the remaining three responding villages (Fig 1). One hotspot village, Miyandhe, is located adjacent to a rocky shore with a steep drop-off in water depth, and thus yielded relatively few snails compared to the remaining nine villages (Table 2).

The number of snails collected varied widely among the sites and among time periods (Figs 2 and 3). The number of all *Biomphalaria* snails collected among the 20 sampling sites for the four sampling times was not significantly different between hotspot villages and responding villages (two-way repeated measures ANOVA; $F_{1,8}$ = 0.346, p = 0.5750); but the number of snails were significantly different across sampling time periods, with an increasing trend over time ($F_{3,24}$ = 64.235; p=0.0155) (Fig 3). As the majority of snails collected were *B. sudanica*, their numbers also did not differ between hotspot villages and responding villages ($F_{1,8}$=0.41, p=0.5399), and were also significantly different across sampling time periods ($F_{3,24}$ =4.971; p=0.008). However, as shown in Fig 4, the mean number of *B. choanomphala* (337.7) recovered from hotspot villages was 14 times higher than from responding villages (23.5) ($F_{1,8}$ =48.44, p=0.0001; time $F_{3,24}$ =0.845; p=0.4826). In fact, *B. choanomphala* was collected at each of the hotspot villages, but only one of the four responding villages.

Table 2: The 20 sampling sites and their GPS coordinates, total number of *B. sudanica* and *B. choanomphala* collected from each, and the number of each species positive for *S. mansoni*.

Village	Habitat	Latitude	Longitude	*Biomphalaria sudanica*	No. of snails (+) for *S. mansoni*	*Biomphalaria choanomphala*	No. of snails (+) for *S. mansoni*
Hotspot villages							
MINYA	Kochilo beach	$00^0$14'10.96"S	$034^0$14'45.79"E	522	0	96	0
	Luthi beach	$00^0$14'04.58"S	$034^0$13'58.74"E	758	1	282	0
AGOK	Gudwa beach	$00^0$21'26.52"S	$034^0$19'48.37"E	209	1	238	2
	Kogoye beach	$00^0$20'51.94"S	$034^0$20'74.32"E	220	6	85	0
MIGIRO	Uyawi beach	$00^0$12'18.58"S	$034^0$09'18.36"E	728	1	89	0
	Kadede beach	$00^0$12'12.09"S	$034^0$09'11.74"E	132	0	100	0
MIYANDHE	Kamumbo beach	$00^0$11'49.04"S	$034^0$10'28.17"E	48	0	228	0
	Daraja beach	$00^0$11'46.65"S	$034^0$09'44.11"E	103	0	71	0
KANYIBOK	Anyanga beach	$00^0$05'22.49"S	$034^0$05'09.34"E	897	28	380	1
	Ndiara beach	$00^0$05'27.33"S	$034^0$05'06.24"E	407	0	161	0
USENGE	Police beach	$00^0$04'34.80"S	$034^0$03'24.74"E	833	19	4	0
	Usenge beach	$00^0$04'21.49"S	$034^0$03'35.84"E	489	4	292	0
***B. sudanica* 60/5,346 (1.12%), *B. choanomphala* 3/2,026 (0.15%)**							
Responding villages							
KOTIENO	Kotieno beach	$00^0$21'09.07"S	$034^0$40'03.52"E	674	2	0	0
	Maguti beach	$00^0$21'17.57"S	$034^0$39'32.50"E	649	16	0	0
SEKA DOK	Seka beach	$00^0$21'18.58"S	$034^0$40'59.95"E	462	2	0	0
	Kagwa beach	$00^0$21'19"S	$034^0$40'52.12"E	834	1	0	0
ST. DOUGLAS WETA	Kobala beach	$00^0$21'05"S	$034^0$45'31.21"E	735	0	0	0
	Alara beach	$00^0$21'08"S	$034^0$45'16.01"E	584	0	0	0
MUMBO	Kabuong beach	$0^0$20'20.06"S	$034^0$21'04.09"E	576	1	93	0
	Mumbo beach	$0^0$20'0.99"S	$034^0$21'33.03"E	389	1	1	0
***B. sudanica* 23/4,903 (0.47%), *B. choanomphala* 0/94 (0%)**							

Fig 2: Variation in number of *B. sudanica* (blue bars) and *B. choanomphala* (black bars) collected at each of the four sampling times (April and August 2016, January and May 2017) from the 6 hotspot villages on the top versus the 4 responding villages on the bottom. Numbers over bars indicate number of *S. mansoni* shedders/total number of snails found. Numbers shown within mouse Figs represent the number of worms recovered from sentinel mice at that time point.

Fig 3: **Number of all *Biomphalaria* snails collected from the 20 sampling sites for the four collection times was not significantly different for hotspot villages (red bars) and responding villages (green bars), error bars indicate standard error.**

Fig 4: Mean number of *B. choanomphala* snails collected over the four times points was significantly different for hotspot villages (red bars) and responding villages (green bars), error bars indicate standard error.

Pearson correlation analysis indicated no significant relationship between the total abundance of *Biomphalaria* snails at a given site and infection prevalence of *S. mansoni* among school children at the villages (prevalence measured after 5 years of prior MDA) (Pearson's r=0.3861, p=0.1352) (Fig 5). However, it was noted that one of the hotspot sites, Miyandhe, with its rocky, wave-swept lakeshore habitat and low abundance of sampled snails, may be an outlier due to a small number of snails at the sampling habitats. Indeed, if this site is removed from the analysis, the correlation becomes significant (Pearson r=0.6337, p=0.0334). In this case, the correlation accounts for 40% of the variation in the data. Alternatively, the responding sites and hotspot sites appear to have very different linear relationships (Fig 5) and if only the hotspot sites are considered, the correlation accounts for 69% of the variation in the data, and Miyandhe appears to be much less of an outlier (Pearson r=0.8329, p=0.0198).

Fig 5 Relationship between the total number of *Biomphalaria* snails collected from habitats at each of ten villages and prevalence of *S. mansoni* in school children after 5 years of MDA. Red indicates hotspot villages and green indicates responding villages. The star indicates the village of Miyandhe, a potential outlier in the analysis.

