Chapter 16
Time Makes You Older, Parasites Make You Bolder — Toxoplasma Gondii Infections Predict Hyena Boldness toward Definitive Lion Hosts

Eben Gering†, Zachary Laubach†, Patricia Weber, Gisela Hussey, Julie Turner, Kenna Lehmann, Tracy Montgomery, Kay Holekamp and Thomas Getty

Abstract There is growing interest in the alteration of host behaviors by parasites, yet crucial gaps remain in our understanding of its ecological and evolutionary significance. Here, we present the first evidence that the enhanced boldness of infected intermediate hosts of *Toxoplasma gondii* can increase their risk of mortality by the parasite's definitive feline hosts. In a long-term study of hyenas in Kenya's Masai Mara region, we found that 65% of hyenas were seropositive for *T. gondii* in ELISA IgG assays. Seropositive hyenas approached lions more closely than uninfected counterparts, and also showed longer latencies to approach a simulated conspecific territorial intruder. Lastly, although not significant, the ratio of mortalities caused by lions (vs. other sources) was higher for hyenas that were infected by *T. gondii*. These results accord with a long-standing hypothesis that the manipulation of host boldness and/or ailurophilia evolved to enhance disease transmission. Since hyenas are rarely consumed by lions, however, elevating their boldness toward lions may not be adaptive for *T. gondii*. Instead, it may reflect "collateral manipulation" that evolved to influence homologous mechanisms underlying behaviors of alternative hosts (e.g. rodents). This model is often invoked to explain *T. gondii's* many effects in humans, but is virtually unexplored in natural settings. For *T. gondii*, these effects could feasibly impact both behavior and fitness in a vast array, and significant proportion, of earth's mammals and birds. In addition to characterizing behavioral covariates of infection, we examined spatial and temporal patterns of *T. gondii* prevalence within the Mara landscape. Contrary to our predictions, disease prevalence did not differ 1) at a protected vs. disturbed locality, or 2) over three decades of increasing human activity within the disturbed locality.

Eben Gering, Zachary Laubach, Julie Turner, Kenna Lehmann, Tracy Montgomery, Kay Holekamp, and Thomas Getty
Department of Integrative Biology, and Ecology, Evolutionary Biology, and Behavior Program, Michigan State University, East Lansing, MI 48824, USA

Patricia Weber, Gisela Hussey
College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824, USA

† Equally contributing first authors

W. Banzhaf et al. (eds.), *Evolution in Action: Past, Present and Future*,
Genetic and Evolutionary Computation, https://doi.org/10.1007/978-3-030-39831-6_16

16.1 Introduction

Parasites often influence their hosts' behaviors, and these changes have important consequences for health and survival in wildlife, livestock, and humans. Behavioral changes can arise within infected hosts via numerous mechanisms including 1) effects of host resource depletion, e.g. lethargy, that are not under natural selection to benefit hosts or parasites. 2) host adaptations that reduce infection costs, e.g. grooming, behavioral fever, and self-medication [14]. 3) "host manipulation" that evolved in parasites to facilitate transmission from infected hosts (e.g. [25]). 4) "collateral manipulation" that evolved to manipulate a subset of hosts, but does not increase transmission form a focal, "collateral" host taxon. This last form of behavioral alteration is hypothesized to be a common byproduct of homologies between the hosts in which extended parasite phenotypes evolve, and alternative "collateral" hosts in which parasite-induced behaviors do not promote disease transmission. Of the four mechanisms described above, the first three are well-studied in the biomedical, ecological, and evolutionary literatures. Collateral manipulation, in contrast has attracted very little research interest apart from the many known effects of zoonotic parasites on humans [26]. Among the most important questions concerning collateral manipulation are 1) does it produce similar behavioral phenotypes in 'targeted' and collateral hosts? 2) how does it impact host fitness? 3) what environmental factors control its frequency and significance in nature?

Since the recent discovery of its life cycle in 1970 [6], *Toxoplasma gondii* has become an infamous example of putatively adaptive host manipulation by a parasite. In several studied hosts, including humans, *T. gondii* infections are associated with reduced aversion towards, or even affinities for, the odor of feline urine (e.g. [10, 31]). This "fatal attraction" to an indirect cue of feline presence is hypothesized to have evolved via natural selection on *T. gondii* to facilitate trophic (prey to predator) transmission from a subset of the parasite's non-definitive hosts (e.g. certain rodents), into definitive feline hosts. Only within these definitive feline hosts can *T. gondii* undergo sexual reproduction and produce recombinant, environmentally stable spores called oocysts [7].

Impressively, *T. gondii* can also produce other changes in many of its non-definitive hosts, including the enhancement of behavioral boldness. In rodents and other small mammals and birds, this boldness may further promote trophic (i.e. prey to predator) transmission of the parasite [34]. In humans, which are typically dead-end *T. gondii* hosts, enhancement of risky behavior appears to be non-adaptive for *T. gondii*. Instead, it is usually regarded as a by-product of homologies between human hosts and other mammals in which manipulation has evolved [7]. This view is supported by experimental evidence (esp. from rodents, see [30]) that shows several neural and hormonal processes controlling human behavior are also modified by *T. gondii* infection of other hosts. Correlations between human behavioral phenotypes and infection severity further support this view, altogether indicating *T. gondii* is likely causal of (vs. correlated with) changes in complex behaviors of human hosts. Again, however, we do not currently know if, or how, this type of collateral manipulation affects fitness-related behavior in non-human *T. gondii* hosts.

Among *T. gondii*'s many hosts worldwide, there is also surprisingly little evidence to link behavioral covariates of infection with risks of mortality by feline definitive hosts. This gap reflects the fact that infection-related behaviors, such as attraction to feline urine, have been chiefly investigated in laboratory models and/or in human subjects that are isolated from the risk of mortality by felines. More limited work with wild hosts, e.g. rats and chimpanzees, shows that *T. gondii* can modulate host responses to highly specific olfactory cues of local felines [4, 24]. While these results are highly suggestive of potential fitness costs of reduced ailurophobia (fear of cats), their connection with mortality risks have never been demonstrated in the wild ecosystems where *T. gondii* co-evolves with intermediate and definitive hosts. This is also the case for other infection-associated traits, such as increased host boldness and reduced host neophobia, that could similarly influence host mortality and/or fitness in nature [7]. The first aim of this study (Table 16.1) is therefore to test for relationships between fitness-related behavioral phenotypes and naturally-occurring *T. gondii* infections in wild non-human hosts.

Here, we test for behavioral consequences of *T. gondii* infection in a long lived and highly social host, the spotted hyena (*Crocuta crocuta*), within a natural setting in Kenya where intermediate hosts frequently interact with lions (*Panthera leo*). Several prior studies indicate lions are an important source of hyena mortality. In Namibia, for example, 71% of hyena mortalities were found to result from lions [29]. Lions were also the leading source of mortalities with known causes in an earlier study of Mara hyenas [33]. Despite these apparent costs, hyenas can also benefit from engaging with lions under some conditions - including interactions that function to defend territories, protect relatives, and/or steal food. Tension between benefits and costs of lion interactions may underlie a previous observation of stabilizing selection favoring hyenas with intermediate boldness in the presence of lions [36]. By focusing our study on hyenas, we can therefore characterize relationships between infection status and behaviors with established fitness consequences. Further, because hyenas are somewhat unlikely to transmit *T. gondii* to lions (see discussion), our study can also shed light on the ecological and evolutionary implications of collateral host manipulation.

