SOCIAL CHANGE, PARASITE EXPOSURE, AND IMMUNE DYSREGULATION AMONG SHUAR FORAGER-HORTICULTURALISTS OF AMAZONIA: A BIOCULTURAL CASE-STUDY IN EVOLUTIONARY MEDICINE

by

TARA CEPON ROBINS

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Student: Tara Cepon Robins

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This dissertation has been accepted and approved in partial fulfillment of the requirements for the Doctor of Philosophy degree in the Department of Anthropology by:

J. Josh Snodgrass	Chairperson
Lawrence S. Sugiyama	Core Member
Frances J. White	Core Member
Brendan J.M. Bohannan	Institutional Representative

and

Scott L. Pratt	Dean of the Graduate School

Original approval signatures are on file with the University of Oregon Graduate School.

Degree awarded June 2015

DISSERTATION ABSTRACT

Tara Cepon Robins

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Title: Social Change, Parasite Exposure, and Immune Dysregulation among Shuar Forager-Horticulturalists of Amazonia: A Biocultural Case-Study in Evolutionary Medicine

The Hygiene Hypothesis and Old Friends Hypothesis focus attention on the coevolutionary relationship between humans and pathogens, positing that reduced pathogen exposure in economically developed nations is responsible for immune dysregulation and associated increases in chronic inflammation, allergy, and autoimmunity. Despite progress in testing these ideas, few studies have examined these relationships among populations undergoing the transition from traditional to more market-based lifestyles. The present study tests relationships between economic development and social change, altered infectious disease exposure, and immune function among the Shuar forager-horticulturalists of Amazonian Ecuador, a population undergoing rapid economic change associated with increased market participation.

Using stool samples to assess soil-transmitted helminth (STHs; parasitic intestinal worms) burden, dried blood spot measurement of the inflammatory marker C-reactive protein (CRP), and interviews to evaluate level of market integration (MI; the suite of social and cultural changes associated with rapid economic development) and disgust sensitivity, this dissertation tests the Hygiene and Old Friends Hypotheses.

The first study tests relationships between STH exposure and MI, using geographic

location in relation to the regional market center as a proxy for MI. This study documents lower rates of STHs in people living in more market integrated regions. The second study tests the coevolutionary role that STHs and other pathogens have played in shaping human psychology and behavior. Findings suggest that pathogen exposure has acted as a selective pressure, resulting in evolved disgust sensitivity toward pathogen related stimuli. This study provides evidence that disgust sensitivity is calibrated to local environments, acting to decrease STH exposure. The third study tests the role of STHs in immune function. CRP was positively related to age in uninfected individuals. No relationships existed for more traditionally living or infected individuals. These findings suggest that STH exposure may decrease the risk of developing chronic inflammation and associated diseases with advancing age. These studies provide support for the idea that STHs provide stimuli that decrease chronic inflammation, suggesting that altered intestinal microflora in developed nations may be partially responsible for the development of chronic inflammatory disorders like allergy and autoimmunity.

This dissertation includes previously published and unpublished coauthored material.

CURRICULUM VITAE

NAME OF AUTHOR: Tara Cepon Robins

GRADUATE AND UNDERGRADUATE SCHOOLS ATTENDED:

University of Oregon, Eugene, OR
Marquette University, Milwaukee, WI

DEGREES AWARDED:

Doctor of Philosophy, Biological Anthropology, 2015, University of Oregon
Master of Science, Biological Anthropology, 2009, University of Oregon
Bachelor of Arts, Anthropology and History, 2007, University of Oregon

AREAS OF SPECIAL INTEREST:

Human health and biology

Parasitology and coevolution

Immune dysregulation

PROFESSIONAL EXPERIENCE:

Graduate Teaching Fellow, University of Oregon, 2007 to 2015

GRANTS, AWARDS, AND HONORS:

Juda Memorial Endowment Travel Fund, University of Oregon, 2010, 2014

Doctoral Dissertation Improvement Grant, NSF, 2013

Stern/Barnett Award, University of Oregon, 2013

Health Education Award, University of Oregon, 2008, 2010, 2012, 2013

Anthropology Department Travel Award, University of Oregon, 2008, 2009, 2011, 2012, 2013

Dissertation Fieldwork Grant, Wenner-Gren Foundation, 2012

Sasakawa Young Leaders Fellowship, SYLLF, 2011

PUBLICATIONS:

Madimenos FC, Liebert MA, Cepon TJ, Snodgrass JJ, Sugiyama LS. 2015. Osteoporosis prevalence in a Colono population from rural Amazonian Ecuador. Am J Hum Biol 27: 139-142.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Table Page

CHAPTER I

INTRODUCTION

Early life exposure to parasitic infection is hypothesized to decrease the risk of allergic and autoimmune disorders by regulating immune activity and down-regulating inflammation. Termed the Hygiene Hypothesis, this idea suggests that exposure to certain infectious agents helps prime immune function and prevents the immune system from reacting to harmless or self-produced stimuli. In economically developed and developing nations, allergy and autoimmunity pose a significant threat to health and well-being, while in some developing nations and regions, as well as among traditionally living populations, these disorders are virtually nonexistent. The framework of evolutionary medicine can be used to understand why these seemingly counterintuitive disorders are on the rise.

Evolutionary medicine seeks to explain why humans get sick using the framework of evolutionary theory (Gluckman et al., 2009; Nesse & Williams, 1996; Williams & Nesse, 1991). Natural selection has acted on all living organisms dating back to and including the origin of life itself. From this perspective, many of the diseases and health problems plaguing humans can be attributed to evolutionary constraints, tradeoffs between different physiological or biological features and functions and mismatches between our evolved biology and our current environment (Williams & Nesse, 1991). The environments that humans live in now and the environments we inhabited throughout our evolutionary history are hypothesized to differ in a number of important ways, including in energy balance (Cordain et al., 1998; Eaton & Eaton, 2003; Leonard, 2008),

caloric availability (Eaton, 2006; Eaton & Konner, 1985; Wallace, 2010), chronic psychosocial stress (Dressler & Bindon, 2000; Flinn, 2006), hygiene practices (Strachen, 1989), and access to modern medical care (Eaton et al., 2002). To date, this mismatch paradigm has been used to understand the increase in obesity (Baker et al., 2008; Power, 2012), cardiovascular disease (CVD; Wick et al., 2001; Dressler, 1995), type 2 diabetes (T2D; Neel, 1962; Neel et al., 1998), osteoporosis (Karasik, 2008; Madimenos et al., 2011a, 2012), and chronic inflammatory diseases (Straub & Besedovsky, 2003; Straub et al., 2007) seen in the United States and other developed nations. Although hypothesized relationships between evolutionarily novel environmental conditions and negative health outcomes are complicated and still in the relatively early stages of testing, having only been formally presented in 1991 (Williams and Nesse, 1991), there is a growing body of support for the utility of evolutionary medicine and the mismatch paradigm for understanding human disease (Gluckman et al., 2009; Nesse & Stearns, 2008; Stearns, 2012; Stearns et al., 2010).

One major change in recent human history is the epidemiological transition from infectious to chronic disease as the major causes of morbidity and mortality: in all but the very poorest countries in the world, chronic diseases now surpass infectious diseases as the leading causes of death (WHO, 2014a, b). Even more recently, Western industrialized countries have seen a rapid rise in diseases associated with immune dysregulation, such as allergies and autoimmune disorders (Bach, 2002; Strachen, 1989; von Ehrenstein et al., 2000; von Mutius, 2007). The Hygiene Hypothesis attributes the increased prevalence of these chronic, non-communicable diseases to increased sanitation and decreased exposure

to important immune-stimulating pathogens that the human immune system has come to rely on for proper development and function (Strachen, 1989).

This dissertation focuses on one class of pathogens, soil-transmitted helminths (STHs; intestinal parasitic worms), examining some of the ways that exposure to these pathogens have shaped human immune function, development, and behavior. Humans share a long co-evolutionary history with STHs (Hurtado et al., 2008; Jackson et al., 2008; Wolfe et al., 2007), yet contemporary environments are thought to have greatly reduced human STH exposure and infection. Broadly, this dissertation focuses on some of the outcomes of this long coevolutionary history, asking what it means for human health and well-being, especially in developed nations, when STH infections have been largely eradicated, or severely limited.

In Chapter I, I introduce the important concepts and theoretical background for this investigation, beginning with a discussion of humans as ecosystems and examining host-pathogen coevolution and symbiosis. This is followed by a short introduction to STHs, and a brief explanation of how they are hypothesized to have shaped human behavior and immune function (discussed in more detail in Chapter II). I then move on to discuss the theoretical framework for this research, including evolutionary medicine and the Hygiene Hypothesis. I conclude this chapter by introducing the study population with whom the research for this dissertation was carried out: the Shuar of Amazonian Ecuador.

Humans as Ecosystems

For the 100 years leading up to the end of the 20th century, the importance of microorganisms for human health was largely based on a germ theory of disease, in

which the understanding of microorganisms and their role in human health was focused on their negative impact as the causes of infectious diseases. Humans were thought to be comprised entirely of human cells and the immune system was seen as a gatekeeper, protecting humans from the ever-present threat of attack by outside organisms and removing those that managed to enter the body. A revolution in how we conceptualize our relationships with microorganisms is now underway, based on a growing body of empirical research and showing the human body to be a complex ecosystem, teeming with non-human life that is necessary for normal development and health (Blaser & Falkow, 2009; Costello et al., 2012; Furusawa et al., 2013; Turnbaugh & Gordon, 2009).

For example, recent genomic studies have demonstrated that only 10% of the cells in or on the human body are human cells, the rest are bacterial, viral, fungal, and even some multicellular animals (Bäckhed et al., 2005). The gastrointestinal (or gut) microbiome alone contains over 100 times the number of genes in the human genome (Bäckhed et al., 2005): only ~23,000 genes found within the human body are human, while over >3 million genes belong to bacteria in the gastrointestinal tract. Even many of the genes that are human show remnants of retroviruses and other non-human DNA that managed to insert themselves in our genomes over the millions of years of our evolutionary history. An analysis of the genomes of fruit flies, nematodes, and ten primate species, found that horizontal gene transfer from bacteria and protozoa to their animal hosts has been occurring consistently throughout the animal kingdom (Crisp et al., 2015; Wybouw et al., 2014).

Considering humans as an ecosystem, then, suggests that the immune system functions not as a simple gatekeeper to keep out all outside organisms, but as a regulator

and farmer, choosing which organisms are allowed into the human environment and which are eradicated. For instance, Immunoglobulin A (IgA), an important antibody for mucosal immunity, appears to serve as a crucial mechanism for maintaining and determining complex ecologies of the intestinal microbiota (the collection of bacteria within the gastrointestinal tract, necessary for digestion, among other things; Suzuki et al., 2004). The immune system's role as a regulator and farmer is also supported by a surprisingly small number of differing enterotypes (i.e., classification for living organisms based on their bacterial profiles) of the gut microbiome (Arumugam et al., 2011). Further, an entire branch of the immune system—the T helper 2 [Th2] dominated pathway—responds to one class of multicellular, parasitic organisms called helminths (Fairfax et al., 2012; van den Biggelaar et al., 2000). While the Th2 branch may work to keep helminth infection intensities low, there is evidence that this branch may function to turn down inflammation, regulate immune function, and prevent complete expulsion of the parasite (Blankenhaus et al., 2011; Helmby, 2015; Schopf et al., 2002; Taylor et al., 2005).

The fact that our immune system selects for the survival of specific phyla and species within us suggests that these organisms play an important role in immune health, development, and function. It is evident that if we are to understand human health, we need to more fully understand our relationships with the microbial organisms with whom we share our bodies.

Human-Symbiont Coevolution

Natural selection results in a change in allele frequency based on the allele's effect on heritable traits that alter the differential reproductive success of their bearers within a given environment (Darwin, 1859; Mayr & Provine, 1998; Wright & Dobzhansky, 1946). One major factor influencing differential reproductive success is other organisms living within that environment (Darwin, 1859). An extension of this idea is outlined in the Red Queen Hypothesis, which describes a type of relationship between predator and prey or parasite and host as an interspecific evolutionary arms race (Van Valen, 1973). A change in the population of one species (a predator or parasite), can change selection pressures on another species (prey or host) producing antagonistic co-evolution between the species. A classic example, is that natural selection might select for increased speed in a predator species, which then changes selection pressure on a prey species to increase speed to escape those predators. Similarly, natural selection can produce adaptations in a parasite species to neutralize a host defense, producing selection pressure on the host species to make the defense more robust, or generate other defenses.

These coevolutionary 'Red Queen' processes are common in symbiont-host relationships, especially if the symbiont proves either beneficial or harmful to its host. A symbiotic relationship is one in which an organism spends some or all of its life intimately associated with another organism of a different species (Bogitsh et al., 2005). There are multiple types of symbiotic relationships, including commensalism, mutualism, and parasitism (Bogitsh et al., 2005). In a commensal relationship, the symbiont lives with or around the host, benefitting from the host without being physiologically dependent. Basically, it means that the two organisms "eat at the same table" (from

French via Latin *commensalis*, literally "sharing meal;" Bogitsh et al., 2005: 2). For example, a number of the bacteria that live in the human intestinal tract are called commensals because they digest food particles that would normally go undigested by the human host (Cantarel et al., 2012; Dumas, 2011). In a mutualist relationship, both the symbiont and the host benefit. One example of a mutualist relationship are intestinal bacteria that rely on their host to supply food and, in return, provide digestive enzymes to extract vital nutrients that the host would not otherwise be able to access (Cantarel et al., 2012; Dumas, 2011; Poinar, 2009).

A parasitic relationship is one in which the symbiont is physiologically dependent on the host and provides no benefit to the host, instead causing harm. Many of the multicellular symbiotic organisms that infect animals, like protozoa and multiple types of parasitic worms, are considered parasites, but the human immune system may have co-evolved with them such that they are now vital for immune system stimulation, development, and regulation. Thus, while parasitic in a general sense, these organisms may also have concomitant commensal or even mutualistic relationships with their hosts. In fact, in nature the lines blur between symbiont categories, and often a symbiont can switch from mutualist to commensal to parasitic all within one host.

Evidence suggests that animals have carried symbiotic microorganisms since the evolution of sponges (Hoffmeister & Martin, 2003; Iyer et al., 2004). Parasites may have been critical selection pressures in the evolution of sexual reproduction (Ellison et al., 2011; Hamilton & Zuk, 1982; Hamilton et al., 1990). Further, there is mounting evidence that mitochondria, the 'powerhouse' of cells in living organisms, are the result of a

parasitic relationship between a single celled organism (the precursor to mitochondria) and other single-celled organisms (Cavalier-Smith, 1987; Lang et al., 1999).

In short, it is increasingly evident that symbiotic relationships have been crucial in shaping the evolutionary trajectory of all living things. Humans, and all animals, evolved under selective pressure posed by different types of symbionts, with most selective pressure probably coming from mutualists and parasites. A current topic of research examines bacterial influences on human health and development. However, while the human microbiome is incredibly important, there is another group of organisms that may have also been a critical selective pressure on humans: STHs. At present, very little research examines the effects of our longstanding coevolutionary relationship with STHs.

Soil Transmitted Helminths: "Old Friends" or Foes?

STHs are parasitic worms generally contracted through oral consumption of fecally contaminated soil, food, and water (Bethony et al., 2006). The main exception are hookworm species, which burrow into exposed skin when an individual comes into contact with soil containing larvae (Bogitsh et al, 2005). Members of the phylum Nematoda, extant STHs likely diverged from free-living roundworms at multiple points in their individual evolutionary histories (Bogitsh et al., 2005). The major STHs that infect humans are *Ascaris lumbricoides* (**Figure 1.1;** ascarids), *Trichuris trichiura* (**Figure 1.2;** whipworm), and the hookworms *Necator americanus* and *Ancylostoma duodonale*. These helminths vary in a number of ways, including size, daily egg output, residence location within the human digestive tract, and lifespan.

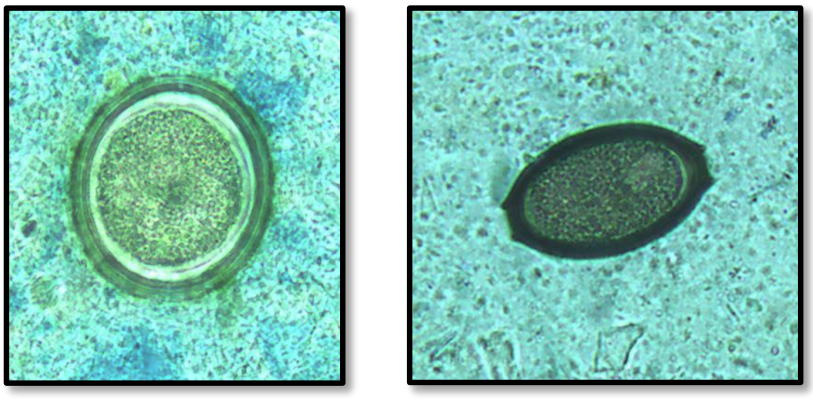

Figure 1.1 (Left). *Ascaris lumbricoides* egg and **Figure 1.2. (Right)** *Trichuris trichiura* egg at 40x magnification. Soil transmitted helminths infections are examined using microscopy and identified by the presence of eggs. Notice differences in shape, color, texture and thickness of cell wall, and presence of other distinguishing features. Photos taken by the author of this dissertation.

Globally, 576-740 million, 807-1,221 million, and 604-795 million people are infected with hookworm, *Ascaris lumbricoides*, and *Trichuris trichiura*, respectively (Bethony et al., 2006; Hotez et al., 2008). *Ascaris lumbricoides* lives in the small intestine and can grow to reach sizes between 150 and 400 mm (**Figure 1.3**). In the course of their one-year lifespan, they lay, on average, about 200,000 eggs per day. This high rate of shedding makes *A. lumbricoides* incredibly easy to spread from person to person. *Trichuris trichiura* are much smaller, reaching adult lengths of 30 to 50 mm with a daily output of 3,000 to 5,000 eggs. They live in the caecum and colon and have a lifespan of about 1.5 to 2 years. Hookworms (*N. americanus* and *A. duodenale*) are the smallest of the STHs, reaching lengths between 7 and 13 mm. They dwell in the upper small intestine and typically live between 5 and 7 years. Their egg output varies between species, with *N. americanus* laying 9,000 to 10,000 eggs per day and *A. duodenale* laying 25,000 to 30,000 eggs per day (Bethony et al., 2006; Bogitsh et al., 2005).

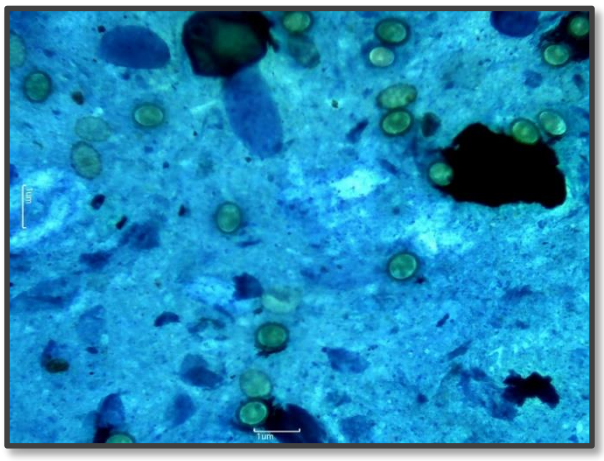

Figure 1.3. Multiple *A. lumbricoides* eggs within a small portion of a single slide observed at 10x magnification. Photo by the author of this dissertation.

Each species derives food from its host in a unique way. *Ascaris lumbricoides* consumes semi-digested food from inside the host, while *T. trichiura* and hookworms attach and consume blood directly from their host at different locations in the digestive tract (Bogitsh et al., 2005). Because each species interacts with its host in a different way, pathogenesis in humans varies between species. Hookworm infections are the most pathogenic because the helminth actually relies specifically on host-derived nutrients, resulting in blood loss, iron-deficiency anemia, abdominal pain, loss of appetite, stunted growth, developmental and mental delays, and death (Bogitsh et al., 2005).

Ascariasis and trichuriasis (as infections with *A. lumbricoides* and *T. trichiura* are called) generally produce no symptoms in humans, especially in light to moderate infections. For instance, 85% of *A. lumbricoides* infections are symptomless (Bogitsh et al., 2005), though intense infections can result in intestinal blockages, due to the large size of the adult worms (Khuroo et al., 1990, Villamizar et al., 1996). This is exacerbated by the fact that *A. lumbricoides* intensity usually peaks between ages 5 to 15 years, when the child is still growing and developing (Bethony et al., 2006). Ascariasis can also result in parasitic pneumonia due to larval migration into the lungs (Gelpi & Mustafa, 1968);

this can be associated with bloody mucus and sputum, resulting in asthma in a small number of infected children (Chan et al., 2001; Sharghi et al., 2001). In the small intestine, ascariasis can cause lactose intolerance and vitamin A deficiencies, as well as several other nutritional deficiencies (Taren et al., 1987). In the long term, ascariasis can result in short stature, impaired physical abilities, cognitive delays, and poor performance and attendance in school (Bethony et al., 2006), making it a disease of poverty from which it is hard to escape.

Trichuriasis can result in inflammation at the adult worm's point of attachment in the caecum and colon, and even the rectum (Bethony et al., 2006). In high intensity infections, this inflammation can lead to colitis, with abdominal pain and diarrhea resembling inflammatory bowel disease (Bundy & Cooper, 1989). These can result in impaired growth and development and anemia. Very heavy trichuriasis can result in chronic dysentery and rectal prolapse.

Estimates of worldwide mortality from STHs vary from 12,000 to 135,000 deaths annually (Bethony et al., 2006; WHO, 2002). This variation in estimates is related to the difficulty of accurately accounting for the impact of STH exposure, especially in hard-to-reach regions, and the synergistic effects of helminths on negative energy balance, poor growth and nutrition, and altered immune function resulting in their role as a secondary cause of mortality associated with countless other diseases (Crompton, 2000; Viteri, 1994). Because STHs cause more disability than death, their impact is often measured in disability-adjusted life years (DALYs). As defined by the World Health Organization, one DALY is equivalent to one year of healthy life lost due to illness and disability, and represents the burden of disease across a population. Worldwide, an estimated 4.7 million

DALYs are lost annually to STH infections, with *A. lumbricoides*, *T. trichiura*, and the two hookworm species resulting in the loss of 1.2, 1.6, and 1.8 million DALYs, respectively (Bethony et al., 2006). Despite this crippling pathogenesis for a number of people around the world, especially those facing extreme poverty in economically developing nations, only 1% of global research dollars are spent to study and prevent helminth infections (Hotez et al., 2008).

STHs are typically grouped with several other infectious diseases as neglected tropical diseases (NTDs). These represent some of the most common infections that affect the poorest people living in Latin America and other developing regions. They are termed "neglected" because they are often ignored by Western medicine due to their eradication in economically developed nations. According to the World Health Organization (WHO, 2015a), there are 17 NTDs present in 149 countries that affect over 1.4 billion people. These diseases include protozoal (Chagas disease, African trypanosomiasis, leishmaniasis), bacterial (Buruli ulcer, leprosy, trachoma, yaws), viral (dengue fever, chikungunya, rabies), and helminthic (cysticercosis/taeniasis, dracunculiasis, echinococcosis, foodborne trematodiases, lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminthiases) infections. Overall, these NTDs account for a total of 534,000 deaths and 56.6 million DALYs lost annually (WHO, 2015a), ranking them second only to HIV/AIDS, and ahead of tuberculosis and malaria, in terms of infectious disease burden. The Latin American and Caribbean regions are heavily affected by these diseases, with a disproportionate occurrence in people from indigenous populations and of African descent due to marginalization and poverty (Hotez et al., 2008).

Despite their negative health outcomes, especially in poorer nations, multiple lines of evidence suggest that STHs share a long, coevolutionary history with humans. The emergence of parasitic helminth species dates back at least 500 million years. In humans, paleoparasitological research has found almost all species of extant STH eggs present in prehistoric human feces and abdominal soil (Hurtado et al., 2008; Jackson et al., 2008) as far back as they will preserve. The oldest evidence of ascariasis in humans was found in human coprolites (fossilized feces) from France dating back to 30,000 years ago (Bouchet et al., 1996), while the oldest evidence of trichuriasis comes from coprolites in South Africa dating back to over 10,000 years ago (Evans et al., 1996). Further, our closest living relatives, the great apes, are infected by similar species of helminths (Wolfe et al., 2007). While caution must be taken in drawing conclusions about STH infection during human evolutionary history based on these findings, they do point to long-term symbiotic relationships between STHs and humans, which likely included earlier hominins.

STHs represent one of just a handful of infection types that could have commonly persisted among hunter-gatherer groups, especially early nomadic human populations who likely lived fairly isolated lives.[1] Population size estimates for pre-agricultural hunter-gatherer groups and earlier human ancestors suggest that these groups were primarily small, with relatively low population densities. Data suggest that these early groups consisted of about 30 individuals each, depending on resource availability, and

[1] Fairly isolated is used here to explain probable day-to-day population sizes. This is, of course, an over-simplification, because many early groups would have had extensive trade networks, with intermarrying and associated visiting relationships, as well as warfare (Marlowe, 2005). Further, as described later, large population centers or "cities" have been shown to exist among hunter-gatherer groups dating back to 25,000 years ago (Nadel & Hershkovitz, 1991; Watkins, 2010; Weiss et al., 2004). Despite these factors, smaller, nomadic groups with only occasional interactions with other groups would have likely been the norm (Kelly, 2013).

that these early groups may have followed fission-fusion patterns depending on resources, climate, and conflict (Marlowe, 2005). Kelly (2013) reviewed population densities among a number of extant hunter-gatherer populations. Population density estimates of hunter gatherer groups on the African continent, calculated as persons per 100 km^2, range from 6.6 people per 100 km^2 among the !Kung of the Kalahari Desert to 17 people per 100 km^2 among the Mbuti of the Democratic Republic of the Congo. For comparison, the present population density of the United States is 340.6 per 100 km^2 and that of the District of Columbia is 40,088.4 per 100 km^2 (United States Census Bureau, 2013). Kelly's (2013) findings suggest that contagious diseases would not have been an important selective pressure on small mobile groups, although it should be noted that the threat of infectious diseases vary by climate and population structure. Diseases that provoked intense immune responses and drastically reduced host fitness, however, would not have been beneficial for a parasite with only a limited number of hosts available (Kelly, 2013; Rook et al., 2014, Wolfe et al., 2007).

Infectious disease virulence, defined broadly as how much damage a pathogen inflicts on its host and reduces host fitness (Read, 1994), is affected by a number of factors. First, if the pathogen is passed vertically (from parent to offspring), then natural selection should favor lower virulence (Alizon et al., 2009; Flint et al., 2000). Vertical transmission relies on the survival of the host and the host's family in order to survive, so high pathogen virulence that significantly harms the reproductive success of its hosts would be selected against (Alizon et al., 2009; Flint et al., 2000). Further, a pathogen that relies on healthy hosts to spread will have more minor effects in order to avoid harming its mode of transmission (Ewald, 1993).

For a pathogen to evolve high virulence, it generally needs a large population of non-related hosts in which to spread, as well as an efficient means of transmission. Otherwise, high virulence will decimate its local host population and limit the pathogen's ability to spread. Pathogens that spread via vectors (like mosquitos) or through contaminated water or are able to jump between different species to exploit multiple hosts are also more likely to become highly virulent (Ewald, 1993). Natural selection also favors greater virulence if multiple pathogen species are present and competing within the host. Called the "short-sighted evolution" explanation of virulence, this idea suggests that if multiple strains are present then the most virulent will outcompete other strains (Levin & Bull, 1994; Read & Taylor, 2001; Ewald, 1993).

While humans are teeming with microbial life, a balanced ecosystem would be one in which all ecological adaptive niches are filled by different species. This is supported by the limited number of microbial enterotypes found in human populations (Arumugam et al., 2011), in which each niche appears to be filled based on digestive or protective functions. A balanced microbiome would limit competition between microorganisms that fill specific niches, while newly introduced microorganisms could lead to competition and increased virulence (Ewald, 1993). STHs, which tend to each inhabit their own ecological niches, do not appear to have very many competitors (Bethony et al., 2006), so competition would likely remain low.

A number of highly virulent viral diseases, like measles, influenza, and Ebola, are not likely to have been endemic to early human groups because population sizes were too small to support their maintenance and spread. Instead, these populations would have selected for symbionts that were less virulent, harmless or at least well-tolerated by the

immune system. These symbionts could more easily survive and be transmitted via small kin groups and networks with periodic contact between them (Babu et al., 2006; Comas et al., 2013; Kelly, 2013; Marlowe, 2005; Rook et al., 2014; Wolfe et al., 2007).

Soil-transmitted helminths, as discussed earlier, are comparatively less harmful than highly virulent viral diseases such as measles, and generally do not cause high mortality or immediate immobility of hosts. Low-virulence parasites would have been tolerated because mounting an immune response to eradicate the pathogen would be more costly than tolerating the parasite, allowing the infection to run its course (Clark, 2008; Hanssen et al., 2004; Little & Killick, 2007). Mounting an immune response entails a number of costs, manifested as damage to surrounding tissue because of errant inflammation, as well as tradeoffs away from energy that could be devoted to growth, development, and reproduction (Little & Killick, 2007; Lochmiller & Deerenberg, 2000).

The energetic costs of an immune response are very high, though little is known about these exact costs, especially among humans. Research among humans has shown that shivering thermogenesis in response to fever, combined with cellular immunity to illness, led to a 300% rise in resting metabolic rate (Chiolero et al., 1997). In general, fever has been shown to increase basal metabolic rate by 10 to 15% for each 1^{o} C rise in body temperature (Roe & Kinney, 1965). A study examining young adult men during and after mild respiratory tract infections documented a mean increase in resting metabolic rate of about 8% during infection, although some men demonstrated increases of greater than 14% (Muehlenbein et al., 2010). Just a subtle increase of interleukin (IL)-6 (associated with upregulated inflammation in response to infection) from infusions into healthy volunteers was enough to increased resting metabolism by 25% (Tsigos et al.,

1997). Even minor immune responses associated with vaccinations have been shown to increase resting metabolic rate by 15-30% (Cooper et al., 1992; Demas et al., 1997; Svensson et al., 1998). What these findings suggest, is that the body should make tradeoffs based on the costs and benefits of mounting an immune response. Because an upregulated immune response is costly to maintain, during light to moderate intensity chronic infections the immune system may have been selected for tolerating low level infections, while preventing more intense infections. This is especially true if that immune response only results in a temporary solution.

STHs generally reproduce rapidly. As noted earlier, adult worms can lay very high numbers of eggs per day (Bethony et al., 2006; Bogitsh et al., 2005). This means that adult worms not only go on living in their hosts for an extended period of time, but they can also lay enough eggs to continually reinfect the host and spread to others within the population. Thus, for intestinal STHs to be successful there is no need for high population density. From the host's perspective, there is no need to do anything but tolerate the infection, while keeping intensity low to prevent severe infection. Because of these tradeoffs and longer term selective pressures, STHs could have been a common, endemic disease in hunter-gatherer populations. This is also why these organisms have been termed 'old infections' or 'old friends' (Wolfe et al., 2007).

Selection for Pathogen Avoidance: The "Behavioral" Immune System

For small, nomadic hunter-gatherer populations, very few mechanisms likely existed to decrease exposure to STH eggs, especially if they were present in high numbers in the soil. The immune response requires a large amount of resources. Because

resources are generally finite, anything that gets devoted to immune function is not available for reproduction, maintenance, growth, or development (Lochmiller & Deerenberg, 2000; Long & Nanthakumar, 2004; McDade, 2003; Schaible & Kaufmann, 2007). An immune response that works to keep STH numbers low, without mounting a full attack, would be beneficial. Further, behavioral responses to reduce exposure to pathogen containing substances would prove useful for preventing the need for a strong immune response.

Humans likely evolved behavioral mechanisms to keep pathogen and other harmful microorganism exposure in check (Curtis & Biran, 2001; Curtis et al., 2004; Fessler & Navarrete, 2003, Oaten et al., 2009; Schaller & Duncan, 2007). In the case of STHs, these behaviors may have worked to naturally limit infection intensity while balancing costs of mounting an immune response. There is no contingent behavior without the central nervous system, so behavioral mechanism are ultimately based on psychological adaptations. One proposed mechanism for pathogen avoidance is the psychological disgust response. Disgust, hypothesized to be a basic human emotion present in all humans, may function to motivate avoidance of fitness-reducing stimuli, activities, and people (Curtis & Biran, 2001; Oaten et al., 2009; Tybur et al., 2009). Three domains of disgust have been identified: sexual, moral, and pathogen (Tybur et al., 2009).

The pathogen disgust response and resultant avoidance behaviors have been called the "Behavioral Immune System" because they provide another, possibly less energetically costly line of defense (Schaller & Duncan, 2007). For example, sheep avoid grazing in areas where they defecate suggesting an avoidance behavior associated with possible pathogen exposure (Cooper et al., 2000). A study among 50 American adults

found that people normally avoid food they associate with parasite contamination (Rozin et al., 1986).

Previous research suggests the existence of three specific categories of pathogen disgust eliciting stimuli (Tybur et al., 2009): 1) bodily excretions and body parts; 2) decay and spoiled foods; and, 3) living creatures recurrently associated with disease. Despite observations hypothetically associated with the disgust response, no studies to date have directly tested the hypothesis that disgust functions to reduce and prevent pathogen exposure and infection. Further, while disgust is hypothesized to be a universal human psychological adaptation, studies to test this have largely been conducted in clinical settings in economically developed nations (Curtis & Biran, 2001; Ekman, 1992).

Because environments differ in the relative costs and benefits of avoidance, to be adaptively functional, disgust sensitivity must be calibrated to those local conditions. Where hygiene, sanitation, and avoidance are relatively less costly, disgust sensitivity should be greater. Conversely, where costs of avoidance of pathogen associated stimuli is higher, pathogen disgust sensitivity should be lower. People in wealthy, developed nations currently live in conditions where ancestral, soil-transmitted disease exposure is reduced and pathogen avoidance is feasible at relatively low cost. Greater disgust sensitivity, and the low-cost means of avoidance, appear to have contributed to the evolutionarily unprecedented hygienic environments in developed nations. This dissertation tests these relationships by exploring the role of disgust in pathogen avoidance among a single indigenous population exhibiting different levels of participation in traditional subsistence and market-based activities and lifestyles.

While pathogen disgust sensitivity may have been beneficial for traditionally living groups of humans and human ancestors with limited means of preventing infection and avoiding infectious agents, it has been suggested that economically developed regions and nations have become reliant on cleanliness and hygiene, creating relatively sterile environments devoid of parasites (Strachen, 1989). It is important to emphasize how recent this phenomenon is even in wealthy, developed nations. As recently as the mid-20th century, hookworm infection was common in the United States (CDC, 2015). In 1947 it was estimated that about 36% of the population of Europe was infected with helminths (Stoll, 1947). Thus, even in the United States and Western Europe, the transition to low or absent parasite infection is very recent. This may be creating one of the most important mismatches between our evolved biology/psychology and our current conditions. High levels of hygiene and sanitation in developed nations have made parasite exposure rare and have led to altered microbial diversity. Changes in pathogen exposure may be associated with the rise in disorders of immune dysregulation and inflammation, like allergy and autoimmunity (Blaser & Falkow, 2009; Elliott et al., 1999; Elliott et al., 2000; Flohr et al., 2008; Hurtado et al., 2008; Strachen, 1989).

Epidemiological Transitions

Humans are currently facing evolutionarily novel types of infectious diseases. Although overly simplistic, this idea is best highlighted by a brief discussion of epidemiological transitions (Omran, 1971). Epidemiological transitions are alterations in health and disease that have occurred (pre)historically and continue to occur to this day in association with subsistence, lifestyle, and population levels changes (Omran, 1971). The

suggested "first" of these transitions occurred between 14,000 and 10,000 years ago (Pollard, 2008), when the archaeological record shows that some groups of humans began switching to food production (agriculture and animal domestication) for subsistence.[2] With this transition, came permanent settlements with storage, food production and the ability to support large numbers of people. The idea that food production is *needed* for permanent settlements, however, is outdated (Watkins, 2010). There is evidence of large, permanent communities of hunter-gatherers in southwest Asia that date to about 25,000 years ago (Nadel & Hershkovitz, 1991; Watkins, 2010; Weiss et al., 2004), well before the documented origin of agriculture. Nevertheless, agriculture and animal domestication were tied to a notable increase in permanent settlements and population numbers, changing the types of diseases afflicting humans, as well as their virulence (Cohen & Armelagos, 2013; Omran, 1971: Pollard, 2008).

A number of factors associated with permanent or semi-permanent settlements resulted in changes in disease exposure, including the need for different measures of sanitation, close proximity to large groups of animals, and manmade alterations to the environment. Sanitation in permanent settlements is harder to maintain, especially as population sizes and densities increased. With pastoralism and animal domestication, came herd diseases in livestock which could mutate to infect humans (Service, 1991). Deforestation, which also accompanies agriculture and permanent settlements, results in increased morbidity and mortality related to parasitic diseases (Patz et al., 2000) by altering vector habitats. Communicable diseases could evolve greater virulence,

[2] It is important to note, here, that not all human groups followed this same pathway. Many populations remained semi-isolated, nomadic hunter-gatherers for thousands of years after agriculture crops up in the archaeological record. Further, handfuls of populations around the world have continued to live these lifestyles until the present day.

spreading between more people, either directly or through contaminated meat, soil and water, or by vector.

A second major epidemiological transition occurred leading up to the 19th century, with the Industrial Revolution (Omran, 1974). This transition resulted in increased urbanization and population densities that far surpassed those of most prior human settlements (Omran, 1974; Pollard, 2008). With increased population densities, greater levels of socioeconomic inequality, and environmental change, came an increased risk for a number of viral and bacterial diseases (Patz et al., 2000; Rook et al., 2014; Wolfe et al., 2007). These new[3] "crowd infections" could be extremely virulent and highly infectious because of an increase in availability and diversity of possible hosts.