Snails recovered with *S. mansoni* infections

The overall number of positive snails and prevalence for *S. mansoni* recovered among all 10 villages examined was higher for *B. sudanica* (83/10,249, 0.81%) than for *B.*

choanomphala (3/2,120, 0.14%). A Z-ratio test indicated prevalence was significantly higher in *B. sudanica* (p = 0.0007). The overall number and prevalence for *S. mansoni* among *B. sudanica* was also higher in hotspot villages (60/5,346, 1.12%) than from responding villages (23/4,903, 0.47%), with prevalence in hotspot villages being significantly higher (Z-ratio test p=0.0001) (Fig 2). However, when each site is considered separately, and time is a factor, there was no significant difference in the number of infected snails between hotspot villages and responding villages (2-way repeated measures ANOVA, $F_{(1,8)}$=0.582, p=0.4766). There was large variance among the prevalence of infected snails among both hotspot sites and responding villages (Figs 2 and 6). It is noteworthy that two of the responding villages with the highest residual post-treatment prevalences of *S. mansoni* in children, Kanyibok and Usenge, had the highest overall prevalence of positive snails (1.57% and 1.42% respectively (Table 2, Figs 2 and 6).

Fig 6: Prevalence of *S. mansoni* infections amongst *Biomphalaria* snails collected from hotspot villages (red bars) and responding villages (green bars).

Infection of *B. sudanica* with other digenetic trematodes

In all 10 villages, the number of trematode infections other than *S. mansoni* recovered from *B. sudanica* (Table 3) exceeded those of *S. mansoni* (totals of 241 and 83, respectively). In hotspot villages 60 of 184 trematode infections were of *S. mansoni* (32%), whereas in responding villages, 23 of 150 (15%) were of *S. mansoni*. Prevalence of trematode infections was similar between hotspot and responding villages (3.5 and 3.1%, respectively), Echinostomes were the most abundant non-*S. mansoni* infections with 112 snails infected from the ten study villages. Echinostomes are of particular interest because their intramolluscan stages ingest schistosome sporocysts, a characteristic which could aid in the control of schistosomiasis. Indeed, the ratio of echinostomes to schistosomes was significantly higher in responding sites vs. hotspot sites (T-test with Welch's correction for unequal variance, t=2.047, p=0.0396), consistent with the hypothesized role for echinostomes in control in schistosomiasis.

Overall, there were significant positive correlations between *S. mansoni* infected snails and echinostome infected snails (Pearson r = 0.6438, p = 0.0445) as well as between *S. mansoni* infected snails and amphistome infected snails (Pearson r = 0.9308, p < 0.0001). Relationships between *S. mansoni* and strigeids and also between *S. mansoni* and xiphidiocercariae were non-significant (strigeid Pearson r = 0.603, p = 0.0650; xiphidicercariae Pearson r = -0.1573, p = 0.6643).

Table 3: Number of *B. sudanica* snails collected from each village and number of snails infected with *S. mansoni* or other trematode cercariae. SM = *Schistosoma mansoni*, ECH = echinostomes, AMPH = amphistomes, STRIG = strigeids, XIPH = xiphidiocercariae, SM:ECH = ratio of *S. mansoni* to echinostomes.

VILLAGE	Total Snails	SM	ECH	AMPH	STRIG	XIPH	Total Trematodes	Trematode Prevalence	SM:EC
				Hotspot villages					
MINYA	1280	1	5	0	0	19	25	2.0	0.2
AGOK	429	7	4	0	1	7	19	4.4	1.7
MIGIRO	860	1	5	0	1	6	13	1.5	0.2
MIYANDHE	151	0	1	0	1	0	2	1.3	0.0
KANYIBOK	1304	28	22	7	4	1	62	4.8	1.2
USENGE	1322	23	10	6	12	14	65	4.9	2.3
TOTAL	5346	60	47	13	19	47	186	3.5	1.2
%		32	25	7	10	25			
				Responding villages					
KOTIENO	1323	18	29	7	17	2	73	5.5	0.6
SEKA DOK	1296	3	10	2	4	1	20	1.5	0.3
WETA	1319	0	13	1	6	15	35	2.7	0.0
MUMBO	965	2	13	1	2	4	22	2.3	0.1
TOTAL	4903	23	65	11	29	22	150	3.1	0.3
%		15	43	7	19	15			

Schistosomes recovered from sentinel mice

In general, adult schistosome recoveries from the sentinel mice were low (53 worms recovered, or 0.066 worms per mouse, or 0.022 worms/mouse/hour of exposure). The greatest number of worms acquired by a single mouse was 7. Of the total number of worms recovered, 40 (75.5%) were from hotspot villages (0.083 worms/mouse) while 13 (24.5%) were from responding villages (0.041 worms/mouse). The mean number of worms recovered per village was consistently higher in hotspot villages than in responding villages during the 4 sampling times, however, this difference was not statistically significant (2-way repeated measures ANOVA $F_{(1,8)}$ =0.5652, p=0.4737) (Fig 8).

The number of infected snails recovered per village was positively correlated with the number of worms recovered in sentinel mice (Fig 7) indicating that the sentinel mice exposures and snail screening give similar results in terms of measuring the force of infection in the water (Pearson r = 0.607, p=0.0326). However, this analysis indicated that at one site, there were positive sentinel mice with no infected snails and another site that showed the opposite, negative sentinel mice and infected snails. Therefore, both measures can underrepresent the force of infection. There was no significant correlation between the total number of snails (infected + non-infected) recovered per village and the number of worms recovered from the sentinel mice (Pearson r = 0.03413, p = 0.347) (Fig 7).

Fig 7: The relationship between sampling snails and sentinel mouse exposure for measuring the force of infection of schistosomes. A. There is a significant positive relationship between the number of infected snails and number of schistosomes recovered from sentinel mice. B. There was no significant relationship between the total number of snails at a site and the number of schistosomes recovered from sentinel mice. Red indicates hotspot villages and green indicates responding villages.

Fig 8: Mean number of worms recovered from sentinel mice from hotspot villages (red) and responding villages (green) during the four sampling times. Error bars indicate standard error.