In addition to examining behavioral covariates of infection, we also explored relationships between focal hyenas' social ranks and *T. gondii* serostatuses. In a subset of its hosts, *T. gondii* causes significant declines in overall condition [7]. If this were the case for hyenas, we would predict infection to be associated with lower social rank.

The second overarching aim of our study was to ascertain if, and how, environmental factors modulate the frequency of *T. gondii* infections in wild populations (Table 16.1). Earlier studies have shown this generalist parasite has long been both geographically and taxonomically widespread in warm blooded African vertebrates (e.g. [2, 27]). There are, however, several ways the continent's growing human population might further increase *T. gondii* prevalence and/or exacerbate its ecological consequences for African ecosystems. Agricultural communities, which are rapidly growing within our study area, represent a large potential reservoir for *T. gondii* due to the high density of vertebrate hosts (livestock, rodents, and humans) that can fa-

cilitate horizontal transmission from prey to predator [12]. Feral and domestic cats are commonly fed potentially infectious scraps of meat in African households to encourage rodent control [22]. Like lions and many other felids, housecats can serve as definitive hosts that propagate *T. gondii* by shedding oocysts into local water and soil (e.g. [20]). In light of these anthropogenic sources of infection, we predicted *T. gondii* prevalence would be positively correlated with human presence in the Mara landscape - both in space and in time.

Table 16.1: Predicted covariates of *T. gondii* infection in spotted hyenas of Kenya's Masai Mara region

H1: *T. gondii* infections alter hyena behavior behavior in a context-dependent manner (predictions 1 & 2), and also reduce overall host condition (prediction 3)
Prediction 1: *T. gondii*+hyenas approach lions more closely than uninfected hyenas Prediction 2: *T. gondii*+hyenas' behavior towards lions differs from their responses toward conspecifics Prediction 3: *T. gondii*+hyenas have lower social ranks than uninfected hyenas
H2: Human activities influence local *T. gondii* prevalence
Prediction 4: *T. gondii* prevalence is lowest within a protected wildlife area[1] Prediction 5: *T. gondii* prevalence is increasing over time in an area undergoing human development[2]
[1]Serena (Eastern side of Masai Mara Reserve) [2]Talek (Western side of Masai Mara Reserve near Talek town)

The results and discussion provided below are somewhat preliminary; they are based on our pilot studies of the data available when an accompanying Festschrift volume was being assembled. As noted in the addendum, we are now compiling and analyzing a much larger dataset of spotted hyena behaviors, life history data, and disease diagnostics. The results will be presented in a forthcoming manuscript.

16.2 Methods

16.2.1 Study Sites in Protected and Developing Regions of the Masai Mara National Reserve

This research uses data and samples from the Mara Hyena Project, a long-term field study of individually known spotted hyenas that have been continuously observed since 1988. Study hyenas are monitored daily and behavioral, demographic, and ecological data are systematically collected and entered into a database. Here, we used data from 4 different hyena groups, called clans, as well as historic in-

formation about ecological conditions in the Masai Mara National Reserve. Taking advantage of a preexisting natural experiment we compared *T. gondii* infection between hyenas living near high versus low human presence by exploiting differing ecological conditions that resulted from two management strategies in the reserve, and a growing human population along one of its eastern borders. More specifically, we classified hyenas from the Serena North, Serena South, and Happy Zebra clans as members of the Serena low human presence group because they live in the remote western side of the Reserve. Here, isolation from pastoralist villages and a strict ban on livestock grazing reduce hyenas' exposures to domestic animals and humans, which are known reservoirs for *T. gondii*. It should also be noted that darting at the Serena site was only recently incorporated into the Masai Mara Hyena project, so all samples from this low human presence location were sampled after 2012. On the eastern side of the reserve are pastoralist villages that have seen increased human population growth, especially around the burgeoning Talek community [16]. We classified hyenas from the Talek clan as experiencing high or low human presence based on livestock counts we conducted inside the reserve. These counts have shown a marked increase in the number of livestock within the reserve beginning in 2000, followed by another livestock increase between 2009 and 2013. These changes coincided with parallel shifts in hyena demography and wildlife community composition [13]. We therefore classified Talek hyenas sampled before 2000 as part of the Talek low human presence group, and after 2012 as part of the Talek high human presence group.

16.2.2 Collection of Demographic, Behavioral, and Biological Samples from Mara Hyenas

We maintained detailed records on the demographics of our study population, including sex, age, and the dates of key life-history milestones such as birth, weaning and death. Spotted hyenas live in matriarchal societies, each structured by a linear social hierarchy. We calculated each hyena's rank within the clan based on wins and losses in repeated agonistic interactions with other clan mates. Rank was updated annually to account for changes in clan demography, and we standardized it as a relative score between -1, the lowest-ranking individual in a clan, and 1, the highest-ranking individual, to allow rank comparisons between clans that vary in size [8].

Hyena and lion interactions occur because of fierce competition over food and considerable overlap in their prey resources [19]. Over the course of our study, we have documented 339 hyena and lion interactions involving individual hyenas for which we also obtained *T. gondii* diagnoses. Because our serodiagnostics were obtained only for the date individuals were darted (and plasma was taken), we cannot know infection statuses of hyenas at the time of each observed hyena-lion interaction. We controlled for this by filtering our data set to include only hyena-lion interactions in which a focal hyena's serostatus was known. For individuals testing

negative, we analyzed only observations that occurred prior to the date of plasma sampling, thus ensuring these represented behaviors of uninfected hyenas. We similarly restricted our analysis of hyenas that tested positive for *T. gondii* to observations that occurred after the date of plasma collection for *T. gondii* diagnosis. To correct for developmental changes in behavior toward lions that are independent of *T. gondii* infection status, these analyses were adjusted for individual hyena ages on the date(s) they were observed interacting with lions (see statistical analyses). For individual hyenas observed multiple times with lions (where serostatus was also known), we analyzed the average of both recorded behaviors (described below) and hyena age (in months) on the dates the behaviors were recorded.

As part of daily behavioral observations, anytime we saw hyena-lion interactions we recorded data about which hyenas were present, their behaviors, their approach distances towards lions [36], and their ranked distance among all hyenas in the observed hyena group. As with social rank, we standardized these ranked distances to permit meaningful comparisons among ranked distances of focal animals in groups of varying sizes. We also recorded information about the group sizes, ages, and sexes of the lions involved in observed hyena-lion interaction [13]. Finally, as part of our long-term data collection, we record sources of mortality whenever we find dead hyenas that we can identify. As part of our necropsy protocol we determine the cause of death when known. Here we were particularly interested in deaths due to lions. All other sources of mortality were binned into a second category (i.e. death by other causes) for analyses in this manuscript.