Because these receding pandemics (Omran, 1971) are relatively recent developments in human evolutionary time, it seems unlikely that the human immune system coevolved with them in the same way it did with STHs and other early symbionts (Rook et al., 2014). While they have played a number of selective roles observed in the human genome, epidemiological evidence suggests that these "crowd infections" are not associated with decreased risk of allergic and autoimmune disorders (Amre et al., 2006; Benn et al., 2004; Bernstein et al., 2006; Bremner et al., 2008; Cardwell et al., 2008; Dunder et al., 2007; Koloski et al., 2008) and sometimes trigger them (Yoo et al., 2007). This relative change in infectious disease exposure may be contributing to what Omran

[3] "New" refers to diseases that became more likely to spread over the last 10,000 years. Although there is evidence that humans have evolved in response to "new" diseases in less than 10,000 years (i.e. CCR5-delta32 mutation [de Silva & Stumpf, 2005], sickle cell mutation [Kwiatkowski, 2005], etc.), these diseases are called "new" because they were likely not as much of a selective force on the immune system during its earliest development (Rook, 2014).

(1971) called the final epidemiological transition: the emergence of chronic, non-communicable diseases.[4]

While these epidemiological transitions are not as universally experienced as Omran originally suggested (1971), they provide important insights into what many people in economically developing nations are currently experiencing. For instance, in less economically developed nations, these shifts from small-scale subsistence to developed, urban populations is occurring extremely rapidly, on the order of years or decades rather than centuries or millennia (Popkin, 2004). Because of the speed of these transitions, we are seeing a very rapid increase in chronic, non-communicable diseases in areas where infectious, parasitic diseases are still common. Many populations in economically developing nations face a double burden of both chronic and infectious diseases (Barrett et al., 1998; Gurven et al., 2009; Prentice, 2006). For example, in some of these regions, infectious disease mortality increased by 40% between 1980 and 1992, a period that brought rapid economic change to many countries (Barrett et al., 1998). So while human groups experienced semi-gradual change in subsistence and disease risk over the course of centuries or even millennia, many populations are experiencing rapid social, economic, and lifestyle changes resulting in a double burden of both infectious and chronic diseases.

[4] This was the last of the epidemiological transitions discussed by Omran in 1971. Since then, other transitions have been discussed, including the re-emergence and drug-resistance of pathogens due to overuse of antibiotics and population change (Barrett et al., 1998) and the double burden of both infectious and chronic diseases observed in a number of developing countries (Barrett et al., 1998; Gurven et al., 2009; Prentice, 2006). More optimistic thinkers like to add a future transition based on behavioral change to fix the problems associated with chronic non-communicable diseases (Popkin, 2002).

Evolutionary Medicine and Market Integration

Evolutionary medicine is the application of modern evolutionary theory to better understand health and disease, focusing on why we are vulnerable to disease. Evolutionary medicine provides a framework for understanding a number of issues related to health, including which symptoms of disease are the products of our immune system's defense mechanisms, which are byproducts, and which are functional effects of pathogens (Williams and Nesse, 1991; Ewald, 1993). Knowledge of the underlying cause of symptomatology is crucial for understanding the costs and benefits of treatment. Evolutionary medicine has also been useful for identifying certain hallmarks or outcomes of infectious disease, previously thought to be non-infectious, such as certain cancers and cardiovascular disease (CVD; Ewald, 2002), as well as explaining a number of the ecological and demographic pressures associated with disease virulence (Ewald, 1993). It has also helped to explain a number of tradeoffs and constraints that lead to features of the human body, including some of the anatomical and developmental compromises we face as bipedal primates with quadrupedal origins. Williams and Nesse (1991) provided the first comprehensive framework and description of evolutionary medicine; the publication of their *Quarterly Review of Biology* article is generally credited as the beginning of the field.

Evolutionary medicine provides a powerful framework for examining the emergence of chronic, non-communicable diseases, especially when looking at changes in health over recent decades. In the simplest terms, this framework for understanding these changes in global health points to the mismatch between evolved human biology associated with small-scale subsistence lifestyles and the current environments in

wealthy, developed nations (Eaton & Konner, 1985; Eaton et al., 1988). It is important to note that there was no single hunter-gatherer or small-scale subsistence way of life in human evolutionary history. Also, many groups continued to live fairly isolated, extremely variable foraging-based lifestyles until fairly recently and some even continue to this day (Kelly, 2013). The major point remains, however, that a number of social, behavioral, and environmental changes have occurred in developed nations and even more rapidly among populations that are currently undergoing the transition away from more traditional lifestyles to more market-based economies.

A number of non-communicable diseases observed in wealthy developed nations today, including obesity (Baker et al., 2008; Power, 2012), CVD (Wick et al., 2001; Dressler, 1995), T2D (Neel, 1962; Neel et al., 1998), osteoporosis (Karasik, 2008; Madimenos et al., 2011, 2012; Stini, 1995), and chronic inflammatory diseases (Straub & Besedovsky, 2003; Straub et al., 2007), are likely associated with adaptations to our ancestral environment that are no longer useful. What were once adaptive traits may have become detrimental in the context of radically different environments and lifestyles (Nesse & Williams, 1999). This may be why chronic diseases are increasing at profound rates in developing nations where both chronic, non-communicable diseases and infectious diseases coexist (Barret et al., 1998; Daar et al., 2007; Nugent et al., 2011; Reddy & Yusuf, 1998; World Bank, 2005).

According to the World Health Organization, 9% of the global population over 18 years of age had type 2 diabetes (T2D) in 2014 (WHO, 2014a) while 1.5 million people died from complications related directly to T2D in 2012 (WHO, 2014b). Importantly, more than 80% of these deaths occur in low- and middle-income countries (WHO,

2014b). The prevalence of T2D among children is also on the rise. In the United States, prevalence of T2D among children increased by 30.5% between 2001 and 2009, (Dabelea et al., 2014). The number of individuals with diabetes is expected to increase by 21.8% between 2013 and 2035 in the United States, while increases of 60.4%, 108.1%, 114.5% and 138.5% are expected in Ecuador, Guatemala, Bangladesh and Ethiopia, respectively, during that same 22 year period (Guariguata et al., 2014).

Cardiovascular diseases are currently the leading cause of death globally and are also on the rise. As of 2005, more than 64 million people living in the United States were afflicted with one or more types of CVD (Cordain et al., 2005), and the prevalence is particularly high among immigrants and minorities in economically developed nations (Awuah et al., 2014; Sorlie et al., 2014). Multiple studies have documented an increase in CVD in populations in developing countries undergoing rapid economic development (Bindon et al., 1997; Chin-Hong & McGarvey, 1996; Dressler & Dos Santos, 2000; Dressler et al., 1987; 1993; Henry & Cassel, 1969; Liebert et al., 2013; McGarvey & Schendel, 1986; Pavan et al., 1999; Snodgrass et al., 2006). Over three quarters of deaths that occur because of CVD are occurring in low- and middle-income countries (WHO, 2015b).

The reason for the increase in chronic diseases in economically developed and developing countries appears to be closely associated with social, economic, and lifestyle changes. This phenomenon can be measured in economically developing countries as the extent of market integration (MI), defined as the suite of social and cultural changes associated with production for and consumption from market-based economies. With more market integrated lifestyles come changes in diet (Eaton, 2006; Eaton & Konner,

1985; Wallace, 2010), physical activity patterns (Cordain et al., 1998; Eaton & Eaton, 2003), socioeconomic disparities (Bindon et al., 1997; Dressler, 1991; 2004; Sorensen et al., 2009), and psychosocial stress (Bindon et al., 1997; Dressler, 1991; 2004; Sorensen et al., 2009). These factors have all been linked to a number of negative health outcomes. For example, changes in diet and physical activity associated with MI have been associated with obesity, T2D, and CVD risk (Leonard, 2009; Wells, 2006; 2009).

This mismatch is based on a number of complex factors, but links have been drawn between these diseases and an energy imbalance associated with over-consumption of calorically dense foods, like saturated fats and refined sugars (Leonard et al., 2008; Martorell, 2002; Pollard, 2008), combined with decreased caloric expenditure from physical activity (Cordain et al., 1998; Gurven et al., 2009; Leonard et al., 2009; Snodgrass et al., 2006). It is hypothesized that physical activity has declined in recent years with the adoption of more sedentary lifestyles (Martorell, 2002), though the change in physical activity with MI appears to have been only moderate (Cordain et al., 1998; Dufour and Piperata, 2008; Eaton & Eaton, 2003; Madimenos et al., 2011b). Thus, diet is likely the main culprit associated with these health changes. It takes only a 2% discrepancy in energy balance to produce obesity in children (Martorell, 2002) and negative health risks are associated with only a small increase in body weight (WHO, 2000), though recent research suggests that being slightly overweight may decrease mortality risk (e.g. Flegal et al., 2013).

Greater discrepancies in socioeconomic status, especially in developed countries, has led to an increase in chronic diseases due to disparities in access to resources and medical care, as well as increased psychosocial stress (Godoy & Cardenas, 2000; Godoy

et al., 2005a; 2005b). Experiencing psychosocial stress in relation to social rank is common among a number of social animals, including non-human primates (Sapolsky, 2004), so this is not unique to humans. The degree of socioeconomic disparities in these developed and developing nations, however, is on a much greater scale than rank differences within social animals. Additionally, hunter-gatherer groups are hypothesized to experience less psychosocial stress due to their more egalitarian societies (Descola, 1994; 1996; Jenny, 2012; Sapolsky, 2004).

The psychosocial stress response, much like the inflammatory response of the immune system, is useful in acute situations where it signals danger. In developed and developing nations, chronic mobilization of energy toward the psychosocial stress response results in altered cortisol (a stress hormone) levels, as well as downstream regulation of immune function in order to devote more energy to immediate survival (McDade et al., 2001; Sorensen et al., 2009). This can lead to intestinal inflammatory problems, like peptic ulcers, ulcerative colitis, and irritable bowel disorder as the adaptive immune system is turned down and certain bacteria that are usually kept in check, like *Helicobacter pylori*, can flourish (Cryan & O'Mahony, 2011; Mawdsley & Rampton, 2005; Sapolsky, 2004). This also leads to decreases in growth, tissue repair, and reproductive function (Sapolsky, 2004), as well as an increased risk of cardiovascular disease (Bindon et al., 1997). Factors that seem to cause chronic psychosocial stress are associated with having no predictability or control over one's life, having no outlets for frustration or social support, and interpreting a stressor as worse than it actually is (Sapolsky, 2004). All of these factors that cause psychosocial stress are associated with

lower socioeconomic statuses in developed and developing nations (Bindon et al., 1997; Chin-Hong & McGarvey, 1996).

Immune System Dysregulation and Inflammation

A common link between obesity, CVD, autoimmunity, and allergy is sub-clinically elevated levels of chronic inflammation (Kumar et al., 2014; Renz et al., 2011). As I will discuss in more detail in Chapter II, inflammation is the immune system's first line of defense and is used to create a barrier around an injury or pathogen to prevent spreading and allow the pathogen to be marked for removal. If inflammation becomes chronic (i.e. lasting for weeks or months; McDade et al., 2012), however, tissue and cell damage can result (Licastro et al., 2005; Renz et al., 2011).

Chronic inflammatory disorders, like asthma, autoimmune diseases, allergies, CVD and some cancers (Kumar et al., 2014; Ungefroren et al., 2011) have been increasing rapidly in recent years in the United States and other developed nations (AAFA, 2012; AARDA & NCAPG, 2011; Eder et al., 2006; Renz et al., 2011; Tobias, 2010). This dissertation focuses on disorders related to immune system hypersensitivity and dysregulation, specifically allergy and autoimmune disorders, which occur when the immune system loses its ability to differentiate between harmless and self-produced antigens, respectively (Clark, 2008; Huether & McCance, 2013). Autoimmune disorders consist of over 80 different diseases, including irritable bowel disorders, multiple sclerosis, rheumatoid arthritis, autoimmune thyroid disorder, T1D, and celiac disease. In these diseases, the immune system becomes unable to differentiate between self-produced antigens and those of pathogenic origin. This causes the immune system to

react to self-cells in the same way it would react to infectious disease cells that could cause the body harm. This response ultimately results in destruction of the specific self-produced cells that are targeted.

Allergies occur when the immune system responds to harmless external antigens (allergens) like dust, pollen, and certain foods as it would a pathogen (Clark, 2008). They activate the Th2 pathway and result in histamine production and increased inflammation (discussed in more detail in Chapter II). Receptors that activate allergic responses are in mucus membranes and skin, thus increasing inflammation in these areas (Clark, 2008). Allergic symptoms range from minor irritation of the skin or mucus membranes to swelling of the airways and possible death (Clark, 2008).

Autoimmune disorders and allergies in the United States, Western Europe, and other developed nations have been increasing rapidly (AAFA, 2012; AARDA & NCAPG, 2011; Eder et al., 2006; Renz et al., 2011; Tobias, 2010). Fifty million people in the United States (1 in 5) have some type of allergy and we have seen a very rapid increase within the last 30 years (Flohr et al., 2008; Tobias et al., 2010). Allergic diseases are extremely rare in developing countries (Flohr et al., 2008). Autoimmune disorders currently affect 8% of the population of the United States, 78% of whom are women, and these numbers are increasing annually (Flohr et al., 2008). Specifically, 201 of every 100,000 adults in the United States have Crohn's Disease, a type of irritable bowel disorder, while 238 of every 100,000 adults suffer from ulcerative colitis (Kappelman et al., 2007). In 2003, 1 in 133 Americans were estimated to have celiac disease, an autoimmune disorder in the small intestine that leads to the immune system attacking gluten protein and resulting in an inflammatory response (Fasano et al., 2003). Recent

data suggest that celiac disease is four times more common now than it was 60 years ago, and affects 1 in 100 people in the United States (Rubio-Tapia & Murray, 2010).

The global distribution of autoimmune disorders leads to some interesting questions. For instance, 16 out of every 100,000 children in the United States, Canada, Western Europe, and Australia suffer from T1D, an inflammatory autoimmune disease of the pancreas (Bach & Chatenoud, 2012). In countries where individuals live more traditional lifestyles, T1D is virtually absent (Bach and Chatenoud, 2012), leading researchers to ask: why are allergy and autoimmune disorders occurring disproportionately in more market-integrated, economically developed countries? One reason may be that these disorders are related to chronic inflammation and immune dysregulation associated with altered infectious disease exposure (Renz et al., 2011; von Ehrenstein et al., 2000; von Mutius, 2007).

The Hygiene and Old Friends Hypotheses

An inverse relationship between infectious diseases and disorders related to immune dysregulation was first suggested by Greenwood, who observed a low prevalence of the autoimmune disorder rheumatoid arthritis in Nigeria, where parasites were present (Greenwood, 1968). In 1989, Strachen proposed the Hygiene Hypothesis based on observations that younger children from larger families were less likely to experience hay fever than those from small families (Strachen, 1989). Strachen hypothesized that children in larger families were exposed to more childhood diseases from unhygienic contact with older siblings, which decreased their likelihood of developing atopic disorders like hay fever, allergic rhinitis, and eczema.

Von Mutius and colleagues (1994a) compared children living in East and West Germany just after reunification to see which area had higher rates of allergy and respiratory disorders. They documented marked differences in sensitization between the two groups. Specifically, children living in what had been West Germany were more sensitive to aeroallergens (allergens that travel by air) and had a greater prevalence of asthma and hay fever (von Mutius et al., 1994a). This research led to questions regarding why children in the more economically developed West Germany would have greater risk for allergy. Originally, this was thought to be associated with environmental pollution (von Mutius et al., 1995), but further research demonstrated that while this did trigger acute allergic responses, it was not responsible for the development of the allergic phenotype seen in more economically developed regions (von Mutius, 2000).

Further research explored the effects of family size and exposure to other children on the development of allergy. Von Mutius and colleagues (1994b) found a decreased skin-prick sensitivity, indicative of lower risk of allergic susceptibility, in children with more siblings. Children who spent large portions of time in daycares, also had decreased risk of allergies (Matricardi et al., 1998; Haby et al., 2000). Additionally, studies comparing urban lifestyles to rural, agricultural environments proved fruitful, finding increased prevalence of allergic disorders in children living in more urban environments and decreased prevalence in rural, agricultural environments (Radon et al., 2004; von Ehrenstein et al., 2000; von Mutius, 2007). Children who were exposed to farming early in their lives, exposed to domesticated animal houses (farms harboring horses, cows, pigs) regularly before the age of 7, and consumed unpasteurized milk in their childhood were less likely to develop disorders associated with immune dysregulation (Radon et al.,

2004). Clearly, there had to be a common link between rural farm environments, large families, and constant exposure to other children that was responsible for decreasing risk of immune dysfunction.

Evidence suggests that increased prevalence of allergy and autoimmunity is related to changes in infectious disease exposure (von Mutius et al., 2000). Allergic diseases are rare in areas where helminth exposure is common, and common in areas where helminth exposure is reduced or absent (Flohr et al., 2008). Von Mutius and colleagues (2000) found that children exposed to *Mycobacterium tuberculosis* were at a reduced risk of developing asthma. Another study found that when areas in Venezuela and Gabon were systematically treated for helminths, incidence of allergic disorders increased (Lynch et al., 1993).

Multiple hypotheses have been proposed to explain the link between altered exposure to infectious agents and the development of chronic inflammation and immune dysregulation seen in developed nations: the HH, the Biodiversity Hypothesis, the Disappearing Microbiota Hypothesis, and the Old Friends Hypothesis. While these hypotheses do have some differences, they converge in attempting to explain the variation in chronic inflammatory diseases that we see with altered environmental exposure. In Chapter II, I will discuss the immune mechanisms that may be responsible for these relationships, but here I focus on the common stimuli driving these relationships.

The Biodiversity Hypothesis emphasizes the loss of biodiversity and climate change as catalysts for altered disease exposure (Haahtela et al., 2013; Hanski et al., 2012). The difference between the Biodiversity Hypothesis and the Hygiene Hypothesis

is that the former focuses specifically on environmental changes regardless of their origins in human behavior (e.g. increased sanitation and hygiene). Studies that test the Biodiversity Hypothesis focus on altered exposure to forests and green places, and a number of other factors related to a loss of biodiverse environments (Haahtela et al., 2013; Hanski et al., 2012). Hanski and colleagues (2012) suggest that the lack of exposure to a specific bacteria called Gammaproteobacteria, which thrive in soil, natural waters, and on plants, may contribute to immune dysregulation associated with decreased anti-inflammatory cytokine interleukin (IL)-10. These environmental effects have been linked to T1D (Bodansky et al., 1992), asthma (Leung, 1996; Rosenberg et al., 1999), and allergies (Kalyoncu & Stålenheim, 1992) in immigrants to wealthy, developed nations.

The Biodiversity Hypothesis is helpful in explaining why immigrants who move from less affluent to more affluent regions develop symptoms related to immune dysregulation despite having developed their immune systems in more rural, less MI regions. If someone is exposed to these gammaproteobacteria throughout their life in more diverse environments and then move to regions where they do not encounter these bacteria, then the immune system might become dysregulated and overreact (Hanski et al., 2012). Relatively recently after moving to urban, more MI regions, immigrants studied acquired allergies and autoimmune disorders (Haahtela et al., 2013). These studies have observed Ethiopian immigrants to Israel (Rosenberg et al., 1999), immigrants from a number of countries who moved to Sweden (Kalyoncu & Stålenheim, 1992), and children who have moved from Turkey to Germany (Grüber et al., 2002), among others (Haahtela et al., 2013). The development of inflammatory disorders among immigrants seems to depend on a number of factors, including age at immigration and

type of disease observed, as most research suggests that U.S. born children are much more likely to develop allergies than children who emigrate to the U.S. after being born elsewhere (Silverberg et al., 2013). However, it should be noted that children of immigrants are just as likely as the host population to develop T1D and multiple sclerosis (Bodansky et al., 1992; Hammond et al., 2000).

The Biodiversity Hypothesis raises interesting ideas regarding why adults develop allergies and autoimmune disorders in new environments. This hypothesis can be examined in the broader sense, as being an offshoot of the Disappearing Microbiota Hypothesis (DMH). The Biodiversity Hypothesis and the DMH have many similarities. However, while the Biodiversity Hypothesis focuses on how we acquire and contact certain bacteria and molecular products in biodiverse environments (Haahtela et al., 2013; Hanski et al., 2012), the DMH suggests that recent changes in human ecology, based on behavioral, lifestyle or environmental change, may be altering the composition of our intestinal bacteria (i.e., our microbiota), thereby reducing vital immune stimulation and programming (Blaser and Falkow, 2009). The DMH is similar to the Old Friends Hypothesis, a broader approach that includes not only microbes, but helminths, protists, and ectoparasites[5] that have coevolved with humans.

To date, altered infectious disease exposure has been linked to many chronic, non-communicable illnesses. A number of viral and bacterial infections have been found that increase the risk for developing certain allergies and autoimmunity. One study found that not only did asthmatics have less diverse bacterial profiles, but 33% of infants that were

[5] Protists or protozoa are single-celled or colonial eukaryotic parasites that can live in a number of niches within the human body (e.g. *Plasmodium falciparum* [malaria], amoebas; Bogitsh et al., 2005). Ectoparasites are multicellular animals that live on the skin or hair (e.g. head, body or pubic lice, fleas; Bogitsh et al., 2005).

found with a pathogenic, non-commensal bacteria had asthma five years later, while only 10% of infants without these pathogenic species developed asthma (Bisgaard et al., 2007). It seems that not all infectious agents are responsible for immune regulation and priming. Ultimately, it may have been those diseases that were able to infect small, nomadic hunter-gatherer groups early in human evolution (e.g. STHs, certain mutual or commensal bacteria), which are still able to turn down inflammation as a way to evade eradication and survive within the hostile digestive tract, that are responsible for the prevention of allergy and autoimmunity. For this reason, there has been a shift away from using the term Hygiene Hypothesis with preference for the Old Friends Hypothesis.

Researchers have observed inverse relationships between STH infection and the development of allergies and autoimmunity. In a study of 1601 schoolchildren aged 6 to 18 in Vietnam, researchers found that individuals who had higher intensity hookworm infection and *Ascaris lumbricoides* infection had reduced skin sensitization to dust mites (Flohr et al., 2006). Individuals using flush toilets rather than no toilet, bush toilets, and pit toilets had increased sensitization (Flohr et al., 2006). If exposure is only important during development, there should be little to no effect on chronic disease burden when STH burden is altered throughout a lifetime. This does not seem to be the case. Studies in an urban "slum" in Venezuela found an increase in the prevalence of allergic disorders in children treated with systemic anthelminthic medication (Lynch et al., 1993). After anthelminthic treatment, children were observed for 22 months, during which helminths were effectively eliminated and serum levels of immunoglobulin E (IgE) and interleukin (IL)-4 significantly decreased. During this time, skin prick sensitivity to allergens increased, as well as levels of specific IgE against environmental allergens (Lynch et al.,

1993). This study provides important support for the role STHs on immune system regulation, and suggests that their presence is important at least through out childhood and not just during the perinatal period, infancy, or very early childhood.

More evidence that STH infection is important throughout the life course, rather than strictly during immune system development, comes from laboratory studies and clinical interventions. Research shows that helminth infections are able to decrease risk and symptomatology of chronic diseases even into adulthood. A mouse-model demonstrated that diet-induced obese mice treated with lacto-N-fucopentaose II (LNFPIII; a glycan found in both human milk and parasitic helminths) improved both glucose tolerance and insulin sensitivity, partly because of an increased production of anti-inflammatory IL-10 (Bhargava et al., 2012). They hypothesized that the presence of helminths turned down inflammation, which is usually increased by obesity (Bhargava et al., 2012). Anti-inflammatory IL-10 is produced in response to STHs when the T helper 2 (Th2) pathway of the immune system is activated in humans. These findings suggest that STHs and their stimulation of IL-10 production could improve glucose tolerance and insulin sensitivity in humans (Bhargava et al., 2012; both associated with the chronic disease T2D and inflammation related to obesity).

In another study examining the role of parasites in immune regulation, mice were exposed to *Schistosoma mansoni* (a trematode) eggs and then treated with trinitrobenzesulfonic acid to induce colitis (Elliott et al., 2004). They found that egg exposure increased production of anti-inflammatory IL-10, suggesting that helminth infection decreased colonic inflammation. Other studies present similar findings between

S. mansoni and autoimmune thyroid disorder (Nayama et al., 2004), T1D (Zaccone et al., 2003), and colonic inflammation (Moreels et al., 2004) in rodent models.

These relationships between parasites and chronic inflammatory diseases carry over to humans as well. A 4.6 year follow-up study of Argentinian adults with multiple sclerosis found that individuals that were infected with intestinal parasites showed fewer symptoms of disease, had no increase of disability from multiple sclerosis, and less evidence of deterioration than individuals that were not infected with parasites (Correale & Farez, 2007). STHs, specifically, appear to play a large role in this process in humans. One such study treated Crohn's diseases patients with hookworm infection (*Necator americanus*), measuring the time it took to observe results (Croese et al., 2006). Infection took 20 weeks to be fully established, after which the patients that had longstanding but inactive Crohn's diseases were in full remission by week 45. Another study of individuals suffering from Crohn's disease were intentionally treated with STH *Trichuris suis* (pig whipworm) every 3 weeks for a 6 month period. Symptoms improved for 72% of the treated patients over this time period (Summers et al., 2005).

In this dissertation, I focus on the Old Friend Hypothesis, specifically examining STHs. Further, I use both the Hygiene Hypothesis and the Biodiversity Hypothesis to explain behavioral, lifestyle, and environmental changes that are altering STH exposure and immune function. This research was carried out among the Shuar of Amazonian Ecuador, whose rapid but variable integration into regional market economies provides a crucial range of lifestyles and disease exposure patterns necessary for testing the relationships between social and economic change, STH exposure, and immune dysregulation.

Study Population: The Shuar

The Shuar are a large indigenous forager-horticulturalist group distributed across over 668 communities in the Cross-Cutucú and Upano Valley regions of the Morona-Santiago and Zamora provinces of Ecuador (CODENPE, 2011). Traditionally, Shuar lived in households scattered across the Paute and Upano River Valley, where they were generally concentrated in the area between the eastern Andean foothills and the Cordeillera de Cutucú. By the 1890s, Shuar began spreading east over the Cutucú mountain range into Achuar territory, and now live on both sides of the Cutucú, and throughout the Upano River Valley (Harner, 1984; Rubenstein, 2001; Stirling, 1938). Population estimates for Ecuadorian Shuar vary from 50,000 to 110,000, but census data for indigenous groups around the world generally underestimate population size (Madimenos, 2011).

Traditional Shuar Subsistence and Health

Shuar subsistence traditionally consisted of blowgun and spear hunting, fishing, and slash and burn horticulture (Descola, 1994; 1996; Harner, 1984; Karsten, 1935; Stirling 1938). Contemporary Shuar living more traditional[6] lifestyles still subsist on hunting, fishing, and horticulture. Fishing is done using hooks and lines, fish poisons, baskets, spears, or by hand. Interestingly, fishing remains an important point of subsistence in the Cross-Cutucú region. Recent interviews conducted by the Shuar Health and Life History Project report declines in fishing yields in the Upano Valley, however,

[6] "Traditional" is used here in reference to reports from Spanish chronicles in the 16th century, early-mid 20th century references, as well as reported historic practices according to the Shuar and as published in the Abya Yala, Mundo Shuar collection compiled by Shuar, missionaries, volunteers and anthropologists from 1975-1983.

making fishing less of a staple and more of a supplemental activity for this region (Madimenos, 2011).

In regions that have not previously been overhunted, hunted game is also an important source of protein (Madimenos, 2011). This is mostly true of the Cross-Cutucú region, where collared peccary, red brocket deer, multiple species of monkey (**Figure 1.4**), tapir, paca, and nine-banded armadillo are the most commonly hunted animals (Zapata-Rios et al., 2009). Other hunted game includes capuchin, coati, and Northern Amazonian red squirrel. Palm grubs and other insects are also used as protein sources. For individuals living in the Upano Valley, hunting has become increasingly difficult and requires at least a six hour walk through the forest. In forested areas near communities, hunting is unproductive (Madimenos, 2011). In these regions, small scale animal-husbandry is more productive as a supplemental protein source.

Figure 1.4. Image of a spider monkey (*Ateles* sp.) taken by the author at a wildlife refuge in a community just outside Sucúa in the Upano Valley.

Shuar households typically have one or more large gardens (*fincas or huertas*; **Figure 1.5**) with staggered planting seasons and intercropping (Descola, 1994). Traditionally, gardens provided the primary source of carbohydrates for Shuar families, and up to 65% or more of dietary calories (Harner, 1984). Staple foods grown in these gardens include manioc, sweet potato, taro roots, and peanuts, as well as non-tuberous food plants like plantains, bananas, and maize. Currently, manioc and plantains comprise the majority of the starches consumed. Other crops grown in Shuar gardens can include oranges, squash, onions, papaya, sugar cane, achiote, chiles, and non-edible plants used for a variety of purposes including fishing, poisons, and medicines. Over 670 documented plant species are utilized across Shuar territory (with regional variation) and 97 of those are non-cultivated, wild growing species (Bennett, 1992). A large number of plants are used for medicinal purposes.

Figure 1.5. Shuar garden in the Upano Valley. Photo taken by the author of this dissertation.

Traditionally, and continuing today in fairly isolated groups, the Shuar would have been in regular contact with soil. For example, manioc and other root crops are dug by loosening the soil with a digging stick or machete and then excavating the soil by hand. Women primarily dig up these tubers, while men dig for barbasco roots used to poison streams for fishing, as well as earth worms for bait. Presence of domesticated animals, particularly hunting and guard dogs, and the use of 'night soil' (human excrements) for fertilizer would have increased rates of soil contamination. This means that the Shuar likely have had fairly continual exposure to STHs. Historically, the Shuar would have relied on interaction with soil for food and medicine. Disease burden and mortality among traditional Shuar communities would have generally been based on infectious diseases, malnutrition, and violent or traumatic injury.

Shuar traditionally believed that diseases were caused by *tsentsak* or "magical blowgun darts" sent by *uwishin* (shaman; Descola, 1996). Tsentsak are believed to have their own spirit and desire to cause harm, so *uwishin* must learn to control them. A rogue *tsentsak* could cause illness to unintended victims as well (Descola, 1996; Incayawar et al., 2009). To cure someone who is ill, the *uwishin* invokes a ritual in which he sucks out the bad spirits of the *tsentsak* and vomits them up. These rituals involve taking a hallucinogenic substance that purges the body by causing vomiting which may also help with purging parasites and other pathogens that cause intestinal illness.

Besides shamanism Shuar culture has a rich ethno-pharmacological array of plants available to combat diseases. Ethnobotanical research compiled a list of at least 120 different wild plant species used by Shuar, of which 16% are used specifically for medicine (Pohle & Reinhardt, 2004). Further, studies of Shuar gardens found a total of

185 wild and cultivated plant species, of which 22% are used for medicine (Pohle & Reinhardt, 2004). Pohle and Reinhardt (2004) documented medicinal uses for wild plant species[7], including diarrhea (*Costus spicatus* [sap of stem from Caña Agria]*; Ficus gomelleira Kunth* [sap of bark from fig tree]*; Piper* species [sap of stem from pepper plant shrub]), intestinal parasites (*F. gomelleira Kunth* [sap of bark from fig Tree]*; Piper stileferum Yunck* [sap of roots from herb]), fever (*Costus spicatus* [sap of stem from Caña Agria]*;Piper cuspidiscum* [leaves of a shrub]), stomach ache (*Piper stileferum Yunck* [sap of roots from herb], *Uncaria tomentosa* [bark, stem and roots from "cat's claw" climber), influenza (*Physalis peruviana* [grapes], *Solanum americanum* [infusion from leaves of the Mortin Shrub]), head aches (*Piper cuspidiscum* [leaves of a shrub]), and immune system strengthening (*Mansoa* spp. [Leaves of wild garlic], *Uncaria tomentosa* [bark, stem and roots from "cat's claw" climber]). Medicinal materials from these plants come largely from the sap, leaves, fruit and bark. Antiparasitic medicines come from certain *Araceae* plant species, like the flowering plant *Anthurium alienatum*. When the fruit from *A. alienatum* are crushed and placed on the skin they are used to kill burrowing insect larvae (Bennett, 1992). Tea can be made from Ericacceae species like *Sphyrospermum buxifolium*, a shrub used to treat stomach aches (Bennett, 1992). Seeds from *Fevillea cordifolia*, often called Javillo or Antidote Cocoon, are used to make medicines to treat internal parasites (Bennett, 1992). Knowledge of this pharmacopeia varied across individuals traditionally, and is maintained by some Shuar to varying degrees. Informants report declining knowledge and use as western medical access becomes available (Sugiyama, personal communication).

[7] Written as *species name* [part used and common name]

Ethnobotanical data suggests Shuar have multiple traditional ways of treating parasite infection (Bennett, 1992; Pohle & Reinhardt, 2004). This could be interpreted as a way of keeping parasite load low even though reinfection is common. Currently, Shuar in the Upano Valley and Cross-Cutucú regions are generally familiar with intestinal infection by "*bichos*" (bugs) and "*gusanos*" (worms), and their role in intestinal discomfort, nausea, and diarrhea, among other symptoms. Shuar participants often report in interviews that they or their children have "*bichos*" or "g*usanos*," and often ask for medicinal ways of treatment (unpublished data). In interviews, a handful of adults have reported seeing worms in their own or their children's feces (unpublished data), which commonly happens with tapeworm segments, as well as in the passing of adult *A. lumbricoides.* Others report themselves or their children coughing up worms, or having a worm crawl out of their child's nose. For instance, one family reported vomiting or coughing up worms after eating "sweets" (probably papaya). This is suggestive of heavy *A. lumbricoides* infection, which causes parasitic pneumonia, coughing and vomiting when the worm larvae travel up the lungs to be re-swallowed and mature into adult worms in the intestines.

Multiple lines of evidence suggest that the environments inhabited by Shuar in the Cross-Cutucú and Upano Valley regions are both highly parasitized. Individuals living in tropical regions, like the Shuar, are said to inhabit NTD "hot spots" (Hotez et al., 2008). For instance, in the Amazon, the weather is hot and humid and skin is often exposed, leading to increased risk of bug bites and vector-borne illnesses. Running barefoot increases rates of hookworm infection. Large bodies of stagnant water allow mosquitos and mosquito-borne diseases to proliferate (Hotez et al., 2008).

There is also a large amount of social and economic disparity in the region. Among the 556 million individuals living in Latin America and the Caribbean, 40% of these individuals live below the poverty line, with 47 million people living on less than $1 USD per day and 74 million more who live on less than $2 USD per day (Hotez et al., 2008). On top of this, rural poverty disproportionately affects indigenous groups, like the Shuar, in the Latin American and Caribbean regions of the world, making exposure more likely due to economic marginalization and making treatment difficult and often unavailable (Hotez et al., 2008). Poverty not only increases rates of infectious disease exposure, but chronic non-communicable diseases as well.

As noted, the Amazon basin and Upper Amazon is home to a number of parasitic and infectious diseases, and intense human migrations and increasing population density may be exacerbating the spread of these diseases. Social, economic, and lifestyle changes occurring among the Shuar as they transition from more traditional subsistence activities to more market-based economies make STH load among the Shuar incredibly important to study in the context of this increasing market integration.

The relationship between social and cultural changes associated with MI and exposure to soil transmitted helminths is complex, yet understudied. In some cases, side effects of MI, like increased consumption of processed food and altered sanitation practices are associated with decreased exposure to certain pathogens (Strachen, 1989). Conversely, other factors such as increased population density, poor water purification, and animal domestication are associated with an increase in pathogen exposure and virulence. Much variation exists regarding infectious disease exposure and MI among transitioning populations (Godoy et al., 2005a; 2005b). Understanding what this variation

looks like among transitioning Shuar will help us better understand and test the Old Friends Hypothesis, while exploring underlying mechanisms of exposure associated with the Hygiene and Biodiversity Hypotheses.

Market Integration and Health among the Shuar

Shuar are currently experiencing varying degrees of social, cultural, environmental, and economic change associated with rapid integration into regional and global market economies (Blackwell et al., 2009; Cepon-Robins et al., 2014; Liebert et al., 2013). The accelerated pace of this change in the last decade, and the wide variability in who is affected provides an important opportunity to explore shifts in chronic and infectious diseases in real time as they occur with MI. Generally, integration into the market economy is producing variation in lifestyles and health in at least two observable ways among the Shuar: 1) inter-regional variability based on differences in travel access or barriers to markets (**Figure 3.1**); and 2) intra-regional variation based on socioeconomic status, age, access to education, and participation in traditional verses MI activities, among others. In the more isolated region east of the Cutucú mountain range (Cross-Cutucú or CC), Shuar maintain more traditional lifestyles based on hunting, fishing, and horticulture, in part due to logistic and travel barriers, including distance, reliability and transport cost, that make frequent market access difficult.

At the time of data collection for this dissertation (2011 through 2013), the Cross-Cutucú communities in this study could access the regional market center of Sucúa after 1.5 to 4 hours via canoe (depending on water level) and an additional travel time of approximately 5.5 to 8.5 hours by bus. In contrast, Shuar living in the Upano Valley

(UV) region could reach Sucúa via bus or truck within an hour, where people can purchase food, produce, materials, visit restaurants, and access the internet, as well as sell lumber, animals, and produce grown in gardens for income. In the UV, Shuar are experiencing greater economic change associated with increased participation in the market economy as well as a potential access to medical and pharmaceutical care (Blackwell et al., 2009; Madimenos, 2011). However, the more economically developed UV environment also leads to increased economic disparity, which may result in marginalization and inequality related to ethnicity, SES, and education. Thus, it is important to understand MI among the Shuar based on regional, familial, and individual levels of variation.

The Shuar Health and Life History Project

The research in this dissertation was conducted in conjunction with the Shuar Health and Life History Project (SHLHP; http://www.bonesandbehavior.org/shuar/). SHLHP is an interdisciplinary collaborative research effort among the Shuar of Amazonian Ecuador. This dissertation and the SHLHP assess MI in two ways. The first is through geographic comparisons based on proximity to the regional market centers of Sucúa and Macas.