Discussion

The SCORE-supported PZQ-based treatment program in and around Lake Victoria in western Kenya recorded significant overall progress in reducing the prevalence and intensity of *S. mansoni* transmission in the 150 villages included in the program [15]. A hotspot comprised of approximately a third of the villages was identified, especially as gauged by 9-12 year old students, who had a relative risk of infection that was nearly 4 times higher than for students outside the hotspot area. The SCORE program is by no means unique in identifying difficulties in reducing prevalence of infection in some villages adjacent to Lake Victoria [21]. The hotspot for *S. mansoni* transmission identified by Wiegand et al. [15] was in Siaya County, Bondo region, of western Kenya, associated with west-facing villages adjacent to the open, unprotected waters of the lake. In contrast, the PZQ responsive villages faced the more protected waters of Winam Gulf. Among the factors postulated by them to be associated with the hotspot of transmission were presence of superspreaders, larger snail populations or presence of different species of vector snails, and worms more refractory to PZQ.

As expected, *B. sudanica* was abundant along the shoreline in nearly all the sampling sites except in the hotspot village Miyandhe where the shoreline was rocky and wave-swept. By contrast, *B. choanomphala,* was most commonly dredged from waters offshore from the west-facing hotspot villages, and was on average 14 times more common in hotspot than responding villages. This species was found only in one responding village, Mumbo, lying close to the mouth of the gulf. *B. choanomphala* was recovered from shallow water in Usenge where it co-occurred with another typically deeper water gastropod species *Gabbiella humerosa*,

suggesting they had both been transported there, possibly by a storm. Previous studies have also shown *B. choanomphala* to be rare or absent within Winam Gulf [9], possibly because the bottom substrate is too muddy, whereas, the substrate found off the shore of the hotspot villages was more a combination of sand and mud, known to be favourable to *B. choanomphala* [22]. Gouvras et al. [23] found *B. choanomphala* to be focally distributed in the Tanzanian waters of the lake and interestingly noted, as did we, a general trend for this species to be more common in western than eastern locations. They considered a possible gradual change in substratum conditions from west to east might be responsible. In contrast to our results for Kenya, Gouvras et al. [23] did note high *B. choanomphala* populations in some more protected inlets and, in general, found more *B. choanomphala* positive for *S. mansoni* than we did: 12.2% of all *S. mansoni* shedders were *B. choanomphala* in their study vs. only 3.5% in our study. In both studies, *B. sudanica* was identified as the major vector for *S. mansoni* in the lake. Many other factors like levels of dissolved oxygen, pollution and seasonal effects like rainfall may also influence *B. choanomphala* populations [23, 24]. There was no indication that *B. pfeifferi* played a role in *S. mansoni* transmission in any of the study villages, as no snails of this species were found in the streams or small impoundments within the study localities. Overall, abundance of all *Biomphalaria* snails did not differ between hotspot and responding villages in spite of the disparities noted for *B. choanomphala*.

With respect to snails positive for *S. mansoni*, the overall prevalence of *S. mansoni* infections in all the *Biomphalaria* snails collected was almost 2-fold higher for the hotspot villages (63/7,732, 0.81%) than for the responding villages (23/4,997, 0.46%), with most of all *S. mansoni* infections coming from *B. sudanica* (96.5%). It was similarly observed that the mean number of worms recovered per sentinel mouse was also 2-fold higher in hotspot (0.0833) as compared to responding villages (0.0406). Although relatively few worms were recovered from the mice so caution is warranted, they do provide a second independent suggestion that the

force of infection from snail to mammal is higher in the hotspot villages. A consequence of this is that the rate of re-infections would be higher in the hotspot villages than in the responding villages, having the ultimate effect of re-establishing a high prevalence rate by one year post-treatment.

We by no means wish to suggest that the differences between hotspot and responding villages were always clear-cut. For instance, we recovered relatively few snails and no *S. mansoni* positive snails from Miyandhe, and only one positive snail each from Migiro or Minya, all hotspot villages. Gouvras et al. [23] also noted localities where human infection rates were high yet no positive snails were retrieved. Conversely, in one of four responding villages (Kotieno) no worms were recovered from sentinel mice, even though infected snails were found on multiple occasions. At St. Douglas Weta, worms were recovered from sentinel mice exposure even though no infected snails were ever collected from the habitats sampled. Once cercariae are shed from infected snails, they can swim or move away under the influence of water currents or waves, and come into contact with a susceptible vertebrate host located away from the shedding snail [25, 26].

Although *S. mansoni* infection rates were generally low among the *B. choanomphala* collected (0.14%), our dredge hauls across the extensive bottom surface of the lake can only be expected to retrieve a small proportion of the snails present and likely grossly underestimate the role of this snail species in perpetuating transmission. Given the vastness of the lake, it seems somewhat improbable that *any S. mansoni* miracidia would find *B. choanomphala* in deep water, especially given there are so many *B. sudanica* close to shore where most human feces would be deposited. At least two factors might facilitate infections in *B. choanomphala*: currents moving from offshore to deeper water, and fishermen who may defecate directly into deeper water from their boats. Another factor potentially favouring involvement of *B. choanomphala* in transmission is that experimental infection studies of Kenya-derived specimens suggest this

species, like *B. pfeifferi*, is inherently more susceptible to *S. mansoni* than *B. sudanica* [27]. With respect to issues of snail susceptibility, including for *B. sudanica*, given the nearly 2-fold higher prevalence of infection in the hotspot villages noted, we cannot rule out the possibility that *B. sudanica* from these localities are simply more susceptible to *S. mansoni* than snails from the responsive villages.

Not unexpectedly, and as reported from previous studies [28-30], *Biomphalaria* snails shedding other trematode cercariae were also frequently collected. Similar to Rowel et al. [28] who found 2.1% of all *Biomphalaria* snails from Ugandan waters of Lake Victoria to be trematode positive, we found an overall prevalence of 2.6% in our Kenyan study. Non-schistosome infections exceeded *S. mansoni* infections by a factor of 2.9 in our study. Rowel et al. [28] also noted excesses of non-schistosome trematodes in Lake Albert (5.33 fold) and Lake Victoria (6.17 fold). As an aside, numbers like this provide an interesting snapshot of the extent of *Biomphalaria* usage by trematodes of human vs. animal origin.