In addition to collecting passive observations, the Mara Hyena Project also conducts behavioral experiments in the field. In the pre-sent study, we analyzed responses from an earlier experiment in which focal subadult and adult individuals with known serodiagnostics ($n = 31$) were presented with a commercially-available model of a full-grown spotted hyena to simulate territorial intrusion by an unfamiliar individual. Intruding hyenas are a common threat that hyenas experience frequently as immigrant males attempt to join new clans. Simulated intrusions therefore permit us to assess a naturally-occurring risk-taking behavior that hyenas would experience in both high- and low-disturbance areas [18]. To simulate territory intrusion, we blocked a focal animal's view of the hyena model with a vehicle while deploying it in the field, then drove approximately 30m away and parked parallel to the simulated intruder. We then recorded behavioral responses with video recorders, commencing when mock intruders were first noticed by focal hyenas as determined by a startle reflect and accompanying pause in other activities. Trials ended when the focal individual either walked $>$ 50m from the model, or lay down for at least five minutes within 50m of the model. We terminated four sessions when focal hyenas attacked the simulated intruder, and a fifth session when other wildlife interfered with a focal animal during the trial. Further details and results from this study will be presented in a forthcoming paper. In the present study, we present data from focal individual's latency to approach the target, and also their minimum distance from the target throughout a given trial.

Finally, we routinely dart the study animals in order to collect biological samples and take morphological measurements. Here we immobilized hyenas using 6.5

mg/kg of tiletamine-zolazepam (Telazol®) in a pressurized dart that is fired from a CO_2 powered rifle. We then drew blood from the sedated hyena's jugular vein into ethylene-diaminetetraacetic acid (EDTA) coated vacuum tubes. After the hyena was secured in a safe place to recover from the anesthesia, we took the samples back to camp where a portion of the collected blood was spun in a centrifuge to separate red blood and white blood cells from plasma. Plasma was aliquoted into multiple cryogenic vials. Immediately, the blood derivatives, including plasma, were flash frozen in liquid nitrogen where they remained until they were delivered to a -80 °C freezer in the U.S.

16.2.3 ELISA Assays of Toxoplasma Gondii Exposure

We diagnosed individual hyenas as seropositive, seronegative, or seroambiguous using the ID-Vet multi-species Toxoplasma gondii ELISA kit. In previously published validation studies, IgG-based ELISAs have yielded concordant results to other diagnostic approach-es in both hyenas [32] and other mammals [1]. This kit contains wells that are precoated with *T. gondii*'s P-30 antigen. If present in tested plasma or serum, IgG anti-bodies to *T. gondii* P-30 bind to the wells. After rinsing the well, bound antibodies are further complexed by the kit conjugate, a cocktail of molecules that target IgGs from a wide array of mammals. These conjugated antibodies produce a color-changing reaction when exposed to the kit's substrate. After a 30-minute incubation, further reaction between the conjugate and substrate are blocked, and a plate read-er is used to measure the color intensity in each well. Colorimetric values are used to infer the presence of conjugated antibody, and to calculate SP ratios (described below) that are used to diagnose prior *T. gondii* exposure.

The SP ratio is the ratio of the colorimetric signal from a sample (S) divided by the positive control (P) after subtracting out the background signal for the plate (i.e. a negative control) from both S and P. The kit manufacturer's criteria for interpreting S/P are: $<$40% = negative result, 40%-50% = doubtful result, $>$50% = positive result. As described in the results section, only a small fraction of hyenas were seroambiguous, falling in the "doubtful" range of ELISA S/P values. We treated these individuals as negative in our analyses, but will also be confirming that excluding them would not have impacted our findings and conclusions using sensitivity analyses.

16.2.4 Quality Control of ELISA Testing

We used a well validated commercial kit (multi-species ID Screen® Toxoplasmosis Indirect, IDVET, Montpellier) for our ELISA assays. Duplicate assays of each individual hyena yielded highly consistent SP ratios and, consequently, a repeat-

able *T. gondii* diagnosis. Among all tested hyenas, the average pairwise differences in SP ratios (indicative of antibody-bound *T. gondii* antigen) was $0.039 + 0.039$sd. This minor, inter-replicate noise would be unlikely to impact categorical *T. gondii* diagnostics of individual hyenas, given the much larger ranges for the kit's positive ($S/P > 50.000$) and negative ($S/P < 40.000$) windows, and the distributions of our observed SP values (Figure 16.1). We also re-tested one focal individual a) on each of our five ELISA plates, and b) in a series of serial dilutions on a single plate. This individual (#653) produced similar SP ratios across all five plates (Table 16.2). In the dilution series, dilution volumes were also strongly predictive of observed SP ratios (results not shown). Altogether, these results suggest that the ELISA kit provided a repeatable and informative index of immunological reactivity to *T. gondii*'s P30 antigen.

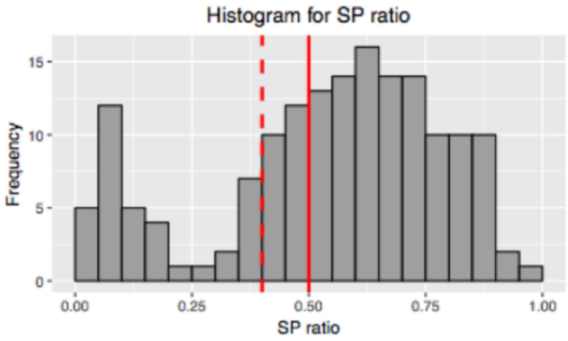


Fig. 16.1: Distribution of SP ratios (from ELISA assays) among spotted hyenas sampled in or near Kenya's Masai Mara National Reserve. The dashed red line is the upper SP ratio cutoff for seronegative and the solid red line is the lower cutoff for serpositive. In between the red lines corresponds to the SP ratio range for seroambiguous.

16.2.5 Statistical Analyses

We selected a subset of hyenas for this study from the Masai Mara Hyena project, with the objective of maximizing power to test our key predictions (Table 16.1). Our final data selection (Table 16.3) reflects an effort to balance representation of hyena age classes and sampling localities, while also selecting individuals for which behavioral observations were available. In our final data selection (Table 16.3), we had 33 samples collected during or after 2012 from Talek hyenas (exposed to high human presence), 21 samples from hyenas living in the remote Serena region of the Mara since 2012 (exposed to low human presence), and 118 samples which were collected from Talek hyenas prior to 2000 (also exposed to low human presence).

Table 16.2: Toxoplasma gondii ELISA plate control. One individual hyena (#653) was tested on all five ELISA plates, along with a positive control of porcine serum included by the test kit manufacturer. Both this individual (#653) and the manufacturer's positive control yielded consistent S/P ratios and, consequently, repeatable *T. gondii* diagnostics across all five plates.

plate_ID	Individual #653 replicate 1	Individual #653 replicate 2	+control replicate 1	+control replicate 2	S/P	Diagnosis
1	0.735	0.836	0.877	0.985	0.844	positive
2	0.864	0.679	1.122	1.062	0.707	positive
3	0.813	0.763	0.969	1.063	0.775	positive
4	0.891	0.864	1.013	0.996	0.874	positive
5-upper	0.907	0.839	1.093	1.087	0.801	positive
5-lower	0.877	0.879	1.093	1.087	0.806	positive

We assessed the background characteristics of this dataset including checking the distributions and reporting raw means and standard deviations for continuous variables (SP ratio, lifetime average minimum approach distance towards lions, lifetime average standardized rank, minimum approach distance toward lions during lion-hyena interactions, minimum approach distance towards a model hyena intruder, latency to approach to the minimum distance towards a model hyena intruder, and cause of mortality. For all nominal categorical variables, including infection status, sex, categorical age, standardized social rank, and human presence, we calculated within group sample sizes and frequency ratios (Table 16.3). Finally, we conducted bivariate analyses to assess for potential confounding variables.

