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**THE DETERMINANTS OF HELMINTH INFECTION IN
BABOONS**

Second Level Proposal

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I. Title:

THE DETERMINANTS OF HELMINTH INFECTION IN BABOONS

Summary Paragraph:

Intestinal parasitic helminths are common in wild primate populations [1, 2] and can impose a significant burden on their host's fitness. Numerous factors can affect the prevalence and diversity of intestinal parasites in natural populations including environmental factors [3, 4], the host's behavior and genetics [5, 6]. How these different factors interact in natural populations remains unclear. This is in all probability due to the fact that previous studies have seldom looked at the prevalence and diversity of parasitic helminths in the same species but in different habitats over periods longer than a one year. I am proposing to conduct a comprehensive analysis of helminth prevalence and diversity in wild non-human primates (NHP), specifically baboons (genus *Papio*) in Ethiopia. *Papio* sp. are common and widespread in Ethiopia and can be found in a wide variety of habitats that range from arid desert regions to rain forests. Their unique distribution across varied topographies affords a rare opportunity to examine the role of the environment on parasitic infestation.

I will test the hypothesis that ecological conditions constitute the major determinant of helminth infestation in baboons in Ethiopia. To achieve this goal I will assess the prevalence and diversity of intestinal helminths in populations of baboons that differ (1) by their ecology, (2) by their genetic composition and (3) by their behavior. I will first perform this analysis on the baboon hybrid zone in Awash National Park because the baboon populations in Awash have been extensively studied for the past 50 years [7, 8] and a vast amount of data on the ecology, behavior and genetic composition

of the troops is readily available. The analysis of troops will be extended to olive baboons (*Papio anubis*) across the entire Ethiopian range of the species. I will compare the prevalence and diversity of helminths in olive baboons inhabiting three main habitats: rain forests, in savannahs and semi-desert. This study will be performed using a combination of microscopic and molecular techniques to insure the most detailed survey possible. The data collected will be analyzed using an ecological niche modeling approach to rigorously assess the relationship between the environment and the distribution of baboon parasites.

While parasite surveys provide useful information they do not inform about the long-term interaction of intestinal parasites and their hosts. We will examine this issue by comparing the molecular evolution of genes involved in parasite resistance between two species with different ecologies and different levels of parasitic infestation: the olive baboon, which is a savannah baboon, and the *hamadryas* baboon, which is a desert baboon. Preliminary analyses suggest that olive baboons carry a greater abundance and diversity of helminth intestinal parasites than *hamadryas*, possibly because the *hamadryas*' habitat is less favorable to the survival of helminth eggs. I will test if this difference in infestation has left a genetic signature on the genome of these two species. To this end, I will amplify and sequence a large number of helminth resistance genes in these two species and assess if selection is acting more strongly on these genes in olive baboons than in *hamadryas* baboons.

A. Background:

All primates (including humans) are infected by intestinal helminths [1, 2]. Because they can significantly affect the fitness of their hosts helminth parasites impose a strong

selective pressure as suggested by the rapid evolution of genes involved in helminth resistance [9]. However, not all primates are equally infected by intestinal worms or have the same levels of resistance to helminth infection. The prevalence and diversity of intestinal parasites can differ greatly between species, between populations and between individuals within species. It remains unclear why some primate populations are heavily infected by a large diversity of helminth parasites while others are only moderately affected by such parasites. Some factors that affect the prevalence of helminths in natural populations include the ecology of the host [3, 4], the behavior of the host and the genetic composition of the primate population [6]. While these factors have been shown to play a role in helminth prevalence, the interaction between these factors and their relative importance remain incompletely understood. The main reason is that most studies consist of parasite surveys over a restricted geographic area (a national park for instance), are usually performed during a single field season and may not compare prevalence between populations.

1. The Ecology of intestinal worm infestation

The success of helminth infection is undoubtedly dependent upon the environmental conditions because most helminth parasites are transmitted to the host from the environment and not through contact between individuals (as is the case for many viral and bacterial infections). The right combination of rainfall, soil composition, surface temperature and altitude can influence the survival of the helminth (eggs and/or larvae), the helminth's ability to successfully infect a novel host [10] and the maintenance of the helminth infections in a population [11, 12]. The proximity of other species, either other primate species [13] or livestock that can act as a source of parasites can affect the diversity of

parasites a species is exposed to [14]. This is exemplified by the higher prevalence of intestinal worms in *hamadryas* baboons living in proximity to humans than in baboons living in more remote habitats [15].