Included among the trematodes found from *B. sudanica* were strigeids, xiphidiocercariae, amphistomes and echinostomes that collectively number approximately 20 species [18]. Such infections are potentially of interest because larvae of other trematode species, particularly the rediae of echnostomes, can actively displace schistosome sporocysts in snails via predation, or may otherwise compete with and either pre-empt or delay cercariae production for *S. mansoni* should they co-occur in the same snail [31]. Consequently, snails infected with other trematode species pose hazards for *S. mansoni* and in many ways are effectively removed from the population of available hosts to infect. Although the preponderance of non-schistosome infections over *S. mansoni* infections was greater in the responding than hotspot villages, it is hard to ascribe a primary effect of these infections on limiting *S. mansoni* infections or contributing to disparities between responding and hotspot villages. This is because the overall prevalence of such infections is relatively low in the lake. There could be as yet poorly

understood secondary effects of their presence however, that bear consideration. One possibility is that some trematodes like echinostome produce cercariae that encyst in other snails as metacercariae. Metacercariae are very commonly recovered from lake-dwelling snails. It is possible that *S. mansoni* miracidia can somehow detect their presence and avoid such snails, further diminishing the pool of available snails to infect. Also, if a proportion of the snails in a population were refractory to all trematode infections, then the competitive interactions among all trematode species - including *S. mansoni* - for the remaining compatible snails might be more intense than currently estimated.

In conclusion, the results of the present study suggest that snail-related factors may be partly responsible for the differences observed in *S. mansoni* prevalence between the hotspot and responding villages. We estimate that the force of transmission in hotspot villages was nearly 2 times higher as compared to responding villages as determined by the mean number of worms recovered per sentinel mouse and number of schistosome-infected snails recovered from the same hotspot villages. However, caution is required as there is variability amongst villages in the magnitude of these effects, and numbers of positive snails recovered at different times were variable even within a village. We noted that *B. sudanica* was present in all the villages we studied and based on the number of positive snails of this species recovered we conclude it is also the major vector for *S. mansoni* in all villages. However *B. choanomphala* was much more frequently found near hotspot villages and in our view play an under-appreciated and more diffuse and difficult to measure role in transmission. Many other trematode species infect *Biomphalaria* snails in Lake Victoria but their overall prevalence is low suggested they do not have a dramatic primary effect on *S. mansoni* prevalence rates in snails, though secondary effects may be greater than appreciated. Control of *S. mansoni* in and around the lake will remain a daunting challenge and argues more persuasively than ever for

implementation of improved sanitation and provision of safe water supplies as both parts of an integrated control approach and as fundamental rights for the people living in the area.

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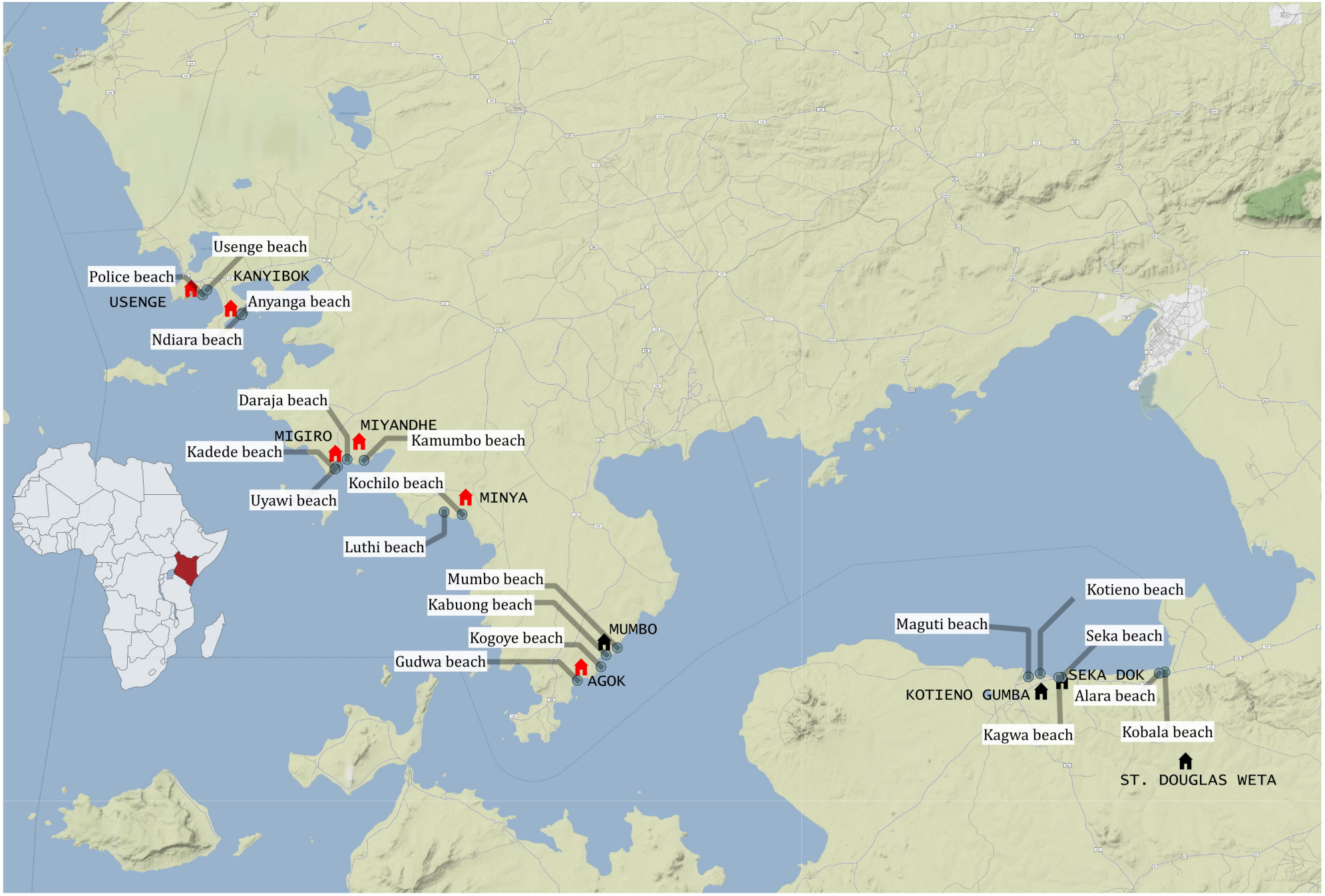