Our formal analyses were carried out in two steps using simple and multiple variable linear regression when the outcome of interest was continuous, and logistic regression when we had a binary outcome. First, in unadjusted linear regression, we modeled the separate effects of standardized social rank, and human presence as potential determinants of toxoplasma ELISA SP ratios, a continuous variable that serves as a proxy for *T. gondii* antibody titers. In our adjusted models we controlled for the potential confounding effect of continuous age (in months). Associations from adjusted models were interpreted as a 1 unit change in SP ratio for each unit of change in standardized social rank or as the difference in SP ratio between each level of human presence. Using the same set of independent variables but replacing the outcome of SP ratio with our binary metric of toxoplasma serostatus (seropositive vs. nonseropositive), we used logistic regression to identify independent variables that predict the odds of infection. Here again, we first examined unadjusted models, then recalculated parameter estimates in adjusted models that controlled for age (in months). The estimates from logistic regression models were exponentiated to transform them from the log odds to the odds scale.

We also used linear regression to assess the effects of *T. gondii* exposure on hyena boldness behaviors obtained from observational and experimfental data. To do this, we fit two sets of models that used ELISA results as predictor variables, expressed categorically (seropositive vs. non-seropositive) vs. continuously (as SP ratios). For

Table 16.3: Background characteristics and *T. gondii* infection of 168 spotted hyenas from Masai Mara, Kenya

Measure of *T. gondii* infection	**%(N)[a] and Mean ± SD**	
Seroprevalence		
Uninfected	35% (58)	
Infected	65% (110)	
SP ratio (n = 168)	0.55 ± 0.26	
Population characteristics: Determination of *T. gondii*	**Uninfected**	**Infected**
Social Rank	17% (8)	83% (39)
Relative rank scale (-1:1)	0.20 ± 0.70	0.18 ± 0.71
Human Presence		
Talek (High; post 2012)	4% (7)	16% (26)
Talek (Low; pre 2000)	28% (47)	39 % (63)
Serena (Low; post 2012)	2% (4)	10% (17)
Population characteristics: Consequences *T. gondii*	**Uninfected**	**Infected**
Boldness towards lions	10% (8)	90% (69)
Lifetime average minimum approach distance (m)	77.81 ± 45.83	37.83 ± 40.78
Lifetime average standardized rank minimum distances	0.24 ± 0.14	0.33 ± 0.19
Boldness towards model hyena intruder	23% (7)	77% (24)
Minimum approach distance (m)	22.00 ± 18.88	32.80 ± 34.10
Latency to approach (min)	1.77 ± 0.67	4.58 ± 2.40
Mortality Source		
Other mortality sources	18% (6)	39% (13)
Lion mortalities	6% (2)	36% (12)

[a] Percentages and Ns may not add up to 100% and 168 individuals respectively, due to missing values.

hyena-lion observations in which interactions were limited to focal individuals with known serostatus (see methods), our response variables were: minimum approach distance to lions (averaged across observations when a hyena interacted with lions on multiple occasions) and standardized ranked distance to lions (similarly averaged when there were multiple measurements per hyena). In our adjusted models we controlled for the focal hyena's age in months at the time of darting because age may affect how hyenas behave towards lions. For the experimental dataset, we modelled focal individuals' minimum approach distance and latency to approach the simulated intruder. Again, because we found a significant age structure in our ELISA assays of *T. gondii* serostatus (see results), we refit each model to adjust for confounding effects of hyena ages (in months) on the date their tested plasma sample was collected.

Finally, we used a logistic regression model to compare the odds of mortality due to lions versus any other known cause between infected and uninfected hyenas. This model was underpowered due to small sample size, and was not adjusted for any confounding variables.

16.3 Results

16.3.1 T. gondii infection Is Common In Spotted Hyenas, And Increases With Age

110 of 168 hyenas (65%) tested positive for IgG antibodies to *T. gondii* by ELISA, indicating prior exposures to the parasite. 37 individuals (22%) tested negative, and 21 hyenas (13%) yielded inconclusive results within the "doubtful/uncertain" range of SP ratios (Figure 16.1). Bivariate analyses revealed that female and male hyenas did not differ in their seroprevalence (OR 0.76 [95% CI: 0.40, 1.46]; $\chi^2 = 0.67, df = 1, p = 0.41$). Hyena cubs had a signifcantly lower seroprevalence than subadults (OR 5.32 [95% CI: 2.18, 13.58) and adults (OR 7.70 [95% CI: 3.52, 17.69]; $\chi^2 = 27.0, df = 2, p < 0.0$).

16.3.2 Seropositive Hyenas Show More Boldness Towards Lions

There was a significant relationship between our *T. gondii* diagnostics and the distances within which hyenas approached lions over the course of their lifetimes. After filtering our observational dataset to include lion interactions where the focal hyena's serostatus was known, and also controlling for age at the time of diagnosis, we found that infected hyenas approached lions more closely (approaching 36.17 meters closer [95% CI: 0.17, 72.16]; t = -1.96, df = 62, p = 0.05). At the group level, we did not detect relationships between *T. gondii* diagnosis and ranked distances from lions (Table 16.4). Lastly, we observed that hyenas which are infected with *T. gondii* had a higher odds, 2.77 (95% CI: 0.52, 21.49); ^{2} = 1.3, df = 1, p = 0.26 of dying due to lions vs. other sources of mortality. This result was not significant, and also likely underpowered due to the small sample size.

Table 16.4: Associations of *T. gondii* infection with boldness behaviors towards lions and lion related mortality among 77 spotted hyenas.

		Lifetime average minimum approach distance (m) β (95% CI)		Lifetime average standardized rank minimum distances β (95% CI)			Lion versus other mortality causes
Measures of *T. gondii* inf.	**N**	**Unadjusted Models**	**Adjusted Models** [a]	**Unadjusted Models**	**Adjusted Models**[a]	**N**	**Mortality Sources OR (95% CI)**[b]
Seropreval.							
Uninfected	8	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	8	0.00 (Reference)
Infected	69	**-39.98 (-70.20, -9.77)**	**-37.17 (-72.16, -0.17)**	0.09 (-0.05, 0.23)	0.09 (-0.04, 0.22)	25	2.77 (0.52, 21.49)
P		**0.01**	**0.05**	0.19	0.18		0.26
SP ratio	77	-22.05 (-68.49, 24.40)	-9.33 (-65.63, 45.96)	0.06 (-0.14, 0.26)	0.08 (-0.12, 0.28)	33	3.84 (0.35, 58.98)
P		0.36	0.75	0.56	0.46		0.29

[a] Adjusted models are controlled for hyena age in months when diagnosed for *T. gondii*.
There may be fewer N in adjusted models due to missing data.
[b] Mortality was assessed as being caused by lions or other when the cause of death was not lions.