2. Host response to infestation

Different helminth's are transmitted to their hosts at different stages in their lifecycle [16] which consists of either the egg, larvae and/or the adult stage [17]. Helminth transmission routes are primarily fecal/oral [5, 18] for example in *ascaris* sp. [19], *enterobius* sp. [20] and *trichostrongylus* sp. [21] and/or transdermal [18] as in *strongyloides*, hookworm and *trichuris* [22, 23]. Because intestinal worms can negatively affect the fitness of their host, a number of mechanisms have evolved to limit the transmission of the parasites or to mitigate their effect on the host's fitness.

a. Behavior

The behavior of an individual may influence the transmission and maintenance of parasitic infections [24]. Depending upon the size and/or location of a population's range [25-28], what type of foragers they are and their food and water sources these behaviors can affect parasite prevalence and patterns of infection in a population [29, 30].

In response to parasitism primates have adaptive behaviors that include avoiding foods that may increase the exposure to parasites and self medication by eating purgative foods to expel them [31-33] *P. hamadryas* may practice a type of parasite avoidance by drinking water solely from self-dug drinking holes [34, 35]. Also, yellow baboons in Amboseli have even been observed practicing a type of parasite avoidance by alternating sleeping sites. In this case a population of baboons rotated their sleeping sites that altered their exposure to contaminated fecal matter [36]. Water sources may also expose baboons

to parasitic infection, as is the case with *schistosoma* where larvae have a lifecycle in water. Baboons drink from various sources of water that include hot springs, random drinking pools and rivers. Baboons may dig watering holes for drinking but baboons also share drinking sources with other NHP (RMonfort observed 2009-2011), wild ungulates [37], livestock from nomadic farmers and even drink from sewage runoffs [38] further exposing them to parasitic infection.

b. Genetics

It is also possible that certain populations may have a genetic advantage that helps them to fight infection and/or the deleterious effects of a helminth infection. The immune response to parasite infection involves two types of mechanisms: innate immunity and acquired immunity. In innate immunity the first step, phagocytosis of foreign bodies, occurs outside of the host cell. Inflammation occurs in the second step when blood flow is increased to the infected areas allowing an influx of phagocytic cells. Finally the complement cascade allows the regulation of the immune response by killing foreign cells and promoting inflammation [39-41]. The innate immunity's modified Th2 response to infection produces IL-4 and IL-13 that in turn directly responds to helminth infections [42-46]. Acquired immunity involves the recognition of antigenic markers while retaining memory of infection. Two types of defense mechanisms may be utilized; cell mediated: where lymphocytes in host cells attack pathogens and humeral immunity that utilizes antibodies in the blood and lymph systems that attack extracellular pathogens [39].

The frequency of alleles involved in the immune response can potentially alter the level of the immune response and hence alter resistance to worm infection since they are

responsible for the immune reaction [47], whether innate or acquired, within the host [39]. Selection can result in a change of allelic frequencies in a population and may be determined by parasitic pressures [39]. MHC genes in rodents [48] and cyprinid fish [49] were shown to have increased polymorphism if helminth parasite diversity is high [50].

3. Standing questions in the field and scope of the study

Evolutionary parasitologists are interested in finding the correlates that are involved in helminth disease occurrence in wild populations. I will address this by looking at factors that can influence helminth intensities and prevalences; specifically the climatic and genetic correlates. Another concern is the immune response to infection and immune heritability. I will address this by making inferences taking into account the habitat, behavior and life history of *Papio* by utilizing the results from the genetic and ecological analysis in this study [40].

Baboons are a good model for this research project because they are ecologically flexible and accessible in many areas of Ethiopia. Baboons are widely distributed in diverse habitats. Baboons are abundant, are omnivores and they are able to adapt to environmental changes whether natural or anthropomorphic. Performing prevalence surveys is achievable because baboons in this region live in sites traversable by foot or in cars and harbor parasites [51]. Further, throughout SSA are areas where baboons may overlap or come into contact with human populations [52] or other taxa (RMonfort observed 2009-2011).