The second method of assessing MI is through structured Style of Life (SOL) interviews. These interviews are typically administered in Spanish, which most Shuar speak fluently. For non-Spanish speakers, a bilingual assistant was provided to translate from Shuar into Spanish. Participants are asked a series of questions drawn from the Material Style of Life (SOL) Index developed by SHLHP for use with the Shuar based on

extensive qualitative research and pre-testing (Liebert et al., 2013). The Material SOL Index was created based on examples used in other populations (Bindon et al., 1997; Leonard et al., 2002). The specific interview conducted by SHLHP consists of a list of market and traditional items. Participants are asked which items they own; SOL scores are then calculated based on the fraction of listed items that a household owns (Scores range from 0 to 1; **Table 1.1**). Two scales are used: a Traditional Style of Life (T-SOL) and a Market-Integrated Style of Life (M-SOL). Individuals with a higher T-SOL owned more traditional goods, while individuals with a higher M-SOL owned more market goods. These two variables are not mutually exclusive or even necessarily inversely correlated and, as suggested in Liebert and colleagues (2013), may both be associated with increased income which allows individuals to buy more goods regardless of their traditional or market-integrated values.

A third SOL variable (Household Style of Life, H-SOL) was created based on six household measures related to household permanence, access to infrastructure, and market participation (**Figure 1.6; Table 1.1**). H-SOL is calculated based on a summation of the scores for each of the six household measures. High H-SOL values are indicative of more market integrated household style/type. Materials included in the SOL scale were selected based on several years of ethnographic observations by the director of the SHLHP, Lawrence Sugiyama. Initial interviews with extensive lists of items were conducted and shortened after pilot testing (unpublished data).

Figure 1.6. Comparison of Shuar houses: More traditional-style Shuar house (left); More integrated wood house with a tin roof and wood floors (middle); Integrated cement homes with cement floors, tin roofs, and glass windows (right). Photo credits: Sugiyama, Liebert, and Madimenos.

Table 1.1. Variables used for calculating T-SOL, M-SOL, and H-SOL.

T-SOL	M-SOL	H-SOL (Scale)
Fishing hook/line	Radio	Floor (0: dirt, 1: palmwood, 2: milled lumber, 3: concrete)
Hunting dogs	Propane stove	Wall (0: dirt, 1: palmwood, 2: milled lumber, 3: concrete)
Blowgun	Mobile phone	Latrine (0: none, 1: pit toilet, 2: outhouse w/ water, 3: indoor toilet)
Firearm	TV	Water Source (0: natural source, 1: well or outdoor pipe, 2: indoor pipe)
Fishing net	Chainsaw	Electricity (0: none, 1: lights, 2: outlets
Canoe	Bicycle	Number of rooms
	Refrigerator	
	Computer	
	Outboard motor	
	Motorcycle	
	Car	
	Truck	

Previous research by SHLHP among the Shuar has documented multiple changes in health associated with MI. Studies to date include relationships between MI and chronic inflammation (McDade et al., 2012), including risk for CVD and metabolic syndrome (Liebert et al., 2013), as well as osteoporosis and physical activity (Madimenos et al., 2011a; 2011b; 2012), and tradeoffs between growth and immune function (Blackwell et al., 2009; 2010; Urlacher et al., 2014, 2015, in press).

Cardiovascular Disease and Metabolic Syndrome. Liebert and colleagues (2013) found that MI results in a number of mixed cardiovascular and metabolic health outcomes. Specifically, Shuar living in the Upano Valley had higher total cholesterol and high density lipoprotein cholesterol, which were positively correlated with MI variables. As predicted, MI affected both lifestyle and diet; people with higher M-SOL and H-SOL consumed more market-based items, like rice, noodles, and soda, as well as more refined carbohydrates in general, and these dietary changes were linked to altered CVD and metabolic health. Conversely, Shuar living in the Cross-Cutucú regions had higher blood pressure (although on average, still at healthy levels). This suggests that while some factors associated with MI result in increased chronic disease burden, other factors may create more favorable health outcomes.

McDade and colleagues (2012) used a repeat measures study design to test whether UV Shuar experience levels of chronic low-grade inflammation based on high-sensitivity C-reactive protein (CRP), similar to that experienced by populations in wealthy, developed countries. No evidence of chronic low-grade inflammation was found, which suggests a low risk of CVD, metabolic syndrome, and other inflammatory

disorders among the Shuar. However, we have since encountered several examples of metabolic syndrome and T2D among UV Shuar living in communities near Sucúa (SHLHP, unpublished data), and the director of the Federación Interprovincial de Centros Shuar (FICSH) health center in Sucúa has identified clusters with high T2D prevalence within some UV villages (Carmen Zúñiga Torres [Enfermera CSU FICSH] personal communication to Sugiyama 2014).

Growth and Immune Function. Blackwell and colleagues (2009) explored height differences between indigenous Shuar and non-Shuar *Colono* children and documented that 40% of Shuar children were stunted. Another study presented important tradeoffs between immune function and growth, likely associated with timing of first helminth infection. Blackwell and colleagues (2011) found that immunoglobulin E (IgE) (an antibody associated with both helminth infections and specific allergens) was negatively correlated with CRP (a pro-inflammatory cytokine involved in nonspecific immunity). Higher levels of IgE were associated with shorter stature, while higher levels of CRP were associated with taller stature. These data suggest that infection during childhood entails trade-offs with growth during times of infection, which may have long lasting results. Urlacher and colleagues (2014, 2015) have recently supported this interpretation, using weekly repeated growth measures and CRP measures to show that growth stops or slows during the week of, and up to a month after, an elevated CRP response. Children who do not experience these tradeoffs rely more heavily on innate immunity, but have higher rates of inflammation throughout later life, which could be related to chronic, non-communicable health problems later in life (Blackwell et al., 2010).

Blackwell and colleagues (2011) also demonstrated higher levels of IgE in general among Shuar compared to industrialized populations, although not as high as indigenous Tsimane of Bolivian Amazonia (Blackwell et al., 2011). IgE is associated with helminth levels, suggesting that the Shuar have greater helminth infection prevalence and intensity than populations in developed nations, but less than Tsimane (Blackwell et al., 2011). A follow-up growth-curve study by Urlacher and colleagues (in press) found that the Shuar follow unique growth curves, with important implications for life history tradeoffs. For instance, just after birth, infants seem to grow rapidly followed by a rapid decrease in growth. This suggests possible immune tradeoffs during infancy and early childhood associated with environmental exposure and immune system priming.

Osteoporosis and Physical Activity. Madimenos and colleagues (2012) used a life history approach to explore bone mineral density and reproduction among the Shuar. This study found important relationships between bone density, age at menarche, and stature, suggesting that there may be important tradeoffs that occur in more traditional, natural fertility populations that are not occurring in more market integrated groups. When compared to non-indigenous *Colonos* living in the same region, the Shuar also had significantly higher bone density (Madimenos et al., 2011a).

Physical activity patterns among an Upano Valley community of Shuar suggest fairly low physical activity levels compared to other non-western groups (Madimenos et al., 2011b). This low expenditure was attributed to a possible focus on long duration, habitual tasks like gathering legumes, clearing weeds, and processing yucca, that do not

require a lot of energy. These findings have interesting implications for our understanding of physical activity differences between hunter-gatherer and the sedentary lifestyles seen in high-income, economically developed nations and regions. More work needs to be done to compare physical activity between the Upano Valley and Cross-Cutucú communities to see how economic change is affecting energy expenditure.

In short, we see evidence of changing health outcomes associated with MI among the Shuar (Liebert et al., 2013; Madimenos et al., 2011a; 2011b; 2012), and a number of these may be related to changing infectious disease burden (Blackwell et al., 2010; 2011; Urlacher et al., 2014, 2015, in press). Diet, physical activity, subsistence strategy, socioeconomic status, psychosocial stress, education, and access to medical care all play important roles in shaping these health changes, either by decreasing exposure to certain infectious disease agents or altering the body's development and function. The Shuar present an interesting opportunity to study these health effects of social and economic change. We have an almost continuous spectrum of more traditional to more integrated lifestyles represented within our study population, which allows us to explore health changes more accurately within this lens of MI.

Dissertation Objectives

Table 1.2 highlights the topics and variables discussed in this dissertation, which explores the relationships among STH infection, MI, evolutionary psychology of the disgust response, and markers of inflammation. Chapter II provides background information on the intricate mechanisms behind the Old Friends Hypothesis (as well as Hygiene and Biodiversity Hypotheses). Chapter III, a version of which was originally

published in the *Journal of Parasitology*, tests the idea that parasite load changes with increased MI, using community (Upano Valley [UV] vs. Cross-Cutucú [CC1, CC2]) as a proxy for economic development. Chapter IV builds on this and explores specific variables associated with market participation and household style as they relate to MI. As such, this chapter presents the first direct test of the disgust response as an avoidance mechanism for pathogen exposure, using mediation modelling to understand the intersection between MI and disgust. Chapter IV contains unpublished coauthored material. Chapter V investigates relationships between STH exposure and inflammatory marker CRP to examine if STH infection is associated with lower levels of chronic inflammation. **Figure 1.7** lays out my proposed mechanisms for the immune dysregulation discussed in Chapter V. In this dissertation, I only focus on the branches of this chart that are associated with market integration, helminth infection, and inflammatory marker CRP, while hypothetically addressing anti-inflammatory effects of interleukin-10 and regulatory T-cells (discussed in more detail in Chapter II). Chapter V is an indirect test of the Hygiene Hypothesis using inflammation as a proxy for immune dysregulation. Chapter V contains unpublished coauthored material. Chapter VI focuses on conclusions and future directions for this research.

Bridge to Chapter II

The present chapter has provided an overview of the theoretical motivations for this dissertation. There are many intricate mechanisms that regulate the relationship between STHs and the human immune system. Chapter II reviews these mechanisms in detail, discussing the development and function of the human immune system, the role of

infectious, parasitic diseases in immune system priming, and the role of chronic inflammation in allergy and autoimmunity pathogenesis. Chapter II also discusses the motivation behind methods used in this dissertation.

Table 1.2. Topics and variables discussed in this dissertation.

Topic	Variables	Description	Chapter
MI	Geographic Region	Upano Valley (UV; Greater market access) Cross Cutucú (CC1, CC2; Less market access)	3
	Style of Life (SOL)	SOL-House (High = more MI house) SOL-Traditional (High = more traditional) SOL-Market (High = more MI lifestyle)	4, 5
Disgust Sensitivity	Factor 1 Factor 2 Factor 3	F1 – Contamination F2 – Raw/Spoiled Food F3 – Pests	4
STH Infection	Infection Status	Infection Status (one or more species present) *Ascaris lumbricoides* infection status *Trichuris trichiura* infection status Co-infection Status (two or more species present)	3, 4, 5
	Infection Intensity	*Ascaris lumbricoides* eggs per gram (EPG) *Trichuris trichiura* EPG	3, 4, 5
Immune Response	C-reactive Protein	Sub-clinically elevated CRP is indicative of chronic inflammatory responses	5
Age	Years	Used to understand change in parasite exposure with age, as well as levels of chronic inflammation associated with age.	3, 4, 5
Control	Body Size	Body mass index (BMI) and weight	5

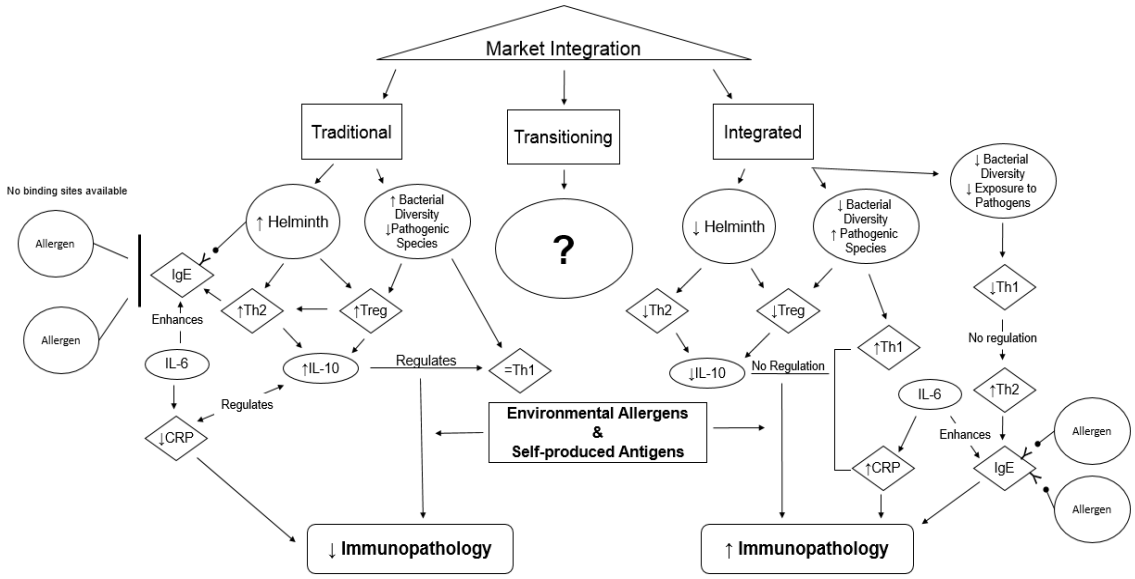

Figure 1.7. Proposed mechanisms of the Hygiene Hypothesis as presented in this research. Specifically, I test STH exposure across the spectrum of MI to understand what this transition looks like for those that are currently shifting from traditional lifestyles to more market integrated lifestyles. Research to date makes hypotheses based on an idealized version of traditional populations or uses data from clinical settings in developed nations, neither of which capture the actual shift in parasite load and immunopathology.

CHAPTER II

MECHANISMS OF THE HUMAN IMMUNE SYSTEM

Underlying the Hygiene Hypothesis and Old Friends Hypothesis is the idea that we co-evolved with pathogens and other microorganisms to the point that we use exposure to these symbionts in the development and regulation of our immune systems. These hypotheses posit that the lack of evolutionarily relevant exposure to these symbionts in contemporary environments leads to immune dysregulation and chronic inflammatory diseases like allergy and autoimmune disorders, among others. This chapter provides basic background on the human immune system, highlighting some of the proposed mechanisms by which infectious agents, particularly soil transmitted helminths (STHs) may affect the development of the immune system. It also covers why a lack of exposure may lead to immune dysregulation and its adverse consequences.

The Human Immune System

The immune system is a specialized system of cells that originate in the thymus and bone marrow and proliferate throughout the spleen, lymph system, and blood. It functions to recognize, control and/or destroy foreign substances and abnormal self-cells (e.g., damaged tissue or tumors; Bäckhed et al., 2005). The human immune system is generally divided into two component parts—the innate immune system and the adaptive immune system. Because of the costs and benefits of mounting an adaptive immune response, relative investment in adaptive vs. innate immunity is regulated by environmental exposures early in life. Thus, exposure to pathogens early in development

has immune consequences into adulthood (e.g., Blackwell et al., 2012; McDade, 2003; McDade et al., 2013). Some details of immune function are particularly relevant to discussions throughout this dissertation, and therefore reviewed in more detail here.

Functions of the Innate Immune System

Innate immunity forms the first line of defense that responds to common classes of microbes (e.g., bacteria, viruses, and fungi). Because the innate immune system lacks specificity and only mounts a general response, it is also referred to as non-specific immunity. Barriers such as skin, saliva and mucus make up the innate immune system, as well as cells geared toward pathogen recognition and inflammation (Clark 2008; Janeway et al., 2001; Murphy 2011). The innate response to infection occurs rapidly, and is geared toward evolutionarily conserved features shared across broad classes of fungi, bacteria, and viruses. This gives the innate immune system the ability to recognize a wide range of infection types. The innate immune response is, however, energetically costly to deploy and may also result in collateral damage to self-cells and tissues (Lochmiller & Deerenberg, 2000; Long & Nanthakumar, 2004; Schaible & Kaufmann, 2007).

Basically, the innate immune system identifies a problem, forms an inflammatory barrier around the problem, recruits immune cells and promotes clearance of dead and foreign cells. It recognizes self from non-self, but has no memory system for recognizing specific pathogens to which it is exposed during an individual's lifetime (Clark 2008; Janeway et al., 2001; Murphy 2011). It thus provides no long-lasting immunity based on a previous specific pathogen exposure. The basic features of the innate immune response are summarized in **Table 2.1**.

Table 2.1. Features of the Innate Immune Response

Feature	Definition	Example
Antigen	Any foreign molecule that initiates an immune system response	Proteins or glycoproteins from bacteria or viruses
Leukocytes[1]	White blood cells that function in ways similar to single-celled organisms to move freely around the body and remove waste, foreign particles, and pathogens.	Natural killer (NK) cells Mast cells Phagocytes (Macrophages, dendritic cells) Neutrophils Eosinophils Basophils
Cytokine[2]	Produced by macrophages, these molecules are important for marking the path to the wound or infection, incite production of more macrophages and neutrophils, and increase temperature to fight infection, stimulate repair cells.	Tumor necrosis factor α (TNF-α) Interleukin-1 Interleukin-6
Acute Phase Response[3]	Conserved inflammatory response that is immediately activated by infection or trauma.	C reactive protein (CRP)
Major histocompatibility complex (MHC)[4]	The MHC represent cell surface molecules for presenting antigens and differentiating between self and non-self.	Class I Class II

[1] Mast cells release histamines, hormonal mediators and cytokines. Phagocytes, including macrophages, eat microbes and dead or injured self-cells. Neutrophils, basophils, and eosinophils release toxic substances to kill or inhibit microbes. Natural killer cells are recognize altered or missing self-cells.

[2] TNF-α, IL-1, and IL-6 are proinflammatory cytokines, activating both local and systemic inflammatory pathways, and are also responsible for signaling that results in chronic inflammation.

[3] CRP production is activated by proinflammatory cytokines. Circulating values of CRP reflect on-going systemic inflammation (Licastro et al., 2005).

[4] Class I are present on all cells except red blood cells and interact with NK cells to trigger cell death. Class II is used by the adaptive immune system to trigger differentiation and production of T cells.

The innate immune system has three basic methods that it uses to identify potential threats. These methods rely on: 1) recognition of "microbial non-self;" 2) recognition of "missing self;" and 3) recognition of "induced or altered self." Recognition of microbial non-self refers to the ability to recognize non-self, based on the presence of molecular properties produced by broad classes of microorganisms. To distinguish non-self, the immune system deploys cells with protein receptors called "pattern recognition receptors" (PRRs). PRRs are toll-like receptors (TLRs) found on cell surfaces of macrophages, dendritic cells, and mastocytes (Clark, 2008; Janeway et al., 2001). These cells circulate looking for pathogen-associated molecular patterns (PAMPs) to bind with. PAMPS are produced by all microorganisms, including bacteria, yeast, viruses, and parasites, regardless of their actual pathogenicity. For example, PAMPs include lipopolysaccharide (a sugar produced by gram-negative bacteria) and peptidoglycan (produced by gram-positive bacteria). PAMPs found on bacterial or yeast cell walls or viral coats are recognized by TLR2 and TLR4, while proteins found on bacterial flagella interact with TLR5 (Clark, 2008). When PRRs attach to a PAMP, the immune system responds. TLRs are also important for triggering the adaptive immune response, accomplished by activating naïve T cells that will respond specifically to antigens (see below).

The recognition of "missing self" uses the lack of cell-surface properties present on one's own cells to identify pathogens. The presence of these surface properties marks the cell as self, so the absence is used to identify potential threats. This pathway is important for differentiating between "normal self" and genetic/microbial products (Medzhitov and Janeway, 2002), and may be why natural killer cells target cells with no

major histocompatibility complex (MHC) class I proteins on the surface (Ljunggren & Kärre., 1990).

The MHC (also called human lymphocyte antigen [HLA] in humans) is a large heterogeneous gene complex critical for the adaptive immune system. It functions to generate molecules that bind to specific foreign proteins (antigens) and present them to either Natural Killer (NK) T-cells that destroy the pathogen, or Helper T-cells that signal other cell systems to attack. Comprised of two primary classes of genes, Class I MHC molecules are present on all cells except red blood cells and interact with NK cells to trigger cell death. The function of NK cells is to eliminate infected or transformed cells, while keeping healthy, normal self-cells alive. If self-cells are manipulated to lack specific MHC class I proteins, the NK cells will attack (Raulet, 2006). In short, recognition of "missing self" is based on cells missing MHC Class I molecules.

Finally, the ability to recognize "altered or induced self" refers to the immune system's ability to recognize self-cells that have been altered by infection or cellularly transformed (Medzhitov and Janeway, 2002; Murphy, 2011). This occurs when a self-cell is infected by an intercellular pathogen, in which case it presents the PAMP on its own surface, to bind with a PRR and be removed. This can also occur for tumor cells, if the mutation changes the MHC, and will activate a T-cell response. In both of these cases, the NK cells play an important role in preventing infection spread or tumor growth. However, if they lose their tolerance for self, immune dysregulation can occur. So, for the immune system to remain properly regulated, it relies on the maintenance and development of these innate mechanisms.

When the innate immune system encounters a non-self or altered-self entity, in other words, a PAMP is detected by a PRR, several responses can occur. Some PRR bearing cells contain microbicides that directly kill the bacteria associated with the detected PAMP (Clark, 2008). These PRRs are found within neutrophils and burrow into the membrane of the microbe, allowing water to leak in and cell contents to escape. This response is also useful for self-cells that have already been infected (Clark, 2008; Janeway et al., 2001; Murphy, 2011). Other PRRs tag detected bacteria, which are then eliminated by specific phagocytic cells.

The innate immune system relies heavily on inflammation to eradicate foreign invaders or irregular self-cells. TLRs on white blood cells, most importantly dendritic cells and macrophages, are responsible for initiating the inflammatory response, which results in redness, heat, swelling, and pain (Clark, 2008; Janeway et al., 2001; Murphy, 2011). This response occurs when a surface-bound TLR binds to a microbial PAMP and results in the release of a number of proinflammatory cytokines (Clark, 2008; Janeway et al., 2001; Murphy, 2011). Some of these cytokines, like prostaglandins, result in blood vessel dilation, while others, like histamine, allow the blood vessels to become more permeable. Both of these responses increase blood flow to the affected area and allow white blood cells to enter to fight the infection. Now, with the blood vessels granting increased access to the site of infection, a family of cytokines, called chemokines, are released in order to guide the white blood cells to the source of infection. Tumor necrosis factor (TNF)-α stimulates local pain receptors in order to tell our brain that there is a problem in the affected region. The innate immune system also acts to activate the adaptive immune system.

Functions of the Adaptive Immune System

The adaptive immune system responds to specific antigens, with a mechanism in place that allows for memory in order to mount faster immune responses against encountered pathogens in the future (Clark, 2008; Janeway et al., 2001; Murphy, 2011). Adaptive immunity requires a prior exposure before mounting an efficient response (Clark 2008; Janeway et al., 2001; Murphy 2011). Further, it takes time to develop and includes the long-term costs of maintaining vigilance toward remembered pathogens.

Mediated by thymus (T) and bone marrow (B) lymphocytes (or T and B cells), adaptive immunity recognizes and targets specific antigens. T and B cells are named after where they mature. Both originate in the bone marrow, but immature T cells leave the bone marrow to mature in the thymus. In the thymus, T cells learn to differentiate between what is self and non-self (Clark, 2008). Thymic maturation of T cells is extremely important. T cells that fail to distinguish between self and non-self is one of the mechanisms behind the development of autoimmunity (Berg et al., 2002; Clark, 2008; Schleinitz et al., 2010). During this period of maturation, at least 90% of the T cells that arise in the bone marrow are destroyed in order to select only for cells that will bind to foreign cells instead of self (Clark, 2008). Mature T cells are responsible for promoting and controlling inflammatory processes against antigens. B cells spend their entire maturation in the bone marrow where they develop to produce antibodies. Once they are fully mature, T and B cells make their way to the lymph nodes and the spleen, where they monitor blood and lymph fluid for anything they recognize as “not self.”

The adaptive immune response begins when the surface receptor of a B or T cell binds to an antigen, resulting in a cascade of events that typically end in the destruction

of the antigen. This can also occur when a dendritic cell from the innate immune response bring ingested bacteria into the lymph node and activate T cells. In general, T cells drive the cell-mediated immune response and are activated by membrane bound receptors associated with the MHC complex (**Table 2.1**). The MHC complex is highly polygenic, polymorphic, and co-dominant, showing evidence of selection for great diversity, and allowing the generation of molecules that bind to a wide array of antigens (Janeway et al., 2001). Upon activation, T cells leave the lymph nodes and search the body for the major source of antigens. When the source is discovered, they lead an attack to remove the microbe or other offending substance from the body. There are many types of T cells, defined largely by the tasks they perform. **Table 2.2** summarizes the types of T cells, as well as other key cells and molecules that make up the adaptive immune response.

When the product of an antigen (literally "antibody generator," defined as any biological entity that results in the production of an antibody) interacts with the membrane bound receptors of the MHC, T helper (Th) cells are activated. Each antigen has multiple binding sites for B-cell receptors, T-cell receptors, and antibodies. Upon binding, naïve helper T (Th) cells differentiate into one of two different subgroups depending on the type of insult they respond to and cytokines they produce: Th1 and Th2 mediated immune pathways. Th1 cells respond to viral and bacterial threats, secreting interleukin-2 (IL-2), interferon (IFN)-y, and TNF-α. These support inflammatory processes, activate macrophages and induce the proliferation of natural killer (NK) cells. This pathway also activates cytotoxic T (Tc) cells, which destroy virally infected cells and tumor cells.

Table 2.2. Features of the Adaptive Immune Response

Feature		Definition	Example
Lymphocytes		White blood cells responsible for adaptive immunity.	T cells B cells
	T cells	Lymphocytes that mature in the thymus. Differentiate into different types depending on pathogen detected. Involved in recognizing and killing infected host cells, releasing cytokines, and regulating the immune response.	T helper cells (Th) T cytotoxic cells (Tc) Memory T cells Regulatory T cells
	B cells	Lymphocytes that mature in the bone marrow. Important for producing antibodies to bind and destroy antigens.	10^9 genetic combinations for B-cell receptors exist in any body
Cytokines[1]		Signaling proteins for white blood cell production, tissue repair, and inflammation. Important for regulating the intensity and length of the immune response.	IL-4 IL-5 IL-10 TNF-α Interferon (IFN)-g IL-2 TGF-b
Immunoglobulins (IGs)		Classes of proteins that function as antibodies. Production is triggered when B receptor cells are bound by an antigen.	IgG IgE[2] IgA IgD IgM

The Th2 response is activated by soil-transmitted helminths (Fairfax et al., 2012; van den Biggelaar et al., 2000), releasing cytokines IL-4, IL-5, and IL-10 to activate B-cell activity. IL-4, results in the production of IgE and IgG by B cells, as well as growth factor for Th2 cells. Production of IL-5 activates eosinophils to fight helminth infections. The Th2 pathway also activates regulatory T (T_{reg}) cells, which produce IL-10 and TGFb, both of which are suppressor cytokines that down-regulate the immune response. Importantly, IL-10 suppresses the Th1 and macrophage responses, regulating the immune response and turning down inflammation (Clark, 2008; Janeway et al., 2001; Murphy,

2011). This is important because if this branch of the immune system fails to turn down inflammation, chronic inflammation and associated cell and tissue damage can occur.

After mature Th cells bind to an antigen, they leave the lymph nodes in search of B cells that have similar surface proteins and bind to them. B cells are responsible for the humoral immune response which increases the specificity and memory of the adaptive immune system. B cells produce antibodies, which are proteins that respond to invasions by foreign bodies (Clark, 2008). An enormous number (10^9) of B cells exist in the body, each with different receptor specificities (Clark, 2008; Janeway et al., 2001; Murphy, 2011). Naïve B cells respond to any potential antigen and then clonally proliferate. There are a number of genes that contribute to B cell receptors and immunoglobulin variability, which results in a large variation in potential responses, demonstrating that B cell diversity was highly selected for.

There are five specific types of B-cell receptors and associated antibodies: immunoglobulin A (IgA), immunoglobulin D (IgD), immunoglobulin IgE (IgE), immunoglobulin G (IgG), and immunoglobulin M (IgM) (Clark, 2008; Janeway et al., 2001; Murphy, 2011). IgA is common in breast milk, tears, saliva, and the intestinal and respiratory mucosa and is involved in the prevention of bacterial and viral adherence to mucous membranes. IgD is very rare and is found on the membrane of immature B cells. IgE is produced in select amounts and responds specifically to helminth infections, as well as harmless antigens (allergens) that result in allergic reactions. IgG is the most abundant antibody and responds specifically to viruses and bacteria. IgM is the first antibody that appears during an infection. Its role is to bind viruses and bacteria in order

to clump them together and mark them for destruction (Clark, 2008; Janeway et al., 2001; Murphy, 2011).

While the innate immune response is responsible for activating inflammation, the adaptive immune response releases cytokines and antibodies that can upregulate or downregulate inflammation. In other words, the adaptive immune response is important for regulating inflammatory activity. Without proper stimuli from certain molecules (like IL-10) and other cells (T_{reg} cells), which are stimulated by specific helminth produced antigens, the immune system may remain in a proinflammatory state and chronic inflammatory disorders may follow.

Inflammation and Immune Dysregulation

There are a number of reasons that disorders associated with immune dysregulation, like allergies and autoimmunity, are hypothesized to occur. One way is a failure of the mechanisms used to distinguish self from non-self (discussed in more detail later). Another is increased tissue damage and subsequent immune response to clear the damage, all associated with chronic, non-acute inflammation. The inflammatory response is important for immune function, but in most cases it also causes a lot of the symptomatology associated with infection (Clark, 2008). For instance, acute inflammation during hepatitis infection causes inflammation of the liver, while colitis is caused by inflammation of the colon during parasitic infection. The inflammatory response needs to remain relatively brief, since acute inflammation can damage surrounding self-cells and self-tissue. This is especially problematic because the innate

immune system is not especially discerning when it is responding to an infection (Clark, 2008).

If the initial immune attack fails to completely clear an infection, these can become chronic. Tuberculosis, for instance, has evolved a way to evade the initial immune response and reproduce. Once the immune system detects the infection, it is too late and an upregulated immune response results in the "liquefaction and cavitation" of the lungs as the host tries to destroy infected macrophages and escaped bacteria (Clark, 2008). Examples of the immune response causing more damage than the actual pathogen also come from cerebral malaria (Clark et al., 2004; Kwiatkowski, 1995; Meyer et al., 2002; Wattavidanage et al., 1999) and viral hepatitis (Clark, 2008).

Under some conditions, including chronic psychosocial stress (Danese et al., 2007; Gimeno et al., 2008; Hänsel et al., 2010; McDade et al., 2006), visceral adiposity and obesity (Gregor & Hotamisligil, 2006; 2011; Medzhitov, 2008), or immune dysregulation (Du Clos, 2003; Ishihara and Hirano, 2002; Kikuchi et al., 2002; Lin, 2002; MacDonald and Monteleone, 2005; Qian et al., 2008; Solem et al., 2005; Vermeire et al., 2004), the inflammatory response can become chronically upregulated, resulting in localized cell and tissue damage (Licastro et al., 2005). This phenomenon has been discussed as a result of the natural aging processes; however long-term, chronic, sub-clinical levels of inflammation throughout the lifespan also increase the rate of aging (Chung et al., 2009; Licastro et al., 2005). The relationship between age and inflammation is complicated and multifaceted, and the directionality of cause and effect is not well understood. Many physiological signs of aging appear because of the accumulation of tissue and cellular damage over time (Singh and Newman, 2011).

Having a strong immune response, in general, may speed the process of aging. Evidence in developed, wealthy nations inextricably links CRP and age-related physiological changes. Whether inflammation in these regions causes aging, is caused by aging, or both is currently unknown (Jenny, 2012).

Increased inflammation may not be universally associated with aging. For instance, chronic, subclinical levels of CRP only seem to increase with age in economically developed nations, with no detectable evidence of chronic subclinical levels of CRP present over multiple weeks among the Shuar (McDade et al., 2012). Chronic elevations in CRP appear to be preventable. Increased physical activity is related to lower levels of inflammation in older adults (Simpson & Guy, 2010). Dietary factors, like antioxidants (Yoshihara et al., 2010), have also been linked to lower levels of inflammation. Western diets low in fruits, vegetables, and fiber and high in processed carbohydrates and fats are related to elevated CRP (Koenig et al., 1999), while diets high in fruits, vegetables, whole grains, fish, and olive oil, and low in red meat, have been associated with lower levels of chronic inflammation (Carter et al., 2010; Pauwels, 2011; Solfrizzi et al., 2011). Robust exposure to important infectious agents early in life may prevent later increases in subclinical levels of CRP (McDade et al., 2011). These studies all suggest that a number of lifestyle factors are likely associated with the subclinical, chronic inflammation seen in wealthy, developed nations.

If inflammation is meant to protect the body in response to infection, helping to rapidly remove the pathogen, how does it become chronic? In all of the examples discussed above (physical activity, diet, body fat composition, infection, etc.) some factors result in immune system dysregulation (Chung et al., 2009; Jenny, 2012), either

triggering an inflammatory response when one is not necessary, or increasing the time it takes to return to baseline function after an infection has cleared. Jenny (2012) provides an extensive review of a number of factors associated with senescence and chronic inflammation, including reactive oxygen species and oxidative stress, mitochondrial damage, chronic over-stimulation of the hypothalamic-pituitary-adrenal axis, sex hormones, telomere attrition, and epigenetic modifications. For the purpose of this dissertation, I only focus on the factors related to immunosenescence.

One proposed mechanism (Jenny, 2012) for chronic inflammation is associated with endogenous reactive oxygen species (ROS). ROS are byproducts of normal function and maintenance in the body (i.e., aerobic respiration), and include oxygen and peroxide molecules that result in oxidative stress. This process breaks down proteins, resulting in molecular, cellular and structural damage over time (Sohal & Orr, 2012). The immune system is responsible for clearing cells damaged by oxidative stress, triggering an inflammatory response (Jenny, 2012). If this cellular destruction from oxidative stress followed by inflammatory responses to clear damage occurs frequently, inflammation can become chronic.

Mitochondrial damage has also been implicated in chronic inflammation (Jenny, 2012). Mitochondria are the ‘powerhouses’ of the cell and are vital for normal cellular maintenance and function. If mitochondria become damaged, less energy is produced and cell turnover slows. This can alter hormone production and decrease function of a number of organs, including ovaries, testis, and adrenal glands (Wei & Lee, 2002). All of these factors combine to alter rates of programmed cell death (apoptosis), which can result in

increased inflammation to clear apoptotic cells and chronic tissue damage (Green et al., 2011).

Combined, oxidative stress and mitochondrial damage seem to result in immune system dysfunction. For instance, the thymus shrinks in size if damaged by these cellular processes, reducing its ability to function normally for T cell development and regulation (Signh & Newman, 2011). Failure to properly produce and regulate T cells may result in increased reliance and chronic activation of macrophages and other pro-inflammatory innate immune cells (Franceschi et al., 2000). Chronic inflammation and a number of associated chronic, non-communicable diseases may follow. Both autoimmune diseases and allergies have been associated with chronically elevated inflammatory processes, measured using CRP (Du Clos, 2003; Ishihara and Hirano, 2002; Kikuchi et al., 2002; Lin, 2002; MacDonald and Monteleone, 2005; Qian et al., 2008; Solem et al., 2005; Vermeire et al., 2004).

Autoimmunity

Autoimmune disorders occur when the immune system, unable to distinguish between self- and non-self, attacks healthy self-cells (Clark, 2008; Murphy, 2011). In turn, this failure to differentiate self from non-self can result in increased levels of chronic inflammation. Autoimmune disorders are distinguished based on the type of cells that are destroyed and their location in the body, though most individuals who are susceptible to one type of autoimmune disorder often have other autoimmune problems (Clark, 2008). Type 1 Diabetes (T1D), for instance, is often associated with pernicious anemia, Grave's disease, and Hashimoto's thyroiditis, among others, suggesting that

autoimmunity usually occurs on a systemic level (Clark, 2008). For this reason, some autoimmune diseases, like ankylosing spondylitis, Crohn's disease, Hashimoto's thyroiditis, and Grave's disease, are considered "relatively tissue specific," while others, like rheumatoid arthritis, scleroderma, and multiple sclerosis are considered "relatively non-tissue restricted" (Clark, 2008).

In some cases, autoreactivity occurs after an infection, as some pathogens use molecular mimicry to evade detection. Molecular mimicry occurs when micro-organisms present surface antigens that resemble those of host-cells (Blank et al., 2007; Damian, 1964). This can result in the creation of memory T cells for this surface antigen and a subsequent response to the similar host cells. Molecular mimicry is used to explain why some autoimmune disorders occur after infection with certain pathogens (Blank et al., 2007). Autoimmunity also occurs due to a breakdown in the system used to detect auto-reactive cells.

There are multiple mechanisms for eliminating B and T cells that have membrane bound receptors for self-produced cells. 50% of B cells and 90% of T cells are eliminated before they leave the bone marrow and thymus in order to prevent self-reactivity. It is possible that this begins to occur during fetal development. At birth, infants often produce a number of antibodies against self (Clark, 2008). During this period of development, the immune system may be testing itself and using self-cells for priming. In general, T-cells are selected for cell death if they do not have Type 1 or Type 2 MHC, which the immune system uses to recognize self (Berg et al., 2002). Negative selection occurs after that, as T cells are presented with MHC bound to antigen presenting cells with self-proteins on the surface. Cells that bind to these compounds are also selected for

cell death. It is when this detection system breaks down that autoimmune disorders occur. NK cells also appear to play some role in some autoimmune diseases, either by becoming autoreactive or by differentially interacting with dendritic cells, macrophages, and T cells. These changes can increase inflammation or hypervigilance (Schleinitz et al., 2010).

Studies on the types of immune cells involved in a number of autoimmune disorders suggest that they are associated with the Th1 branch of the immune system (Axtell et al., 2013; Stromnes et al., 2008). Damage from autoimmunity results from chronic, low-grade inflammation, in ways similar to infection by unresolved infectious diseases (Clark, 2008). The initial immune response is caused by activation of B cells and T cells, while the immunopathology comes from both destruction of self-cells by the immune system and resultant inflammation. For instance, rheumatoid arthritis results from the production of rheumatoid factor, an antibody which attacks other antibodies (Clark, 2008). More antigen/antibody complexes are produced than can efficiently be cleared by macrophages. These immune complexes then settle in the joints where they lead to inflammation, destruction, and arthritis. Another example, T1D, results from an attack on pancreatic β-cell antigens, resulting in β-cell destruction and insufficient sugar uptake.