16.3.3 T. Gondii Seropositive Hyenas Are Less Responsive To Simulated Territorial Intruders

Seronegative hyenas approached a simulated hyena intruder approximately 1.6 times more rapidly (Table 16.5; 2.89 [95% CI: 0.91, 4.86]; t = 2.86, df = 23, p = 0.01). Both seropositive and seronegative individuals ultimately approached within the same minimum distance of the simulated intruder.

Table 16.5: Associations of *T. gondii* infection with boldness behaviors towards a model hyena intruder among 31 spotted hyenas.

Measures of *T. gondii* infection	N	Minimum approach distance (m) β (95%CI)		Latency to approach (min) β (95% CI)	
		Unadjusted Models	Adjusted Models[a]	Unadjusted Models	Adjusted Models[a]
Streovalence					
Uninfected	7	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Infected	24	10.80 (-15.77, 37.38)	3.73 (-25.01, 32.48)	**2.81 (0.99, 4.62)**	**2.89 (0.91, 4.86)**
P		0.43	0.80	0.01	0.01
SP ratio	77	14.37 (-49.67, 78.40)	-8.52 (-76.67, 59.62)	**6.88 (2.56, 11.19)**	**6.73 (2.01, 11.44)**
P		0.66	0.81	<0.01	0.01

[a] Adjusted models are controlled for hyena age in months when diagnosed for *T. gondii*.
There may be fewer N in adjusted models due to missing data.

16.3.4 T. Gondii Prevalence Is Not Predicted By Spatial Or Temporal Variation In Human Presence

Among 19 adult hyenas sampled from the protected Serena part of the reserve, 15 (78.9%) tested positive for *T. gondii*. Among 44 adult hyenas sampled in Talek before 2000, predating subsequent expansions of the local human population, 36 (81.8%) tested positive. Among adult hyenas sampled in Talek after 2012, 12 of 15 (80%) adults tested positive (Figure 16.2). While prevalence initially appeared to change over time at Talek, this effect was eliminated after adjustment for age at diagnosis. Thus, hyenas in our Talek low human presence sample (animals darted pre 2000) had similar odds of infection as compared to the Talek high human presence group (post 2012; see Table 1.5), and the difference between them was not significant. The Serena low human presence group (sampled post 2012) also did not differ from the low disturbance Talek sample, which was collected over the same (pre-2000) time interval (0.51 [95% CI: 0.08, 4.25]; $\chi^2 = 2.5, df = 1, p = 0.12$).

Our sampling design did not allow for meaningful assessments of *T. gondii* prevalence in subadults or cubs from Serena ($n = 0$ and $n = 2$, respectively), nor in cubs from Talek post-2012 ($n = 0$). Thus, while prevalence increases with age, we are presently unable to compare the age structure in prevalence among localities or

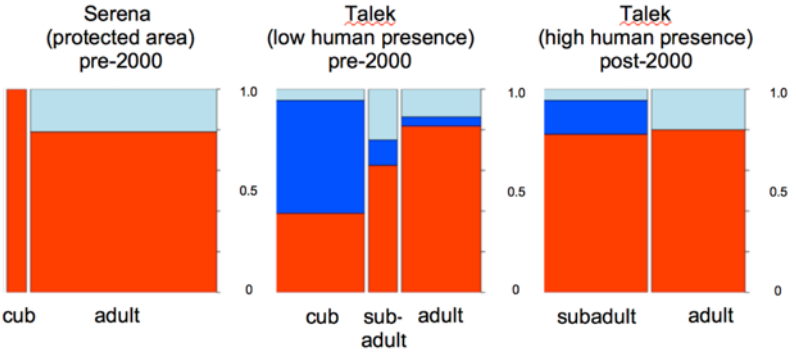


Fig. 16.2: Serodiagnostics for spotted hyenas from a protected area (Serena), and an area that has experienced increasing human disturbance since 2000 (Talek). Red indicates seropositive hyenas, blue indicates seronegative hyenas, and light blue indicates seroambigous "doubtful" diganoses. Serodiagnostics for spotted hyenas from a protected area (Serena), and an area that has experienced increasing human disturbance since 2000 (Talek). Red indicates seropositive hyenas, blue indicates seronegative hyenas, and light blue indicates seroambigous "doubtful" diganoses.

time strata using data from cubs. Among the subadults sampled from Talek, 10 of 16 sampled before 2000 were seropositive (62.5%), and 14 of 18 (77.8%) sampled after 2012 were seropositive. Across these time strata (and concomitant human density changes at Talek), the small difference in subadult seroprevalence did not approach statistical significance (z=-0.9759, p=0.33).

Table 16.6: Social and ecological determinants of *T. gondii* infection in 168 spotted hyenas.

		Unadjusted Models		**Adjusted Models**[c]	
	N	**β (95% CI) SP Ratio**[a]	**OR (95% CI) Seroprevalence**[b]	**β (95% CI) SP Ratio**[a]	**OR (95% CI) Seroprevalence**[b]
Determinants of *T. gondii* infection					
Social Rank					
Relative rank scale (-1:1)	47	0.00 (-0.07, 0.07)	0.96 (0.30, 2.84)	0.02 (-0.06, 0.10)	1.39 (0.34, 5.91)
P		0.93	0.94	0.84	0.64
Human Presence					
Talek (High; post 2012)	33	0.00 (Reference)	1.00 (Reference)	0.00 (Reference)	1.00 (Reference)
Talek(Low; pre 2000)	111	**-0.10 (-0.20, 0.00)**	**0.37 (0.14, 0.88)**	**-0.10 (-0.20, 0.00)**	0.45 (0.16, 1.19)
Serena (Low; post 2012)	21	0.02 (-0.12, 0.16)	1.14 (0.30, 4.92)	-0.01 (-0.19, 0.18)	0.51 (0.08, 4.25)
P		0.04	0.03	0.01	0.12

[a] Significance is based on type I sums of squares F-test or t-test.
[b] Significance is based on Wald Chi-squared test.
[c] Adjusted models are controlled for hyena age in months when diagnosed for *T. gondii*

16.4 Discussion

This is the first study to show that *T. gondii* infected hosts incur greater risks of mortality via felids in nature. As predicted (Table 16.3), infected hyenas approached lions more closely than their uninfected counterparts. This behavior is likely costly for hyenas given that lions readily attack and kill them (e.g. [18, 29]), and represent the primary source of hyena mortality in many populations [33]. A lion is roughly four times as large as a spotted hyena, and a single swipe from a lion's paw can maim or kill an adult hyena. As a definitive host for *T. gondii*, lions can also shed recombinant, environmentally stable *T. gondii* spores into the local environment. These "oocysts" can infect a multitude of warm-blooded host species, inclusive of hyenas, that inhabit the Masai Mara landscape. Nonetheless, hyenas are relatively unlikely to transmit *T. gondii* to lions because lions rarely consume hyenas after killing them [15]. Because this makes lions unlikely to ingest infectious hyena tissues, hyena boldness towards lions most likely reflects "collateral manipulation" by *T. gondii*. Here, we introduce this term to describe extended parasite phenotypes that evolved to manipulate a subset of hosts, but which are not under positive selection (i.e. do not enhance transmission) in a focal alternative host.