I propose executing a research project that connects field data, genetics, behavior and ecology while examining the frequency of pathogen resistant alleles in wild populations of baboons in several localities in Ethiopia. Additionally I will address the effects of human proximity and habitat diversity between *anubis* populations in Ethiopia.

B. Specific Aims

1. Aim 1: Assess the factors affecting intestinal worms' prevalence in wild baboon populations in Ethiopia.

a. Study of the Awash Hybrid Zone

i. Rational and Hypotheses

I began my investigation of intestinal worm infestation in baboons by studying baboons from Awash National Park. The baboons of Awash have been studied for more than 50 years and a large amount of behavioral, genetic and ecological data pertaining to these baboons is available. Two species are found in Awash, the olive baboon (*P. Anubis*) and the hamadryas baboon (*P. hamadryas*). These two species hybridize in Awash and a stable hybrid zone is found in the South East portion of the park. When the Awash hybrid zone was first discovered in the 1960's it contained at least 180 hybrids and currently the hybrids appear to have a high level of fecundity [53]. When compared to the ranges of the parent species the hybrid zone exhibits differences in its environment that make the zone unique [54]. Hybrids can be found just east of *anubis* populations in the southern areas of the park along the Awash River and directly after the falls heading in a northeasterly direction. Beyond the Awash falls downstream is a deep canyon. The hybrid zone extends at least 20km to the eastern edges of the park, just South of the *hamadryas* populations near the town of Awash. The Awash hybrid zone is primarily dominated with thorn scrub along the cliffs of the canyon and gallery forests along the Awash River downstream from the falls. In the hybrid zone the climate is not as arid as the north areas where the hamadryas live. Like the *anubis*' habitat the hybrid's range has intermittent sections of gallery forests.

Although the hybrids do not seem to suffer from sterility and in fact seem to be thriving in their habitat, the stability [53] and the narrowness of the hybrid zone suggests

that something is limiting the spread of the hybrid phenotypes. It is possible that the hybrids do in fact have a lower fitness than their parental species (the tension zone model, Table 1). Under this model the balance between selection against the hybrids and the dispersal of the parental species maintains the hybrid zone [55]. Alternatively, it is possible that hybrids have a higher fitness than the parental species in the environmental conditions of the hybrid zone, (bounded hybrid superiority model) [56-58]. When an increased level of fitness of a hybrid population occurs in the zone of contact this can be defined as hybrid vigor [58, 59]. This increased level of hybrid fitness occurs in a specific geographic area where the hybrid is more fit than either parent species [60].

Table 1: Hybrid Zone Models [61].

Hybrid zone models	Exogenous	Endogenous	Fitness of hybrid	Fitness of parent species
Tension zone	No	Yes	Low, selected against	High
Bounded hybrid superiority	Yes	No	High	Low
Temporal hybrid superiority	Yes	No	Can fluctuate in space and time	Can fluctuate in space and time
Evolutionary novelty model	Yes	Yes	If high will move to novel space and may replace parental genotypes	Genotype dependent

One problem with assessing fitness in the hybrid zone is that it is difficult to measure fitness in natural populations. A way to address this is to compare the parasite prevalence of the parental populations to the parasite prevalence in the hybrid population. I will then be able to assess the fitness of the hybrids and the two parental species using parasitic infection as a proxy for fitness [62].

Finally, in order to fully understand the dynamics of the hybrid community multiple sites across multiple years will be analyzed [61]. This is because parasite prevalences have been shown to be cyclical and may change in value from year to year or from site to site as shown in *daphnia* sp. [63, 64] and *xenopus* sp. [65]. To date we are not aware of any survey of the parasites in the Awash hybrid zone that has been performed and no survey of parasite infestation over multiple years has ever been done in any primate system.

i. Experimental approach

1- Microscopical survey of helminth parasite infestation: Fecal samples were collected from three wild populations of baboons, (*P. anubis*, *P. hamadryas*) and hybrids (*P. anubis* x *P. hamadryas*) over a four-year period. 20-30 *P. anubis* samples were collected

Figure 1: Map of Collection sites in Awash National Park, Ethiopia.