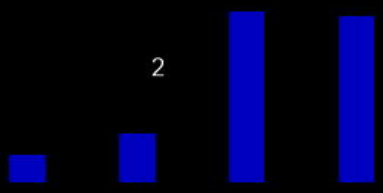
Non Responding

Responding

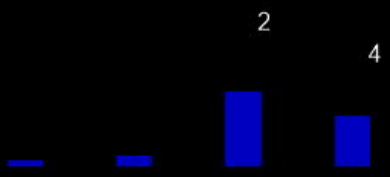
10 0 10 20 30 40 km

Hotspot Villages

Minya



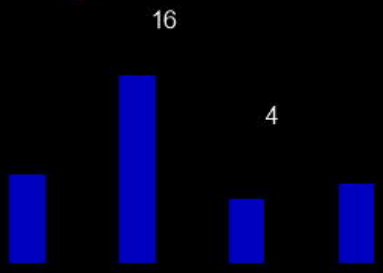
Agok



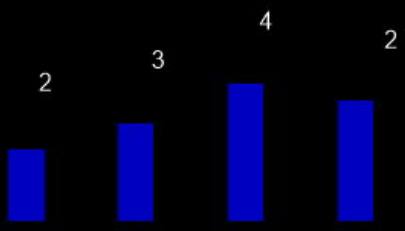
Migiro

Miyandhe

Kanyibok



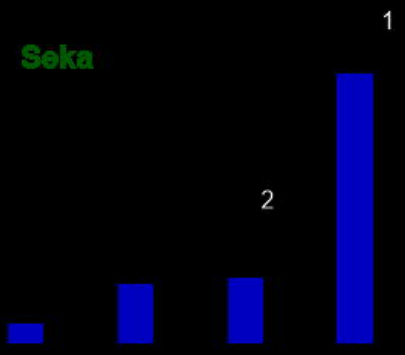
Usenge



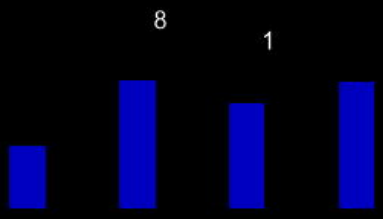