Effects of collateral manipulation on host behavior are virtually unexplored in wild hosts. There is, however, an array of indirect evidence of their ubiquity and significance in mammalian hosts of *T. gondii*. In humans, which are dead end hosts for the parasite, infections are correlated with changes to neural, hormonal, and behavioral phenotypes that are also seen in experimentally-infected rodent models despite these hosts being separated by $\sim$100 million years of evolutionary divergence [7]. In sea otters, encephalitis caused by *T. gondii* is also associated with elevated risks of shark predation [17]. It is unclear, however, if this stems from the manipulation of otter boldness, or merely declines in condition that impede shark evasion and/or force otters to engage in riskier foraging. For hyenas, however, declines in condition are unlikely to explain behavioral covariates of *T. gondii* infections. Infected hyenas have shown otherwise normal behavior in our extensive field observations. And unlike sea otters, which can also die from *T. gondii*-induced encephalitis, disease was rarely determined to be the cause of death in extensive necropsies of Mara hyenas. Given the high prevalence of *T. gondii* in Mara hyenas ($>$ 80% of tested adult hyenas), this suggests the parasite causes low morbidity for the focal population. As described below, we also failed to see any relationship between hyena ranks and infection statuses, which we predicted would occur if infections reduce overall condition (Table 16.3). Finally, a prior study of spotted hyenas found that latent *T. gondii* infections were common in a zoo population, yet did not involve any symptoms of ill health [32].

We also did not find a relationship between *T. gondii* serostatus and the ranked distance of individual hyenas within groups that we observed interacting with lions. This may reflect our inability to control for the serostatuses of other individuals within these groups, and/or additional factors that influence a focal individual's boldness within a group. Another limitation of the present study is that we could only obtain hyena serostatus on the date when individuals were tranquilized to col-

lect plasma samples. This limits the power of our behavioral comparisons, but also makes them conservative. Thus, effects of *T. gondii* on hyena behavior might be even more pronounced and/or multifaceted than those suggested by our present dataset. In the near future, we hope to pursue serial diagnostics of focal individuals to pinpoint sources, timing, and consequences of parasitic infections of wildlife in the Masai Mara National Reserve.

Because only 33 of our focal individuals have died of known causes at the time of this writing, we are currently unable to draw informative comparisons between mortality rates and/or causes in uninfected vs. infected hyenas. Nonetheless, we noted a non-significant pattern of higher mortality by lion (vs. other sources) in hyenas that were seropositive for *T. gondii*. This suggests it will be fruitful to diagnose a larger panel of individuals with known mortality causes. Meanwhile, given previously-established links between hyena boldness toward lions and fitness [36], the present study provides the clearest evidence to date that enhanced feline proximity can incur fitness costs for an intermediate *T. gondii* host.

As predicted (Table 16.3), and similar to findings from other hosts, *T. gondii*'s effects on hyena boldness are also context-dependent. In the absence of lions, we found that seropositive hyenas were slower to respond toward a simulated territorial intruder. In these trials, and in contrast to the pattern found for lion interactions, infected hyenas and uninfected hyenas did not differ in how closely they approached the behavioral stimulus (here, a model intruder).

Absent further studies, it is difficult to ascertain the reason(s) for the observed differences in approach latencies of infected vs. uninfected hyenas during simulated territorial intrusion. A simulated intruder would likely be regarded by focal animals as an unfamiliar individual. Longer response latencies might, therefore, reflect reduced neophobia in the presence of a novel threat. Reduced neophobia has also been correlated with infection of other hosts, e.g. rodents, that are commonly predated by felines [34]. This same effect could explain infected hyenas' closer approaches to lions, though we cannot exclude olfactory modulation by the parasite, nor other mechanisms of reducing ailurophobia, without further experimental studies.

Regardless of their proximate causes(s), the different behavioral outputs of seropositive hyenas in the presence of lions (i.e. closer approaches) vs. toward simulated intruding conspecifics (i.e. slower approach to within an equal distance) reveals an intriguing context-specificity in *T. gondii*-associated host traits. This echoes findings from other social mammals, in which *T. gondii*'s highly specific modulation of responses to olfactory cues imply fine-tuned manipulation mechanisms (e.g. [30]). For example, in other intermediate hosts, *T. gondii* selectively modulates response to urine odors of wild (vs. domestic), and/or to local (vs. allopatric) felines. These effects have been interpreted by previous authors as evidence of adaptive host manipulation that facilitates local transmission (e.g. [24, 28]). If hyenas are indeed collaterally manipulated by *T. gondii*, our results reveal remarkable similarities in the extended phenotypes of targeted and collateral hosts, despite these hosts' striking evolutionary distances and ecological dissimilarities.

Our ELISA tests revealed that a high proportion of adult hyenas in the Masai Mara have been exposed to *T. gondii*. Our finding of age structure in disease preva-

lence is not surprising since, with very few known exceptions, infected hosts remain seropositive throughout their lives ([23] outlines possible exceptions). Observed *T. gondii* prevalences in our study are very similar to findings from a recent, independent study of infection prevalence in a smaller number of spotted hyenas at nearby field sites [9]. Altogether, these observations likely reflect the aggregate influence of several non-exclusive routes of exposure within African landscapes including 1) *T. gondii*'s prevalence within the diverse wild prey hyenas consume, 2) a rich community of indigenous felids that can serve as definitive hosts, 3) increasing presence of domesticated non-definitive hosts (e.g. sheep, goats and swine) [13], which can act as reservoirs and/or sources of trophic infection, and 4) the popularity of housecats (*Felis domesticus*) within Kenya for household rodent control [22].

While the relative role(s) of these potential infection sources for hyenas are not entirely clear, the current study and earlier work offer several informative insights. Since hyenas rarely migrate between Talek and Serena, we conclude *T. gondii* is currently well-distributed throughout the Mara. We further suspect that infection often originates from ingested oocysts in this ecosystem, given the high prevalence of *T. gondii* in Africa's native and domesticated herbivorous mammals [27], which are unlikely to consume infected animal tissues. Trophic transmission from wild and/or domesticated prey is also likely contributing to *T. gondii* prevalence in Mara hyenas, given that these and other generalist carnivores have higher infection rates than sympatric herbivores, and also that grazing herbivores would, most likely, ingest oocysts at equal or higher rates than carnivores [2].

Counter to our predictions (Table 16.3), temporal increase in human presence was not associated with rising seroprevalence in Talek hyenas. Human activities may still be impacting the parasite's contemporary evolution (e.g. by facilitating strain recombination), but do not appear to be necessary to produce high prevalence of *T. gondii* in wild hyena hosts.

Finally, while several non-definitive hosts can also sustain *T. gondii* through congenital infection of their offspring [7], this does not appear to be the main source of hyena infections. We found that seroprevalence increases in hyenas after weaning, when subadults transition to carnivorous diets. This again suggests that environmental routes of infection, i.e. oocysts and/or trophic transmission, are probably common sources of infection in the Mara's spotted hyenas.