ted from each of 1-4 troops located upstream of the Awash River, near the Awash River Falls and just downstream of the Awash Falls near the Awash Gorge (Figure 1). 20-30 *P. hamadryas* samples were collected from each of 1-2 troops on the Filoha and/or Wassaro cliffs near Filoha. 10-20 hybrid samples were collected from each of 1-2 troops in the Awash gorge near Kereyou lodge and in the hybrid zone near the Geda campsite. All samples were collected in the morning and immediately preserved for further testing. Collection sites varied in aridity, elevation and human exposure. Samples were preserved in 15% formalin and analyzed using a modified McMaster flotation technique [66] where helminth eggs float to the top of a ZnSO₄ flotation solution to a specific gravity of 1.18. Two grams of the preserved fecal sample were strained into a beaker containing 60ml of ZnSO₄. After 15 minutes fluid from the beaker was swirled and pipetted into a McMaster slide where I identified and photographed eggs under an inverted microscope at 10x

and/or 40x magnification. This slide analysis consisted of identifying helminth eggs by morphology [67] and recording their incidence per sample [68]. From this detailed analysis I was able to determine the most common helminth species within these populations in the first year of the study.

2- Microscopical survey of helminth parasite infestation in the field: Fecal samples were collected and analyzed in the field using a flotation method where helminth eggs float to the top of a specimen vial called a Fecalyzer®. In this stage of the study and in subsequent years we focused on the most common helminth species found in the first experimental approach. The Fecalyzer® tube was opened and I used the green insert to collect the fecal specimens that were then placed back into the Fecalyzer® tube. The Fecalyzer® tube was then filled with Fecasol® solution (a commercially prepared sodium nitrate solution with a specific gravity of 1.2) and the green insert was rotated to break up the fecal matter. Additional Fecasol® solution was then filled into the Fecalyzer® tube until a meniscus formed on the top. I then placed a cover slip on top of the specimen vial for 15-20 minutes. After the specified time I placed cover slip onto a microscope slide and analyzed the slide under a light microscope at 10x and/or 40x magnification. The slide analysis consisted of identifying helminth eggs by morphology [67] and recording their incidence per sample [68].

3- Data analysis: Descriptive statistical analysis for all samples was conducted using the statistical analysis program Quantitative Parasitology 3.0 [68]. Descriptive statistics including the prevalence, mean and median to obtain the intensity of infection and the variance of the mean were calculated. The variance within and between troops was analyzed to determine significant differences along with additional statistical analysis.

Bootstrap values for the mean intensity, degrees of freedom and when applicable the Chi square (comparing prevalences), Fisher's exact test (comparing prevalences) and Mood's median test (mean intensity) were also computed. A confidence limit for 90% was obtained. Prevalences were compared by year over a four-year period and these prevalences were compared to infection scenarios to see what hybrid zone model may best fit the current Awash hybrid zone as discussed in the preliminary data section at the end of the proposal.

b. Survey of helminth infestation of *anubis* baboons in relation to the environment.

i. Hypothesis

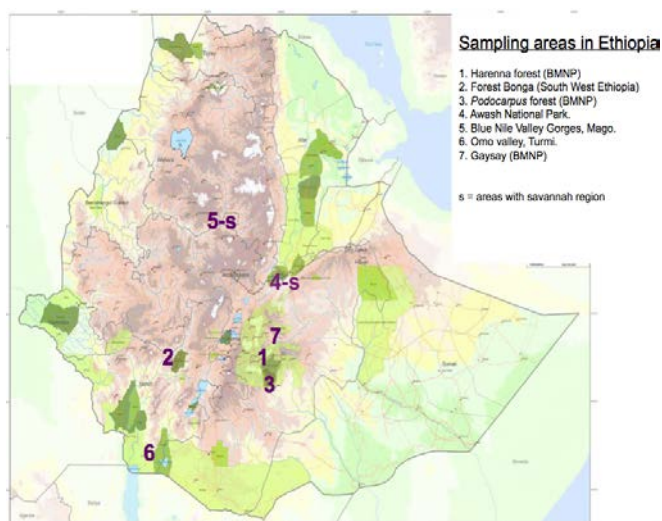
It is commonly accepted that the environment primates live in has an important impact on parasitic infestation. Previous studies have shown that species that live in areas that are warm and moist tend to harbor more parasites than species in areas that are hot and arid [69]. However, most studies are comparing prevalence in a small number of habitats (most often 2) and do not take into account the diversity of parasites. Surveys are limited in their identification of helminth eggs due to the similarity of egg morphology and often studies present results with only genus names (e.g. *Trichuris* sp., *Strongyloides* sp.). Therefore primate populations in different locations may be parasitized by different helminth species not discernible by microscopy alone. I intend to thoroughly test the hypothesis that primates inhabiting warm and moist areas have a higher prevalence and a wider diversity of parasites than primates living in dry environments. To this end, I will assess the diversity and prevalence of helminth parasites in a species of primates that can be found in a diversity of habitats, the olive baboon (*P. anubis*). I will combine the traditional method of microscopic fecal analysis with DNA barcoding to refine the identification of helminth species. I will then analyze the parasitic survey using the tools