Very little is known about the underlying reasons for why cellular immune dysregulation associated with autoimmunity occurs, though evidence points to both sex- and genetics-related predispositions toward autoimmunity. Most autoimmune disorders occur disproportionately in females (Shoenfeld et al., 2012), and this appears to be either chromosomally or sex-hormone linked, because males with Klinefelter's syndrome (an

extra X chromosome) also experience higher rates of autoimmunity (Seminog et al., 2015).

On top of sex differences, other genetic predispositions appear to be at play, with further levels of differential gene expression leading to symptomatology (Clark, 2008). A recent study on genetic variations in immune cell production found a surprisingly extensive genetic component was responsible for the numbers and variation of immune cells produced, including those that increased risk for autoimmunity (Orrù et al., 2013). Further, genotype variations associated with the MHC response seem to come into play. While there is a genotype that predisposes for autoimmune activity, it appears that not all individuals with this genotype develop autoimmune disorders. Autoimmunity appears to run in families, and even within identical twins with differential pathogenesis (in other words, one twin with clinical autoimmunity and one without), both twins have the associated antibodies (Clark, 2008).

Another study observed changes in gene expression that were independent of disease activity, duration, therapeutic history, and type of autoimmune disease, that altered signaling pathways essential for self-tolerance (Maas et al., 2002). If these pathways do not function properly, lymphocytes that attack self are allowed to leave the bone marrow and thymus and proliferate, increasing the likelihood of autoimmune diseases. This suggests an environmental stimuli for disease pathogenesis as well, and many links have been made between psychosocial stress and emotional trauma on the onset of autoimmune symptomology (Clark, 2008). Taken together, research on autoimmunity points to a complicated milieu of genetic predispositions, developmental function, and environmental factors that combine to produce autoreactivity.

Allergy

Allergic disorders stem from a hypersensitive immune response related to over-activity of the Th2 branch of the immune system. Allergens are antigens that selectively activate the Th2 response, causing B cells to selectively produce the antibody IgE (Clark, 2008). B cells that produce IgE are found in the skin, lungs, and intestines, rather than the lymph nodes and spleen. IgE triggers histamine production, which causes blood vessel vasodilation and increased permeability in the region where the antigen is encountered, precipitating an inflammatory response. IgE can be very specific, only reacting to certain allergens. It is still unclear why common allergens, like certain chemicals, dust, microbial spores, animal dander, fibers, pollen, and insect parts, lead to an immune responses while others do not (Clark, 2008; Janeway et al., 2001; Murphy, 2011).

Allergens are typically relatively small proteins that are also highly soluble. Solubility is important because when the allergen meets with mucosa, where IgE producing B-cells are commonly found, the allergen is easily diffused. Allergic reactions usually occur after a second exposure to an allergen, due to memory associated with the adaptive immune response, suggesting that the first exposure primes the immune system to respond. This response can be inhibited by Th1 activation and production of IFN-y. However, even relatively low doses of allergens, even a single peanut protein, can trigger Th2 activation over Th1 (Clark, 2008; Janeway et al., 2001; Murphy, 2011).

Like autoimmunity, allergy susceptibility has a clear genetic component (Clark, 2008; Janeway et al., 2001; Murphy, 2011). For example, variation in the promotor region of the gene NFKBIA has been associated with increased likelihood of developing asthma and other inflammatory diseases of the respiratory tract (Ali et al., 2013). Studies

looking at hypersensitivity to environmental allergens suggest that individuals from susceptible families have variable regions on chromosomes 5q and 11q (Janeway et al., 2001; Murphy, 2011). The variable gene on chromosome 11 encodes for high-affinity IgE receptors, while a cluster of genes on chromosome 5 appear to be linked to IL-3, IL-4, IL-5, IL-9, IL-12, IL-13 and granulocyte macrophage colony-stimulating factor production, all associated with hypersensitive immune reactivity (Janeway et al., 2001). A second type of heritable variation related to the IgE response is associated with a class of MHC (class II), and seems to be associated with responses to specific allergens (Janeway et al., 2001; Murphy, 2011). Not all individuals who are genetically susceptible develop allergy and it may be the size of the dose or timing of first exposure that ultimately determines pathogenesis (Clark, 2008).

Both allergic and autoimmune disorders result from the breakdown of immune pathways that normally protect the body from overreaction to harmless and self-produced stimuli. Symptomatology of these disorders, rather than susceptibility, is inextricably linked to chronic levels of low-grade inflammation (Clark, 2008; Du Clos, 2003; Ishihara and Hirano, 2002; Kikuchi et al., 2002; Lin, 2002; MacDonald and Monteleone, 2005; Qian et al., 2008; Solem et al., 2005; Vermeire et al., 2004). While susceptibility to allergies and autoimmunity may have always been present genetically and may have conveyed a selective advantage in highly infectious environments (Clark, 2008), we are now seeing an increase in symptomatology in wealthy developed nations that may be linked to proinflammatory processes that would have been down-regulated under different circumstances.

The Old Friends Hypothesis

The mismatch between the evolved biology of the immune system and our current lifestyles may be responsible for the increased incidence of autoimmune and allergic reactions. The first mechanism to be suggested was an imbalance between the Th1 and Th2 dominated immune responses (Mosmann & Coffman, 1989). The Th1 response is triggered by viral and bacterial infections, so it was originally thought that if these infections were not encountered early during development, then the allergy-promoting Th2 response would be upregulated and allergies would occur (Mosmann & Coffman, 1989). This hypothesis, however, proved to be unsupported because autoimmune disorders, which were rising in conjunction with allergies, were dominated by the Th1 response, which would not be the case if dysregulation was driven by an imbalance with Th2 (Bettelli et al., 2007; Stene & Nafstad, 2001).

Now, research has shifted to trying to understand what is driving the pro-inflammatory phenotype that results in immune dysregulation (Helmby, 2015). While some studies are exploring the role of microbial diversity (the Disappearing Microbiota Hypothesis) within the human ecosystem on inflammation and immune dysregulation (Blaser & Falkow, 2009; Haahtela et al., 2013; Hanski et al., 2012), this dissertation examines the role of macro-parasitic infection from soil-transmitted helminths (STHs) to test whether STH infection is associated with reduced levels of chronic, low-grade inflammation (**Figure 1.7**).

The host body constitutes the environment where parasites live, and this relationship exposes the host's immune system to antigenic substances of parasitic origin (Bogitsh et al., 2005). STHs have coevolved with their hosts species. When surface

proteins from helminths bind the Th2 pathway, the inflammatory response is down-regulated, making the intestinal tract more habitable for the worms. Just like we are able to turn on or off the heat in our homes to make our lives more comfortable, STHs have adapted mechanisms to manipulate our immune systems to turn down inflammation. Specifically, *Ascaris lumbricoides* secretes two molecules once inside their host: 1) Pepsin inhibitor-3 (PI-3), and 2) phosphorylcholine (PC) (Bethony et al., 2006). PI-3 inhibits production of pepsin, which protects the worms from digestion (Ng et al., 2000), while PC suppresses lymphocyte proliferation, thus protecting *A. lumbricoides* from immune system detection (Deehan et al., 2002). *Trichuris trichiura* also secretes specific molecules, including *Trichuris trichiura* 47 (TT47), excretory secretory products (ES products), and *Trichinella spiralis* macrophage inhibitory factor (TsMIF; Bethony et al., 2006). TT47 creates pores in host epithelial cells, which allow the whipworms to attach (Drake et al., 1994). ES Products promote the Th2/T_{reg} response and decreases localized inflammation in the intestines, ultimately stimulating both IL-6 and IL-10 secretion from intestinal epithelial cells (Parthasarathy & Mansfield, 2005; Summers et al., 2005). TsMIF competes with the host's macrophage inhibitory factor to keep blood cells from migrating to the area of infection (Tan et al., 2001). These helminth products have been suggested for a number of uses including vaccination against parasite infections, as well as therapy for certain inflammatory disorders like Crohn's disease and ulcerative colitis (Bethony et al., 2006).

As parasites invade their hosts they secrete enzymes to break down host proteins. These enzymes allow the parasite access to the host's tissue, but they also activate and promote the Th2 response (Flohr et al., 2008; Maizels et al., 2004; 2014; van den

Biggelaar et al., 2000). These antigenic substances, usually proteins from the parasite body, are able to dampen the host's immune response and reduce pathogenic effects. Helminth infections, specifically, are sustained based on an immune-modulatory network that results in increased T_{reg} activity and elevated levels of IL-10 (Gale, 2002; van den Biggelaar et al., 2000) and TGFb (Flohr et al., 2008). The production of T_{reg} cells and the release of IL-10 have an immunotolerant or suppressive effect which turn off T cell-mediated immunity and suppress any autoreactive T cells that pass the thymus. Because of these reactions, it seems that T_{reg} cells suppress the immune response and are useful for preventing allergy and autoimmunity (Bach, 2005; Flohr et al., 2008). Referred to as the initial "spark," helminths are able to suppress initial alarm signals of their infection in order to decrease early inflammatory responses (Maizels et al., 2004; 2014). This spark, however, must receive necessary feedback from Th cells and an associated dampening of the innate immune response (Maizels et al., 2004; 2014).

In response, it is hypothesized that our immune system has adapted to allow it, because mounting a strong immune response may have proven more detrimental to differential reproductive success and survival (Blankenhaus et al., 2011; Helmby, 2015; Schopf et al., 2002; Taylor et al., 2005). Studies on mice have shown that those individuals that do not turn off inflammation using IL-10 in response to STHs suffer higher mortality and morbidity associated with infection (Helmby, 2015; Schopf et al., 2002). Mice that have no T_{reg} response completely clear their infection and show increased associated pathology (Blankenhaus et al., 2011; Helmby, 2015; Taylor et al., 2005). The presence of both IL-10 and T_{reg} highlight an important evolutionary balance or stalemate between the needs of the host and the needs of the parasite (Helmby, 2015).

The presence or absence of helminths helps to determine the ultimate immunological phenotype. At birth, newborns are oriented toward a Th2 response, based on the presence of types of cells and antibodies found in both the infant and the mother (Gale, 2002; Prescott et al., 1998; Raghupathy, 1997). Perhaps, if helminths are not encountered, this continued phenotype may result in production of antibodies that bind and respond to allergens instead. Where helminth exposure is reduced or absent, we see a Th2 cytokine dominated phenotype continue to develop which, especially when combined with genetic predisposition, can lead to risk of clinical allergy (Gale, 2002). Individuals with helminth infections, however, have a T_{reg} dominated phenotype and appear to be protected from mast cell degranulation and other inflammatory responses (Flohr et al., 2008), which seems to be related to those molecules secreted by the helminth itself. This is also influenced by the number of parasites present, the species of parasite, and the physiological condition of the host (Bogitsh et al., 2005). Further, research has found that perinatal exposure to significant levels of parasite antigen may create long-term hypo-responsiveness of the immune system, suggesting that these antigens and environmental allergens have cross-reactivity (Flohr et al., 2008). This is supported by the existence of IgE, which is produced in response to helminth exposure, but also binds allergens.

A number of studies have documented relationships between STH infection and hypersensitivity. Nyan and colleagues (2001) demonstrated an inverse relationship between STH infection and atopy (hypersensitivity to skin allergens associated with an IgE-mediated reaction) in an urban/rural comparison in Gambia, with mean IgE levels highest in atopic compared to non-atopic individuals. Similar results were found in an

urban/rural comparison of children in Ethiopia, where children with high intensity *A. lumbricoides* infections had fewer cases of wheeze in the past year (Dagoye et al., 2003). A third study examined atopy among children in rural Ecuador and found that helminth infection was associated with decreased allergen skin test reactivity (Cooper et al., 2003). These results all support the idea that helminth infection prevents chronic inflammatory allergic disorders like atopy, but do not look at the underlying, chronic inflammatory response, nor do they explore these relationships based on economic development and age-related changes in CRP.

For instance, infection with the whipworm *Trichuris trichiura* (Faulkner et al., 2002) results in increased TNF-α and IFN-g with age, suggesting a relationship between increased chronic inflammation throughout the life-course and parasite infection. IgE is negatively correlated with infection intensity and positively correlated with host age, suggesting that IgE production may be associated with the age-dependent decrease in *T. trichiura* infection intensity (Faulkner et al., 2002). Using data from the Shuar Health and Life History Project, Blackwell and colleagues (2010) hypothesized that immune response to helminths may be determined by age at first infection, with earlier infections resulting in a balanced adaptive immune response and later first infections associated with increased reliance on innate immunity and inflammation. Tradeoffs among IgE, CRP, height, and age in the Shuar (Blackwell et al., 2010) suggest that individuals exposed earlier rely more heavily on their adaptive immune response, have lower chronic inflammation, but also slower growth and smaller adult height do to the energetic costs of adaptive immunity. Those exposed later seem to rely on the innate immune system to fight infection and have higher levels of inflammation.

The ability to 'turn off' inflammation is good for the parasite, and the host may have evolved in response, using these reactions with helminth-produced antigens for its own regulation. For instance, hypo-reactivity can reduce infection severity in the host. Malaria pathogenesis provides one of the best examples for this. TNF-α is heavily involved in fighting malaria after it is detected. It is also implicated in providing innate immunity against malaria (Clark et al., 2004; Kwiatkowski, 1995). TNF creates a proinflammatory response, so children who are homozygous for a specific TNF allele (TNF-308A) have increased susceptibility to the most deadly form of malaria, cerebral malaria (McGuire et al., 1994). This type of malaria results in increased damage to the brain and nervous system by the immune system as it responds to infection. Overall, these individuals have increased susceptibility to severe infectious diseases in general because of more elevated inflammatory responses (Wattavidanage et al., 1999; reviewed in Kwiatkowski, 2005). All of this shows that sometimes the immune system itself can cause more destruction than the pathogen.

The Importance of Infection Timing for Immune Regulation

If STHs have an important regulatory effect on the immune system, then determining the importance of infection timing is critical. Immune system development begins early in gestation. T cells appear in the thymus at 7 to 9 weeks and enter circulation after 12 to 14 weeks, reaching adult levels by 15 to 18 weeks (McDade, 2003). Immature B cells are present in the blood and bone marrow by 12 weeks, with adult levels present by 15 to 18 weeks (McDade, 2003). This means that the fetus is

capable of recognizing antigens at 12 weeks, though specific immunity is depressed throughout gestation so more energy can be devoted to development (McDade, 2003).

At birth, the infant relies on IgE and IgG from its mother (McDade, 2003). At this period, nutrition is extremely important, as undernutrition may preselect for downregulated adaptive immune function, impaired antibody responsiveness, and reduced thymic hormone production (McDade, 2003). It is during early childhood, when passive immunity from the mother's breastmilk ceases as a result of weaning, that the child becomes more reliant on its own immune function. By adolescence, sex hormones kick in that also alter immune function. During this period, the thymus peaks in activity and specific immunity becomes incredibly important. Once it develops fully, the adaptive (specific) immune response functions more readily and with hypothetically fewer energetic costs (McDade, 2003).

During the developmental period, immune system development and regulation is context dependent, driven by the intensity and diversity of antigen exposure. The body is paring down B cells and T cells, because there is no reason to continue producing something that will not be encountered or used (McDade, 2003). Maizels and colleagues (2014) explore the importance of infection timing (during infancy/childhood vs. throughout adulthood) on immune regulation. Research suggests that helminth infection is most important early in life when the immune system is developing. The importance of this exposure extends as far back as the prenatal period, where maternal infection and probiotic exposure seems to decrease newborn risk of allergic eczema, even into childhood (Ege et al., 2011; Elliott et al., 2005; Mpairwe et al., 2011).

While data point to the importance of helminth exposure early on, there are also negative side effects at this stage. Vaccinations appear to be less effective in infected individuals, and efficacy increases when anthelminthic treatment occurs (Cooper et al., 2000; 2001; Elias et al., 2001; Labeaud et al., 2009). McDade and colleagues (2011) demonstrated that early-life nutritional and microbial exposures are important for promoting the development of the antibody-mediated immune response, as well as controlling levels of chronic low-grade inflammation into adulthood. This is because helminths increase immune tolerance. Infection, however, has to occur in the individual and cannot rely on the mother for priming, as anthelminthic treatment of pregnant women had no effect on the efficacy of their children's vaccines (Webb et al., 2011). Research on vaccination efficacy and childhood infections are important for demonstrating effects on parasites on immune tolerance during development, as well as the importance of controlling these parasites in regions where other infectious diseases are common.

There is some evidence that infection throughout adulthood can still have an important effect on the mature immune system. Research on helminth therapy, for instance, is finding that helminths may be effective treatments for chronic inflammatory disorders. A study of multiple sclerosis patients in Argentina found that adults who had asymptomatic gastrointestinal helminth infections remained in remission, while uninfected individuals suffered relapses over the course of 5 years (Correale & Farez, 2007). This was associated with an increase in IL-10 and TGFb in infected individuals. When a few of the infected patients took anthelminthic medication, their multiple sclerosis pathogenesis returned (Correale & Farez, 2011). In another case-study, a patient

that self-infected with *Trichuris trichiura* found relief from ulcerative colitis symptoms (Broadhurst et al., 2010; Maizels et al., 2014).

Other studies on the effects of helminths on dampening the symptoms of autoimmunity in adulthood have focused on the effects of *Trichuris suis* and *Necator americanus* on Crohn's disease (Weinstock & Elliott, 2013), celiac disease (McSorley et al., 2011), and multiple sclerosis (Fleming, 2013). Specifically, *N. americanus* was able to decrease the immune response in patients with celiac disease, but this still did not alter symptomatology significantly (Daveson et al., 2011; McSorley et al., 2011). Furthermore, a study exploring the effects of helminths on allergic rhinitis proved to be unsuccessful, as worms provided no benefits to patients (Bager et al., 2010; Croft et al., 2012). So while there is some evidence that STHs can decrease inflammation and immune dysregulation in adulthood, this evidence is far from consistent across all studies. More work needs to be done to understand what role helminths play in immune regulation, which helminth species are most important, and whether there is a critical time period for infection to prove beneficial. While clinical studies are helpful for determining if STHs can provide important immune priming at different points throughout the life-course, they miss out on the opportunity to test hypotheses like the Old Friends Hypothesis as they occur in real time.

To date, no studies have explored factors associated with the Hygiene and Old Friends hypotheses in a single population experiencing the range of relevant conditions needed to test these hypotheses. Most studies compare helminth exposure (Knopp et al., 2010; Phiri et al., 2000) or immune dysregulation (von Ehrenstein et al., 2000; Von Mutius et al., 1994a; Yemaneberhan et al., 1997) between urban and rural populations,

and very few studies have expanded this to look at both immune dysregulation and altered STH exposure (Cooper et al., 2003; Dagoye et al., 2003; Nyan et al., 2001). While these latter studies do link STH infections to decreased hypersensitivity responses, they do not look specifically at relationships with underlying inflammatory processes across a range of ages. These issues are addressed throughout this dissertation.

Bridge to Chapter III

Chapter III follows with a regional comparison among the Shuar. One area where research is lacking is among populations experiencing rapid social and economic change associated with the transition from subsistence to more market based production and consumption. Little is known about how parasite load changes in transitioning groups. Before exploring specific factors related to this transition, it is important to first document STH prevalence and infection intensity among the Shuar and then see if the Shuar demonstrate similar "urban/rural"[8] relationships as previous studies. If the Hygiene Hypothesis is correct, we would expect to see decreased STH infection prevalence and intensity associated with increased participation in market based economies. To test this, Chapter III uses geographic location as a proxy for MI. As discussed in Chapter I, Shuar living in the Cross-Cutucú region have limited regular access to larger market centers like Sucúa and Macas, while those living in the Upano Valley region can access these centers after relatively short bus or truck rides. Chapter III tests whether this market access alone, without looking specifically at SOL variables, accounts for variation in parasite load.

[8] "Urban/rural" is in quotes here, because the Shuar in the UV region in this study are not living in an urban environment, but do have greater access to a more urbanized, market center than Shuar living in the CC region, as discussed in Chapter I.

CHAPTER III

SOIL-TRANSMITTED HELMINTH PREVALENCE AND INFECTION INTENSITY AMONG GEOGRAPHICALLY AND ECONOMICALLY DISTINCT SHUAR COMMUNITIES IN THE ECUADORIAN AMAZON

A version of this chapter has been published by Tara J. Cepon-Robins, Melissa A. Liebert, Theresa E. Gildner, Samuel S. Urlacher, Alese M. Colehour, J. Josh Snodgrass, Felicia C. Madimenos, and Lawrence S. Sugiyama (2014) in the Journal of Parasitology (pp. 598-607). The author of this dissertation provided the study design and was responsible for collection and analyses of variables related to STHs, as well as statistical analyses and write-up. Liebert, Gildner, Urlacher, Colehour, and Madimenos assisted in data collection. Sugiyama is the field site director for the Shuar Health and Life History Project and provided editorial help. Snodgrass is the academic adviser for this dissertation and also provided editorial assistance.

Introduction

Soil-transmitted helminths (STHs), like *Trichuris trichiura* (whipworm), *Ascaris lumbricoides* (roundworm), and *Necator americanus* (hookworm), can cause negative health outcomes, including stunting, wasting, diarrhea, organ failure, nutritional deficiencies, mental and developmental retardation, and death (Ahmed et al., 2011; Bethony et al., 2006; Blackwell et al., 2010; Dold and Holland, 2011; Francis et al., 2012; Hurtado et al., 2008; Tanner et al., 2009). In 2000, more than a third of the world's population was estimated to harbor infection by one or more helminths (Ahmed et al.,

2011; Elliott et al., 2000). Worldwide, STH infections are also estimated to result in 12,000 to 135,000 deaths annually (WHO, 2002), with an additional loss of about 39 million disability-adjusted life years (DALYs; 1 DALY is used to represent the loss of one healthy year of life) (Coulibaly et al., 2012; Hotez et al., 2006; WHO, 2002). To put this in perspective, traffic accidents, HIV/AIDS, and ischaemic heart disease result in the loss of 41.2, 58.5, and 62.6 million DALYs, respectively (WHO, 2008).

STH infections tend to follow an "overdispersed" pattern in endemic communities, characterized by high worm burdens in a few individuals and light or no infections in the rest of the population. Those with high worm burden tend to be clustered within households or families (Bethony et al., 2006; Dold and Holland, 2011). Infection with one type of STH increases the likelihood of infection with others, most commonly between *T. trichiura* and *A. lumbricoides* (Needham et al., 1998). While age is one of the key factors associated with STH exposure, with most infections occurring in school-aged children (Galvani, 2005), many studies document high levels of infection among adults, amongst whom infection also shows an overdispersed pattern (Blackwell et al., 2011; de Silva et al., 2003; Dold and Holland, 2011; Needham et al., 1998). However, few studies (Fitton, 2000; Godoy and Cardenas, 2000; Tanner et al., 2009) have been conducted that test these patterns among indigenous populations experiencing social and cultural change associated with rapid economic development and market integration (MI; the emergence of and increased dependence on market-based systems of exchange, resulting in increased consumer goods ownership, processed food consumption, and changes to housing structure and materials).

STHs are grouped with other parasitic, bacterial, viral, and fungal infections, all closely associated with poverty, as neglected tropical diseases (NTDs). These diseases are known to contribute to the risk of poverty due to their debilitating, chronic nature. Further, NTDs occur disproportionately in "hotspots" that are already experiencing adverse conditions within developing countries (Hotez et al., 2008). Together, Latin America and the Caribbean regions make up one of these hotspots, with extremely high economic disparities; an estimated 40% of the approximately 556 million people in this region live below the poverty line (Hotez et al., 2008). Within the Latin American and Caribbean region, the Amazon basin is one of the most heavily infected regions, with individuals in indigenous communities often simultaneously experiencing co-infection with STHs, river blindness, leishmaniasis, and other NTDs (Hotez et al., 2008).

It remains unclear to what extent this heavy infection amongst Amazonian groups is affected by rapidly changing socioeconomic conditions. Because MI often results in pronounced disparities in socioeconomic status, access to healthcare, and availability of protective barriers against pathogen exposure (McDade & Nyberg, 2010), it creates a framework for studying how disease burden is affected by economic, dietary, sanitary and healthcare-related change. There is some indication that there is a large amount of variation regarding infectious disease exposure and factors related to MI among transitioning populations (Fitton, 2000; Godoy et al., 2005a; 2005b), yet this issue has not been systematically studied using the multidimensional approach offered by MI. Some effects of MI, such as increased consumption of processed food and altered sanitation practices (e.g., boiling water, hand-washing, separate bathroom facilities), are associated with decreased exposure to certain pathogens, while other factors, including increased

population density, poor water purification, and animal domestication, are associated with an increase in pathogen exposure and virulence (Strachen, 1989).

Populations experiencing MI often face a double burden associated with increased rates of both infectious and chronic diseases (Barrett et al., 1998). To date, most studies have focused on chronic diseases associated with MI, such as obesity, cardiovascular disease (CVD), autoimmune conditions, and type 2 diabetes (T2D) (Bindon, 1995; Bindon et al., 1997; Cassel et al., 1960; Cepon et al., 2011; Dressler, 1985; 1999; Dressler and Bindon, 2000; Liebert et al., 2013; Snodgrass et al., 2007a). The effects of MI on infectious disease exposure, specifically STHs, have been largely ignored, even though understanding this relationship is critical for a more complete understanding of the effects of MI on health and more targeted, effective public health interventions.

The present paper examines helminth exposure among the Shuar, an indigenous neo-tropical group of the Ecuadorian Amazon experiencing rapid economic development and social change. Although helminth infection has not been previously studied among the Shuar, four lines of evidence suggest that STH infection is a major contributor to negative health. First, previous surveys among non-indigenous Ecuadorian populations show *A. lumbricoides* to be present in between 25% and 45% of the population, while hookworm and *T. trichiura* both have a prevalence of 5% to 25% (de Silva et al., 2003). Second, our previous research among the Shuar has shown that 40% of Shuar children are stunted, a much higher prevalence than is found among other indigenous and non-indigenous children living in the same area (Blackwell et al., 2009). Third, our research on immunoglobulin E (IgE; a class of antibody closely associated with parasitic worms) documented overall high IgE levels among Shuar compared to industrialized populations,

as well as negative correlations with stature in both children and adults (Blackwell et al., 2010). Finally, we have shown marked variation in MI within and between Shuar communities in association with geographic distance from a centralized market location (Liebert et al., 2013) with relevance to a variety of health conditions, including cardiovascular disease risk (CVD; Liebert et al., 2013) and bone density (Madimenos et al., 2011, 2012).

The objectives of the present study are threefold. First, we report infection prevalence and intensity of STH among the Shuar. We predict that as a group, Shuar will have moderate to high prevalence and intensity of STH infection based on standard definitions (Montresor et al., 1998) due to their local ecology and geographic location within NTD hotspots (Hotez et al., 2008). We also expect to see an overdispersed distribution with a few individuals harboring most of the population's worm burden, due to the proposed nature of STH infection (Bethony et al., 2006; Dold and Holland, 2011; Needham et al., 1998).

Second, we compare STH infection patterns in geographically and economically separated Shuar communities at different levels of MI. We hypothesize that less market integrated CC communities—those located deeper within the Amazon rainforest and further from roads allowing market access—will have higher prevalence of STH infection, based on less ability to buffer exposure through housing and sanitation barriers that would confer protection from pathogens (McDade & Nyberg, 2010).

Third, we explore STH infection prevalence and intensity as it relates to age and sex distribution in each of the three communities. We predict negative relationships between age and infection, based on higher rates of childhood behaviors associated with

fecal-oral contamination and close proximity with other infected children (Nwaneri and Omuemu, 2012).

Materials and Methods

Study Population

The present study was conducted as part of the Shuar Health and Life History Project (SHLHP; www.bonesandbehavior.org/shuar). The Shuar are a large indigenous forager-horticulturalist group (~46,000 individuals in over 668 communities) concentrated in the Cross-Cutucú (CC) and Upano Valley (UV) areas of the Morona-Santiago and Zamora provinces of Ecuador (**Figure 3.1**). Traditionally, horticulture, hunting, and fishing were the foundations of the Shuar economy (Harner, 1984; Karsten, 1935; Stirling, 1938). Now, the accelerating pace of Shuar integration into the regional market economy provides an excellent opportunity to study the health effects associated with social and lifestyle changes. In the isolated region east of the Cutucú mountains (Cross-Cutucú or CC), Shuar continue to follow more traditional lifeways based on hunting, fishing, and horticulture, while those in the Upano Valley (UV) are experiencing greater economic change associated with increasing participation in the market economy (Blackwell et al., 2009; Madimenos et al., 2011). Within the UV region, road access and resource depletion has decreased reliance on hunting and fishing, although production of traditional crops such as manioc and plantains continues to provide the dietary staples (Liebert et al., 2013). These staples are supplemented by raising chickens, and purchasing foodstuffs with money earned from selling cows, agricultural goods (e.g., plantains and papayas), and timber, or through wage labor or government jobs.

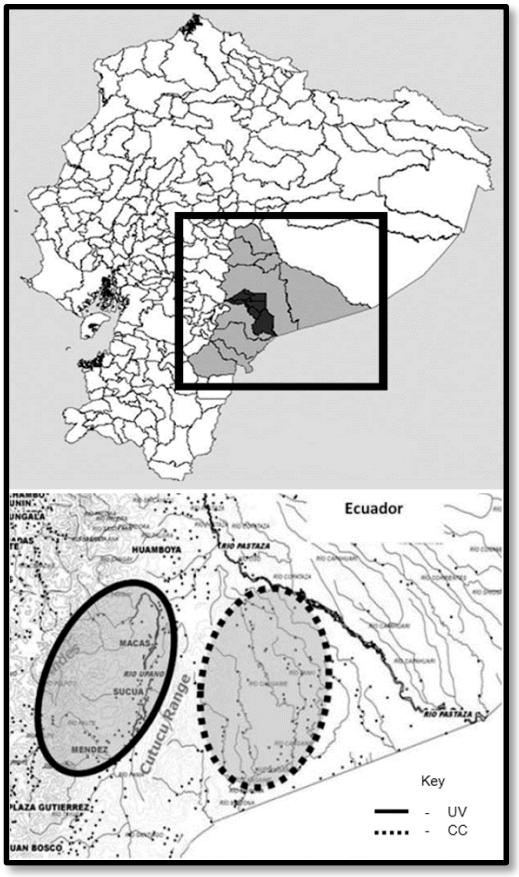

Figure 3.1. Map of Ecuador emphasizing Shuar territory. For this study, Shuar territory is divided into two geographically and economically isolated areas: the Upano Valley (UV) and the area across the Cutucú mountain range (CC). Figure adapted from Liebert and colleagues (2013).

The present study bases MI measures on geographic location, a key determinant of market access and availability, with UV individuals residing within walking distance of the main road where trucks allow transport to the town of Sucúa within about 45 minutes to 1.5 hours. Sucúa is the local market center, with restaurants, stores, and potential access to medical and pharmaceutical care. In contrast, at the time of data collection, the CC communities in this study could access Sucúa via 1.5 – 3 hours by motorized canoe (depending on water level) and an additional effective travel time of approximately 5.5 – 8.5 hours by bus. The CC communities in this study thus have significantly less regular access to markets than the UV sample community, but greater

access than most CC communities. Further, CC sample communities in this study have access to a small health center, staffed by a nurse practitioner, where limited medicines and services for minor injuries and illnesses are available. Previous research based on household level data found significant differences in indices of MI between UV and CC communities, with UV Shuar being significantly more market integrated than CC Shuar (Liebert et al., 2013).

Participants and Sampling

The present study design was cross-sectional and employed a geographic comparative approach with an age-stratified sample of juveniles (<15 years) and adults (≥15 years). Data were collected over two field seasons (August-September 2011 and August-September 2012). A total of 211 volunteers ages 0 through 86 (116 female and 95 male participants) from 3 communities—one UV community of comprised of about 350 individuals (n = 89; 52 females, 37 males) and 2 CC communities of ~60 and ~300 individuals respectively (CC1: n = 55, 29 female and 26 male participants; CC2: n = 67, 35 female and 32 male participants)—participated in this study. Although conditions in these villages prevented us from obtaining a random sample of residents, we made every effort possible to enroll a sample of participants that was representative of the community in terms of age, socioeconomic status, and style of life (i.e., traditional vs. acculturated lifestyles). Participants were recruited on a voluntary basis based on word of mouth and information distributed at community meetings. Although useful for recruiting participants, this method only recruits individuals who are open to health and physiological research, and may over-enroll participants concerned about their health.

In this paper, we compare prevalence and intensity of STH infections across three sample communities (UV, CC1, and CC2), rather than collapsing CC1 and CC2 and doing a simple UV vs. CC comparison. This was necessary because CC1 was somewhat divergent compared to other CC communities we have worked in previously. CC1 was comprised of a subset of people from a larger community, who recently established CC1 (within the year prior to data collection) when their previous village flooded. It included an extended family who had invested heavily in education, including a very large number of adults who commuted to other communities to work as school teachers and earn government wages, putting them in a socioeconomic level above other CC communities. Many in CC1 have close social connections to individuals in the larger towns of Sucúa and Cuenca, and visited and/or worked in these places regularly. Though not as market integrated as UV communities (SHLHP, unpublished data), CC1 represents a middle ground and demonstrates the complex nature of, and intra-regional variation in, the rapidly increasing process of MI as it spreads throughout the CC region.

Ethics Statement. Informed verbal consent was obtained from adult participants; for participants under 15 (the local age of consent), parental verbal consent and child assent were obtained. Individuals were informed that they could choose not to participate, participate only in individual portions of the study, or participate in the full study. The study and consent procedures were approved by the Institutional Review Board (IRB) of the University of Oregon, and the *Federacion de Centros Shuar* (FICSH or Shuar Federation) authorized this research.

Field and Laboratory Procedures

Stool collection and analysis. Fresh stool samples were collected and processed based on methods established by Raso et al. (2004). A pre-packed plastic bag containing an empty stool container marked with the name and identification number of the participant was provided the evening before collection. Participants were instructed to either defecate directly into the cup, or onto a clean, broad leaf or sheet of paper in which case a sample of the feces could be transferred to the container using the clean tongue depressor provided. Participants were asked to only take feces that had not touched another surface (e.g., the ground, dirt, grass). Containers were then returned with a small amount of fresh stool to a centralized village location within an hour of passage, where they were processed within an hour of sample drop off. A single Kato-Katz thick smear (Katz et al., 1972) was prepared from each specimen using a 42 mg plastic template (Vestergaard Frandsen, Lausanne, Switzerland). Kato-Katz thick smears were examined for soil transmitted helminth eggs at 10x and 40x microscopy (Cole-Parmer, Vernon Hills, Illinois) in the field by a single trained observer (TJC). Though we looked specifically for evidence of all intestinal parasitic infections that could be found in stool, with the exception of one case of tapeworm, only *Ascaris lumbricoides* and *Trichuris trichiura* were present. Helminth egg presence, species presence, species-specific eggs per gram (EPG) of feces, and species-specific infection intensity were recorded. Individuals with greater EPG of feces are infected with a larger number of adult worms and are characterized as having a higher intensity infection. Infection intensity levels were classified based on EPG cutoffs according to WHO standards (Montresor et al., 1998): *A. lumbricoides* light (1-4,999 EPG), moderate (5,000-49,999 EPG) and heavy

(≥50,000 EPG) intensity infection; *T. trichiura* light (1-999 EPG), moderate (1,000-9,999 EPG), and heavy (≥10,000 EPG) intensity infection.

Market Integration. For the present study, geographic location was used as a proxy for MI. In addition to ethnographic observation of overall differences in MI between UV and CC communities, previous analyses show that economic and dietary indices of MI differ by study community location as predicted, with UV Shuar being significantly more market integrated than CC Shuar (Liebert et al., 2013; SHLHP, unpublished data). This comparison was made based on structured interviews used to create variables associated with ownership of goods purchased within a market economy, goods used in traditional subsistence activities, and measures of household construction and access to water/electricity (see Liebert and colleagues [2013] for a more in-depth description of this analysis). Further, these variables associated with MI have been shown, in a preliminary, single community study to be significantly related to STH infection and intensity (Cepon et al., 2012).

Age Estimation. Ages were determined by birthdates on government identification cards and extensive genealogical information collected by SHLHP, including birth order within families. Overlapping genealogies were collected and cross-checked among multiple informants to ensure accurate age information (Blackwell et al., 2010; Liebert et al., 2013). The split between child (<15) and adult (≥15) was made based on previous research (Blackwell et al., 2010) and local age of consent, or the age at which an individual is considered and treated as an adult within these communities.

Statistical Analysis

Data analyses were conducted using SAS (SAS Institute Inc., Cary, North Carolina) and BIOMStat Version 4.0 (Exeter Software, E. Setauket, New York). Infection prevalence, *A. lumbricoides* prevalence, and *T. trichiura* prevalence were calculated based on the proportion of participants with any helminth eggs and respective species specific helminth eggs in their stool. Coinfection was the proportion of participants with both *A. lumbricoides* and *T. trichiura* in their stool.

Because of the overdispersed nature of EPG values, extreme outliers were not excluded from the sample because this would have removed data points of particular interest. The Shapiro-Wilk test was used to test for normality in EPG variables before comparing statistics by age and by community. EPG variables were $\log_{10}$-transformed due to non-normal distributions. This resulted in normally distributed variables with skew and kurtosis both between ± 1. Only parametric tests with transformed data were used for analysis of these variables. One-way ANOVAs compared log *A. lumbricoides* and log *T. trichiura* EPGs between adults and children with communities combined. Correlations were used to determine relationships between species-specific EPGs in infected males and females in each of the three communities (UV, CC1, CC2).

Likelihood ratio G-tests were used to compare infection prevalence, coinfection prevalence, and species specific prevalence for males and females between the three communities. Three-way ANOVAs explored the effects of age, sex, and community on log *A. lumbricoides* and log *T. trichiura* EPGs. Replicated goodness of fit tests were used to determine if men and women differed significantly in *A. lumbricoides* infection prevalence in each of the three communities. Replicated goodness of fit tests with a priori

comparisons were also used to determine differences in *A. lumbricoides* infection prevalence between adults and children in each of the three communities.