Further discoveries lie ahead concerning the direct and indirect costs of latent *T. gondii* infections for hyenas in nature. Though non-significant, our observed pattern of hyena mortality sources (lion vs. other; Table 16.3) hints at possible fitness costs of collateral manipulation by *T. gondii*. Territorial behavior, which covaries significantly with *T. gondii* infection (Table 16.6), also plays a critical role in the organization of hyena societies. In view of the parasite's multiplicative effects on fitness-related behaviors, we suggest collateral manipulation by this parasite can have important and dynamic consequences for the fitness of Mara hyenas. For example, hyenas at Talek compete more intensely with lions for food [33], and this may change both the costs and benefits of boldness in lion presence. However, these costs and benefits may also be shifting rapidly, since Talek's lions are more sensitive than hyenas to the growing human population in the area [13]. Clearly, further

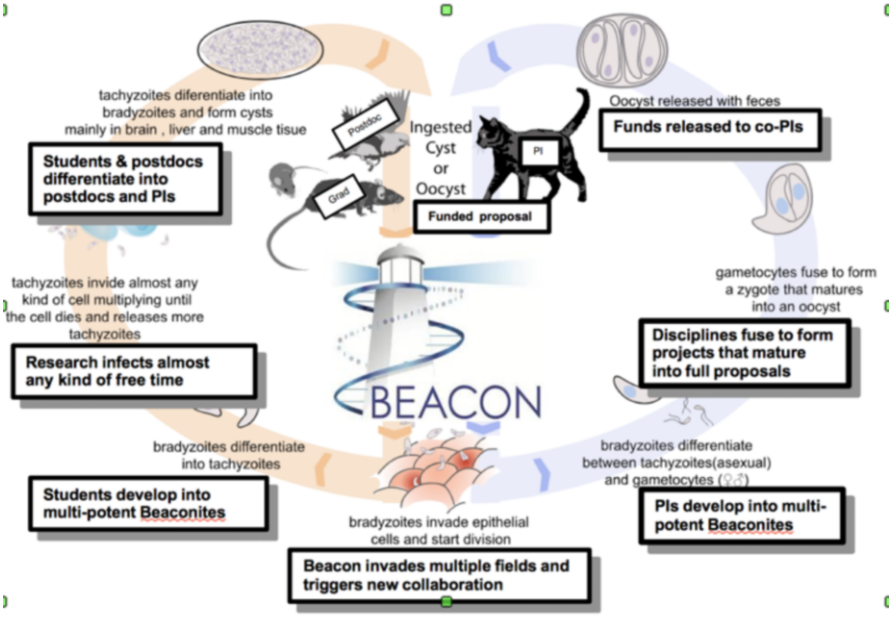
work will be needed to understand the dynamic feedbacks between human activities, disease prevalence, and the behavior and fitness of interacting *T. gondii* hosts.

Our finding of high *T. gondii* prevalence in hyenas should be of general concern to conservationists of Africa's remarkable and threatened biodiversity. Acute *T. gondii* infections are contributing to declines of many endangered species (e.g. [35]), and there is growing concern that increasing *T. gondii* abundance and connectivity facilitates recombination between strains that differ in infectiousness and virulence [5]. Circulating strains are also more diverse and virulent in South America and Africa than other parts of the world, and Kenya has the second highest recorded *T. gondii* diversity among African nations [11].

T. gondii's presence in areas of both human and wildlife activity, such as the Masai Mara, are further concerning in light of rapidly increasing HIV infections in Africa's human population. Latent *T. gondii* infections are often benign in healthy humans, though infection is a risk factor for a growing list of physical and psychological diseas-es [21]. In contrast, *T. gondii* often causes debilitating or lethal symptoms in individuals living with AIDS or other immunodeficiencies [3]. Preventing human *T. gondii* infections is thus an especially important public health objective in Africa, where $\sim$25 million people live with HIV; it also requires a detailed understanding of the parasite's local mechanisms of transmission. Prior studies have suggested only limited exchange between the sylvatic (wildlife) and anthropogenic (urban) populations of *T. gondii* [12], but this may differ in the Mara where wildlife, livestock, and humans interact with atypically high frequency. These considerations suggest that both public health and wildlife conservation in Kenya will benefit from further research into the behavioral and ecological drivers of local transmission between humans, domestic animals, and wildlife. And lastly, further studies of host manipulation by parasites will continue to boldly advance our understanding of animal behavior, ecology, and evolution.

Acknowledgements This material is based in part upon work supported by the National Science Foundation under Cooperative Agreement No. DBI-0939454. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. The authors wish to thank Chiara Bowen for help in the lab. Kathryn Fiedler, Kay Chalkowski, Dan Nunez, and Whitman the cat have helped to inspire their interest in Toxoplama gondii. Countless MSU undergraduates, Holekamp lab graduate students, and Masai field workers contributed to collection of plasma and behavioral data included in this study. Getty and Holekamp lab members also contributed to the development of project aims, and offered feedback on the analyses and writing of this manuscript. Finally, we thank Erik Goodman, director of the NSF-BEACON center, along with many BEACON staff, for facilitating the collaborative boldness needed to embark on this work.

ADDENDUM



Similarities between life cycles of *T. gondii*, the protist which causes toxoplamsosis, and *B. eacon*, which causes beaconosmosis.

It has not escaped our notice that Toxoplama gondii exhibits several biological similarities with a newly discovered superorganism: *Boldnessbuilding eacon*. This is somewhat surprising, since *T. gondii* is a carbon-based apicomplexan protist whose life cycle was discovered in 1988. In contrast, *B. eacon* is an MSU-based research center directed by Dr. Erik Goodman that invaded East Lansing in 2010. Despite these phylogenetic, biological, and historical distinctions, both organisms have remarkably similar, potentially fitness-altering impacts on their many hosts. Specifically, individuals infected with either organism exhibit greater boldness in the face of unknown and/or formidable challenges.

In the near future, we will begin experimental computational research, supported by two beaconosmosis experts (Hintze and Adami), to test whether simulated *T. gondii* infections promote risky cooperation at the group level in co-evolving digital hosts. The execution of the research reported above has already shown that this is true for *B. eacon*, which significantly emboldened the co-authors of the brain-altering manuscript you have now almost finished reading.

Our studies of Kenyan hyenas document, for the first time, how parasitically-enhanced boldness elevates risk of predation by felids in *T. gondii*-infected hosts.

It remains to be seen whether *B. eacon*-enhanced boldness will incur similar risks, e.g. attacks from lionesque reviewers. Regardless, we are confident many readers will eventually acquire beaconosmosis through consumption of the infectious ideas embedded herein. This need not concern anyone, however, since *B. eacon* is only known to have enhancing effects on the intellects of its many grateful hosts.