and concepts of ecological niche modeling, which should provide a more accurate assessment of the relationship between habitats and parasite infestations.

Although it is usually described as a savannah baboon, the olive baboon is distributed across a wide range of habitats. In Ethiopia, olive baboons can be found in semi-desertic habitats, in savannahs, in riverine habitats, in tropical rain forests, in tropical dry forests and in high elevation grasslands. Based on personal observations during travel in Ethiopia in 2009, 2010 and 2011 or based on observations of colleagues, I have identified a number of locations where olive baboons can be found and where I intend to collect fecal samples. Of course, additional locations might be discovered at the time of collection. The following locations will be the focus of the survey (Figure 2):

Rain forest habitat: Haremma forest (Bale National Park), the forest near the town of Bonga, (South West Ethiopia). Tropical dry forest: *Podocarpus* forest (Bale National Park). Riverine habitat: Awash National Park, Blue Nile Valley Gorges, Mago River in Mago National Park. Savannahs: (ANP, BNVG). Semi-desertic habitat: Omo valley, near the town of Turmi. High elevation grasslands: Gaysay (Bale National Park).

Figure 2 Collection sites in Ethiopia



ii. Experimental approach

Survey of helminth parasite infestation:

I will use a combination of microscopic and molecular analysis. The microscopy method I will use is identical to the one described in aim 1. An alternative approach would entail examining samples that I collected that have not been looked at from previous years.

DNA Barcoding. Parasite DNA will be extracted from stored RNALater samples using a protocol for pathogens in stool (QIAamp® DNA Stool kit) which will then be amplified using PCR [70] and sequenced. DNA barcoding will be utilized to identify nematode species utilizing markers that are conserved at the species level, have sufficient flanking regions and are the right length for DNA extraction and sequencing. MtDNA (COI) [71-73] and rRNA (family delineation) internal transcribed spacers (ITS-1 and ITS -2) are examples of appropriate markers [74]. There are mtDNA sequences readily available on GenBank (nucleotide search) that include complete mitochondrial genomes for *trichuris*, *necator americanus*, and *enterobius vermicularis*. Mitochondrial DNA and/or internal transcribed spacers (ITS1 and ITS2) [72] will be amplified using applicable universal primers, degenerated nematode primers, and species-specific primers [74]. Primer sequences will be acquired from previous publications; NemAtol and/or GenBank for helminth parasites *schistosoma*, *ascaris*, *trichuris*, *enterobius*, *strongyloides* and *trichonstroglyoides* [74, 75]. PCR products will be cloned using TA cloning and sequenced by Sanger sequencing using BigDye terminator cycle sequencing [76]. If funding is available, PCR products will be directly sequenced using Next Gen Roche 454 sequencing. Sequences will be aligned with ClustalW or MUSCLE. Nucleotide BLAST will be used to compare sequences of unknown samples to those stored in NCBI. DNA

barcodes can be used to aid further investigation in the population genetic structure of the helminth populations in *anubis* by looking at the genetic diversity of the species according to sample size.