Results

Overall, 65% of the 211 individuals sampled were infected with at least one STH species, and 25.1% of the sample had coinfections with both STH species found. *Ascaris lumbricoides* eggs were present in 48% of all individuals sampled, while *T. trichiura* was present in 38% of individuals sampled. One individual harbored a tapeworm. No evidence of any other intestinal parasites, including hookworm, was found in any of the fecal samples. **Table 3.1** presents descriptive statistics for males and females in UV, CC1 and CC2.

Based on WHO standards (Montresor et al., 1998), most of the 102 individuals infected with *A. lumbricoides* had moderate intensity infections (51%) while only 4% had heavy intensity infections. Similarly, most of the 81 individuals infected with *T. trichiura* had light intensity infections (91%), a few had moderate intensity infection (9%) and none had heavy infections (**Table 3.2**). One-way ANOVA tests demonstrated that both log *A. lumbricoides* EPG ($p < 0.01$) and log *T. trichiura* EPG ($p < 0.01$) differed significantly between adults and children, with children having significantly higher EPG values in both cases.

To understand the extent to which STH infection follows an overdispersed pattern in this sample, we first determined what percent of the worm burden was harbored by individuals with the highest EPG for each species. Of the 102 individuals infected with *A. lumbricoides*, the person with the highest intensity infection harbored 9% of all EPGs.

The five most infected individuals harbored 26% of all *A. lumbricoides* EPGs. Similarly, of the 81 individuals infected with *T. trichiura*, the individual with the highest intensity infection harbored 15% of all *T. trichiura* EPGs, while the top 5 individuals with the highest *T. trichiura* EPGs harbored 36% of all *T. trichiura* EPGs.

Table 3.1. Descriptive statistics for age and STH infection for UV, CC1, and CC2.

	UV		**CC1**		**CC2**	
	Females (N = 52)	**Males (N = 37)**	**Females (N = 29)**	**Males (N = 26)**	**Females (N = 35)**	**Males (N = 32)**
Age (years)	19.16 (2.67)	19.50 (3.20)	21.07 (3.53)	20.35 (3.19)	18.14 (2.84)	21.65 (3.66)
Infection prevalence (%)	53.85	59.46	44.83	61.54	88.57	84.38
Coinfection prevalence (%)	28.57	9.09	30.77	31.25	45.16	59.26
***A. lumbricoides* prevalence (%)**	28.85	27.03	37.93	50.00	77.14	81.25
***A. lumbricoides* EPG**	5366.40 (1643.79)	2997.60 (2180.84)	10843.64 (4239.20)	11928.00 (3813.02)	24564.44 (5357.29)	13040.31 (3155.51)
Log *A. lumbricoides* EPG	3.14 (0.27)	2.65 (0.32)	3.49 (0.30)	3.76 (0.18)	3.96 (0.16)	3.74 (0.14)
***T. trichiura* prevalence (%)**	36.54	37.84	20.69	30.77	48.57	53.13
***T. trichiura* EPG**	289.26 (69.83)	336.00 (95.37)	196.00 (55.91)	162.00 (66.62)	439.06 (112.62)	616.94 (285.42)
Log *T. trichiura* EPG	2.22 (0.12)	2.31 (0.13)	2.22 (0.10)	1.91 (0.20)	2.34 (0.15)	2.19 (0.18)

Age and EPG data are presented as mean (SE). Prevalence data is the percent of individuals infected with at least one species (infection), specific species (*A. lumbricoides* and *T. trichiura*), and both species together (coinfection).

Table 3.2. Percent of individuals with light, moderate, and heavy intensity infections across three communities using WHO (Montresor, 1998) Standards.

STH Type	Sample	Light	Moderate	Heavy
Ascaris lumbricoides	Total (N=102)	45% (N=46)	51% (N=52)	4% (N=4)
	UV (N=25)	68% (N=17)	32% (N=8)	0% (N=0)
	Combined CC (N=77)	38% (N=29)	57% (N=44)	5% (N=4)
	CC1 (N=24)	37.5% (N=9)	62.5% (N=15)	0%† (N=0)
	CC2 (N=53)	37.7% (N=20)	54.7% (N=29)	7.6% (N=4)
Trichuris trichiura	Total (N=81)	91% (N=74)	9% (N=7)	0% (N=0)
	UV (N=33)	91% (N=30)	9% (N=3)	0% (N=0)
	Combined CC (N=48)	92% (N=44)	8% (N=4)	0% (N=0)
	CC1 (N=14)	100% (N=14)	0% (N=0)	0% (N=0)
	CC2 (N=34)	88% (N=30)	12% (N=4)	0% (N=0)

Next, we tested the relationship between species specific infections among infected individuals. Log *A. lumbricoides* and log *T. trichiura* EPG values were significantly positively correlated with each other ($p < 0.001$), but when participants were divided based on sex and community, it was clear that this positive correlation was driven only by males in CC2 ($p < 0.05$). For males and females in UV and CC1, and females in CC2, there were no relationships between log *A. lumbricoides* and log *T. trichiura* EPGs.

Log-linear likelihood ratio (G) tests were used to compare infection prevalence between communities for each sex. For females, there were no significant differences between UV and CC1, but CC2 did differ significantly in general infection prevalence ($G(2) = 17.10$, $p < 0.001$) and *A. lumbricoides* infection prevalence ($G(2) = 20.95$, $p <$

0.001). The same was true for males: CC2 was significantly different from both of the other communities in general infection prevalence (G (2) = 5.99, $p < 0.05$) and *A. lumbricoides* infection prevalence (G (2) = 21.04, $p < 0.001$). *Trichuris trichiura* was not significantly different for either sex across communities. Coinfection prevalence differed significantly between males in all three communities, with significantly more men coinfected than not coinfected in CC2, but significantly fewer men were coinfected than infected in UV and CC1 (G (2) = 14.17, $p < 0.001$). Coinfection prevalence did not significantly differ between the three communities for females.

Tables 3.3 and **3.4** show three-way ANOVAs for the effects of Age, Sex and Community on species specific EPGs for *A. lumbricoides* and *T. trichiura* respectively. Both sex and community had significant effects on log *A. lumbricoides* EPG (**Table 3.3**). Further analysis with linear regression found that there was a negative relationship between age and log *A. lumbricoides* EPG (F [26, 1] = 4.42, $R^2 = 0.15$, $p < 0.05$) and log *T. trichiura* EPG (F [16, 1] = 6.17, $R^2 = 0.29$, $p < 0.05$) only for females in CC2. There were no significant relationships between age and species specific EPG for males in any community, or females in UV or CC1.

Table 3.3. Three-way ANOVA of the effects of Age, Sex, and Community on log *A. lumbricoides* EPG

	df	**F**	***p***
Age	42	1.860	0.078
Sex	1	5.060	0.037*
Community	2	7.470	0.004**
Age x Sex	13	0.950	0.528
Age x Community	14	0.800	0.660
Sex x Community	2	1.216	0.320
Age x Sex x Community	1	0.282	0.602

* $p < 0.05$, ** $p < 0.01$, $R^2 = 0.892$

Table 3.4. Three-way ANOVA of the effects of Age, Sex, and Community on log *T. trichiura* EPG

	df	F	*p*
Age	30	1.347	0.291
Sex	1	0.095	0.763
Community	2	2.796	0.098
Age x Sex	10	0.664	0.739
Age x Community	15	1.390	0.279
Sex x Community	2	0.021	0.979
Age x Sex x Community	2	1.569	0.245

$R^2 = 0.853$

Replicated goodness of fit tests were used to see if males and females significantly differed in *A. lumbricoides* infection prevalence in each community (**Table 3.5; Figure 3.2**). Females and males did not significantly differ in their ratio of infected to non-infected individuals in any of the three communities, though patterns of infection did differ significantly in each community. In the UV, more individuals are uninfected than infected, but this prevalence ratio did not differ significantly between sexes. In CC1, both female and male participants were just as likely to be infected as non-infected. In CC2, significantly more individuals were infected than not infected and the ratio did not differ significantly between sexes. Because the ratio of infected and uninfected individuals does not vary significantly between males and females in each community, the sexes were collapsed into one sample to analyze infection ratios for *A. lumbricoides* by age groups.

Table 3.6 shows replicated goodness of fit tests comparing *A. lumbricoides* infection prevalence between children (0 to 14 years) and adults (15+ years) in each of the three communities. Both children and adults in the UV were significantly more likely to be uninfected than infected, and this ratio did not differ significantly between the two groups. In CC1, children were equally likely to be infected as they were to not be

infected, but adults were significantly more likely to not be infected. The ratios between adults and children differed significantly, suggesting that children were more likely to be infected than adults. In CC2, children and adults were both more likely to be infected than not and this ratio did not differ significantly between the groups (**Figure 3.3**).

Table 3.5. Replicated goodness of fit tests comparing *A. lumbricoides* prevalence between females and males in each community

		G	df	*p*
UV	Females	9.516	1	0.002
	Males	8.004	1	0.005
	Heterogeneity	0.035	1	0.851
CC1	Females	1.678	1	0.195
	Males	0.000	1	1.000
	Heterogeneity	0.813	1	0.367
CC2	Females	10.740	1	0.001
	Males	13.269	1	<0.001
	Heterogeneity	0.171	1	0.679

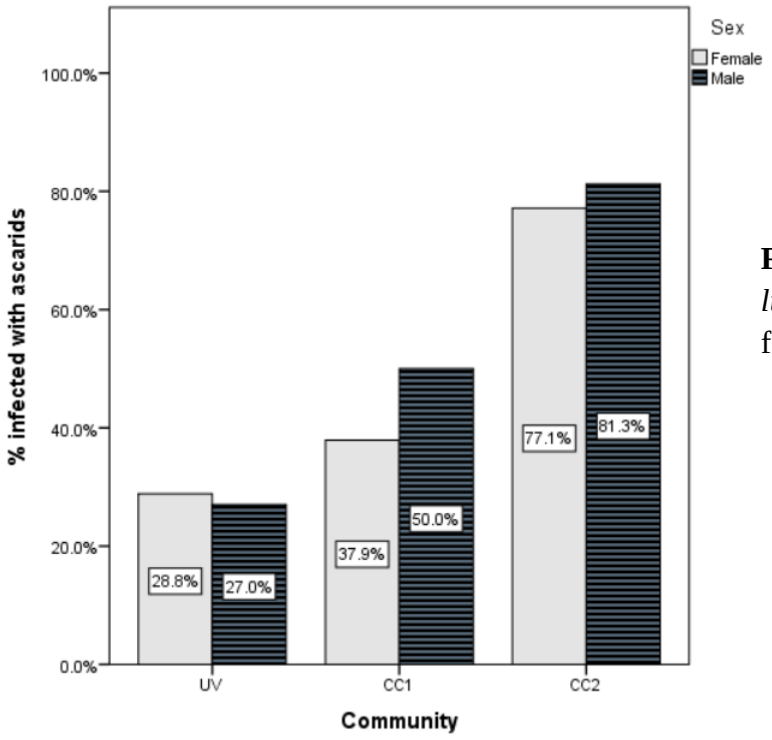

Figure 3.2. Ratio of *A. lumbricoides* by sex for each community

Table 3.6. Replicated goodness of Fit Tests Comparing *A. lumbricoides* Prevalence between Adults and Children in Each Community

		% Infected	G	df	*p*
UV	Children	24.5	14.287	1	<0.001
	Adults	33.3	4.022	1	0.045
	Heterogeneity		0.816	1	0.367
CC1	Children	60	0.987	1	0.321
	Adults	30	4.856	1	0.028
	Heterogeneity		5.050	1	0.025
CC2	Children	76.9	11.779	1	0.001
	Adults	82.1	12.320	1	<0.001
	Heterogeneity		0.272	1	0.602

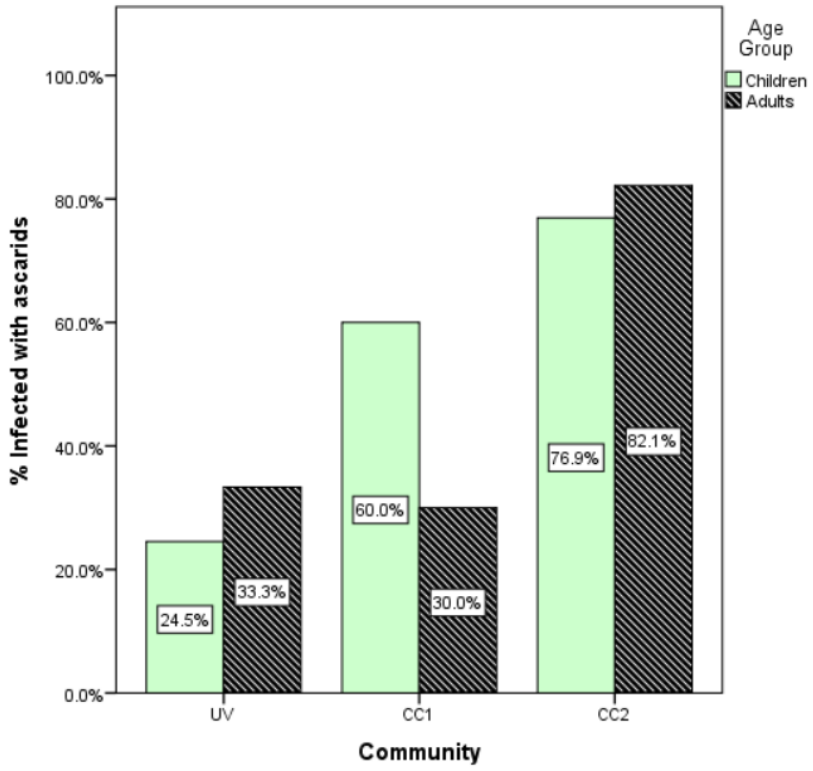

Figure 3.3. Ratio of *A. lumbricoides* infection by age group for each community.

Discussion

Figures 3.4 and **3.5** present comparative data between this study and other studies of indigenous subsistence-based (SB) populations (Blackwell et al., 2011; San Sebastian and Santi, 2000; Scolari et al., 2000; Tanner et al., 2009), rural (R) populations (Francis et al., 2012; Needham et al., 1998; Sackey et al., 2003; Saldiva et al., 1999), urban (U)

populations (Francis et al., 2012; Nwaneri and Omuemu, 2012; Scolari et al., 2000), and regional surveys (RS; de Silva et al., 2003). When compared to previous studies conducted among all age groups, the present study shows much higher rates of infection with both *A. lumbricoides* and *T. trichiura* than most other populations, with the exception of a Vietnamese farming population (Needham et al., 1998; **Figure 3.4**). The Vietnamese population lives in an STH hotspot, with livelihoods dependent on interaction with soil (Needham et al., 1998), increasing their likelihood of infection.

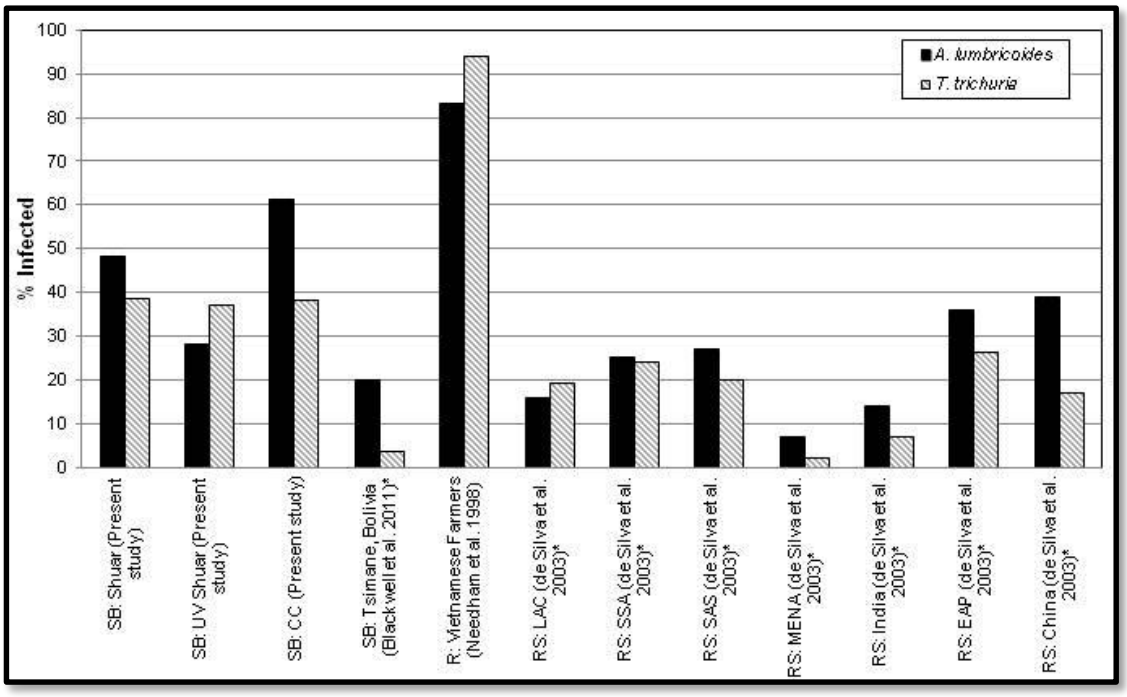

Figure 3.4. *Ascaris lumbricoides* and *T. trichiura* infection by population/region (all age groups).

Note: Methods used in some studies (*) differed from the Kato-Katz method used here.

Abbreviations: SB, Subsistence-based; R, Rural; RS, Regional survey; LAC, Latin America and the Caribbean; SSA, sub-Saharan Africa; MENA: Middle East and North Africa; SAS, South Asia; EAP, East Asia and the Pacific Islands

In a comparative study, de Silva et al. (2003) used data from a number of regions to show the global distribution of STHs. Latin America is home to many of the world's NTDs and many individuals suffer related morbidity (Hotez et al., 2008), yet de Silva's estimations for the Latin American/Caribbean (LAC) region is relatively low and fails to capture the high infection rates and intensities experienced by a sampling of its residents in the Amazon (present study).

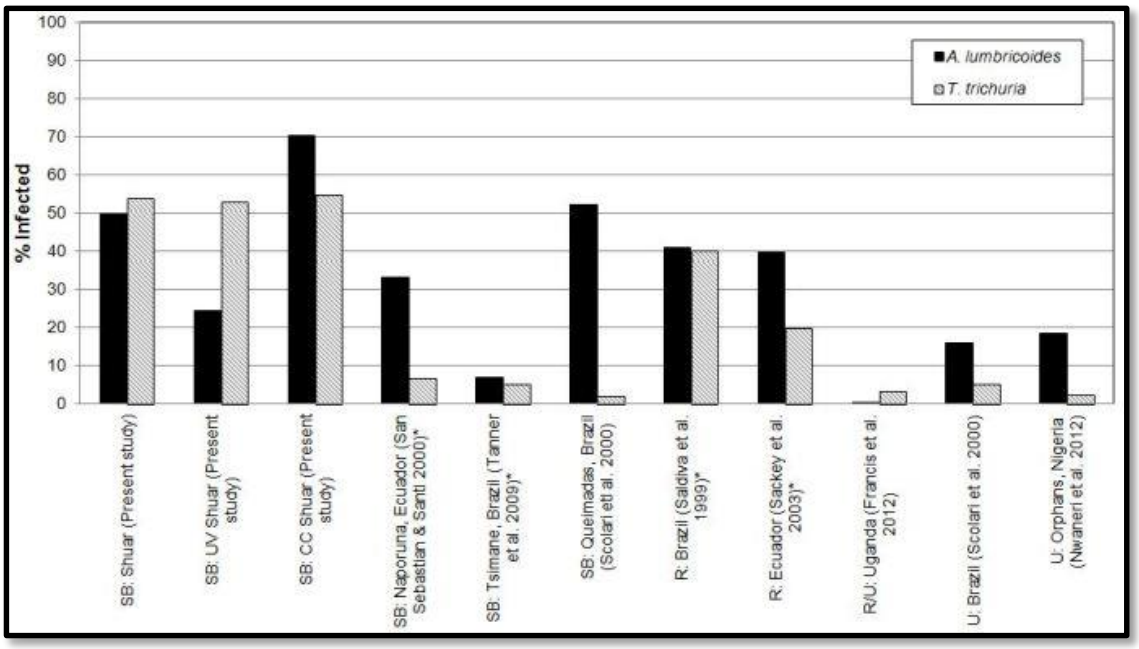

Figure 3.5. *Ascaris lumbricoides* and *T. trichiura* infection by population/region (children only).

Note: Methods used in some studies (*) differed from the Kato-Katz method used in the present study.

Abbreviations: SB, Subsistence-based; R, Rural; U, Urban.

Compared to infection rates of children from other populations, the present study demonstrated that Shuar children had very high infection rates, particularly children from the CC region (**Figure 3.5**). Interestingly, UV children had similar *A. lumbricoides* infection rates to the other studies and, as a group, a smaller percent of Shuar children were infected with *A. lumbricoides* than subsistence-based Queimadas Children of Brazil (Scolari et al., 2000). Only when CC children (CC1 and CC2 combined) were observed alone did this study find comparatively high infection rates. This is not true for *T. trichiura* infection rates. UV, CC and the combined sample had the highest prevalence of *T. trichiura* of all studies included in this review.

Based on WHO standards (Montresor et al., 1998), the present study found mostly light to moderate intensity infections among Shuar of all ages for *T. trichiura* and *A. lumbricoides* respectively. As predicted, we found an overdispersed infection distribution, with a few individuals harboring the majority of eggs. This overdispersed distribution pattern is further demonstrated by the positive correlation between log *A. lumbricoides* EPG and log *T. trichiura* EPG in CC2 men, suggesting that individuals with high EPG of one species were more likely to be more heavily infected with the other species. Interestingly, *T. trichiura* infection seemed to be largely associated with age, while *A. lumbricoides* infection was associated with community. The fact that they were closely related has interesting implications for research on how and when different helminth species infect human hosts.

Interestingly, no evidence of hookworm infection was documented, even though it is fairly common in other populations around the globe and in Latin America. In comparison, hookworm prevalence among the Tsimane of Bolivia is 45.4% (Blackwell et

al., 2011). Of the STHs, hookworm is most closely associated with anemia (Ezeamama et al., 2008; Stoltzfus et al., 1997; Tatala et al., 1998; Tsuyuoka et al., 1999). The absence of evidence for hookworm infection here is consistent with our findings that Shuar hemoglobin levels are primarily in the normal range, with relatively low rates of anemia compared to other South American populations that have been studied (SHLHP, unpublished data). It is important to note, however, that the absence of hookworm could in part be related to issues with the Kato-Katz method. While this method is considered the most useful and recommended method available for field studies (WHO, 1991), concerns about its ability to detect hookworm eggs have been raised (Tarafder et al., 2010; WHO, 1991). The Kato-Katz method has been shown to be less sensitive to hookworm infection due to the rapid degeneration of delicate hookworm eggs (Tarafder et al., 2010). While it is possible that the degeneration of hookworm eggs contributed to the lack of hookworm in the sample, it is unlikely that we would see no evidence of hookworm eggs in any of the sampled individuals if the infection were present. This, combined with hemoglobin data, supports the idea that very little or no hookworm is present in this sample and these communities.

The present study found significant regional differences in the prevalence and intensity of STH infections. Both males and females from our CC2 sample were more likely to be infected with *A. lumbricoides* than the other two communities. Analyzing differences in all three communities, rather than just dividing regionally into UV and CC regions showed the importance of small-scale studies to capture intra-cultural and inter-regional variation within Ecuador and around the world. Simply reporting Ecuadorian

data, Amazonian Ecuador data, or even Shuar data fails to capture the experiences of individual communities and can result in suboptimal targeting of public health resources.

Though we found significant differences in infection prevalence between UV and CC communities, UV and CC1 did not differ significantly in any of the infection variables. Most of the differences between the three communities were a result of high infection prevalence and intensity in CC2. As previously discussed, CC1 is an unusual CC community: newly established, relatively small with a high proportion of residents earning government wages as schoolteachers, as well as more intensive ties to the larger towns in the UV. CC1 and CC2 are in short walking distance from each other and use the same river as a water source, suggesting that more than geographic and ecological variability is at play and pointing toward a role for MI in parasite exposure variation.

CC1 may represent a middle ground between UV and CC2 on the spectrum of MI, facing decreased exposure to STHs based on an increased reliance on market goods/processed foods, sanitary and architectural protective barriers, and less interaction with soil during subsistence based activities (Strachen, 1989). This is supported by differences in infection ratios between the three communities. In the UV, individuals are more likely to be uninfected than they are to be infected, while in CC2, individuals are more likely to be infected than uninfected. CC1, on the other hand, did not have significantly different proportions of infected and uninfected individuals.

Age ratios also suggest that CC1 represents a middle ground between UV and CC2. In the UV, both adults and children are more likely to be uninfected. In CC2, both adults and children are more likely to be infected. In CC1, however, children are just as likely to be infected as not infected, while adults are significantly more likely to be not

infected. This suggests that adults are better avoiding infection in CC1, either through behavioral or infrastructural changes, while children in CC1 continue to expose themselves to STHs. However, the exposure among children in CC1 is still on a lesser scale than in CC2.

If the community level differences in parasite load documented in the present study are related to differences in MI, this research has major implications for understanding recent changes in public health, specifically the rise of allergies and autoimmune disorders. Multiple studies now link the increase in several allergies and autoimmune disorders in developed nations with the concomitant decrease in early life exposure to parasites (Butcher, 2008; Elliott et al., 1999; Fleming and Cook, 2006; Hurtado et al., 2008; Nagayama et al., 2004). The present study suggests a suite of lifestyle based factors (i.e., MI) contributing to this decrease in parasite exposure and opens doors for public health researchers to understand the social, cultural, and economic changes that may ultimately result in many negative health outcomes associated with MI.

This study has several important limitations. First, the sample size was relatively small and participants were volunteers, which limits the generalization of our findings, by biasing our data toward individuals who were more interested in or concerned about their health. Second, this study uses regional disparities as a proxy for MI. This approach does not allow us to identify specific factors associated with MI that are related to STH infection and conclusions about MI and parasite load are preliminary. In the future, methods used by Liebert and colleagues (2013) to measure MI based on style of life variables will be used to explore relationships between specific lifestyle factors associated with MI and parasite load among these regionally separated communities.

Third, very little data are available on access to anti-helminth treatments, making it impossible to consider if healthcare availability is a driving force in the differences in STH infection rates between UV and CC communities. Both communities report periodic access to anti-helminth medications, especially among school-aged children, but school attendance, documentation of treatment, and self-report of treatment are all inconsistent and sporadic. According to the WHO's Preventative Chemotherapy and Transmission Control Database (2012), the last large scale chemotherapeutic intervention in Ecuador occurred in 2009, when a reported "100%" of school aged children were treated with 2 rounds of albendazole and mebendazole. It is not clear if those treated include indigenous and rural populations, especially those sampled in this study. The lack of large-scale intervention among young children and adults, as well as repeated treatment for school-aged children, results in rapid re-exposure. There is evidence that individuals with predispositions for heavy infection tend to return to pre-treatment levels relatively soon after treatment (Dold and Holland, 2011), suggesting that the differences we see between UV and CC, especially in moderate to heavy infection, would be similar regardless of access to healthcare provided medication was not taken immediately prior to sample collection, though more studies are needed to clarify this important issue.

To summarize, the present chapter found moderate intensity STH infections that followed an overdispersed pattern among the Shuar. Importantly, we found significant differences in rates and intensities of STH infection using geographic distribution as a proxy for MI. Participants from the more remote CC communities had higher STH infection prevalence and infection intensities than more market-reliant UV communities, suggesting that MI decreases risk of parasite exposure and/or increases access to

treatment. This has important implications for understanding health changes associated with MI. In conjunction with decreased STH infection, we are seeing a heightened risk of chronic diseases among the Shuar with increasing MI (Liebert et al., 2013). Clearly, the relationship between MI and disease risk is complicated, resulting in both negative and positive health outcomes. Although preliminary, this paper represents an important step in understanding how parasite exposure is altered by MI and how this alteration may affect overall health.

Bridge to Chapter IV

Chapter III found significant differences between more traditional Shuar living in the Cross-Cutucú region of Ecuador and more market integrated Shuar living in the Upano Valley. While these differences may have been based on geographic proximity, SOL data, discussed in more detail in Chapter IV, does suggest that these differences are likely driven by MI. If humans share a long, coevolutionary history with pathogens, as the Old Friends Hypothesis suggests, we should see both behavioral and physical protective mechanisms in place to naturally limit infection to prevent high intensity infections. Chapter IV explores avoidance behaviors, including the disgust response, to understand if evolutionary psychology plays a role in pathogen avoidance. These behavioral mechanisms would be important, evolutionarily, because people living in highly parasitized environments would be at a constant risk for reinfection. Even if reinfection with STHs is unavoidable in these environments, mechanisms that allow for some avoidance would keep infection intensity low and prevent parasite related pathogenesis.

CHAPTER IV

PATHOGEN DISGUST FUNCTIONS TO REDUCE PARASITE LOAD WITH REGULATOR MECHANISMS THAT CALIBRATE SENSITIVITY TO ENVIRONMENTAL FACTORS: EVIDENCE FROM AN INDIGENOUS AMAZONIAN POPULATION

This chapter contains unpublished, co-authored material and has been prepared with some assistance from Theresa E. Gildner, Melissa A. Liebert, Samuel S. Urlacher, Felicia C. Madimenos, J. Josh Snodgrass, and Lawrence S. Sugiyama. The author of this dissertation is responsible for the study design, collection and analysis of stool samples and disgust data, data analyses, and all write up for this paper. Gildner, Liebert, Urlacher, Madimenos and Sugiyama aided in data collection in the field. Sugiyama is the field site director. Snodgrass is the academic adviser for this dissertation and provided editorial assistance.

Introduction

Over the past several decades, evolutionary approaches to studying human psychology, behavior, and health have made dramatic advances in our understanding of the human condition. These approaches, however, remain poorly integrated across fields. For example, hypotheses derived from principles of evolutionary psychology predict the information-processing mechanisms designed to solve recurrent adaptive problems in ancestral environments, generating behavioral outcomes in response to environmental cues (Tooby & Cosmides, 1990, 2005). In these cases, while the predicted psychological

design features have been well tested, the hypothesized downstream effects of these mechanisms on fitness-related outcomes have not been tested directly. Conversely, hypotheses in human population biology examine how social and environmental variables affect physiological responses, but rarely consider the information-processing adaptations that interpret environmental cues and alter physiology. The present study links these approaches to test whether variation in the sensitivity of an evolved psychological emotion (disgust) impacts a predicted fitness-related outcome (parasitic worm [helminth] infection).

Disgust is hypothesized to be a basic, universal human emotion that evolved to motivate avoidance of fitness reducing activities, stimuli, or individuals (Curtis & Biran, 2001; Ekman, 1992; Oaten et al., 2009; Tybur et al., 2009). Three functionally distinct domains of disgust psychology have been identified: 1) pathogen disgust, associated with avoiding exposure to disease causing agents; 2) sexual disgust, associated with avoiding fitness reducing mate choice and sexual activities; and, 3) moral disgust, associated with avoiding dishonest or otherwise harmful individuals. In turn, pathogen disgust appears to respond to three classes of stimuli: bodily excretions and body parts, decay and spoiled foods, and living creatures recurrently associated with disease (Tybur et al., 2009).

Further, pathogen disgust sensitivity (PDS) varies between individuals. While too high or too low disgust sensitivity will be maladaptive (Blackwell et al., 2010; Hurtado et al., 2008; Jackson et al., 2008), within reaction norms, those individuals with higher disgust sensitivity are predicted to be more likely to avoid stimuli exhibiting cues recurrently associated with pathogens under evolutionary conditions, and therefore have lower prevalence and intensity of infection (Tybur et al., 2009). Past research shows that

pathogen disgust responds to hypothesized cues of potential pathogen harboring stimuli (Curtis & Biran, 2001; Curtis et al., 2004; Oaten et al., 2009; Tybur et al., 2009), but until now, no study has directly tested the hypothesized adaptive benefit of this mechanism in lower infection prevalence and intensity.

Across evolutionary history, local environments differed in likelihood of pathogen exposure, thus the costs and benefits associated with pathogen avoidance would vary based on likelihood of exposure. Therefore, in addition to individual differences in PDS, we should also see PDS calibrated to other environmental stimuli based on cues associated with trade-offs between the costs and benefits of avoidance. These trade-offs should be associated with likelihood of encountering a pathogen, types of pathogens encountered, probable fitness-reducing costs of exposure, and the ability to avoid those threats. For example, if an animal defecates on the floor, avoiding fecal contamination of food is much less costly, and more effective, if the floor is wood and cooking surfaces elevated on a table. If the floor is dirt and cooking is done by fire on that floor, then contamination is much more likely and would be much less difficult to completely avoid. Further, if one is nutritionally stressed, eating meat that is beginning to spoil entails potentially greater relative benefit to cost than if one is well fed. Because the relative costs and benefits of avoidance change locally for individuals throughout their lives, there should be relatively short-term re-calibration or regulation of disgust sensitivity.

Because immune function is costly and varies by individual, the importance of avoidance behaviors may also differ based on the relative individual costs of immunity based on phenotypic condition, and across the lifespan. Developmental calibration and age-related changes in disgust sensitivity may occur, with various researchers suggesting

that it comes online early and declines with advanced age (Curtis et al., 2004; Oaten et al., 2009). Sex has also been shown to impact sexual disgust sensitivity, as predicted by the relatively greater benefit to women than men of avoiding poor quality mates or risky sexual behavior (Fessler & Navarrete, 2003; Tybur et al., 2009). It is possible that there are sex differences in pathogen disgust as well, because women may face greater relative fitness costs of infection, for example during pregnancy (e.g., nausea and vomiting during pregnancy and food aversion [Sherman & Flaxman, 2002]), yet this issue has attracted surprisingly little attention.

Pathogen disgust is predicted to yield adaptive benefits by decreasing infection with evolutionarily relevant pathogens, not necessarily modern or recently emerging ones. Humans share a long coevolutionary history with parasitic helminths (Hurtado et al., 2008; Jackson et al., 2008; Wolfe et al., 2007). Typically contracted via fecal-oral contamination, Soil-transmitted helminths (STHs) such as *Trichuris trichiura* (whipworm), *Ascaris lumbricoides* (roundworm), and *Necator americanus* (hookworm) can entail fitness-related costs including stunting, wasting, diarrhea, organ failure, nutritional deficiencies, mental and developmental retardation, and even death (Ahmed et al., 2011; Bethony et al., 2006; Blackwell et al., 2010; Hurtado et al., 2008). Despite these negative health outcomes, the immune system has co-evolved with these parasites (Hurtado et al., 2008; Jackson et al., 2008; Wolfe et al., 2007). Until quite recently in evolutionary history, the complete avoidance of STHs would have been difficult or impossible (Hurtado et al., 2008; Jackson et al., 2008), but behaviors that regulated exposure and kept infection levels low would have been beneficial. STH infection

therefore provides an evolutionarily valid basis for testing the proposed ultimate function of pathogen disgust.

Research among populations with intra-cultural variation in features relevant to pathogen exposure and avoidance is useful for testing the hypothesis that PDS is calibrated to local environments. While the Shuar of Ecuadorian Amazonia share a recent genetic and cultural history, rapidly increasing MI has resulted in intra-cultural variation in housing structure and material, water sources, cooking methods, and sanitation infrastructure, among others. For example, housing varies from traditional structures with thatched walls and ceilings and dirt floors, to wood-board walls and floors with tin-roofs, to, very recently (within the last three years), government provided cinder-block structures, often within the same community. This variation provides an opportunity to examine how different environmental living conditions lead to differences in PDS and pathogen exposure.

Past studies have shown that in some cases factors associated with MI, like sanitation and cooking practices (e.g., use of outhouses or latrines, cooking on stoves instead of ground fires), have been associated with decreased exposure to pathogens (Godoy et al., 2005a; 2005b). In other cases, factors associated with MI, including greater population density, were associated with an increase in pathogen exposure and virulence (Godoy et al., 2005a; 2005b). MI, therefore, may cause changes in the cue structures to which pathogen disgust mechanisms respond. For instance, where disgust elicitors must regularly be encountered in order to acquire food, or where living conditions are such that some elicitors are more costly to avoid (e.g., exposure to human and animal waste, dirt cooking floors, and contaminated water associated with the absence of clean water

systems and plumbing) disgust motivated avoidance and cleanliness is expected to be more costly, so PDS should be down-regulated (Tybur et al., 2009).

Among the Shuar, MI is associated with changes to housing construction, cooking surfaces, water source, and latrine types/locations. Traditional houses have dirt floors, thatched roofs, and palmwood slat walls. Water is sourced from rivers, streams, or ponds, and cooking is done on a fire on the dirt floor of the house or adjacent cooking area roofed with palm thatch. Domestic animals, particularly guard and hunting dogs but also chickens or ducks if the owners have them, often wander inside the house, and may defecate on the floor, and contaminate food. Infants and toddlers also urinate or defecate on the floor (or begin to before adults or older siblings pull them outside), and it is common when drinking chicha (*nihamanch* or manioc beer) for adults to spit the more fibrous residue on the floor. Urination occurs at the edge of the house clearing, or under the eaves of the structure if it is raining, and defecation occurs nearby, wherever privacy given by vegetation is available. In order to prevent some contamination, floors of the home are swept to bare dirt using traditional fiber brooms.

More market integrated households, on the other hand, typically have houses with wood board walls and floors, and tin roofs, though some have government funded cinderblock houses with cement floors, tin roofs, and glass windows. Primarily cooking is done on a table top using a propane stove. Pit toilet latrines, or outhouses with a toilet and water access are common. Water is often obtained from spigots connected to a pipe bringing water from a spring, or pulled from a well. Entry of dogs and other domestic animals into the home is more variable because house structure tends to prevent entry

except through the door. Floors of the home are typically washed with water as well as swept.

Given these and other differences, we expect that the costs of pathogen cue avoidance are higher, and the ability to successfully mitigate contamination are lower, among less market integrated Shuar households. However, more market integrated housing types would not necessarily be expected to have an effect on STH infection, unless people were motivated to clean them and use protective barriers. We therefore predict that the effect of MI on STH infection will be mediated by an increase in PDS.