References

1. Al-Adhami, B., Gajadhar, A.A.: *A new multi-host species indirect ELISA using protein A/G conjugate for detection of anti-Toxoplasma gondii IgG antibodies with comparison to ELISA-IgG, agglutination assay and Western blot*. Veterinary Parasitology **200**(1-2), 66–73 (2014)
2. Bakal, P.M., Karstad, L., Veld, N.I.T.: *Serologic evidence of toxoplasmosis in captive and free-living wild mammals in Kenya.* Journal of Wildlife Diseases **16**, 559–564 (1980)
3. Basavaraju, A.: *Toxoplasmosis in HIV infection: An overview*. Tropical Parasitology **6**(2), 129–135 (2016)
4. Berdoy, M., Webster, J.: *Fatal attraction in toxoplasma-infected rats: A case of parasite manipulation of its mammalian host*. Proc R Soc B. **267**(1452), 1591–1594 (2000)
5. Dardé, M.L.: *Toxoplasma gondii "new" genotypes and virulence*. Parasite **15**(3), 366–371 (2008)
6. Dubey, J.P.: *History of the discovery of the life cycle of toxoplasma gondii*. International Journal for Parasitology **39**(8), 877–882 (2009)
7. Dubey, J.P.: *Toxoplasmosis of animals and humans*. CRC Press (2016)
8. Engh, A.L., Esch, K., Smale, L., Holekamp, K.E.: *Mechanisms of maternal rank "inheritance" in the spotted hyaena, Crocuta crocuta*. Animal Behaviour **60**, 323–332 (2000)
9. Ferreira, S.C.M., et al.: *High prevalence of anti-Toxoplasma gondii antibodies in free-ranging and captive African carnivores*. International Journal for Parasitology: Parasites and Wildlife **8**, 111–117 (2018)
10. Flegr, J., Lenochoyá, P., Hodný, Z., Vondrová, M.: *Fatal attraction phenomenon in humans-cat odour attractiveness increased for toxoplasma-infected men while decreased for infected women*. PLoS Neglected Tropical Diseases **5**(11), e1389 (2011)
11. Galal, L., Ajzenberg, D., Hamidović, A., Durieux, M.F., Dardé, M.L., Mercier, A.: *Toxoplasma and Africa: One Parasite, Two Opposite Population Structures*. Trends in Parasitology **34**(2), 140–154 (2017)
12. Gilot-Fromont, E., et al.: The life cycle of *Toxoplasma gondii* in the natural environment. In: O. Djurkovic-Djakovic (ed.) Toxoplasmosis — Recent Advances, pp. 1–36 (2012)
13. Green, D.S., Johnson-Ulrich, L., Couraud, H.E., Holekamp, K.E.: *Anthropogenic disturbance induces opposing population trends in spotted hyenas and African lions*. Biodiversity and Conservation **27**(4), 871–889 (2018)
14. Hart, B.L.: *Behavioral adaptations to pathogens and parasites: Five strategies*. Neuroscience & Biobehavioral Reviews **14**, 273–294 (1990)
15. Holekamp, K.: Personal communication
16. Kolowski, J.M., Holekamp, K.E.: *Spatial, temporal, and physical characteristics of livestock depredations by large carnivores along a Kenyan reserve border*. Biological Conservation **128**(4), 529–541 (2006)
17. Kreuder, C., et al.: *Patterns of mortality in southern sea otters (Enhydra lutris nereis) from 1998-2001*. Journal of Wildlife Diseases **39**, 495–509 (2003)
18. Kruuk, H.: *The spotted hyena: A study of predation and social behavior*. University of Chicago Press, Chicago (1972)
19. Lehmann, K.D., et al.: *Lions, hyenas and mobs (oh my!)*. Current Zoology **63**(3), 313–322 (2017)

20. Miró, G., Montoya, A., Jimnez, S., Frisuelos, C., Mateo, M., Fuentes, I.: *Prevalence of antibodies to Toxoplasma gondii and intestinal parasites in stray, farm and household cats in Spain*. Veterinary Parasitology **126**, 249–255 (2004)
21. Ngô, H., Zhou, Y., Lorenzi, H., Wang, K., Kim, T., Zhou, Y., El Bissati, K., Mui, E., Fraczek, L., Rajagopala, S., Roberts, C.: *Toxoplasma modulates signature pathways of human epilepsy, neurodegeneration and cancer*. Scientific Reports **7**, 11,496 (2017)
22. Ogendi, E., Maina, N., Kagira, J., Ngotho, M., Mbugua, G., Karanja, S.: *Questionnaire survey on the occurrence of risk factors for Toxoplasma gondii infection amongst farmers in Thika District, Kenya*. Journal of the South African Veterinary Association **84**, 00–00 (2013)
23. Opsteegh, M., Swart, A., Fonville, M., Dekkers, L., Van Der Giessen, J.: *Age-related Toxoplasma gondii seroprevalence in Dutch wild boar inconsistent with lifelong persistence of antibodies*. PLoS One **6**, e16,240 (2011)
24. Poirotte, C., Kappeler, P.M., Ngoubangoye, B., Bourgeois, S., Moussodji, M., Charpentier, M.J.: *Morbid attraction to leopard urine in Toxoplasma-infected chimpanzees*. Current Biology **26**(3), R98–R99 (2016)
25. Poulin, R.: *Parasite manipulation of host behavior: An update and frequently asked questions*. In: H. Brockmann (ed.) Advances in the Study of Behavior, vol. 41, pp. 151–186. Academic Press, Burlington (2010)
26. Poulin, R., Levri, E.P.: *Applied aspects of host manipulation by parasites*. In: D. Hughes, J. Brodeur, F. Thomas (eds.) Host Manipulation by Parasites, pp. 172–194. Oxford University Press, New York (2012)
27. Riemann, H.P., Burridge, M.J., Behymer, D.E., Franti, C.E.: *Toxoplasma gondii antibodies in free-living african mammals*. Journal of Wildlife Diseases **11**(4), 529–533 (1975)
28. Tenter, A.M., Heckeroth, A.R., Weiss, L.M.: *Toxoplasma gondii: From animals to humans*. International Journal for Parasitology **30**(12-13), 1217–1258 (2000)
29. Trinkel, M., Kastberger, G.: *Competitive interactions between spotted hyenas and lions in the Etosha National Park, Namibia*. African Journal of Ecology **43**(3), 220–224 (2005)
30. Vyas, A.: *Mechanisms of host behavioral change in Toxoplasma gondii rodent association*. PLoS Pathogens **11**(7), e1004,935 (2015)
31. Vyas, A., Kim, S.K., Giacomini, N., Boothroyd, J.C., Sapolsky, R.M.: *Behavioral changes induced by Toxoplasma infection of rodents are highly specific to aversion of cat odors*. Proceedings of the National Academy of Sciences **104**(15), 6442–6447 (2007)
32. Wait, L.F., Srour, A., Smith, I.G., Cassey, P., Sims, S.K., McAllister, M.M.: *A comparison of antiserum and protein A as secondary reagents to assess Toxoplasma gondii antibody titers in cats and spotted hyenas*. The Journal of Parasitology **101**(3), 390–392 (2015)
33. Watts, H.E., Holekamp, K.E.: *Ecological determinants of survival and reproduction in the spotted hyena*. Journal of Mammalogy **90**(2), 461–471 (2009)
34. Webster, J.P.: *The effect of Toxoplasma gondii on animal behavior: Playing cat and mouse*. Schizophrenia bulletin **33**(3), 752–756 (2007)
35. Work, T.M., Massey, J.G., Rideout, B.A., Gardiner, C.H., Ledig, D.B., Kwok, O.C.H., Dubey, J.P.: *Fatal toxoplasmosis in free-ranging endangered 'Alala from Hawaii*. Journal of Wildlife Diseases **36**(2), 205–212 (2000)
36. Yoshida, K., Van Meter, P., Holekamp, K.: *Variation among free-living spotted hyenas in three personality traits*. Behaviour **153**, 1665–1722 (2016)