GIS and ecological niche modeling of parasites. After conducting the survey of parasite infections in wild populations of baboons I will overlay the GPS information on sample collection prevalence and location in Ethiopia with the temperature, rainfall, elevation and aridity using satellite and/or weather station information over the affected areas from information obtained from the Ethiopian Meteorological Association and from Worldclim [77]. ArcGIS (ESRI v.9.3.1) will be used to create a projected and the current niche model for *anubis*. The outcome may say that the weather does or does not play a role in the prevalence of disease infections. Possible problems include limited access to atmospheric data. An alternative is to use data collected by the United States on Africa and create an analysis in ArcGIS or use the Maxent computer program to process the data for the projected and current niche model [78].

2. Aim 2: Assess the long-term interactions between baboons and their parasites.

i. Hypothesis

Pathogens, particularly helminthes, can influence the evolution of allele frequencies at loci involved in fighting infestation [79]. Changes in allele frequency occur when a gene associated with the immune system is selected for and/or against during the immune response. The two types of selection that may occur from host–pathogen interactions are positive directional selection [80] and balancing selection [50, 81-84]. In the case of balancing selection multiple alleles are maintained within a population while heterozygotes have an advantage over homozygotes. Balancing selection can be maintained by frequency dependent selection and/or heterozygote advantage [85].

While evolutionary histories of pathogen infection are being elucidated the complexity of the molecular evolution of the immune response to pathogens is not fully understood. This is a two-fold process. The ecology may first influence parasite species richness at a specific locality and then indirectly influence host loci selection within the host. *Anubis* baboons in ANP have a higher parasite species richness than *hamadryas* and we believe that this is because of the wetter conditions in the southern portion of the park along with greater exposure to livestock, humans, human refuse and other primate taxa. Climatic fluctuation affects the prevalences in *hamadryas* more than the *anubis* and the hybrids. Pathogen resistance genes in *anubis* populations may be under stronger selection than pathogen resistance genes in *hamadryas* populations.

One way to address this is to look at allele frequencies in a natural population of baboons and determine if there is a correlation between location, parasite prevalences, climate and allele frequency. I also propose to look at the molecular evolution of pathogen resistant loci in baboon populations and discover if there is any evidence of selection for these loci in baboons. Looking at allelic frequencies has allowed researchers to make inferences about the infection history of certain pathogens within a population. For example lack of MHC variation in chimps suggested that there may have been previous pandemic of SIV [39].

ii. Experimental Approach

Baboon Pathogen Genetic Analysis: Determination of the frequency of pathogen resistant alleles. Pathogen genes will include those that are involved in the immune response for helminth, virus and protozoa. Genes of interest include but are not limited to innate

immunity and acquired immunity genes for example TLR1, TLR4, and TLR5, cytokine production and Th2 response (IL-4, IL-10, IL-5 and IL-13), T-regs (CD200R11 and STIM2), helminth diversity (CTLA4), and intergrin regulation and MHC genes [86],[87].

PCR and sequencing: 20 Candidate genes will be chosen from existing literature and The Immunology Database and Analysis Portal, <https://www.immport.org> or IMMPORT.

This analysis will be conducted on DNA samples from two captive primate populations (*P. anubis* and *P. hamadryas*) and three wild NHP populations (*P. anubis*, *P. hamadryas* and their hybrid *P. anubis* x *P. hamadryas*) from samples currently in the Boissinot Lab.

- a. Coding sequences of genes will be retrieved from BLAST/GENE Bank and UCSC's Genome browser that contains the complete macaque genome and can be utilized to create primers. PCR products will be sequenced using the Genomic Center at the University of Washington, followed by a haplotype analysis using PHASE [88] and further haplotype analysis by amplifying long fragments if needed. An alternative to this strategy would be to sequence the complete exome of both olive and hamadryas baboons using exome capture followed by Illumina sequencing. This alternative approach will be used if funding becomes available. Statistical and population genetics analysis: Alignment will be performed using Geneious [89]. Nucleotide diversity will be calculated using parameters π and Watterson's θ . Tajima's D [90], the HKA test [91] and the MK test [92] will be used to determine the effects of selection. The TMRCA of alleles will be computed using GENETREE software.