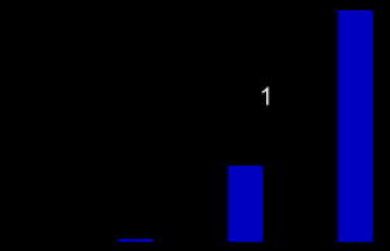
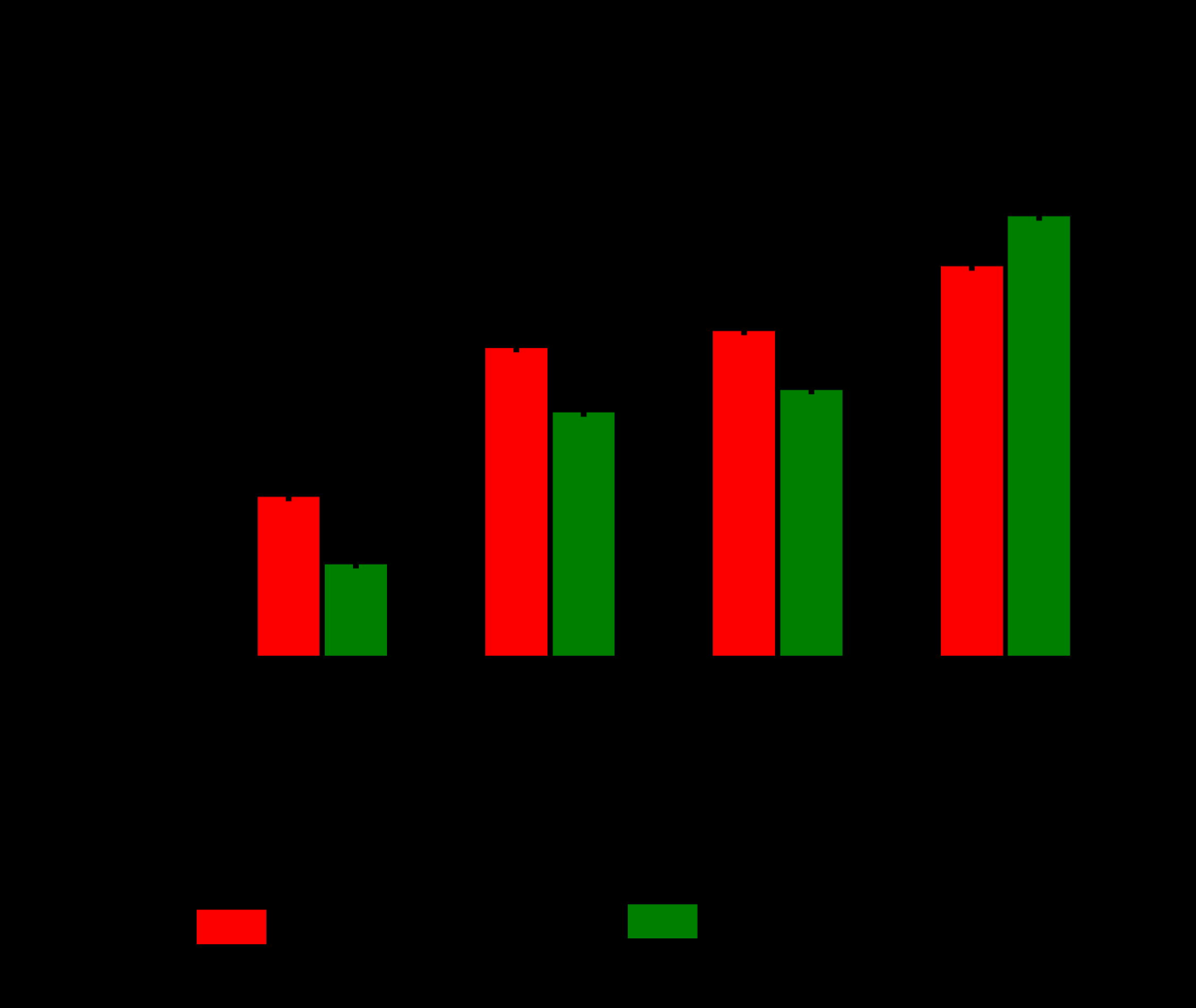
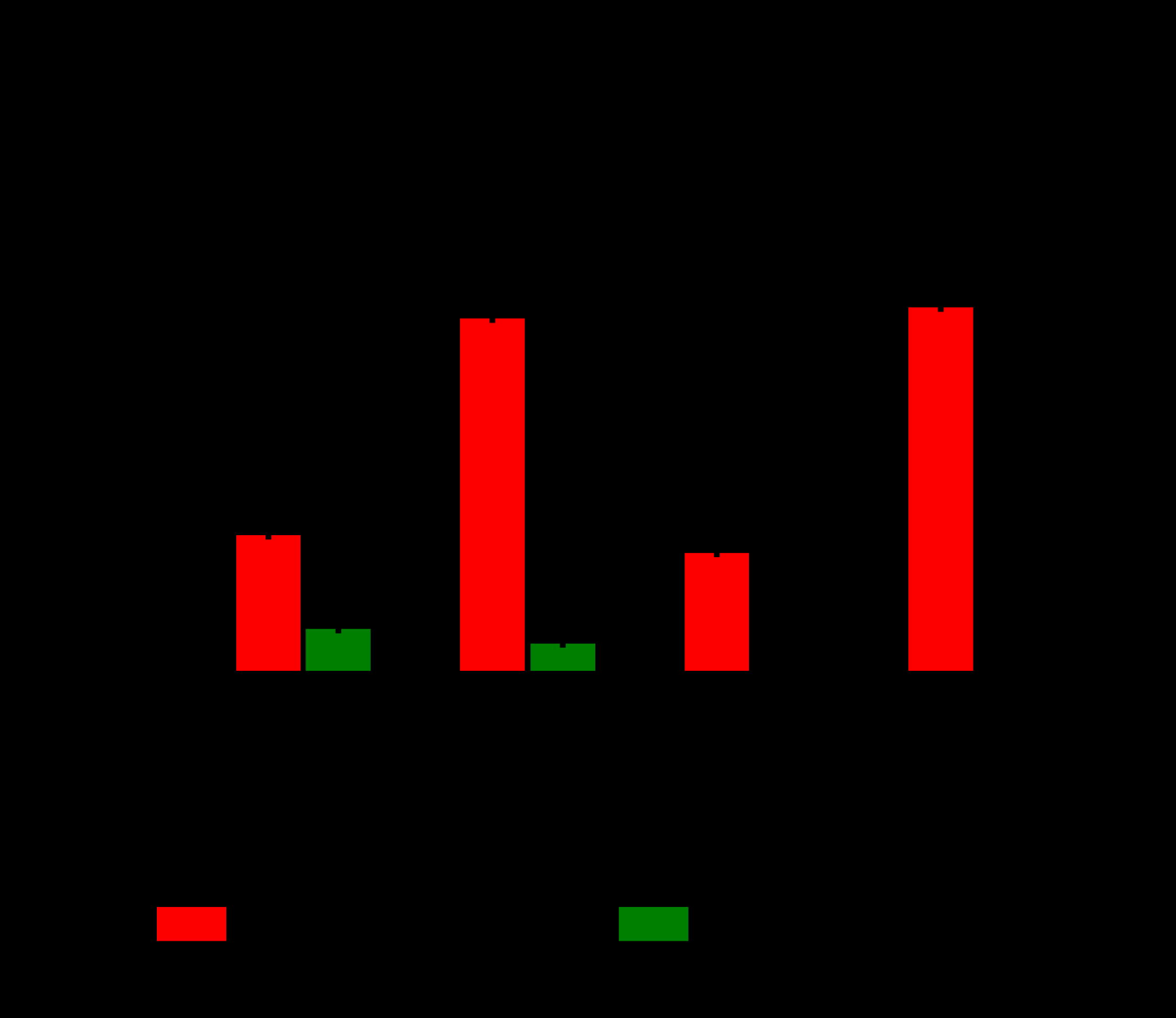
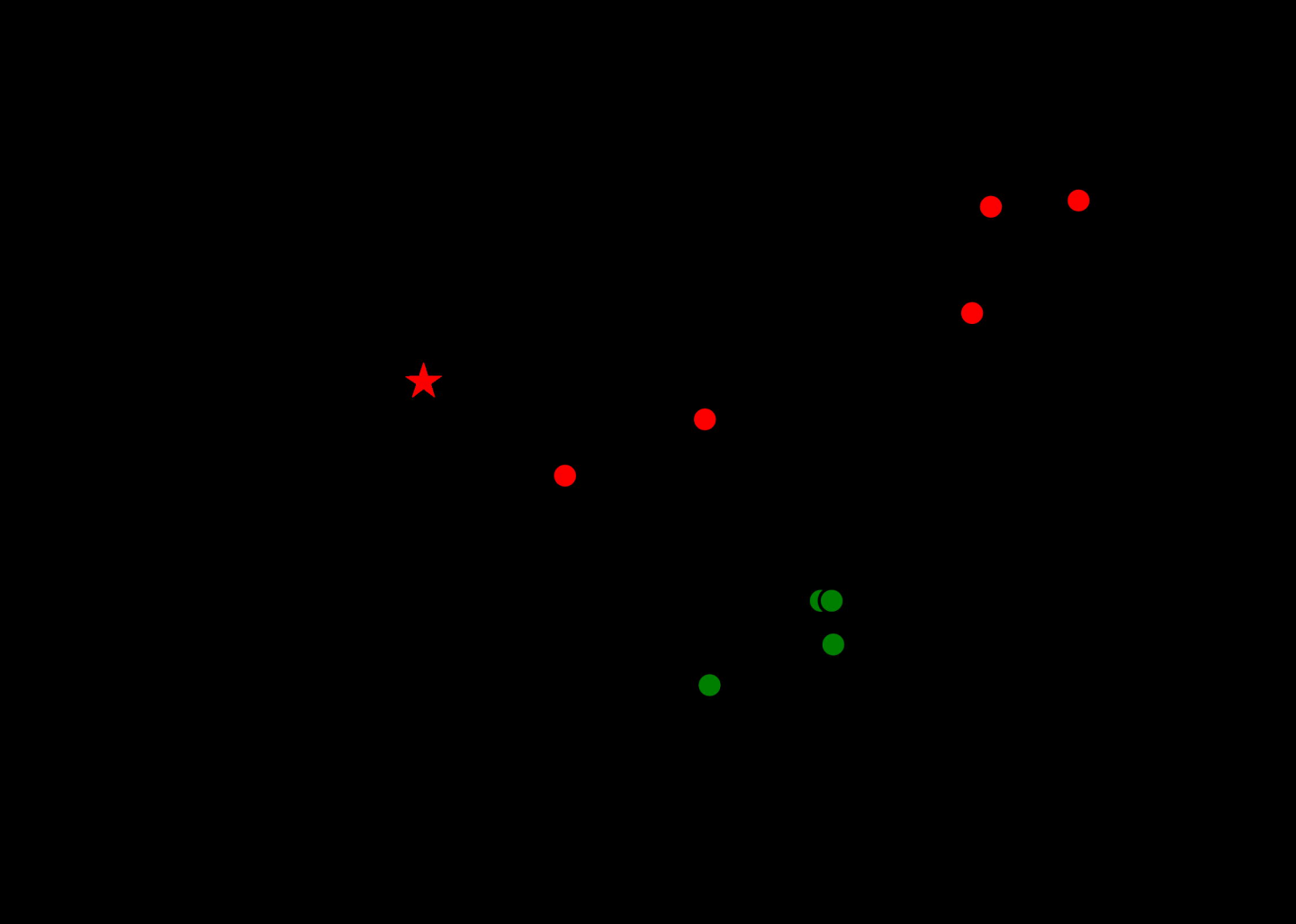