To test these relationships, we administered a disgust questionnaire and material style of life (SOL) interview and collected stool samples from Shuar participants. We hypothesized that: 1) Individuals with greater PDS will have lower prevalence STH infection and intensity; 2) Individuals with greater MI will also have lower prevalence and intensity of helminth infection; 3) Individuals with greater MI (i.e., those for whom avoidance has relatively lower cost to benefit ratio) will exhibit greater PDS; and, 4) the relationship between MI and helminth infection will be mediated by (i.e., in part a function of) the effect of higher PDS.

Study Population

This study was conducted as part of the Shuar Health and Life History Project (SHLHP; www.bonesandbehavior.org/shuar). The Shuar are a large indigenous forager-horticulturalist group (>50,000 individuals in well over 668 communities), concentrated in the cross-Cutucú (CC) and Upano Valley (UV) areas of the Morona-Santiago and Zamora provinces of Ecuador. Traditionally, horticulture, hunting, and fishing were the

foundations of Shuar economy (Harner, 1984; Karsten, 1935; Stirling, 1938). In the more isolated region east of the Cutucú mountains (CC), Shuar continue to follow more traditional lifeways based on hunting, fishing, and horticulture, while those in the UV are experiencing greater economic change associated with increasing participation in the market economy (Blackwell et al., 2009; Liebert et al., 2013; Madimenos et al., 2011). Within UV Shuar communities, road access and resource depletion have decreased reliance on hunting and fishing, although horticultural production of traditional crops such as manioc and plantains continues to provide the dietary staples (Liebert et al., 2013).

UV participants in this study resided within 60 minutes (via truck or bus) of Sucúa, the local market center with commercial, medical, and pharmaceutical access. In contrast, at the time of data collection CC sample communities could access Sucúa via 1.5-3.5 hours by motorized canoe (depending on water levels) and an additional effective travel time of approximately 5.5-8.5 hours by bus. The CC study communities thus had significantly less regular access to markets than UV sample communities. These CC communities had access to a small health center, staffed by a nurse practitioner, where they could receive limited medicines and services for minor injuries and illnesses.

Previous research has documented that UV Shuar have significantly higher levels of MI than CC Shuar (Liebert et al., 2013). It is important to note, however, that while these differences are closely associated with ownership of market goods and general housing structures, they are moderate in comparison to the full range of variation in MI seen across cultures. By Western standards, CC and UV communities are at the less-market integrated end of the spectrum. Further, both UV and CC communities have an

array of housing types that range from traditional thatched roof/dirt floor houses to cement floor/tin roof houses, as described above. UV and CC communities both rely on traditional cultigens for the bulk of their diets, and own various domesticated animals with notable exposure to these animals in and around households. Further, a large portion of individuals from both communities spend significant time in occupations that bring them into direct contact with soil (e.g., subsistence horticulture). In general, however, homes in the UV are more likely to have wood or cement floors, separate cooking areas, propane gas stove, and access to piped water, all features that lower the costs of maintaining hygienic barriers to STH infection, if the owners are sufficiently motivated to do so.

Materials and Methods

Participants and Sampling

The present study design was cross-sectional, with an age-stratified sample of juveniles (<15 years) and adults (≥15 years). Data were collected over two field seasons (August-September 2011 and August-September 2012) in one UV community (population ~350 individuals) and two CC communities (combined population ~360 individuals). A total of 155 participants (UV n = 75; CC n = 80) aged 5-59 years old completed the disgust questionnaire, of which 98 completed the household interview used to determine degree of MI, or style of life (SOL), and 75 also provided a stool sample (UV n = 30; CC n = 45). Because of this relatively small sample size, UV and CC were analyzed together in this study.

Disgust Sensitivity Interviews

A commonly used disgust scale (Haidt et al., 1994; Olatunji et al., 2007) was adapted for relevance to Shuar culture and administered in Spanish, which most Shuar speak fluently. A bilingual (Spanish/Shuar) assistant translated or clarified questions for those participants who were not fluent in Spanish. The questionnaire measured disgust sensitivity to 19 items, using a 5 point Likert scale on which higher values indicate greater disgust (1: "not disgusting" or "*no me da asco*" to 5: "very disgusting" or "*me da mucho asco*"). Moral and sexual disgust items were not included on the questionnaire.

Lifestyle Measures

Structured interviews were administered in Spanish to collect basic demographic and lifestyle information with members of each household. A bilingual assistant translated for those participants not fluent in Spanish. Participants were asked a series of questions from a version of the Material Style of Life (SOL) Index (Bindon et al., 1997; Leonard et al., 2002), modified for use with Shuar (Liebert et al., 2013). The SOL Index is a list of market and traditional items used to assess ownership of goods purchased within a market economy and goods used in traditional subsistence activities (Liebert et al., 2013). The selection of items in the Shuar SOL Index was based on extensive ethnographic observations and pilot testing by one author (LSS) (unpublished data). Two scales were created from the SOL index: Traditional Style of Life (T-SOL) and Market-Integrated Style of Life (M-SOL). The final T-SOL scale contained six items reflecting investment in a foraging lifestyle (fishing hook/line, hunting dogs, blowgun, firearm, fishing net, canoe), while the M-SOL scale included 12 items reflecting investment in a

market economy (radio, propane stove, mobile phone, TV, chainsaw, bicycle, refrigerator, computer, outboard motor, motorcycle, car, truck). Individual scores for M-SOL and T-SOL were calculated as the fraction of list items owned (range 0-1).

Six household measures were also incorporated in the SOL questionnaire to capture household construction, access to water and electricity, market participation, and pathogen risk. These included [in order of increasing MI: floor (0: dirt, 1: palmwood, 2: milled lumber, 3: concrete), wall (0: palmwood, 1: milled lumber, 2: cinder block), latrine type (0: none, 1: pit toilet, 2: outhouse with water, 3: indoor toilet), water source (0: river/stream/spring pond, 1: well or outdoor pipe, 2: indoor pipe), electricity (0: none, 1: lights, 2: outlet) and number of rooms in house. A Household Style of Life (H-SOL) value for each individual was computed based on a summation of the scores (Liebert et al., 2013). High T-SOL values represent greater participation in subsistence activities, high M-SOL values indicate greater participation in market activities, and high H-SOL values indicate greater number of non-traditional household features. It is important to note that individual households could be high on all scales, low on all, or any combination. Therefore, separate analyses based on the T-SOL, M-SOL, and H-SOL scores were conducted.

Stool Collection and Analysis

Fresh stool samples were collected and processed based on methods established by Raso and colleagues (2004). A pre-packed plastic bag containing an empty stool container marked with the name and identification number of the participant was provided the evening before collection. Participants were instructed to either defecate

directly into the cup, or onto a broad leaf or sheet of paper and transfer a portion of feces to the container using the clean tongue depressor provided. Participants were asked to only take feces that had not touched another surface (e.g., the ground, dirt, grass, leaf, paper). Containers were then returned with a small amount of fresh stool to a centralized village location within an hour of passage, where they were processed within an hour of drop-off. A single Kato-Katz thick smear (Katz et al., 1972) was prepared from each specimen using a 42 mg plastic template (Vestergaard Frandsen, Lausanne, Switzerland). Kato-Katz thick smears were examined at 10x and 40x microscopy (Cole-Parmer, Vernon Hills, IL) in the field by a single trained observer (TJC). Helminth egg presence, species presence, species-specific eggs per gram (EPG) of feces, and species-specific infection intensity were recorded.

Infection prevalence, *A. lumbricoides* prevalence, *T. trichiura* prevalence and coinfection prevalence were calculated based on the proportion of participants with any helminth ova, *A. lumbricoides* ova, *T. trichiura* ova, or ova from both species, respectively, in their stool. The Shapiro-Wilk test was used to test for normality in EPG variables before comparing statistics by age and by community. EPG variables were $\log_{10}$-transformed due to non-normal distributions. Because of the overdispersed nature of EPG values, extreme outliers were not excluded from the sample because this would have removed data points of particular interest. Parametric tests with transformed variables are reported in this study. Individuals with greater EPG of feces are likely infected with a larger number of adult worms and are characterized as having a higher intensity infection (Ahmed et al., 2011; Dold & Holland, 2011; Montresor et al., 1998).

Statistical Analyses

Data analysis was conducted using SPSS version 20.0. To determine relationships between disgust sensitivity, age, and sex. Separate two-way ANOVA tests were used to determine the effects of sex and age on PDS scores (F1, F2, and F3). Pearson correlations were used to determine the relationships between age (as a continuous variable) and disgust scores.

To explore the hypothesis that disgust sensitivity and factors associated with MI are related to reduced STH infection and intensity (Objectives 2 and 3), one-way ANOVA tests were used to compare the disgust and SOL scores of infected (with *A. lumbricoides* and/or *T. trichiura*) and non-infected individuals. Pearson correlations were used to determine relationships between log-transformed EPG scores, and disgust and SOL scores. To understand the role that MI plays in disgust sensitivity, Pearson correlations were used to determine the relationships between disgust scores and the various SOL measures.

Mediation analyses were conducted for the 75 individuals who had participated in all three parts of this study (SOL, PDS, STH) to consider whether PDS acts as a mediator between MI and parasite infection (Baron & Kenny, 1986; Preacher & Kelley, 2011; Shebl et al., 2010). Regression analyses were run to test relationships between (a) SOL variables and PDS variables, (b) PDS variables (controlling for SOL variables) and STH variables, (c) SOL variables and STH variables, and (c') SOL variables (controlling for PDS variables) and STH variables (Baron & Kenny, 1986). A mediated effect was calculated by multiplying the coefficient from (a) by the coefficient from (b). Sobel tests were used to test the significance of the mediated effect (Sobel, 1986).

Results

Principal Components Analysis (PCA) with varimax rotation was conducted on the 19-item Disgust Questionnaire, to reduce items into components for further analyses. Three disgust component factors were identified using Eigenvalues greater than 1.0 **(Table 4.1)**. Together, these three factors explained 54% of the total item variance **(Table 4.2)**. They were not significantly correlated, and the Kaiser-Meyer-Olkin (KMO) measure for sampling adequacy was meritorious at 0.893, indicating sample size for PCA was appropriate. Variables that loaded heavily on Factor 1 (F1; Contamination) were most closely associated with direct contact with pathogen containing fluid (e.g., vomit or mucus) as well as contamination of water and food. Factor 2 (F2; Spoiled/Raw Food) was closely associated with spoiled or raw food, or touching a dead animal (but not freshly killed game). It is important to note that in this tropical climate, raw food spoils quickly, as does cooked food that is not thoroughly cooked or re-cooked adequately. Variables that loaded on Factor 3 (F3; Pests) were associated with animals that bite and potentially carry diseases.

Table 4.1. Principal components analysis of disgust stimuli

	Factors		
Variable	**1**	**2**	**3**
Finding a worm in your food.	**0.759**		
Drinking chicha made by someone who has no teeth.	**0.714**		
Someone coughing in your face.	**0.673**		
Drinking chicha made by someone who is ill.	**0.655**		
Stepping in feces with bare feet.	**0.650**		
Finding a cockroach in your food.	**0.649**		
Someone vomiting on your shoes.	**0.588**		
Knowing someone hasn't showered in three days.	**0.557**		
A dog licking your face.	**0.533**		
Drinking brown, dirty water.	0.433		
Eating raw fish.		**0.852**	
Eating raw chicken.		**0.847**	
Eating raw beef.		**0.791**	
Eating meat that has gone bad.		**0.699**	
Picking up a dead animal with your hands.	0.404	**0.527**	
Not washing your hands before eating.		0.438	
Finding a spider in your house.			**0.814**
Seeing a rat in your kitchen.		0.348	**0.665**
Coming into contact with someone else's blood.			**0.654**

*Only those factors with eigenvalues of at least 1.5 were included. Values represent loading scores for individuals factors, which were included in the factor if they were at or above 0.3. Items with loadings greater than 0.5 are shown in bold.

Table 4.2. Disgust factors

#	**Factor**	**Eigenvalue**	**# of variance**
1	Contamination	4.473	23.54
2	Raw/Spoiled Food	3.58	18.85
3	Pests	2.25	11.83
Total variance explained:			**54.22**

With the sample pooled, two-way ANOVAs compared disgust scores by sex and age (**Table 4.3**). Age was significantly associated with F2 (Spoiled/Raw Food), though this was not a linear relationship: partial correlations controlling for sex found no significant relationships between age and disgust factors.

Table 4.3. Three-way ANOVA of the effects of age and sex on PDS

	df	F	*p*	R^2
F1: Contamination				0.377
Age	36	1.511	0.055	
Sex	1	0.353	0.554	
Age x Sex	11	0.772	0.667	
F2: Raw/Spoiled Food				0.422
Age	36	1.763	0.014	
Sex	1	2.114	0.149	
Age x Sex	11	1.770	0.068	
F3: Pests				0.262
Age	36	0.788	0.791	
Sex	1	2.262	0.136	
Age x Sex	11	0.705	0.732	

We hypothesized that greater MI (Household Style of Life [H-SOL], Traditional Style of Life [T-SOL], and Market-integrated Style of Life [M-SOL]) and higher PDS should be associated with decreased STH infection prevalence and intensity. To test these hypotheses, a series of one-way ANOVA tests were used to compare disgust and SOL variables based on general STH infection status (infected or not), coinfection status (infected with at least two types of helminths or not), and species-specific infection status (infected with *A. lumbricoides* or *T. trichiura* respectively, or not). No significant differences in the PDS or SOL factors were associated with general STH infection status. There were significant differences based on *A. lumbricoides* infection, *T. trichiura* infection, and co-infection. *Ascaris lumbricoides* infected individuals showed a trend toward lower disgust sensitivity associated with contamination ($p = 0.058$), a lower H-SOL ($p < 0.08$) and had significantly lower M-SOL ($p < 0.001$) than non-infected individuals. A high H-SOL is indicative of more market integrated household construction, access to water and electricity, and MI. A high M-SOL represents increased

ownership of consumer/market goods. *Trichuris trichiura* infected individuals had significantly higher disgust sensitivity toward raw/spoiled foods ($p < 0.05$). Individuals who were co-infected with both *T. trichiura* and *A. lumbricoides* had significantly lower H-SOL ($p < 0.01$) and M-SOL ($p < 0.01$), and higher T-SOL ($p < 0.05$) than those who were either not infected or infected with only one type of helminth.

PDS factors and MI variables were also associated with infection intensity. *Ascaris lumbricoides* EPG was negatively correlated with F1 (Contamination) ($p < 0.05$) and H-SOL ($p = 0.01$), and positively correlated with T-SOL ($p < 0.01$). This relationship between *A. lumbricoides* EPG and F1 (Contamination) was no longer significant when we controlled for SOL measures.

We hypothesized that PDS would have a positive association with MI. We found that with all subjects combined, Factor 1 (Contamination) was positively correlated with H-SOL ($p = 0.001$) and M-SOL ($p < 0.05$), and negatively correlated with T-SOL ($p < 0.01$). Factor 2 (Spoiled/Raw Food) was also positively correlated with H-SOL ($p < 0.05$) and M-SOL ($p < 0.01$). No significant relationships were found for F3 (Pests).

Finally, we hypothesized that PDS would play a mediating role between increased MI and decreased STH infection prevalence and intensity. Only *A. lumbricoides* variables were used in the mediation model because they were the only infection variables associated with both MI and PDS. For the model to suggest mediation, all three relationships (see **Figure 4.1** and **Table 4.4** for a breakdown of relationships a, b, and c) should be significant or trending. One then tests whether c', which controls for the mediating variable, becomes non-significant. If c' is non-significant or less significant,

then the controlled variable may act as a mediator between the independent and dependent variables.

Table 4.4 shows proposed relationships between variables in the simple mediation model, as well as linear regression and logistic regression data for each relationship. Though multiple models were tested to account for all MI, PDS, and STH variables, only two models met the initial criteria that relationships a, b, and c are all significant. Model 1 explored the role of F1 (Contamination) as a mediator between H-SOL and *A. lumbricoides* intensity (LogEPG). Relationship a: Linear regressions show that for every unit increase in H-SOL, F1 (Contamination) increased by 0.094 (p = 0.001). Relationship b: For every unit increase in F1 (Contamination), LogEPG decreased by 0.395 (p = 0.064) when controlling for H-SOL. Relationship c: For every unit increase in H-SOL, LogEPG decreased by 0.12 (p = 0.015). Relationship c': When F1 (Contamination) was included as a mediator in the model, every unit increase in H-SOL only yielded a decrease of 0.083 in LogEPG (p > 0.1). A full mediation relationship is present because including F1 (Contamination) in the model reduces the relationship between H-SOL and LogEPG to non-significant. Overall, the mediated effect was -0.037 with a Sobel Statistic of -1.668 (p = 0.095).

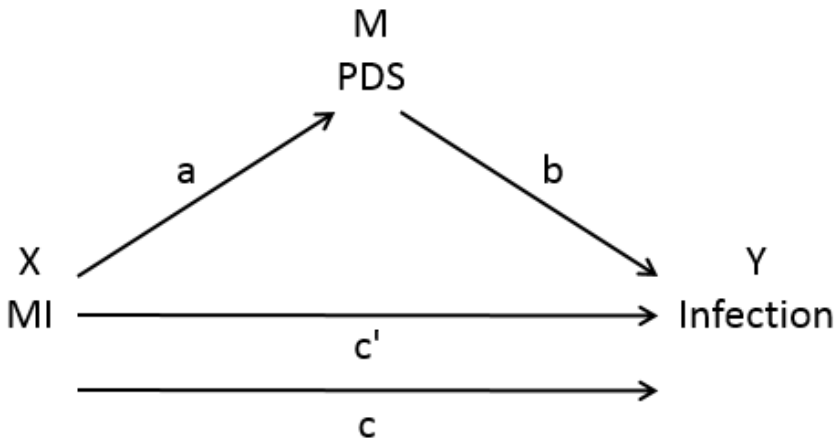

Figure 4.1. Illustration of the relationships tested in the mediation model.

Table 4.4. Simple regression and mediation results for each pathway of the mediation model

	Coefficient (SE)	***P***	**Mediated Effect (SE) a * b**	**Sobel Statistic**
Model 1:		0.095	-0.037 (0.02)	-1.668
a: HSOL → F1	0.094 (0.03)	0.001		
b: F1 (HSOL) → LogEPG	-0.395 (0.21)	0.064		
c: HSOL → LogEPG	-0.12 (0.05)	0.015		
c': HSOL (F1) → LogEPG	*-0.083 (0.05)*	*0.109*		
Model 2:		0.147	-0.766 (0.53)	-1.452
a: MSOL → F1	2.373 (0.85)	0.007		
b: F1 (MSOL) → LogEPG	-0.323 (0.19)	0.098		
c: MSOL → LogEPG	-5.732 (1.42)	0.000		
c': MSOL (F1) → LogEPG	-4.965 (1.47)	0.001		

Only models that were significant (or trending at $p < 0.1$) for a, b, and c are shown here.

Model 2 explored the role of F1 (Contamination) as a mediator between M-SOL and *A. lumbricoides* intensity (LogEPG). Relationship a: For every unit increase in M-SOL, F1 (Contamination) increased by 2.373 ($p = 0.007$). Relationship b: When controlling for M-SOL, LogEPG decreased by -0.323 ($p = 0.098$) for every unit increase in F1 (Contamination). Relationship c: LogEPG decreased by 5.732 for every unit increase in M-SOL ($p < 0.001$). Relationship c': When F1 (Contamination) was included as a mediator in the model, LogEPG decreased by 4.965 ($p = 0.001$). While significance was slightly reduced by including F1 (Contamination) in the model, this did not reduce c' to non-significance. F1 (Contamination) is not a mediating variable in the relationship between M-SOL and *A. lumbricoides* infection intensity.

Discussion and Conclusions

The present paper is the first to examine and directly test the relationship between PDS and pathogen infection as well as the effects of environmental calibration of disgust sensitivity. We provide cross-cultural evidence of the three pathogen disgust domains from a small-scale, non-Western society, and document important relationships between disgust responses, their environmental calibration, and the risk of parasite exposure. These findings support claims that disgust, as an emotion, evolved to provide a protective barrier from environmental pathogens. Previous research on disgust response grouped pathogen disgust stimuli into three distinct categories: 1) bodily excretions and body parts; 2) decay and spoiled food; and 3) particular living creatures (Tybur et al., 2009). These categories correspond well with the three disgust factors (F1 [Contamination], F2 [Spoiled/Raw Food], F3 [Pests]) identified via PCA in the present study, providing

support for these as basic psychological categories in a non-Western population representing multiple points on the spectrum of MI.

We found no significant relationship between sex and disgust, which is consistent with other research that found significant differences between sexes only in the sexual disgust domain (Tybur et al., 2009; Lieberman et al., 2003; Fessler & Navarrete, 2003). Further, disgust did not vary consistently by age among this sample, contrasting with earlier hypotheses suggesting decreased sensitivity during older adulthood (Oaten et al., 2009, Curtis et al., 2004). Because most previous studies of disgust have been conducted among college-aged adults, there is very little information on age variation across a wider spectrum. Although the results of the present study are preliminary, the lack of predictable linear age effects on disgust sensitivity, coupled with significant differences in sensitivity associated with MI, suggests that children begin calibrating disgust sensitivity based on relevant cues in their social and ecological environment very early on (prior to 5 years of age). This calibration continues to shift throughout their life in relation to their contemporary environment. Due to our small sample size of older adults and the use of methods that could not illicit disgust sensitivity from very young children, we cannot address this hypothesis here. Future studies should examine the developmental psychology of disgust sensitivity, and the particular cues it uses in generating changes in sensitivity throughout the life course as environmental and social conditions change. Furthermore, follow-up studies of social and economic change through the regions tested here could illuminate how PDS is calibrated to environmental change within a longitudinal sample.

The significant relationships between MI variables and *A. lumbricoides* infection prevalence, intensity, and co-infection occurrence, suggests that factors related to higher MI reduce STH infection and intensity. As discussed above, likely reasons include protective barriers related to house construction such as wood instead of dirt floors, use of latrines, and access to water piped in from fresh water springs. Higher disgust sensitivity related to contamination was related to decreased prevalence and intensity of *A. lumbricoides* infection, but these relationships were less significant than effects of MI. It is likely that barriers associated with MI confer relatively more protection from pathogens in this context than avoidance behaviors associated with increased disgust sensitivity alone. However, these avoidance behaviors related to the disgust response, especially those associated with contamination elicitors, do appear to provide some protection from pathogens, as predicted, particularly where contamination is more easily avoided (e.g., less traditional housing construction).

Although not captured in the PDS scale used in the present study, we propose that expectations of cleanliness and motivation to clean associated with disgust sensitivity could be key mediating factors in the relationship between MI and infection. Future studies should directly test questions related to desire to clean and time spent cleaning. For example, although wood floors are easier to clean than dirt floors, contaminated mud is easily tracked inside and almost all house types allow for entry of domesticated animals. Increased disgust sensitivity would result in increased sanitation around the house that, in a more market integrated easier-to-clean house, would combine to decrease pathogen exposure. This idea is supported by the positive significant relationships between elevated MI and disgust variables. SOL variables measuring higher MI (M-SOL

and H-SOL) were positively associated, and traditional SOL (T-SOL) negatively associated, with PDS, again confirming our hypothesis that disgust sensitivity among the Shuar increases with factors associated with MI. The fact that H-SOL (our measure of MI-related house construction) was positively correlated to PDS related to contamination suggests that more MI household structures give at least perceived protection from disgust elicitors associated with contamination. This is unsurprising since wood or cement floors and walls, access to piped water from clean springs, and latrines or plumbing reduce the relative costs of keeping cockroaches, rodents, feces, and other vectors of infection away from food and other possible sources of contamination. For instance, wood floors are more easily cleaned of fecal matter (or mud containing fecal matter) than dirt floors, reducing risk of contamination.

Furthermore, M-SOL (associated with increased market participation) was positively related to disgust sensitivity to contamination and uncooked/spoiled food factors, while T-SOL (associated with participation in more traditional activities) was related to decreased disgust sensitivity associated with contamination. Again, this makes sense since cooking over portable gas stoves (one of the M-SOL variables) reduces the relative labor costs of boiling water and cooking or re-cooking food, compared to cooking with firewood. Taken together, these results suggest that greater MI likely increases the ability to avoid disgust elicitors and thus increases disgust sensitivity to these stimuli.

Results also suggest that this calibration of disgust to the local environment begins at a relatively young age based on cues associated with the likelihood of exposure, the ability to avoid disgust elicitors, and social learning associated with hygiene. We

deduce this early timing of disgust calibration based on our data, which found no difference in disgust sensitivity based on age. This suggests that by the time participants were old enough to answer the disgust questionnaire (~5 years old) they already had similar PDS to the adults. Recalibration also appears to occur throughout the lifespan, as evidenced by the fact that in this sample of individuals experiencing rapid MI, there was higher disgust sensitivity more MI individuals, and yet no age differences were documented in sensitivity.

The hypothesis that disgust sensitivity mediates the relationship between MI and STH infection is supported by this study, with F1 (Contamination) mediating the effects of H-SOL on *A. lumbricoides* infection intensity. However, this was not the case with Model 2, which examined F1 (Contamination) as a mediator between M-SOL and *A. lumbricoides* infection intensity. These different relationships make sense based on our hypothesis that elevated disgust sensitivity motivates pathogen avoidance behavior which, when combined with the means of avoidance provided by elevated MI, decreases exposure to pathogens.

M-SOL represents items used and purchased within a market-based economy. These items are indirectly associated with parasite exposure because they represent an overall change in subsistence associated with decreasing interactions with contaminated materials and increasing access to health care. H-SOL, on the other hand, represents housing construction, water purification, and other means of sanitation. Items associated with higher H-SOL could easily be manipulated by individuals with heightened disgust toward stimuli associated with F1 (Contamination) to provide barriers against parasite exposure, which would reduce overall intensity of infection. Higher H-SOL introduces

the means for increased sanitation and protective barriers, while F1 (Contamination) may act to motivate individuals to clean. Thus, we see F1 (Contamination) acting as a mediator because heightened PDS alters behavior and increases avoidance. While it seems that likelihood of infection is largely related to MI, increased PDS seems to reduce frequency of exposure and reinfection, thus reducing infection intensity and associated risk of pathogenesis.

Conclusions

This study is the first to document associations among disgust sensitivity, socio-ecological variation, and parasitic disease burden. As predicted, higher pathogen disgust sensitivity and more participation in market based economies are associated with decreased prevalence and infection intensity of STHs. Further, factors related to a greater ability to avoid pathogen related stimuli (higher MI) are associated with greater disgust sensitivity, supporting the idea that disgust is calibrated to the cost-benefit tradeoffs in the local environment.

Overall, this study points to the kinds of tradeoffs faced in the relative costs and benefits of disgust sensitivity and provides evidence for its importance as a potential evolutionary strategy for avoiding pathogens. In developed nations, heightened disgust sensitivity may prove problematic as the means to avoid pathogens result in limited exposure to helminths and bacteria. This change in disease exposure may ultimately be responsible for allergies and autoimmune disorders (Hurtado et al., 2008; Jackson et al., 2008).

Further, this study demonstrates the utility of conceptual unity and methodological integration across different branches of human evolutionary research—in this case evolutionary psychology and human biology—to elucidate how environmental differences are processed by psychological adaptations to produce variation in fitness-related outcomes. Ultimately, such integration promises to connect the causal chain running from ancestral environments to present behavior, from evolved psychological mechanisms and their neurobiological substrates to the different cue structures of the environments in which they operate and their ultimate behavioral outputs and fitness effects. Integration across all these links is not only analytically powerful but also necessary if we are to fully understand the human condition.

Bridge to Chapter V

Chapter IV demonstrated that humans, under selective pressure from parasites and other pathogens, evolved psychological and behavioral mechanisms to avoid stimuli associated with pathogens. Too high of disgust sensitivity in wealthy, economically developed nations may be resulting in the associated decrease in parasite exposure and loss of microbial diversity seen in these regions. As discussed in Chapters I and II, this loss of microbial and parasitic stimuli may be responsible, at least in part, for immune dysregulation and the development of allergy and autoimmune disorders. Chapter VI further examines the long coevolutionary history between humans and helminths, looking for evidence that STH exposure regulates immune function. Specifically, Chapter VI tests relationships between MI, STH infection, and immune function using C-reactive protein levels as indicators of sub-clinical chronic inflammation.

CHAPTER V

THE EFFECTS OF ECONOMIC DEVELOPMENT AND SOIL-TRANSMITTED HELMINTH EXPOSURE ON CHRONIC INFLAMMATION AMONG THE SHUAR

This chapter contains unpublished, co-authored material and has been prepared with some assistance from Theresa E. Gildner, Melissa A. Liebert, Samuel S. Urlacher, Felicia C. Madimenos, Lawrence S. Sugiyama, and J. Josh Snodgrass. The author of this dissertation is responsible for the study design, collection and analyses of stool samples, data analysis, and all write up for this paper. Gildner, Liebert, Urlacher, Madimenos and Sugiyama aided in data collection in the field. Sugiyama is the field site director. Snodgrass provided lab facilities and support for C-reactive protein analysis, is the academic adviser for this dissertation, and provided editorial assistance.

Introduction

The Hygiene Hypothesis and the more specific and aptly named "Old Friends Hypothesis" posit that the increased prevalence of inflammatory diseases related to immune dysregulation (i.e., allergic and autoimmune disorders) in economically developed nations stems from decreased exposure to certain relatively harmless pathogens and commensals (Strachen et al., 1989; Wolfe et al., 2007). Soil transmitted helminths (STHs) are considered to have a central role in this hypothesis due to their ability to manipulate the host immune system to evade detection and reduce inflammation (Bethony et al., 2006; Deehan et al., 2002). STHs, like *Trichuris trichiura*, *Ascaris*

lumbricoides, and *Necator americanus*, are parasitic worms generally transmitted through orofecal contamination. Chronic infections with STHs are generally asymptomatic, although they can lead to stunting, wasting, nutritional deficiencies, and diarrhea; in extreme cases, organ failure and death can occur (Ahmed et al., 2011; Bethony et al., 2006; Blackwell et al., 2010; Dold and Holland, 2011; Francis et al., 2012; Hurtado et al., 2008; Tanner et al., 2009).

Humans and STHs share a long coevolutionary history, with STHs being among a limited number of infectious agents that were able to persist within relatively small human hunter-gatherer groups (Kelly, 2013; Rook et al., 2014; Wolfe et al., 2007). This is why STHs, along with commensal and environmental bacteria, have taken center stage in the Old Friends Hypothesis. In contrast, highly virulent "crowd" diseases that rely on high population density, like measles and other viruses, are a relatively recent phenomenon and have a shorter coevolutionary history (Wolfe et al., 2007). Because virulence that incapacitated its host would not be supported well within small, kin-based hunter-gatherer societies, STHs likely evolved a way to go undetected, while continuing to benefit from their hosts.

At light to moderate levels of intensity, STHs are generally harmless, or at least cause less harm than certain types of intense immune responses against them (Clark, 2008). Further, they reproduce rapidly. Adult worms can lay very high numbers of eggs per day (Bethony et al., 2006; Bogitsh et al., 2005). This means that the adult worms can not only go on living in their hosts for an extended period of time, but they can also lay enough eggs to constantly re-infect the host and others within the population on a continuing basis. For intestinal STHs to be successful, there is no need for high

population density. Immune responses that completely eradicate worms would have been pointless due to high likelihood of reinfection, leading to unnecessary energy expenditure and tissues/cell damage (Rook et al., 2014).

The idea that tolerance toward low to moderate level worm burden would be selected for over complete immune vigilance is supported by data on the high cost of mounting an immune response. As discussed in Chapter I, upregulated thermogenesis and fever, combined with cellular immunity to illness, results in up to a 300% rise in resting metabolic rate (Chiolero et al., 1997). Fever, in which the body temperature to is elevated decrease habitability for the pathogen, has been shown to increase basal metabolic rate by 10 to 15% for each 1^{o} C rise in body temperature (Roe & Kinney, 1965). Even a subtle increase of interleukin (IL)-6 (associated with upregulated inflammation in response to infection) from infusions into healthy volunteers increased resting metabolism by 25% (Tsigos et al., 1997). Further, slight immune responses associated with respiratory infections and vaccinations have been shown to increase resting metabolic rate by 8 to 14% and 15-30%, respectively (Cooper et al., 1992; Demas et al., 1997; Muehlenbein et al., 2014; Svensson et al., 1998). It is important to emphasize, however, that very little data exist on the exact costs of the immune response (Muehlenbein et al., 2014), especially in response to helminth infections. Research that investigates tradeoffs related to STH infections would be especially useful for understanding human-parasite coevolution.

In order to evade the immune response, antigens released by STHs trigger the Th2 branch of the immune system, which increases regulatory T cell (T_{reg}) activity and production of interleukin-10 (IL-10; an anti-inflammatory cytokine), both of which

suppress inflammation and downregulate immune system activity (Bach, 2005; Flohr et al., 2008; Gale, 2002; Rook et al., 2014). This makes the host environment more habitable for the parasite. In turn, the host immune system may have evolved to use this down-modulation of inflammatory processes in its own development and regulation. A lack of STH exposure may be one of the reasons for the increase in diseases associated with chronic inflammation, like cardiovascular disease (CVD), allergy, and autoimmunity, in economically developed nations (AAGA, 2012; AARDA & NCAPG, 2011; Bindon, 1995; Bindon et al., 1997; Cassel et al., 1960; Dressler, 1985; 1999; Dressler & Bindon, 2000; Tobias, 2010).

C-reactive protein (CRP) is a pro-inflammatory protein found in plasma that plays an important role in normal immune responses to pathogens following macrophage and T-cell induced activation of its predecessor interleukin-6 (IL-6; McDade et al., 2012; 2013; Miller & McDade, 2012). Though important in acute immune responses, wealthy nations such as the US are seeing an increase in chronic inflammation associated with low-level elevations of CRP (McDade et al., 2012, 2013). Chronic inflammation associated with sub-clinically elevated plasma CRP, measured using high-sensitivity assays, has been shown to increase with age in developed nations (Ferrucci et al., 2005; Wener et al., 2000). Further, chronic inflammation has been implicated in the development of a number of chronic degenerative diseases including CVD (Ridker & Haughie, 1998), type II diabetes (T2D; Pradhan et al., 2001), and depression (Gimeno et al., 2009), as well as disorders associated with immune system dysregulation like allergy and autoimmunity (Du Clos, 2003; Ishihara and Hirano, 2002; Kikuchi et al., 2002; Lin, 2002; MacDonald and Monteleone, 2005; Qian et al., 2008; Solem et al., 2005; Vermeire

et al., 2004). Interestingly, the increase in CRP with age seems to be related to an increased likelihood of developing CVD (Ferrucci et al., 2005), due to damage to arterial walls that result in increased arterial plaque buildup and atherosclerosis (Ross, 1999). Further, CRP tends to be associated with measures of body mass, including body mass index, body fat percentage, waist circumference, and waist-to-stature ratio (Ford, 1999; Greenfield et al., 2004; Snodgrass et al., 2007b; Sorensen et al., 2006), although these relationships appear to be sex-specific depending on location of adiposity (Snodgrass et al., 2007; Sorensen et al., 2006).

The immune system of individuals experiencing chronic inflammation may be primed to respond to pathogens or cellular damage in a proinflammatory manner (McDade et al., 2013). Low levels of stimulation by certain pathogens during immune system development may result in greater inflammatory responses to later infections, as well as to harmless or self-produced antigens (Bach, 2005; Gale, 2002). Without proper priming and stimulation associated with STH infection, inflammatory pathways associated with CRP production are enhanced in response to infectious agents like viruses and bacteria, as well as chronic physical and psychosocial stress (Blackwell et al., 2010), which may be a cause of the recent increase in allergic and autoimmune disorders in wealthy regions and nations.

Allergy and autoimmunity result when the immune system overreacts to harmless or self-produced cells, respectively. According to the Allergy and Asthma Foundation (2012), 20 million Americans—mostly children—currently suffer from asthma. Further, 50 million Americans are estimated to have an autoimmune condition (Tobias, 2010). These prevalence rates for both allergies and autoimmunity have been increasing rapidly

over the past few decades in the US (AAGA, 2012; AARDA & NCAPG, 2011; Tobias, 2010). Specifically, people living in wealthy nations are seeing an increase in chronic diseases associated with inflammation (Bindon, 1995; Bindon et al., 1997; Cassel et al., 1960; Dressler, 1985; 1999; Dressler & Bindon, 2000).

Evolutionary medicine provides a useful approach for understanding the increase in chronic inflammatory diseases. Specifically, these diseases may be associated with the mismatch between evolved human biology and the contemporary environments and lifestyles of people living in economically developed and developing regions (Nesse & Sterns, 2008; Williams & Nesse, 1991). One major change that has occurred is a rapid decrease in exposure to "old friends," or those infectious agents, like STHs, that were ubiquitous in ancestral hunter-gatherer populations and shaped immune function (Rook et al., 2014; Wolfe et al., 2007).

If STH exposure has provided crucial immune priming throughout human evolutionary history, as the Hygiene Hypothesis suggests, research efforts should be focused on economically developing nations where populations are currently undergoing transitions from more traditional lifeways to those with increasing reliance on regional and global market economies. This transition alters exposure to STHs by shifting subsistence activities, food sources, and population densities, among a suite of other factors (Hurtado et al., 2008). Wealthy nations, for instance, generally provide no exposure to STHs, though this is a relatively recent phenomenon that has only occurred within the past 30 to 50 years (CDC, 2015; Hurtado et al., 2008; Stoll, 1947; Strachen, 1989).

Changes in lifestyle associated with economic, social and health transitions in developing nations can be understood under the framework of market integration (MI). MI is the suite of social and economic factors associated with production for and consumption from market economies. MI generally results in pronounced disparities in socioeconomic status, access to health care, and exposure to animals and fecally-contaminated soils. Higher MI, using geographic location as a proxy for market involvement, has been linked to lower infection prevalence of STHs (Chapter III), as well as increases in chronic diseases like obesity, CVD, T2D, and allergy and autoimmunity (Cassel et al., 1960; Dressler, 1985; Bindon, 1995; Bindon et al., 1997; Dressler, 1999; Dressler & Bindon, 2000; Snodgrass et al., 2007; Cepon et al., 2011; Liebert et al., 2013).