C. Timeline:

Year	Accomplishments: Aim 1 a is completed
2012	Aim 2 Fall 2012

2013	Aim 2 Spring 2013. Aim 1 b- Summer 2013 (collection of samples), Fall 2013 (sample analysis)
2014	Aim 1 b January 2014 and Spring 2014 (possibly with local help). Fall Graduation

D. Preliminary ecological data: 2008-2011.

342 baboon fecal samples were analyzed in the field for parasite prevalence. Descriptive statistical analysis was conducted using the statistical analysis program QP 3.0 [68]. Prevalence for six species of parasites identified: *Trichostrongylus spp.*, *Enterobius spp.*, *Trichuris spp.*, *Strongyloides sp.*, hookworm and *Schistosoma spp.* was calculated where applicable. The *anubis* populations exhibited a consistently high prevalence (75% or greater) from 2008-2011. When comparing the prevalence among the four troops of *anubis*, a p value of 0.709 indicates that there is no significant difference in prevalence among troops. The prevalence was significantly lower in the hybrids (35-45%) than in *anubis* for each year. For hybrids G5-G6/G7, a p value of 0.145 also indicates no significant difference in their prevalence's. For the *hamadryas* 2004 (preliminary samples) and 2008 samples the results indicate no significant difference in prevalence. In 2009 and 2011 *hamadryas* have higher prevalences (~70%) when compared to 2008 (less than 10%) and 2010 (40%) and are statistically different. The extreme droughts that occurred in 2009 and 2011 may have modified the behavior and range of *hamadryas* baboons. The changes of behavior include expanding their range into areas that have greater populations of humans, more interaction with other wildlife and greater access to food and water harboring parasites. *Hamadryas* baboons that must to range longer and farther in search for more food and water sources may also be under greater stress thereby weakening their immune response to parasitic infection.

When we compare all three baboon populations, *anubis*, hybrid and *hamadryas*, we get significant differences in prevalence for 2008, 2009 and 2011. *Hamadryas* and hybrids had a similar prevalence level in 2010. We have confirmed that the infection scenarios [93] of baboons in Awash National Park do vary from year to year (Table 3) which indicates that it is the environment that is primarily controlling prevalences in lieu of the genetic composition of the baboons (RMonfort 2008-2011). Years 2009 and 2011 support the bounded hybrid superiority hybrid zone model while 2008 and 2010 may support the evolutionary novelty hybrid zone model.

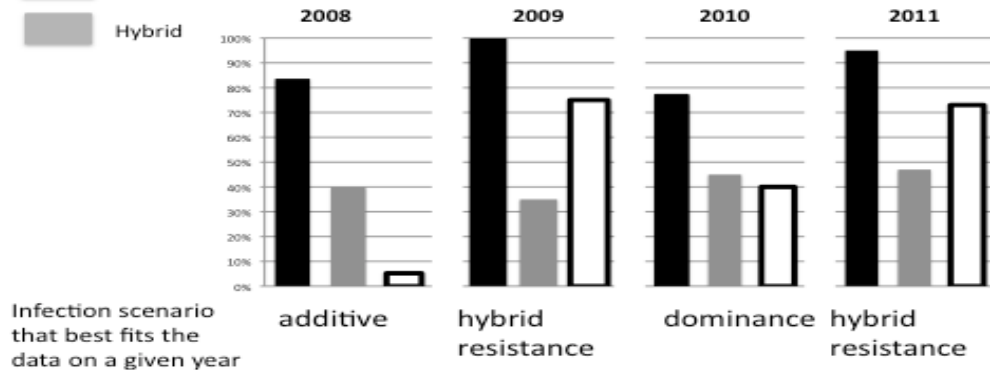
Conclusion: There are significant differences in helminth infestation among primate taxa with *anubis* having the highest prevalence, hybrids have an intermediate prevalence and *hamadryas* have a variable prevalence. The ecology is the driving force in prevalence rates and the outcomes in this study are supported by previous studies. The prevalence rates for *hamadryas* are greatly influenced by the changes in climate conditions. Multiple year studies are needed to make informative inferences regarding fitness, prevalences and behavior changes in wild populations. Finally, this study indicates that the utilization of parasite prevalence as a proxy for hybrid fitness may not be an ideal choice.

Table 3: Parasite prevalence 2008-2011

Parasite Prevalence 2008-2011

Legend:

- Olive baboon
- *Hamadryas*
- Hybrid



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