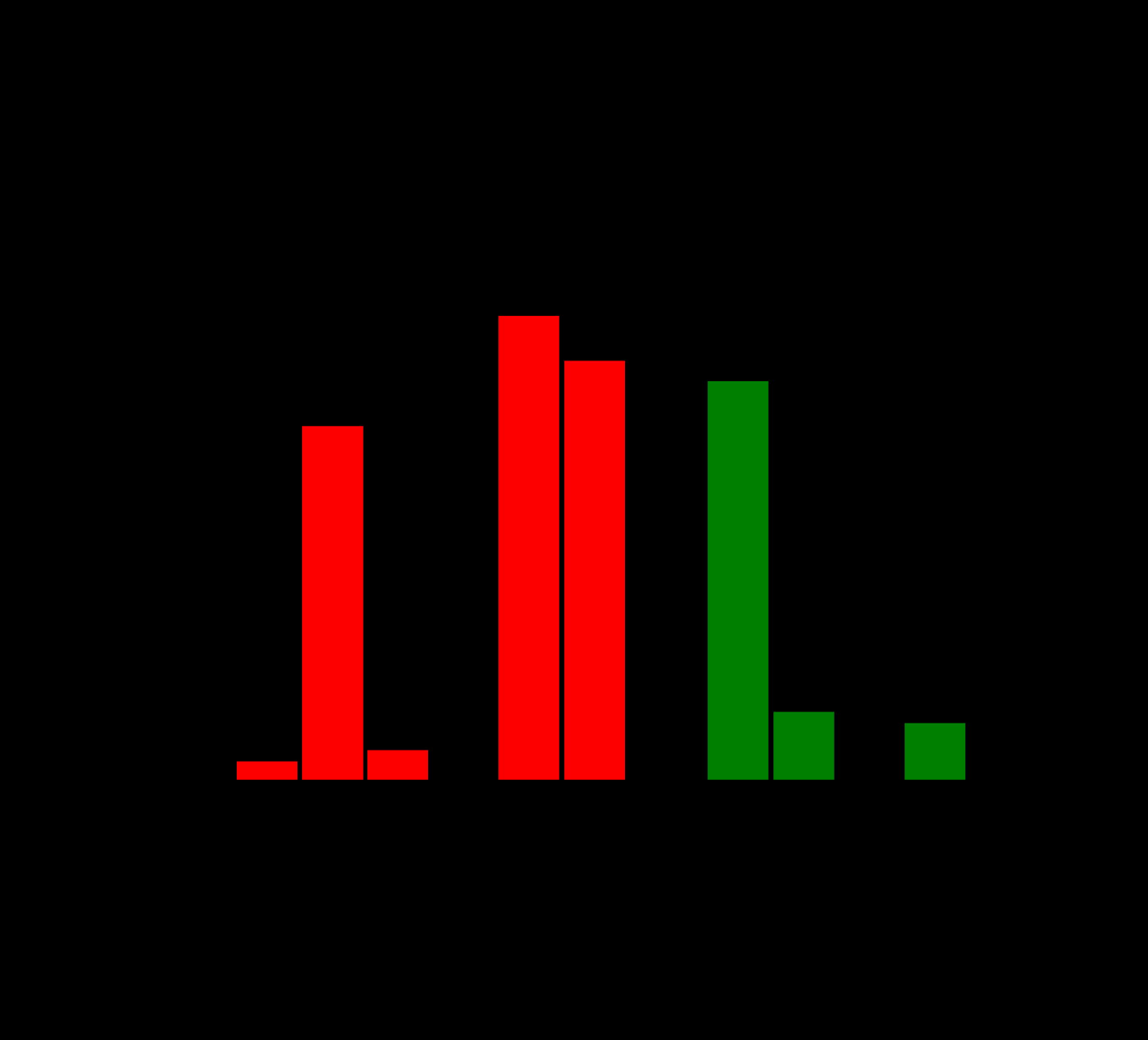
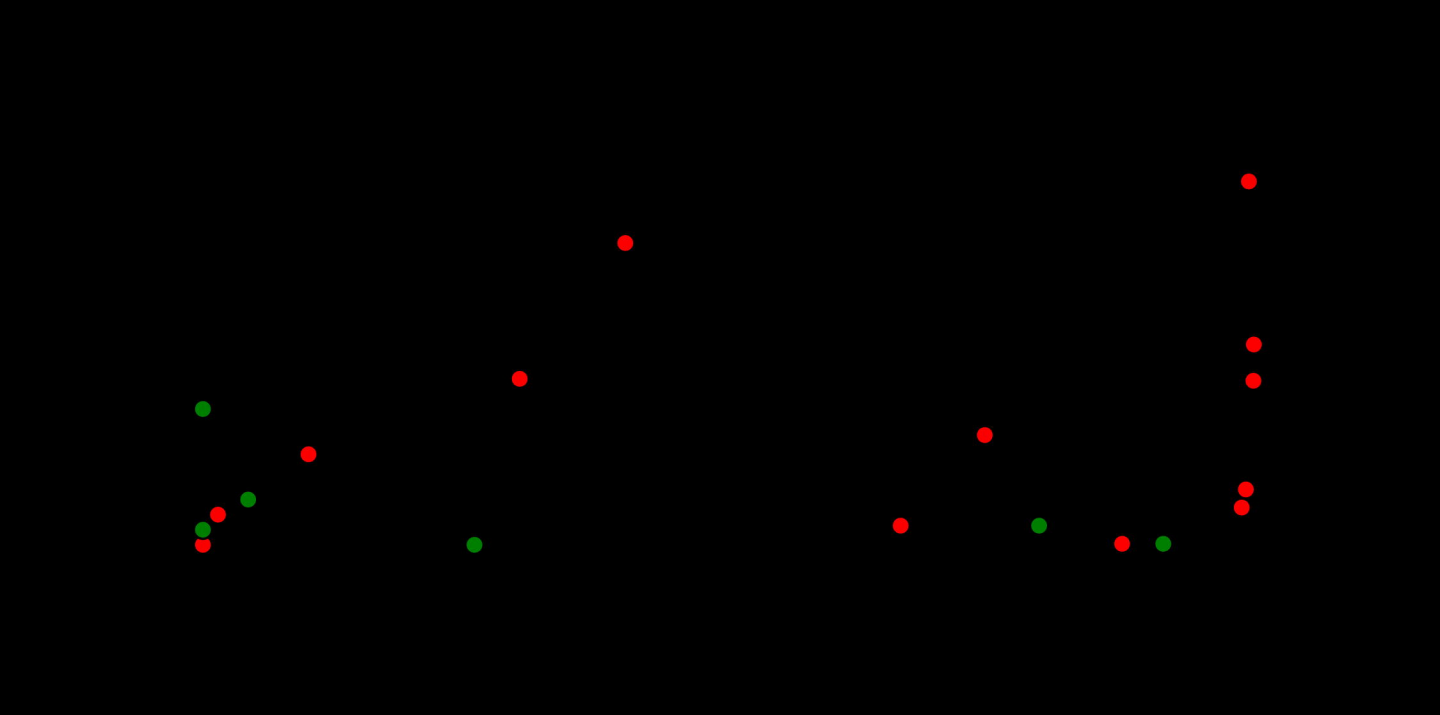
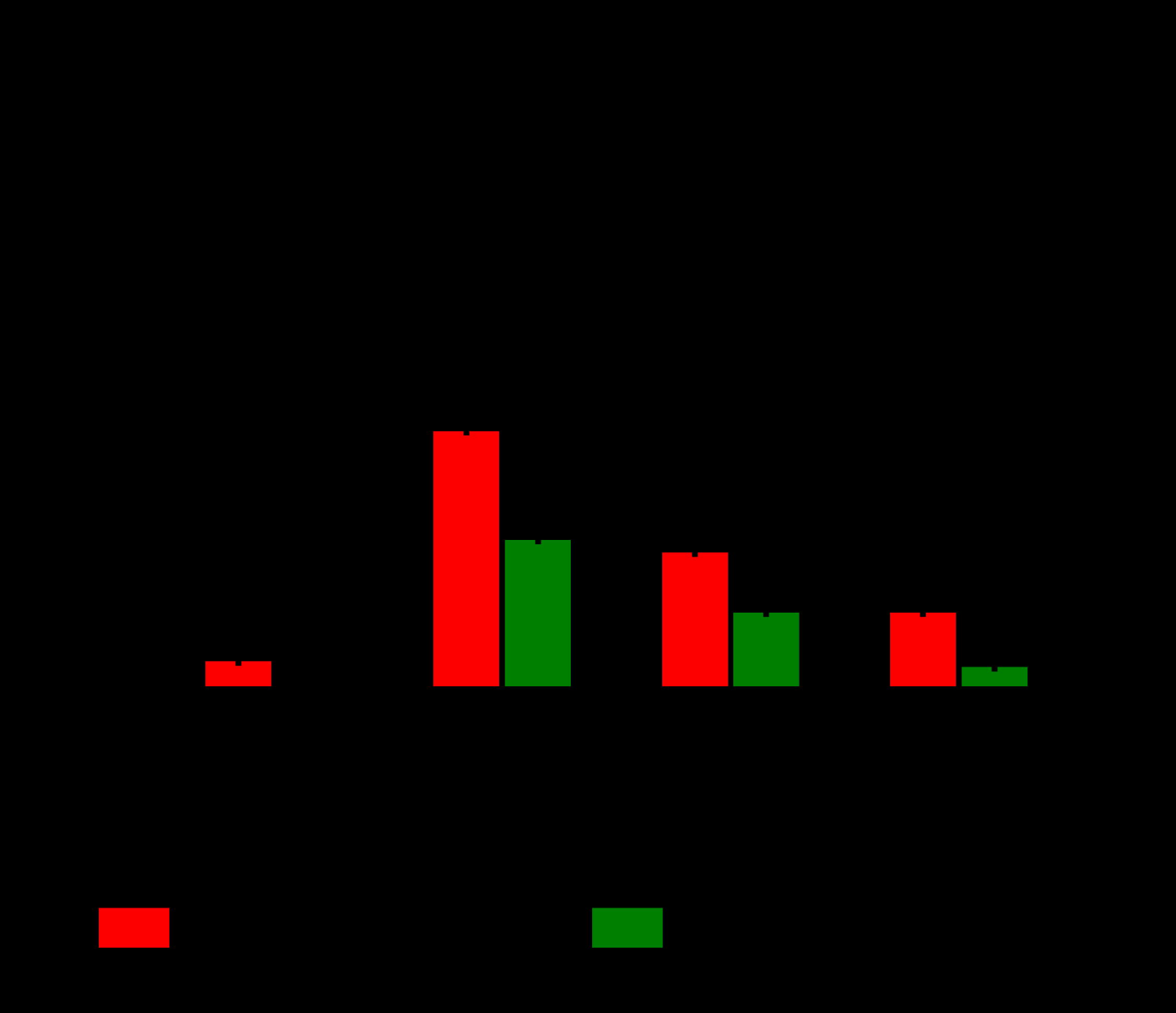
Responding Villages

Kotieno

Seka



Weta



Mumbo