The present study investigates differences in STH infection and inflammation among Shuar participants at multiple points along the spectrum of MI. It is important to stress that MI exists as a continuum, even though most studies about the Hygiene Hypothesis to date have relied on comparisons between hypothetical ends of the spectrum. At the most traditional end, research proposes the presence of heightened levels of infectious and parasitic diseases and commensal microbes with limited chronic disease burden. In contrast, the most wealthy, developed, market integrated end of the MI spectrum presents with elevated rates of chronic diseases, some infection by extremely virulent "crowd" diseases like viruses, and no contact with STHs and other environmental microbes (Fitton, 2000; Godoy et al., 2005a; 2005b; Rook et al., 2014). In the middle, especially in developing nations, a double burden of disease has been documented, with both infectious and chronic, non-communicable diseases (Barrett et al., 1998; Gurven et al., 2009; Prentice, 2006).

To date, most studies that explore the effects of MI on health have focused their attention on chronic diseases like CVD and T2D (Cassel et al., 1960; Dressler, 1985; Bindon, 1995; Bindon et al., 1997; Dressler, 1999; Dressler & Bindon, 2000; Liebert et al., 2013; Snodgrass et al., 2007). This provides an incomplete picture, given the role that altered infectious disease burden may have, not only on morbidity in developing nations, but also on the development of chronic disease. Research that simultaneously examines both the prevalence of infectious diseases like STHs and chronic inflammatory diseases among a population experiencing rapid economic development is especially important because it can document changes in disease exposure and the resultant experience of immune dysregulation related to inflammation. The present study tests relationships among MI, STH infection, and CRP in the Shuar forager-horticulturalists of Amazonian Ecuador. The Shuar are currently undergoing profound social, economic, and environmental changes associated with MI. These changes are leading to altered helminth exposure (Chapter III) and CVD risk (Liebert et al., 2013).

This study tests the Hygiene Hypothesis in order to observe relationships between altered infectious disease burden and chronic inflammation between more traditional and more market integrated individuals. This study tests the Hygiene Hypothesis, with the following objectives:

Objective 1: Anthropometrics, MI, and CRP

Objective 1 is to examine relationships among measures of body mass, MI, and CRP. Chronic inflammation is often associated with measures of overweight/obesity and these tend to hinge on factors associated with increased participation in the market

economy, especially those factors related to consumption of energetically dense diets and increased sedentary behavior. If these variables have begun to affect chronic inflammatory patterns among the Shuar, we should see elevated CRP associated with both more market integrated lifestyles and altered body mass.

Objective 2: MI and STHs

Objective 2 is to test relationships between individual level of MI and STH infection and intensity. More market integrated individuals are predicted to have lower STH infection prevalence and intensity than individuals living more traditional lifestyles.

Objective 3: STHs and CRP

Objective 3 is to observe the relationship between STH infection and subclinical elevations of CRP. If STHs have an anti-inflammatory effect, there should be lower CRP in infected compared to uninfected individuals and an inverse relationship between CRP concentration and STH infection intensity. CRP concentration should be positively associated in individuals that are not currently infected with STHs. There should either be a negative relationship (or no significant relationship) between CRP and age in participants infected with STHs.

Study Population

This study was conducted among the Shuar, a large indigenous forager-horticulturalist group (~46,000 individuals in over 668 communities), in conjunction with the Shuar Health and Life History Project (SHLHP; www.bonesandbehavior.org/shuar).

The Shuar are concentrated in the cross-Cutucú and Upano Valley regions of the Morona-Santiago and Zamora provinces of Ecuador. While the Shuar traditionally practiced subsistence horticulture, hunting, and fishing (Harner, 1984; Karsten, 1935; Stirling, 1938), they are currently experiencing social, cultural, environmental, and economic change associated with rapid integration into regional and global markets. The accelerated, yet uneven, pace of this change provides an important opportunity to examine shifts in chronic and infectious diseases as they occur with MI.

Integration into market economies among the Shuar is producing variation in lifestyles and health in two observable ways: 1) regionally, based on travel availability and barriers to market access; and 2) within regions based on socioeconomic status, access to education and medical care, among other factors. In the more isolated region east of the Cutucú mountain range (Cross-Cutucú or CC), Shuar maintain more traditional lifestyles based on hunting, fishing, and horticulture, in part due to travel barriers that make market access difficult on a regular basis. At the time of data collection in the present study (August-September of 2011, 2012, and 2013), the CC communities could access the regional market center of Sucúa in 1.5 to 3.5 hours via canoe (depending on water level) and an additional travel time of approximately 5.5 to 8.5 hours by bus.

Shuar living in Upano Valley (UV) communities could reach Sucúa via bus or truck within 60 minutes. In the UV, Shuar are experiencing greater economic change associated with increased participation in the market economy as well as greater access to medical and pharmaceutical care (Blackwell et al., 2009; Madimenos et al., 2011). However, the more economically developed UV environment leads to increased economic disparities, which may result in marginalization and inequality related to

ethnicity (non-Shuar *Colonos* live in the UV region as well, often with better jobs and more access to resources) and SES. While these variables of ethnicity and SES are not directly measured in this study, they are important because they are deeply interwoven in MI and result in health disparities. The present study examines individual differences in MI, rather than conducting a community-based comparison. This is because MI variables allow us to assess different features associated with MI across both regions.

Previous research by SHLHP documented a number of relevant, health-related issues among the Shuar, validating the need for studies that explore both infectious and chronic disorders. First, we have documented significant differences in STH infection prevalence and intensity between the UV and CC regions (Chapter III). This is also evident in elevated levels of immunoglobulin E (IgE; a class of antibody closely associated with parasitic worms) among Shuar, especially compared to industrialized nations (Blackwell et al., 2010). Further, 40% of Shuar children are stunted, a much higher prevalence of stunting than is found among other indigenous and non-indigenous children living in the same area (Blackwell et al., 2009; Urlacher et al., in press). Stunting is often associated with life history tradeoffs that shift resources toward immune function and maintenance and away from growth in highly pathogenic or resource scarce environments (Blackwell et al., 2010). STH exposure and immune priming among the Shuar is further supported by the fact they show no evidence of chronically elevated CRP, at least among a sample of three communities from the UV region (McDade et al., 2012). This does not, however, mean that immune dysregulation and chronic inflammation are not an issue, as our previous research has demonstrated increased risks for CVD associated with MI (Liebert et al., 2013).

Materials and Methods

Participants and Sampling

The present study design was cross-sectional, with data collection occurring during three field seasons (August-September 2011, August-September 2012, and August-September 2013). A total of 310 volunteers, ages 0 to 86 years old, participated in the study. Although conditions in these communities prevented drawing a random sample, every effort possible was made to enroll a representative sample in terms of age, socioeconomic status, and style of life (traditional vs. market integrated lifestyles). Information about the study was distributed at community meetings and through word of mouth, and participants were selected based on those interested in participating in the study.

Ethics Statement

Informed consent was obtained from all adult participants. Parental verbal consent and child assent were obtained for all participants under 15 years of age (the local age of consent). Individuals were informed that they could choose not to participate, to participate only in individual portions of the study, or to participate in the full study. This study was approved by the Institutional Review Board (IRB) of the University of Oregon, and the *Federacion de Centros Shuar* (FICSH or the Shuar Federation) authorized this research.

Field and Laboratory Procedures

Anthropometrics. Body weight and height were measured using standard techniques (Lohman et al., 1988). Stature was measured to the nearest 1.0 mm using a field stadiometer (Seca Corporation, Hanover, MD). Body weight was calculated to the nearest 0.1 kg using a Tanita bioelectrical impedance analysis (BIA) scale (Tanita Corporation, Tokyo, Japan). Body mass index (BMI) was calculated as weight (kg)/height (m^2). BMI was used as a continuous variable with sexes and age groups (children/adults) compared separately. Relationships with weight and CRP were also tested within these groups.

Age Estimation. Ages were determined based on birthdates on government-issued identification cards, as well as using extensive genealogical information collected by the SHLHP. Overlapping genealogies were collected from multiple informants and cross-checked to ensure accuracy (Blackwell et al., 2010; Liebert et al., 2013). The sample was divided into children (<15 years) and adults (15+ years), as well as six 10-year age groups for comparison across ages (0-10, 11-20, 21-30, 31-40, 41-50, and >50 years).

Stool Collection and Analysis*:* Stool samples were collected and processed based on methods established by Raso et al. (2004) and previously described by SHLHP (Chapter III). Helminth egg presence, species-specific eggs per gram (EPG) of feces, and species-specific infection intensity were recorded in the field from a single Kato-Katz thick smear (Katz et al., 1972) for each participant (Vestergaard Frandsen, Lausanne, Switzerland) at 10x and 40x microscopy (Cole-Parmer, Vernon Hills, Illinois). Higher

EPG represents the presence of more adult worms, suggesting a higher-intensity infection.

C-reactive Protein (CRP). Capillary whole blood samples were collected and preserved on filter paper (dried blood spots [DBS]; Whatman #903, GE Healthcare, Pascataway, NJ) from a finger prick following standard methods (McDade et al., 2007). Samples were dried for approximately 4 hours and stored in a portable freezer at -20^{o} C until completion of each field season, when they were express shipped to the US on dry ice and stored at -30^{o}C until analysis. Samples were analyzed in the Snodgrass Human Biology Research Laboratory at the University of Oregon following a modification of a validated high-sensitivity enzyme immunoassay protocol (McDade et al., 2004), modified for use with different coating (Biodesign #M86005M) and detection (Biodesign #M86284M) antibodies, since the antibodies from the original protocol are no longer available for purchase in the U.S. This modified protocol was validated against the published protocol, as well as venipuncture-obtained plasma samples (Blackwell et al., 2010; McDade et al., 2012).

All samples were run in duplicate and all between assay coefficient of variances (CV; SD/mean) were <10%. Raw DBS values were converted to serum-equivalent CRP concentrations based on the formula used by McDade and colleagues (2012): serum (mg/l) = 1.84 X DBS (mg/l). All analyses were performed using $\log_{10}$-transformed raw (i.e., DBS) values for CRP.

Market Integration Measures. Structured interviews administered in Spanish were used to collect information on basic demographics and lifestyle for each household. Bilingual assistants translated for those individuals who did not speak Spanish (i.e., those who only spoke Shuar) or for those not fluent in Spanish. A modified version of the Material Style of Life (SOL) Index was used to assess ownership of specific objects purchased within a market economy and objects used in traditional subsistence activities (Bindon et al., 1997; Leonard et al., 2002; Liebert et al., 2013). The modified SOL was based on extensive ethnographic observations and pilot testing by a key collaborator (LSS) to apply specifically to Shuar culture. Two scales were created from the SOL index with the goal of representing where individuals and households fall in their involvement in traditional subsistence activities and market production/consumption. The traditional Style of Life (T-SOL) scale contained six items reflective of investment in traditional foraging activities (fishing hook/line, hunting dogs, blowgun, firearm, fishing net, and canoe). The Market-Integrated Style of Life (M-SOL) scale contained 12 items associated with investment in a market economy (radio, propane stove, mobile phone, TV, chainsaw, bicycle, refrigerator, computer, outboard motor, motorcycle, car truck). Scores for each of these variables were calculated as the fraction of the list items owned (range 0-1), with higher T-SOL reflecting more involvement in traditional subsistence activities and higher M-SOL indicating more participation in regional and global markets.

A final SOL variable (Household SOL [H-SOL]) used six household measures to capture household construction, access to water and electricity, and environmental barriers against pathogen exposure. Items and associated scores (in order of increasing market integration) included: floor (0: dirt, 1: palmwood, 2: milled lumber, 3: concrete),

wall (0: palmwood, 1: milled lumber, 2: cinder block), latrine type (0: none, 1: pit toilet, 2: outhouse with water, 3: indoor toilet), water source (0: river/stream/spring pond, 1: well or outdoor pipe, 2: indoor pipe), electricity (0: none, 1: lights, 2: outlet) and number of rooms in a house. H-SOL was computed based on a summation of these scores (Liebert et al., 2013). High H-SOL values are indicative of a greater number of non-traditional household features. Separate analyses based on the T-SOL, M-SOL, and H-SOL scores were conducted because each variable represents a different facet of MI. Individual households could be high on all scales, low on all scales, or any combination.

Statistical Analyses

Hypothesis testing. Data analysis was conducted using SAS (SAS Institute Inc., Cary, North Carolina) and BIOMStat Version 4.0 (Exeter Software, E. Setauket, New York). Prior to analysis, variables were tested for normality to confirm that all variables met standard parametric assumptions. Variables that were skewed were $\log_{10}$-transformed and retested for normality. Retested log-transformed variables were normal, with a skew and kurtosis between ± 1. Log-transformed variables were calculated and used in analyses for EPG values and CRP.

Objective 1: MI, Anthropometrics, and CRP. The sample was divided by age group and sex to see if relationships existed between body mass variables (BMI and weight) and logCRP. Separate linear regressions tested the effects of BMI and weight on logCRP for girls (<15), boys (<15), women (15+) and men (15+). Linear regressions were also used to test relationships between logCRP and SOL variables.

Objective 2: MI and STHs. With sexes and age groups (children/adults) combined, individuals were divided into one of four infection classes: 1) not infected; 2) infected with *A. lumbricoides* only; 3) infected with *T. trichiura* only; and 4) coinfected with both *A. lumbricoides* and *T. trichiura*. Likelihood ratio (G) tests compared percentage of men and women and women in each of these groups to see if they differed by sex. A single class ANOVA with multiple comparisons was run to compare SOL variables based on infection status. Comparisons were made for each SOL variable based on the following criteria: 1) infected vs. non-infected; 2) singly-infected vs. coinfected; and, 3) *A. lumbricoides* vs. *T. trichiura* infected individuals. Pearson's correlations were used to test relationships between infection intensity (EPG) and SOL scores as continuous variables.

Objective 3: STHs and CRP. Two-way ANOVA tests were used to test the effects of general infection status (yes/no) and age group (both child vs. adult and 10-year age group [0 through 10, 11 through 20, 21 through 30, 31 through 40, 41 through 50, and 50+]) on logCRP. ANOVA contrasts were used in the case of the latter, to see which 10-year age groups were associated with infection and logCRP.

Results

Objective 1: Anthropometrics, MI, and CRP.

Table 5.1 displays descriptive statistics for anthropometric, STH, CRP and MI variables for males and females. The sample was divided by age group (child vs. adult) and sex (female vs. male) and regressions were run to see if anthropometrics (BMI and

weight) were significantly related to logCRP. BMI significantly affected logCRP in girls (<15 years), but not in boys or adults of either sex (**Table 5.2**). Weight was not significantly associated with CRP for adults or children of either sex. Because BMI was only a factor for girls (females, <15), and not for any other group, these variables will not be used as controls.

Linear regression tests were used to determine the relationship between logCRP and SOL variables (**Table 5.3**). There were no significant relationships between any of the SOL variables and logCRP for girls, boys, women or men.

Table 5.1. Descriptive Statistics for Females and Males

	Females		**Males**	
	n	**Mean (SE)**	***n***	**Mean (SE)**
Age (years)	167	19.27 (1.30)	147	18.84 (1.45)
BMI (kg/m^2)	94	21.44 (0.44)	76	20.64 (0.48)
Weight (kg)	96	41.18 (1.79)	77	40.75 (2.51)
Not Infected (%)	167	56.90	147	59.90
Ascaris only (%)	167	29.90	147	25.2
Trichuris only (%)	167	12.00	147	15.60
Both Ascaris and Trichuris (%)	167	15.00	147	19.00
A. lumbricoides intensity (EPG)	167	6131.21 (1155.89)	147	4072 (818.77)
T. trichiura intensity (EPG)	167	85.22 (18.09)	147	127.84 (37.76)
Serum-equivalent CRP (mg/L) [a]	106	0.46 (1.41)	88	0.35 (1.08)
M-SOL (Score)	161	0.18 (0.01)	141	0.17 (0.01)
T-SOL (Score)	161	0.59 (0.04)	141	0.63 (0.04)
H-SOL (Score)	161	8.90 (0.34)	141	8.41 (0.35)

Results presented as mean (SE), except prevalence data which are presented as %

[a] Because of non-normal distribution, results are presented as median (IQR).

Table 5.2. Linear Regression: Body size and logCRP divided by sex and age group.

		df	F	*p*	R^2
BMI x logCRP					
	Girls	(33, 1)	5.58	0.025	0.148
	Boys	(35, 1)	0.25	0.621	0.007
	Women	(42, 1)	1.70	0.199	0.041
	Men	(23, 1)	0.580	0.455	0.026
Weight x logCRP					
	Girls	(33, 1)	2.72	0.109	0.078
	Boys	(35, 1)	2.33	0.136	0.064
	Women	(42, 1)	2.22	0.144	0.052
	Men	(23, 1)	2.90	0.103	0.117

Table 5.3. Linear regression: SOL variables and logCRP divided by sex and age group.

		df	F	*p*	R^2
H-SOL					
	Girls	(57, 1)	0.06	0.813	0.001
	Boys	(61, 1)	3.27	0.076	0.052
	Women	(46, 1)	0.04	0.840	0.001
	Men	(25, 1)	0.580	0.455	0.026
T-SOL					
	Girls	(57, 1)	0.05	0.821	0.001
	Boys	(61, 1)	0.43	0.513	0.007
	Women	(46, 1)	0.11	0.741	0.003
	Men	(25, 1)	1.27	0.271	0.050
M-SOL					
	Girls	(57, 1)	0.36	0.551	0.006
	Boys	(61, 1)	0.00	0.999	0.000
	Women	(46, 1)	2.97	0.092	0.062
	Men	(25, 1)	0.03	0.869	0.001

Objective 2: MI and STHs

Likelihood ratio (G) tests compared men and women based on the four infection categories (not infected, only *A. lumbricoides* infected, only *T. trichiura* infected, and coinfected). There were no significant differences in any of these categories between the sexes (G (3) = 2.347, $p = 0.504$). Age groups and sexes were combined to see if STH infection status varied with MI. SOL variables were compared across infection status (**Table 5.4**). *H-SOL:* Infected individuals had significantly lower H-SOL than non-infected individuals ($p < 0.001$; **Figure 5.1**). Coinfected individuals had much lower H-SOL than individuals only infected with one type of helminth ($p < 0.001$). H-SOL was not significantly different between those infected with *A. lumbricoides* and *T. trichiura.* *T-SOL:* There were no significant differences in T-SOL based on infection status (**Figure 5.2**). *M-SOL:* Individuals who were coinfected had significantly lower M-SOL than individuals infected with only one type of STH ($p = 0.001$; **Figure 5.3**). There were no other relationships between infection status and M-SOL.

Table 5.4. Single-class ANOVA with multiple comparisons for the effects of infection status on SOL variables.

	df	F	*p*	R^2
H-SOL model	3	12.03	<0.001	0.109
Infected vs. non-infected	1	15.23	<0.001	
Single infection vs. coinfection	1	20.60	<0.001	
Ascaris vs. *Trichuris* Infected	1	19.86	0.268	
T-SOL model	3	1.49	0.216	0.014
Infected vs. non-infected	1	3.20	0.075	
Single infection vs. coinfection	1	0.02	0.878	
Ascaris vs. *Trichuris* Infected	1	2.03	0.155	
M-SOL model	3	4.11	0.007	0.040
Infected vs. non-infected	1	0.50	0.480	
Single infection vs. coinfection	1	10.95	0.001	
Ascaris vs. *Trichuris* Infected	1	2.09	0.149	

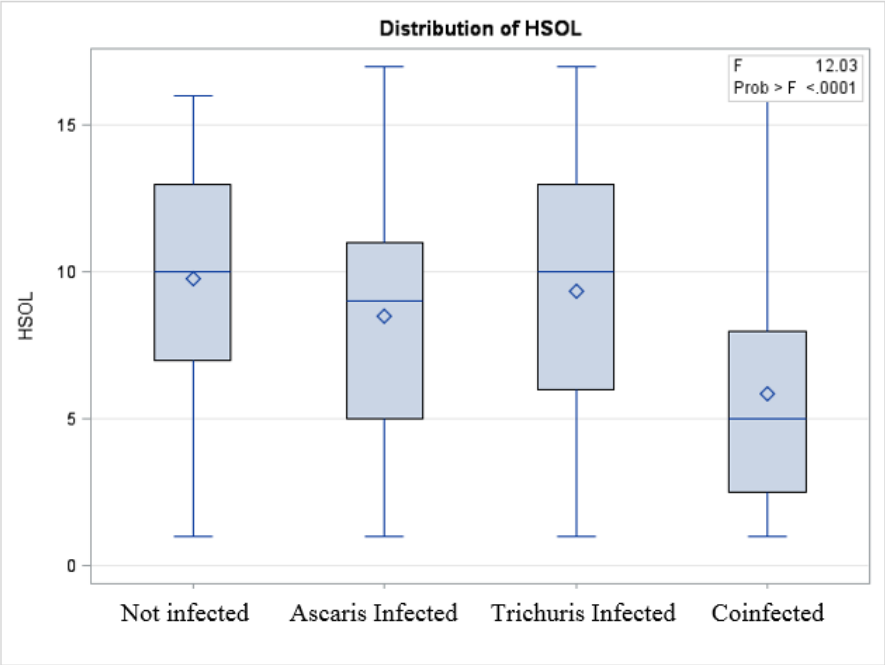

Figure 5.1. Distribution of H-SOL based on infection status.

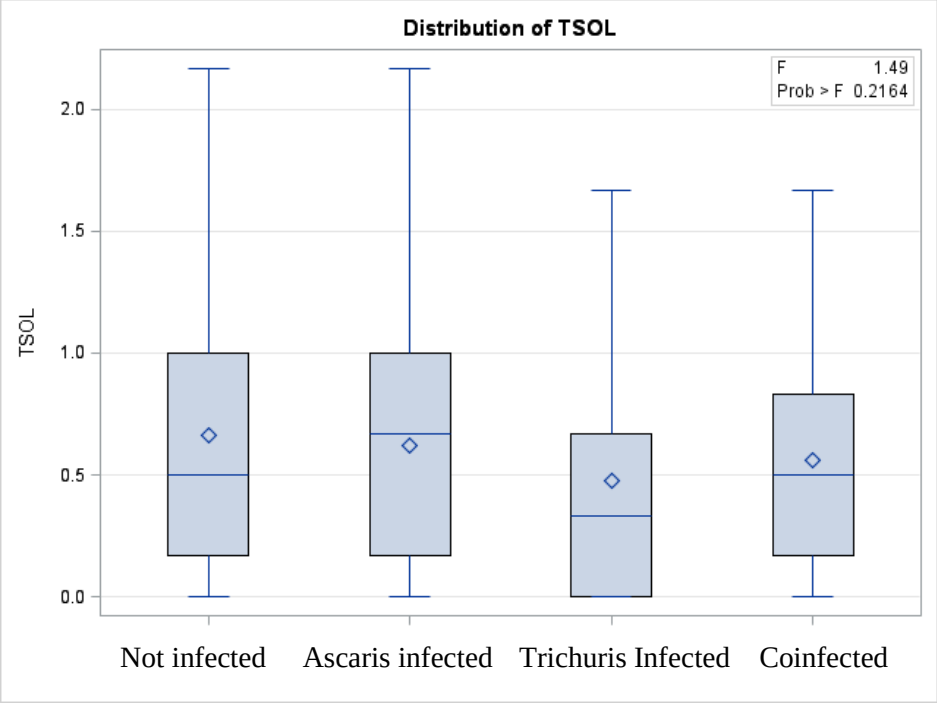

Figure 5.2. Distribution of T-SOL based on infection status.

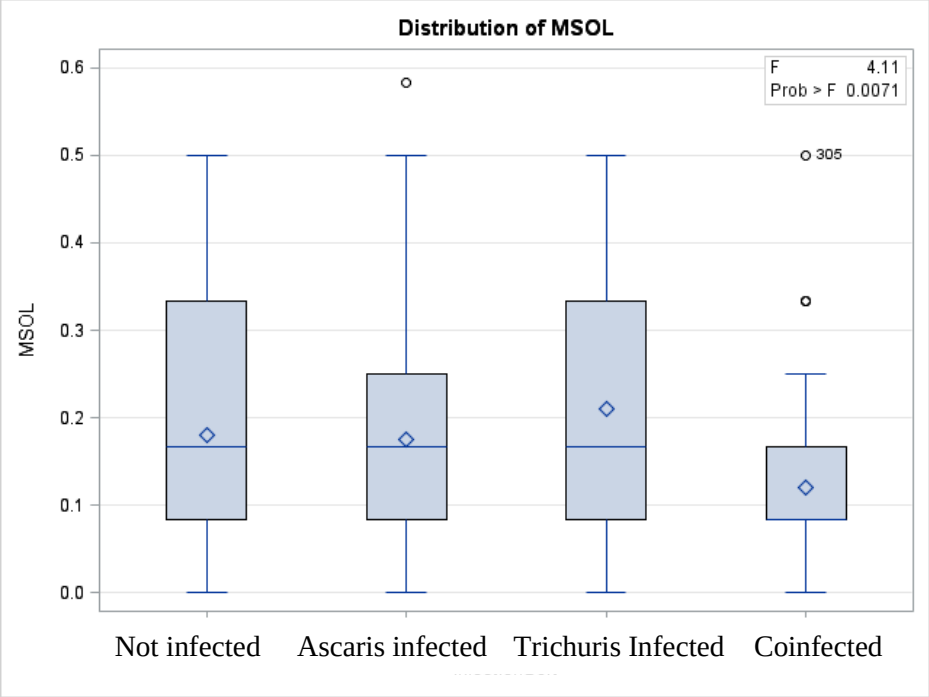

Figure 5.3. Distribution of M-SOL based on infection status

Pearson correlations explored relationships between species-specific STH intensity and SOL variables (**Table 5.5**). Log *A. lumbricoides* EPG values were significantly negatively correlated with H-SOL ($p < 0.001$) and M-SOL ($p < 0.05$), meaning that more market integrated household structures and ownership of more market integrated goods were both associated with lower intensity infections. Log *T. trichiura* EPG values were significantly negatively correlated with H-SOL ($p = 0.001$) and T-SOL ($p < 0.05$), meaning that more market integrated housing structure and owning more traditional goods were associated with lower infection intensity.

Table 5.5. Pearson correlations between STH infection intensity and SOL variables.

	Log Ascaris EPG	Log Whipworm EPG
H-SOL	-0.319 (< 0.001)	-0.190 (= 0.001)
T-SOL	0.012 (= 0.836)	-0.131 (= 0.022)
M-SOL	-0.140 (= 0.015)	-0.064 (= 0.268)

Values presented as Correlation coefficient (*p*)

Objective 3: STHs and CRP.

A two-way ANOVA was used to test the effects of age group and infection status on logCRP (**Table 5.6**). There was a significant difference between adults and children within this model ($p < 0.01$) and a trend toward significance in the interaction between infection status by age group ($p = 0.07$). These relationships were plotted (**Figure 5.4**). The differences in logCRP between children and adults only existed in individuals who were uninfected with STHs and individuals who were only infected with *T. trichiura*. There were no differences in logCRP between age groups for individuals infected with *A. lumbricoides* or coinfected.

Table 5.6. Two-way ANOVA testing the effects of age group and infection status on logCRP.

	df	F	*p*	R^2
Model	7	2.70	0.011	0.092
Infection Status	3	1.50	0.217	
Age Group	1	7.21	0.008	
Infection Status x Age Group	3	2.39	0.070	

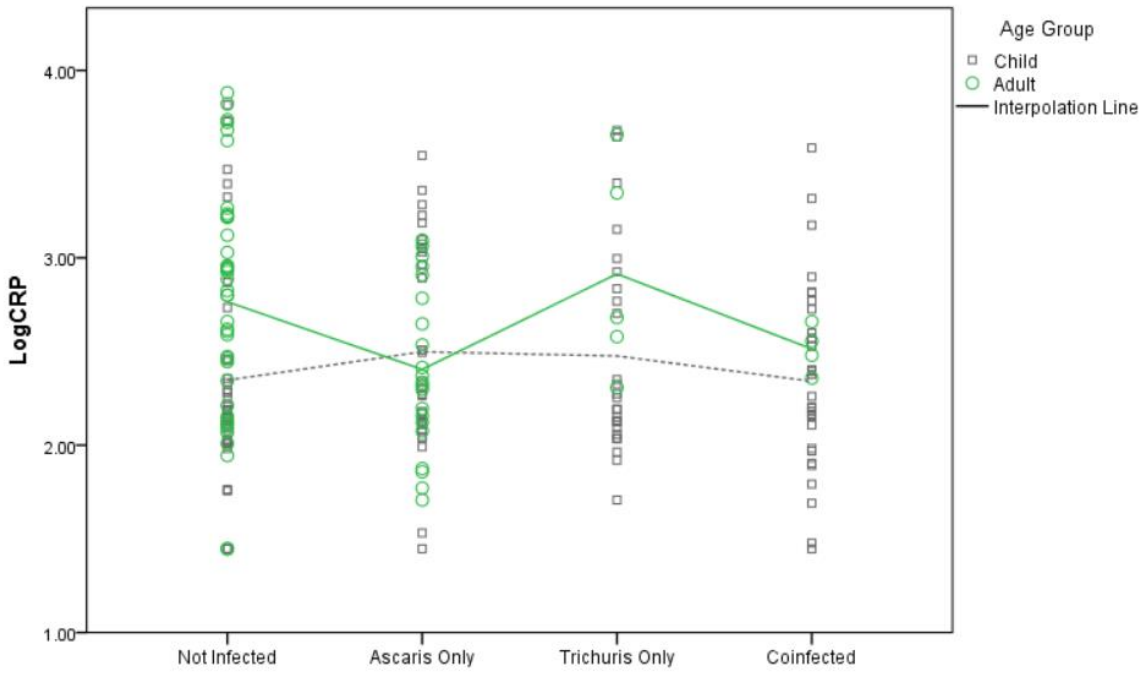

Figure 5.4. Plot comparing logCRP between children and adults based on infection status.

To understand where these major effects associated with age are located, age was divided into 10-year age groups (0 through 10, 11 through 20, 21 through 30, 31 through 40, 41 through 50, and 50+). A two-way ANOVA determined the effects of these age groups and general STH infection status (yes/no) on logCRP (**Table 5.7**). While the model was significant ($p < 0.05$), only the interaction term between infection status and 10-year age group was significant ($p < 0.05$). A priori comparisons were used to see which age groups were driving this variation. Only the comparisons between individuals under 20 years of age (the first two 10-year age groups) to those over 20 (the last four 10-year age groups) was significant ($p < 0.05$; **Table 5.7**). This relationship was plotted to

see the relationship between these variables (**Figure 5.5**). For individuals ages 20 years or younger, logCRP did not differ based on infection status. For individuals 21 years of age or older, however, CRP was significantly higher in uninfected individuals than in infected individuals. In fact, these infected adults had logCRP levels that were similar to the levels seen in younger individuals.

Table 5.7. Two-way ANOVA testing the effects of 10-year age groups and infection status on logCRP (with age group contrasts).

	df	**F**	***p***	**R^2**
Model	11	2.04	0.027	0.110
Infection Status (yes or no)	1	2.53	0.113	
Age Group (10 year)	5	1.55	0.177	
Infection Status x Age Group (10 year)	5	2.44	0.036	
Contrasts				
0 to 21 vs 21+	1	4.34	0.039	
0 to 11 vs 11 to 20	1	0.00	0.980	
20 to 41 vs 41+	1	1.44	0.231	
20 to 31 vs 31 to 40	1	0.34	0.558	
40 to 51 vs 51+	1	1.01	0.316	

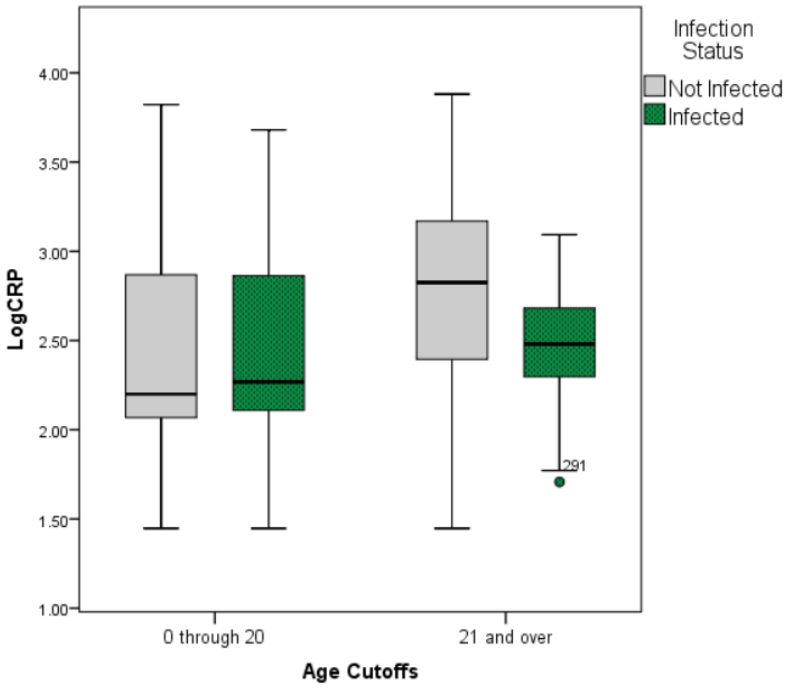

Figure 5.5. LogCRP based on age group and STH infection status.

Discussion

This study tested relationships among MI, STH infection and inflammation using the inflammatory marker CRP. The Hygiene Hypothesis provides this basis for exploring these variables. MI is hypothesized to decrease exposure to parasites, like STHs. This is important because research has demonstrated an anti-inflammatory effect for STHs in clinical settings. If STHs have an anti-inflammatory effect outside of a clinical setting and within general populations, this may suggest that the elevated rates of chronic inflammation and associated disorders that we see in economically developed nations is related to increased hygiene and decreased exposure to STHs.

To test this hypothesis, we first examined the relationship between CRP and MI to examine whether integration into the market economy was driving variation in CRP among the Shuar. This hypothesis was not supported. The present study found no significant relationships between CRP and any of the SOL variables for any sex or age group. This was surprising, since a number of factors associated with increased MI, including diet and physical activity have been linked to elevated levels of chronic inflammation and associated disorders (Ford, 1999; Greenfield et al., 2004; Snodgrass et al., 2007b; Sorensen et al., 2006).

Next, we tested relationships between MI and STH infection, to see if they are associated in ways predicted by the Hygiene Hypothesis. We demonstrated that infected individuals lived in more traditional houses, in other words had lower H-SOL, than non-infected individuals. Coinfected individuals had even lower H-SOL than individuals infected with only one type of STH. Infection intensities, based on EPGs, were negatively correlated with H-SOL as well. Based on these findings, we can conclude that factors

associated with housing structure (H-SOL) play an important role in STH exposure. This makes sense, because more traditional houses usually have dirt floors, which are harder to clean. They also often do not have running water or bathroom facilities, which lead to easier contamination. Cooking areas in more traditional homes are usually not separated from the rest of the living area and domestic animals are often hard to keep out of the cooking space. All of these factors are associated with increased parasite exposure and should be examined in more detail among the Shuar in future studies to understand what specific variables are playing the largest roll in STH exposure.

Coinfected individuals also had significantly lower M-SOL than individuals infected with only one type of STH. *Ascaris lumbricoides* EPGs were also negatively correlated with M-SOL, meaning that more market integrated individuals had lower intensity infections. These results may suggest that coinfected individuals represent the most traditionally living participants, since they own fewer market-based items. This could also suggest that these individuals are of lower socioeconomic status (SES) and are exposed to more STH infections due to minimal access to healthcare and sanitation, which may be reflected indirectly by low M-SOL. Future studies will examine the role of SES on STH exposure among the Shuar, to see how factors related to economic disparities, access to resources, and marginalization affect health.

The final objective of this study was to consider whether STH infection was associated with lower levels of inflammatory marker CRP. We were especially interested in determining relationships between these two variables and age, since CRP has been shown to be associated with age in economically developed nations. Because CRP was not associated with MI variables, MI was not considered in these analyses. We found

significant differences in CRP between children and adults based on infection status. **Figure 5.4** plots these relationships, showing that adults who are uninfected and those infected with whipworm alone had significantly higher CRP than children. Interestingly, however, adults infected with *A. lumbricoides* alone or coinfected had mean logCRP that were similar to or lower than children in the same category. These findings provide support for the anti-inflammatory effects of *A. lumbricoides*, since *A. lumbricoides* infection was present in both groups of adults with lower CRP. *Ascaris lumbricoides* surface proteins bind the T-helper 2 (Th2) branch of the immune system, triggering the release of anti-inflammatory cells that prevent removal or digestion of the parasite (Bethony et al., 2006; Deehan et al., 2002; Ng et al., 2000). *Trichuris trichiura* does not appear to have an anti-inflammatory effect, since adults infected with this helminth had higher levels of CRP. This may be because even though *T. trichiura* triggers the release of anti-inflammatory cells and cytokines, it survives by attaching to the intestine and thus has a proinflammatory effect as well (Bethony et al., 2006; Drake et al., 1994; Parthasarathy & Mansfield, 2005, Summers et al., 2005).

To see exactly where this age effect associated with STH infection and inflammation lies, we compared CRP across 10-year age groups between individuals who were infected with at least one type of STH (either *A. lumbricoides*, *T. trichiura*, or coinfected) and those who were uninfected. Interestingly, the only significant contrast was in individuals under 21 years of age and those 21 and over. When these relationships were plotted, we saw that regardless of infection status, individuals under 21 had similar levels of CRP. Individuals over 21, however, had significantly higher CRP if they were not infected with STHs. These findings suggest that STH infection may have an anti-

inflammatory effect and may prevent the development of chronic inflammation associated with age in developed nations. Taken another way, this could mean that the increase in disorders related to chronic inflammation, including cardiovascular disease, allergies, and autoimmunity, may in part be driven by a decreased exposure to STHs.

This brings up important questions regarding infection timing within the Hygiene Hypothesis. Research suggests that helminth infection is most important early in life when the immune system is developing (Maizels et al., 2014). Hypotheses about when infectious disease exposure is most useful for proper immune development extends prenatally. Specifically, studies suggest that maternal infection and probiotic exposure decrease newborn risk of allergic eczema, the effects of which can last into childhood (Ege et al., 2011; Elliott et al., 2005; Mpairwe et al., 2011). Vaccination also appears to be less effective in infants and children infected with helminths, and efficacy increases with anthelminthic treatment (Labeaud et al., 2009; Cooper et al., 2000; Cooper et al., 2001; Elias et al., 2001). Based on these data, it appears that very early exposure to certain infectious diseases in general and helminths specifically, may increase immunotolerance, which can decrease the inflammatory responses throughout the life course and protect from chronic inflammatory diseases later in life.

There is also some evidence that infection at any age can increase immunotolerance. In many cases, helminths have been used to treat inflammatory autoimmune disorders in adults, with successful outcomes for multiple sclerosis (Correlae & Farez, 2007, Fleming, 2013), ulcerative colitis symptoms (Broadhurst et al., 2010; Maizels et al., 2014), Crohn's disease (Weinstock & Elliott, 2013), and celiac disease (McSorley et al., 2011).

The findings presented in this study lend some support the idea that helminth infection remains important throughout the life course. Economic development among the Shuar is a relatively recent occurrence and even those individuals living in the more integrated Upano Valley region would have had extremely limited access to market purchased goods as recently as 15 to 20 years ago. This means that adults in both regions would have likely grown up and developed their immune systems under similar STH burdens. That we see such pronounced differences in CRP infection with age between infected and uninfected individuals suggests that current infection status and current lifestyle play more important roles on immune system regulation and tolerance than early (prenatal, postnatal, and childhood) environments.

Study Limitations

This study has many important limitations. First, the data are cross-sectional and from a relatively small sample size of 310 individuals. This results in even smaller groups when divided by SOL or infection status, and especially small groupings when the six age groups are also considered. While analyses were adequately statistically powered, larger and more representative sample sizes would have allowed us to consider other explanatory factors and to draw more generalizable conclusions.

Second, this study only used a single measure of CRP, making specific interpretations of chronic inflammation tentative at best. CRP levels fluctuate with injury and illness. Thus, these are not representative of long-term average CRP values. Further, we cannot definitively state that the CRP measures here are not associated with acute immune responses. Similarly, STH levels were deduced from a single stool sample. Rate

of shedding can vary between helminths, suggesting that infection intensity based on EPG could vary throughout the day. However, we controlled for this to the best of our abilities by collecting fecal samples passed in the early morning from each participant.

A further limitation was the difficulty of controlling for body size when considering CRP across age groups for both adults a children. BMI is a complex, age-dependent measure in children, thus underweight, normal, overweight, and obese categories normally need to be calculated based on growth curves that consider sex and age. These groups, however, are not comparable to adult BMI groups. For this reason, weight was tested as well in order to account for overall body size, although this is also problematic because it does not allow specific consideration of height, age, or body fat. While these measures of body size (BMI and weight) were not significantly related to CRP in the present analyses, future studies will use recently calculated Shuar growth curves (Urlacher et al., in press) to create relevant and comparable groups between adults and children. This will allow us to see if body size plays a role in inflammation among the Shuar. Larger sample sizes, including recently collected data from 2014 that is not included in this dissertation, will also allow for more accurate divisions between adults and children based on BMI categories in order to control for age and body size while investigating chronic inflammation.

Conclusions

Chapter V considers evidence for the Hygiene and Old Friends Hypotheses. Specifically, we were able to illustrate changes in STH infection with economic development, supporting the hypothesis that MI reduces STH burden. We also found

compelling, though preliminary, evidence for the role of STH infection in immune system regulation and anti-inflammatory responses. CRP was negatively associated with STH infection status and intensity, and this was especially evident when we explored relationships between CRP and age based on infection status. Especially intriguing, this study led to new questions regarding the timing of infection and subsequent immunoregulatory benefits. Based on our findings, we can conclude that current infection status may be as important as early-life STH exposure in regulating inflammatory processes. This has important implications for understanding the development of chronic, non-communicable diseases associated with inflammation, including CVD, metabolic syndrome, allergies, and autoimmunity.

Bridge to Chapter VI

Chapter VI provides a synthetic overview of the studies discussed in this dissertation, integrating them and discussing what this dissertation says about evolutionary medicine and the Hygiene/Old Friends Hypotheses. The findings presented in this dissertation provoke a series of new questions that require exploration. For this reason, I will conclude with a discussion of how future research will answer these questions, including specific future directions for this project.

CHAPTER VI

CONCLUSIONS AND FUTURE DIRECTIONS

The chapters in this dissertation test the Old Friends Hypothesis, as well as the mechanisms of exposure discussed by the Hygiene Hypothesis and Biodiversity Hypothesis. To recap, the Old Friends Hypothesis explores the role of soil-transmitted helminths (STHs) in human immune system priming and regulation (Flohr et al., 2006; 2008; Lynch et al., 1993; Rook et al., 2014). The Hygiene Hypothesis postulates that allergies and autoimmunity (disorders associated with immune system dysregulation) result from improved hygiene and lifestyle factors decreasing exposure to certain infectious agents (Strachen, 1989; von Mutius et al., 1994), while the Biodiversity Hypothesis attributes altered infectious disease exposure to environmental and climate change, rather than hygiene and behavior (Haahtela et al., 2013; Hanski et al., 2012).

In this dissertation, I take an evolutionary medicine approach to explore these hypotheses among the Shuar of Amazonian Ecuador. Specifically, I explore changes in lifestyle associated with the transition from traditional subsistence-based lifestyles to more market-integrated lifestyles associated with economic development. Based on the mismatch paradigm discussed by evolutionary medicine (Eaton et al., 1988; Nesse & Williams, 1999; 1998; Williams & Nesse, 1991), this transition appears to be creating a disconnect between our evolved biology and the lifestyles that populations in economically developed nations currently live. Reconstructed human evolved biology involves reliance on STH infections for immune system regulation and priming. Current lifestyles in wealthy nations, on the other hand, involve minimal exposure to the soil and

other environmental surfaces potentially contaminated with STHs, as well as increased hygiene practices and a better ability to avoid pathogenic substances (Bach, 2002; Strachan, 1989; von Ehrenstein et al., 2000; von Mutius, 2007). It is this mismatch that is hypothesized to be related to elevated rates of allergy and autoimmunity over the past several decades in wealthy nations by not providing necessary immune stimuli, priming, and anti-inflammatory support to which our immune systems appear to rely (Flohr et al., 2006; 2008; Haahtela et al., 2013; Hanski et al., 2012; Strachen, 1989; von Mutius, 1994).

Synthetic Overview

This dissertation investigates STH infection prevalence and intensity among the Shuar, and specifically tests three hypotheses drawn from the Old Friends Hypothesis:

Hypothesis 1. Addressed in Chapters II, IV, and V, I hypothesized that lower levels of STH infection prevalence and intensity should be associated with increased access to and participation in market based economies;

Hypothesis 2. If helminths have exerted a large selective pressure on human health, we should see adaptations in psychology and behavior that work to moderate exposure to pathogens (for instance, keeping STH infection intensity low or moderate to prevent associated morbidity). These behavioral adaptations should be calibrated to the local environment and ability to avoid fitness reducing activities. This hypothesis was tested in Chapter IV;

Hypothesis 3. Altered STH exposure associated with market integration (MI) and behavioral changes should be related to changes in immune function and increased chronic inflammation (measured using CRP) throughout the life course. Chapter V tested this hypothesis.

Chapter III reported STH infection prevalence and intensity among the Shuar, as well as differences between the more traditional Cross-Cutucú region and the more market integrated Upano Valley region. Overall, the research presented in this dissertation documented light to moderate infection intensity among the Shuar based on WHO standards, though infection prevalence was high compared to other populations. When adults and children were considered together, Shuar in this study had higher rates of infection with both *A. lumbricoides* and *T. trichiura* than other samples from subsistence-based, rural, and urban populations (**Figure 3.3**; Blackwell et al., 2011; de Silva et al., 2003; Needham et al., 1998). The exception was a Vietnamese farming population (Needham et al., 1998), also residing in a neglected tropical disease hotspot and spending large portions of time in contact with the soil. Shuar children, especially those living in the Cross-Cutucú region, had higher STH infection prevalence than other groups (**Figure 3.4**). Those in the Upano Valley region had similar STH prevalence than other populations for which data are available (Francis et al., 2012; Nwaneri & Omuemu, 2012; Sackey et al., 2003; Saldiva et al., 1999; San Sebastian & Santi, 2000; Scolari et al., 2000; Tanner et al., 2009). These findings highlight the importance of studying STH infection among the Shuar.

High prevalence of STH infection among the Shuar, especially among children, compared to other populations could have several important health implications that warrant further study, especially for growth and development (Blackwell et al., 2011; Bogitsh et al., 2005). Importantly, Shuar children harbor higher intensity infections than adults, meaning that children that are infected suffer from a higher worm burden. Higher intensity infections, when combined with smaller body sizes and developing digestive tracts, can result in intestinal blockages (Khuroo et al., 1990; Villamizar et al., 1996). In general, high worm burden in children can result in nutritional deficiencies, and mental and developmental retardation.

The fact that infection among the Shuar follows an overdispersed pattern, with a few individuals harboring the majority of eggs, calls for studies that examine who is most at risk for negative health outcomes associated with STH infection. According to previous helminth studies, high worm burden tends to cluster within households or families, suggesting genetic and environmental influences on helminth exposure, infection, and intensity (Bethony et al., 2006; Dold & Holland, 2011). Too much immunotolerance, dictated by developmental and genetic factors, toward helminths can lead to high levels of infection, while limited tolerance can completely eradicate an infection. It seems that maintaining low to moderate infection levels may be best for immune system development, while still avoiding the negative health outcomes associated with high intensity infections. Future research should look for similarities in immune function and parasite load within Shuar families, based on where family members were raised and where they currently live.

The patterns of helminth infection discussed in Chapter III provide preliminary insights into the specific factors that result in population variation in infection status and intensity. Children, having higher infection prevalence (in the hypothetically moderately-integrated CC1) and intensity (with communities combined) than adults in this study, probably engage in more activities that put them in contact with fecally contaminated soils and foods, including play, farming, and interactions with other infected children. Further, children from large families and those who attend school would come into contact with other children more often and thus share exposures and infections.

The physiology of immune system development should also be considered. The adaptive immune system forms memory cells for every antigen it acquires throughout the lifespan. Older children are more likely to have been exposed to more pathogens (because of their longer life), so they are more likely to have already formed specific memory cells to a variety of pathogens and thus can respond more quickly when infection is first detected. This is believed to keep worm burden low without expending too much energy mounting an immune response from scratch. If, on the other hand, the body has not encountered a specific pathogen, then the burden is likely to increase until the immune system can produce the proper response. This should explain some of the variation seen in infection intensity across age groups. Further research should address factors that increase exposure risk, immune development and infection intensity across ages and sexes, while also examining pathogenesis and health outcomes among the Shuar.

Testing Hypothesis 1

Chapter III provides a test for Hypothesis 1, employing an age-stratified geographic comparison to see if market access, measured by approximate travel time from the community of residence to the market center, was associated with lower STH infection prevalence and intensity. STH infection variables did differ significantly between individuals in the Cross-Cutucú and Upano Valley regions. An especially useful comparative sample came from the inclusion of a Cross-Cutucú community that differed from other Cross-Cutucú communities on a number of levels. This community was made up of an extended family[9] who worked a series of government jobs, taught school in other communities, and regularly visited the market center of Sucúa. They represent a higher socioeconomic status and educational level than other Cross-Cutucú communities in this study, and provide an interesting intermediate between Cross-Cutucú and Upano Valley along the spectrum of MI. Chapter III presents analyses that document significant differences in infection prevalence and intensity between the more traditional Cross-Cutucú and more integrated Upano Valley regions, with the outlying Cross-Cutucú community lying between the two regions.

There was one major limitation in Chapter III that made deducing the role of MI in STH exposure difficult. Using geographic location as a proxy for MI may be problematic, especially for drawing conclusions about how economic development changes pathogen exposure. There are many environmental factors that have not been

[9] It is important to note that the interrelatedness of this community, as well as a number of families within other communities, results in a non-random sample. This makes it difficult to deduce differences at the individual and family level and leads to statistical non-independence for individual participants in this study. Future studies should examine the effects of interrelatedness on STH exposure and overall health outcomes.

measured that may differ between the two regions, including soil type, specific factors associated with water source origins (including direction and speed of the current for rivers used for bathing or drinking), temperature, and rainfall (de Silva et al., 2003; Brooker et al., 2006). All of these factors can effect as likelihood of coming into contact with STH eggs.

Our previous research (Liebert et al., 2013) has shown marked differences in MI using Style of Life (SOL) variables between the Upano Valley and Cross-Cutucú two regions, so it is very likely that MI is driving these differences, at least to some degree. Further, the presence of the transitioning Cross-Cutucú community helped to visualize for these differences. This community was located directly across the river from the second Cross-Cutucú community and, as a result, soil type, water source, and climate were all similar. Yet, these two communities still differed in helminth infection prevalence and exposure in predictable ways based on actual MI variables. While evidence points to MI as a driving factor in these differences in helminth exposure, the actual extent of this relationship cannot be determined without also testing the geographic variability.

Chapters IV and V provide additional tests of Hypothesis 1, this time using specific SOL variables indicative of house style (H-SOL) and participation in market-based (M-SOL) and subsistence-based (T-SOL) economies. These variables allow more direct testing of the role of MI in STH exposure and permit comparison across geographic locations. Both of these analyses found relationships between these variables and STH infection prevalence and intensity. H-SOL seemed to have the biggest effect on parasite exposure, which is unsurprising. H-SOL reflects a summary measure of house construction materials, water source, latrine location, number of rooms, and food

preparation location. Having a larger house would prevent crowding and decrease exposure to infectious agents. Having latrines (either a separate area of land, a pit toilet, or an entire bathroom facility) away from the kitchen and garden would keep contaminated feces away from the food and cooking spaces and make it less likely to be tracked into the home. Cement floors and walls are easier to clean than dirt floors and usually provide more of a barrier to keep wild and domesticated animals and insects out of the home. All of these factors can prevent helminth eggs being brought into the house and decrease exposure risk, even if individuals do not spend a lot of time working or playing in or around contaminated soil.

M-SOL had the second strongest associations with parasite load. This variable is a composite measure of the ownership of market purchased goods and indirectly indicates a subsistence pattern that relies more on market exchanges based on wage labor, and less on hunting, fishing, and horticulture. These individuals likely do not spend as much time gardening, farming, hunting, or fishing (all activities that increase risk of contamination) and live in more market integrated, permanent housing structures that are easier to clean. They also have increased access to market purchased, processed food, which is usually prepackaged and harder to contaminate.

Results of analyses with the T-SOL were less straightforward to interpret. While this variable is expected to be a proxy for participation in subsistence-based activities, like hunting, fishing, foraging, and gardening, it ended up being positively correlated with M-SOL. Individuals with high M-SOL also tended to have high H-SOL, meaning that they owned more market goods *and* traditional items. This suggests that, instead of being indicative of traditional lifestyles, T-SOL is actually related to higher

socioeconomic status, which results in expendable income for purchasing both MI and traditional goods. Future studies should examine what effect individual variables within T-SOL and M-SOL have on parasitic disease exposure and if they are more closely related to socioeconomic status, measured based on household income considering family size, than they are to lifestyle factors. This is an important distinction because, while more traditional lifestyles should be indicative of increased risk of parasite exposure, higher levels of poverty, even within economically developed regions and nations, are also associated with elevated parasite exposure. So individuals could appear to be more market integrated, but be of low socioeconomic status, which will still increase their likelihood of exposure.

The results of these analyses all support Hypothesis 1, providing evidence that parasite load changes with increased participation in the market-based economy. The results, however, also raise some important questions. The key variables appear to be those that provide protection from pathogens and pathogen containing elements (e.g., houses that are easier to clean or keep animals out, factors that reduce likelihood of tracking contaminated feces into the house, etc.). These factors may be associated with increased hygiene, though hygiene itself is driven by a number of behavioral factors. If humans and parasites share a long coevolutionary history, then we should see psychological and/or behavioral mechanisms in place that keep parasite load to low or moderate levels. This is important because complete avoidance (especially of STHs) would be difficult (discussed in Chapters I and II), so mechanisms that keep infection intensity low would be beneficial for preventing pathogenesis associated with high worm

burden. To avoid pathogens, a person has to be motivated to avoid them and this motivation should be calibrated to the environmental and physical ability do so.

Testing Hypothesis 2

To address the behavioral component of parasite avoidance (Hypothesis 2), Chapter IV provides data on the disgust response and its associations with helminth load. Pathogens have provided an important selective pressure during human evolution, and there should be psychological and/or behavioral aptitude toward avoiding these pathogens and associated stimuli (Curtis & Biran, 2001; Oaten et al., 2009; Tybur et al., 2009). Further, given the wide range of environments that humans inhabit, this response should be calibrated to local conditions (Curtis et al., 2004; Lieberman et al., 2003; Oaten et al., 2009). For instance, high disgust sensitivity would theoretically reduce fitness if the individual lives in an environment where pathogen containing stimuli are difficult to avoid. If subsistence relies on unavoidable contact with fecally contaminated soil, either through farming or animal domestication, then being disgusted by soil or insects often found in soil would not be a beneficial response and would make subsistence activities more difficult. Avoidance behaviors should exist at the edge of a sustainable realm. This is important because in wealthy nations avoiding pathogen-related stimuli is relatively easy for most people, disgust sensitivities reach extremes that can completely eradicate parasite exposure, which may have negative implications for immune system development.

To date, several studies have tested the cross-cultural universality of the disgust response (Curtis & Biran, 2001), as well as determined general types of associated

stimuli (Curtis & Biran, 2001; Curtis et al., 2004; Fessler & Navarrete, 2003; Lieberman et al., 2003; Oaten et al., 2009; Tybur et al., 2009); however, most of these studies were conducted among college students in wealthy nations. Chapter IV provides the first study to directly test the role of disgust sensitivity in pathogen avoidance, and its related environmental calibration, demonstrating that higher levels of disgust sensitivity are related to lower STH exposure. Evidence from this study suggests that disgust itself is calibrated by MI, playing a mediating role between MI and parasite exposure. For instance, individuals living in easier to clean cement homes may spend more time cleaning their homes, and both behavioral and structural factors will reduce exposure. These findings highlight the likely ancient, fitness-reducing role that high intensities of infectious disease agents played in human evolution. Specifically, humans responded by adopting environmentally calibrated, emotional responses to stimuli that could contain pathogens (Curtis & Biran, 2001; Curtis et al., 2004; Fessler & Navarrete, 2003, Oaten et al., 2009; Schaller & Duncan, 2007).

The results from Chapter IV raise a number of new questions. First, how does the disgust response affect exposure to other infectious agents? For example, exposure to a diverse array of bacteria is extremely important for human health, playing a large role in digestion and immunity. Bacteria are also much harder to avoid than STHs, and are generally common on all living things and surfaces, even in wealthy, developed nations (Fraune & Bosch, 2010; Turnbaugh & Gordon, 2009; Hooper et al., 2002; Mazmanian et al., 2008). Exposure to a diverse array of bacteria throughout development and across the life course is crucial for proper immune and digestive system development and function (Blaser and Falkow, 2009) Avoidance behaviors associated with disgust, under most

cases, would probably only slightly reduce bacterial exposure, especially in highly pathogenic environments. Wealthy nations, however, have a number of means to reduce bacterial exposure, including antibiotic medicines, soaps, and surfaces. In these cases, it may be that our sensitivity to disgust eliciting stimuli can be taken too far, severely limiting important bacterial diversity, with serious implications for health, digestion, and physical and cognitive growth/development. Future studies should explore these relationships across the spectrum of MI, to see if disgust sensitivity limits microbial diversity, and what effects this has on health.

Testing Hypothesis 3

Chapter V provided a preliminary test of Hypothesis 3, supporting the role of helminths in proper immune function and health. As previously discussed, a number of published studies have shown that chronic inflammation (measured using the biomarker C-reactive protein [CRP]) typically increases with age in economically developed nations, but our work among the Shuar show limited evidence of this happening in this more traditionally living, non-western population (McDade et al., 2012). Chapter V observed differences in chronic inflammation occurring within the Shuar based on STH infection, especially *Ascaris lumbricoides*. Age appeared to be a driving factor in subclinical elevations in CRP, but this relationship only existed for adults who were not infected with parasites. In the future, the Shuar Health and Life History Project (SHLHP) will repeat these analyses with a larger sample size and with repeat measures to look at differences in CRP between age groups (and using age as a continuous variable)

comparing individuals infected with STHs with those who are not. Breaking it down to age groups within MI distinctions will help to understand which factors (STH infection or lifestyle variables associated with MI) play a larger role in increased chronic inflammation with age.

Chapter V raised important questions about the timing of STH exposure and subsequent immune regulation. As discussed in Chapters I and II, helminths activate the anti-inflammatory Th2 pathway of the adaptive immune system, increasing the release of T_{reg} cells and interleukin-10 which downregulate inflammatory immune responses (Mahanty et al., 1996; Parthasarathy & Mansfield, 2005; Summers et al., 2005; Wammes et al., 2012). This response increases immunotolerance and may prevent the immune system from overreacting to harmless (allergens) or self-produced (auto) antigens. A critical debate lies in whether exposure to helminths is only important early in life, like during infancy and childhood when the immune system is just developing (Djuardi et al., 2011; Ege et al., 2011), or if later infection throughout late childhood and adulthood can also have effects on immune regulation (Fleming, 2013; McSorley et al., 2011; Weinstock & Elliott, 2013).

Chapter V provides support for STH exposure, especially *A. lumbricoides*, having an effect on inflammatory responses throughout the life course (Hypothesis 3), although these are only preliminary conclusions. Shuar in this study come from fairly rural environments in the Upano Valley and Cross-Cutucú regions. Most participate in some level of traditional subsistence, so there is no clear line between more traditional and more integrated lifestyles, as MI operates on a continuum. On top of this, MI has occurred very rapidly in the past 10 to 20 years. Because of these factors, it cannot be

assumed that the adults that are uninfected now were not infected with helminths during their early childhoods. This would have resulted in their immune systems being primed for the Th2 response. It may be the long-term removal of helminths at any point during the life-course that has the potential to result in a heightened inflammatory response. This is supported by studies that find that infected individuals and individuals that are desensitized to allergens both show an increase in immunoglobulin G4 (IgG4; an antibody associated with the Th2 immune pathway) production. However, when helminth infection is cleared these individuals rapidly lose their IgG4 phenotype and allergic and autoimmune reactivity are exacerbated (Adjobimey & Hoerauf, 2010; Kwan-Lim et al., 1990; Larche et al., 2006). Maintaining helminth infection appears to remain important throughout the lifespan, even if infection occurred during crucial points in development.

Future Directions

This dissertation provides support for the Old Friends Hypothesis (as well as the associated Biodiversity/Hygiene Hypotheses) based on research conducted among the Shuar as they are transitioning from a more traditional subsistence lifestyles to lifestyles based on participation in the market economy. Despite progress in documenting relationships among MI, STH, and immune function among the Shuar, many important questions remain unanswered. My future research will address the following topics:

Effects of Specific Lifestyle and MI Variables on STH Exposure

To date, the SHLHP has used SOL variables to test relationships between MI and health (Cepon-Robins et al., 2014; Liebert et al., 2013), without looking at the individual

component parts of SOL such as specific market related variables (like processed food consumption), factors associated with daily domestic animal exposure (including number of animals owned and whether animals are allowed inside or around the house), and other variables related directly to subsistence culture (farming, fishing and hunting implements). This is important for understanding what factors are driving helminth exposure differentially across Shuar communities and individuals.

Preliminary analyses (not included in this dissertation) on a moderately integrated village in the Upano Valley found that individual variables associated with SOL scores were differentially related to STH exposure depending on sex (Cepon et al., 2012). For males of all ages, helminth exposure and infection intensity were associated with larger household sizes. For females of all ages, helminth exposure and infection intensity were related to the number of domesticated animals owned, and whether or not the animals were kept near the home. However, these analyses were preliminary and only based on a single Shuar community. Future research will hone in on these individual features across regions and communities, to determine which factors related to MI are driving STH exposure. To do this, a larger sample size is required and it must represent greater variation across the MI spectrum, which will also allow consideration of the level of family interrelatedness. Future field seasons will collect data with these questions in mind.

Research that examines specific variables related to MI has important implications for health, as well as physical and cognitive development, among Shuar children. Research among other populations has shown that schoolchildren are disproportionately infected with STHs and these infections tend to be passed easily

between classmates (Bundy, 1988; Blackwell et al., 2011). Chapter III documented disproportionate levels among Shuar children compared to adults as well. Despite the potential benefits of low to moderate intensity STH infections for immune regulation, high intensity infections can have serious pathological and developmental consequences for many children, especially early in ontogeny (as discussed in Chapters I and II). Other studies have documented decreased vaccination effectiveness in STH infected children (Cooper et al., 2000; 2001; Elias et al., 2001; Labeaud et al., 2009), suggesting that these infections should be reduced before vaccination occurs in order to prevent infection with more contagious, virulent infections. Children are also easily reinfected after anthelminthic treatments (Bundy, 1988), which could have negative consequences for immune sensitivity against serious pathogens, as well as decreased effectivity of vaccinations (Labeaud et al., 2009; Cooper et al., 2000; 2001; Elias et al., 2001). Understanding the MI variables that differentially influence this exposure may help prevent high intensity infections, which could increase health and well-being, as well as physical growth and development for Shuar children, as well as other children living in highly parasitized environments.

Importance of STH Infection Timing on Immune Regulation

STH infection creates an important challenge. On the one hand, these infections are associated with a number of serious negative health outcomes (Bethony et al., 2006; Bogitsh et al., 2005; Gelpi & Mustafa, 1968; Khuroo et al., 1990; Villamizar et al., 1996), yet complete eradication of helminths is likely associated with increased levels of chronic inflammation and disorders associated with immune dysregulation (Flohr et al., 2008;

Maizels et al., 2014; van den Biggelaar et al., 2000). Future studies need to examine the importance of infection timing and duration, as well as specific STH species, on immune system development and regulation so that attempts can be made to balance the benefits and consequences of infection with STHs. Chapter V showed that reduced helminth exposure, even among people who were probably exposed during development, was associated with elevated inflammation with age. As described above, research has found that treating helminth infections decreases specific antibodies related to the Th2 pathway, and exacerbates allergic and autoimmune symptoms (Adjobimey et al., 2010; Kwan-Lim et al., 1990; Larche et al., 2006). If this is true, not only is original timing of infection important, but maintaining some level of infection is important as well. This could explain why immigrants (discussed in the context of the Biodiversity Hypothesis [Haahtela et al., 2013; Hanski et al., 2012]) that move into more urban environments experience an increase in allergy and autoimmunity.

More research needs to be aimed at understanding the timing of initial infection, as well as the importance of continuous infection across the life course. This kind of data could be useful for optimizing the timing of interventions, like determining when to deliberately infect someone with predisposition to allergy or autoimmunity with harmless helminths to thwart a pro-inflammatory response. It can also be used to understand how and when to treat STH infections among indigenous groups in order to prevent helminth-related pathogenesis, while still allowing the immune system to develop properly.

Life History Tradeoffs and Health

All living organisms, humans included, experience tradeoffs between growth, reproduction, and maintenance based on finite availability of energy and nutrients (Charnov & Schaffer, 1973; Gadgil & Bossert, 1970; Hill & Hurtado, 1996, Hill & Kaplan, 1999; Lessels, 1991; Stearns, 1976). Mounting an immune response is costly to maintain, but very little research has calculated specific metabolic costs associated with immune responses, especially to STHs. Blackwell and colleagues (2011) showed tradeoffs between immune function and growth among the Shuar. In this case, tradeoffs with growth were likely associated with early priming and maintenance of the adaptive immune response, and this was measured through IgE. This seems to show that children who are infected with helminths early during development may have better primed and regulated immune systems, but are often stunted. Another study by Blackwell and colleagues (2008) found that, among the Shuar, 38% of female and 41% of children were stunted[10], compared to 16% of male and 20% of female non-Shuar *colonos*. Future research should examine these relationships as they relate to helminth infection and growth.

Helminth infections may also be related to tradeoffs in reproductive success, though this has also been understudied, especially in humans. Non-human animal studies have shown that testosterone decreases immune function. As a result, parasites act as major selective pressures, especially in males with high testosterone (Folstad et al., 1992; Sundberg, 1995). These studies have been conducted largely among birds, demonstrating that parasite have selected for mechanisms that signal immune health and have led to

[10] In this case, stunting was measured as Z-scores for height-for-age ≤ -2 (Blackwell et al., 2008).

selection for brighter coloring, more elaborate songs, and other gratuitous mating behaviors in order to demonstrate immunological fitness to potential mates (Folstad et al., 1992; Sundberg, 1995). Bright coloring, for instance, demonstrates that the male is able to invest resources in feather growth and vibrancy, either due to having a healthy, well-primed immune system or by not needing to invest in immune function due to low parasite burden, thus signaling for lower parasite burden. While most studies have been on non-human animals, this has important implications for humans, leading many to wonder if humans have similar signaling strategies related to parasite burden and/or immune function.

Signaling reproductive fitness is especially important, because high parasite burden may decrease energy that can be devoted to reproduction. Similar non-human animal studies, also on birds, have found that parasite load decreases reproductive fitness for females (Gustafsson et al., 1994; Marzal & Navarro, 2005). Thus, females would want to avoid highly parasitized environments, while choosing to reproduce with males with strong immune function. Future research that studies the tradeoffs between growth, reproduction, and immune function are extremely important, especially research that explores specific helminth infections. Future studies among the Shuar will combine STH infection data with information on reproductive success (number of surviving offspring) for both men and women, while also including important immune markers and other measures of health for men, women, and their children.

Expanding the MI Spectrum

This dissertation presented data collected among Shuar communities in the Upano Valley and Cross-Cutucú regions of Amazonian Ecuador. In summer of 2014, I also collected data from the market center of Sucúa, which is located in the Upano Valley. Though these data have not been included in analyses for this dissertation, they expanded our measures of MI to include individuals living in more urbanized environments. These data will allow us to test a number of hypotheses related to economic and social change.

Future field seasons in Ecuador will also collect data among non-Shuar *Colonos* living in Sucúa. *Colonos* are an unrelated, non-indigenous group living in the same geographic region as the Shuar, particularly in the Upano Valley. The non-Shuar *Colonos* moved into the Morona Santiago region during the oil boom of the mid-20th century (Madimenos et al., 2011). Even though they occupy the same regions, the Shuar and *Colonos* remain socially and politically divided (Rubenstein, 2001). *Colonos* typically engage in wage labor, attend school, and participate in very few subsistence activities, though in rural areas, they do engage in animal husbandry and agriculture (Madimenos et al., 2011). Including *Colonos* in future comparative studies will give us a greater understanding of which factors associated with parasite exposure are geographic, socioeconomic, based on marginalization and poverty, or related to other behaviors and lifestyle variables associated with MI. Being able to compare two unrelated ethnic groups living in the same region can also give us basic insights into behavioral, biological, and environmental factors that affect immune system development.

Future research by the SHLHP will also examine these relationships among Shuar and non-Shuar Ecuadorian immigrants to the United States. Immigrant studies will allow

us to answer questions about how parasite exposure and immune function changes when individuals, both adults and children, move to environments that are different from where they developed. This will allow for more specific tests of the Biodiversity Hypothesis, as it relates to allergic development in adulthood. Further, these data have the potential to provide the most extensive representation of MI across a single ethnic group (traditional, transitioning, market integrated, and immigrant among the Shuar), as well as comparative data for an unrelated ethnic group living in the same regions (*Colonos* and Ecuadorian immigrants to the United States).

Direct Biomarkers of Autoimmune Disorders

One area of research on the relationship between STH infection and immune function that is severely lacking involves a consideration of specific antibodies responsible for allergic and autoimmune responses on indigenous traditional and transitioning populations. This type of data is very difficult to get. Collecting serum samples from participants via venipuncture is intrusive and, in remote field settings, very hard to preserve (in compact spaces) and store. A lot of headway has been made in recent years to validate many of the same immunoassay kits for use with dried blood spots, which are less intrusive, coming from a single finger prick (McDade et al., 2007) and require less storage space. For instance, our blood spots are stored in a small, portable freezer attached to solar panels in the field and shipped back to the U.S. on dry ice. The shortcomings, however, is that very few direct markers of autoimmunity have been validated for use with dried blood spots. Future studies with SHLHP will validate kits for detection of antibodies associated with Autoimmune Thyroid Disorder (anti-thyroid

peroxidase antibody; TPOAb) and Irritable Bowel Disorders (IgA and IgG). While clinical cutoffs may not be useful for an indigenous transitioning population, small changes in these antibodies could be associated with immune dysregulation.

Inflammatory bowel diseases (IBDs)—including Crohn's Disease—and autoimmune thyroid disorders (AITDs) are two important types of autoimmune disorders to look at in association with parasite exposure and infection. In the United States alone, about 1 to 2 million people have Crohn's Disease or ulcerative colitis (Weinstock and Elliott, 2009), while 20 million people in the United States have thyroid disorders, a leading cause of which is AITDs (American Thyroid Association, 2000). Intestinal parasites IBDs are more directly related than other autoimmune disorders due to localization and prevention of the innate inflammatory immune response, as well as immune priming. This relationship has been well supported, though no study has traced this relationship among a population as they transition from traditional to market integrated lifestyles. AITDs, on the other hand, do not occur in the same location as intestinal parasites, and have a much more complex physiological relationship. Relationships between intestinal parasites and AITDs provide further support for the idea that helminths play an important role in general immune system development. No studies have examined the development of AITDs as they relate to parasites within the context of MI, though previous research has found that AITDs are related to social and economic change among the Yakut (Sakha) of northeastern Siberia (Cepon et al., 2011). Being able to combine SOL and STH infection data with specific markers of autoimmunity would provide for a valuable test the Old Friends Hypothesis.

Testing the Disappearing Microbiota Hypothesis among the Shuar

There is a growing body of research suggesting that bacteria and humans have an important symbiotic, coevolutionary relationship (Fraune & Bosch, 2010). The average healthy human intestinal microbiome—the collective genomes of all microorganisms residing in an individual's intestine—is made up of a very diverse collection of bacterial species, with members of the phylum Bacteroidetes normally outweighing Firmicutes in healthy human guts (Fraune & Bosch, 2010; Turnbaugh & Gordon, 2009). Particular species compositions of the intestinal microbiome have been shown to aid in nutrient and vitamin absorption, tissue development and upkeep. Specifically, studies have shown an individual's microbiota to be important for digestion and metabolism (Hooper et al., 2002), as well as immune system development and function (Mazmanian et al., 2008). Unfortunately, previous studies have been: 1) based on small sample sizes (often fewer than 10 individuals), and 2) largely clinically focused and conducted among populations with very low parasite burden.

To date, 300 samples have been processed for bacterial DNA extraction and are awaiting sequencing for 16s RNA strains to analyze microbial diversity. These samples are from individuals that we have STH, MI, and blood spot data on, allowing for extensive testing of the Disappearing Microbiota Hypothesis, as well as the other hypotheses discussed in this dissertation.

This work among the Shuar will be the first study of its kind to directly test associations between microbial diversity and intestinal bacterial profiles across a nuanced spectrum of MI. If the intestinal microbiome is being affected by social changes associated with MI (including antibiotic use access to medical care) then more

traditionally living Shuar should have a more diverse array of bacterial species present in their intestine, especially those phyla and species associated with proper digestive and immune system development. The extensive database of SOL variables, SES data, family and genealogical information, and other biomarkers will allow us to test a number of hypotheses associated with microbiome studies in more detailed and specific ways than any study to date.

STH and Microbial Coexistence

Because intestinal helminths and bacteria reside in the same general region of the human body, it is important to understand how they interact and affect each other, and how they, in turn, affect immune function (Mideo, 2009). Research on animal models has shown that *H. pylori* pathogenesis, seen clinically in gastritis and inflammation-mediated gastric atrophy, is greatly reduced when helminths are present (Fox et al., 2000; Martin et al., 2010). This may be related to competition for access to important resources, especially iron and red blood cells, which typically limit population sizes of helminth and bacterial species. This relationship has been shown to be detrimental for bacterial species when an anemia causing helminth is present (Graham, 2008). Helminths can also take up surface area needed for binding by other parasites (Graham et al., 2008; Roberts-Thomson et al., 1976) and both groups compete in an evolutionary arms race by altering the host immune system to release cytokines that target their competitors (Abbas et al., 1996; Brown et al., 2009; Cox, 2001; Graham et al., 2008). Few studies, however, consider the interaction between bacteria and helminths, and even fewer do this in conjunction with information on host immune function. Research that considers both

STHs and the microbiome would provide information on all members of our microflora as they relate to immune dysregulation, in order to capture the complex co-evolutionary relationship between parasites/bacteria and the immune system, and understand what happens when environmental change alters these relationships.

Conclusions

In conclusion, this dissertation provides support for the Old Friends Hypothesis, as well as the Hygiene and Biodiversity Hypotheses. There were significant differences in helminth exposure associated with level of MI. This dissertation presented evidence that the disgust response provides a mechanism for avoiding pathogen related stimuli, which may be especially effective when combined with MI. Further, important preliminary support for the idea that decreased exposure to STHs is associated with altered levels of chronic inflammation, especially when this is compared across age groups, was also provided. More than just supporting the Old Friends Hypothesis, these studies also support the exposure mechanisms proposed by both the Hygiene and Biodiversity Hypothesis. An interpretation in favor of all of these hypotheses is that MI increases hygienic behavior due to increased ability to avoid pathogens. This, combined with lifestyle changes in subsistence behavior and depleted biodiversity due to a number of factors like slash-and-burn agriculture, irrigation, different housing construction, and decreased forested areas can result in complete eradication of STHs. This depletion of STHs from the human ecosystem appears to be related to altered immune function that favors the inflammatory pathways and decreased immunotolerance, resulting in the immune system responding to harmless and self-produced antigens. Research that

documents these relationships, parsing out the nuanced variables associated with infection and immune regulation, is beneficial for preventing pathogenesis related to helminth exposure while decreasing risk of immune dysregulation associated with allergies and autoimmunity.

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