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METAGENOMICS AND SOCIAL INCLUSION IN PERU

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF ANTHROPOLOGY

BY

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Abstract

Metagenomics is the study of all the genetic material found in an ecosystem. Each part of the human body is an ecosystem with a distinct bacterial profile or microbiota whose metagenomic signature is called a human microbiome. The study of human microbiomes is a promising new area of biomedical research. Certain bacterial species are directly associated to specific diseases, suggesting their potential as biomarkers or even as therapeutic alternatives. As it has happened with genetics and genomics applications, most microbiome research is focused on urban human populations, thus providing a very biased set of study populations, both human and bacterial.

The developing world harbors biological phenomena absent in the developed world. The study of traditional communities from developing countries provides unique opportunities for scientific advancement. At the same time, the knowledge obtained might impact public health programs and help to build research capacity in these countries. However, including traditional communities from developing countries in research has proved to be a sensitive matter.

Research with indigenous populations carries the stigma of being exploitative, neocolonialist, and imperialist. Engaging communities in research can reduce the risk of exploitation and increase protection for these vulnerable populations. The biggest challenge to establishing meaningful partnerships with indigenous populations is distrust. The history of marginalization and constant friction between indigenous communities and outsiders has eroded any potential predisposition to trust. In absence of trust, any attempt to engage a community will be futile, superficial or pretend

community engagements without any real participation from the community. Real community engagement requires open dialog that results in real partnership which is impossible without a relationship of trust.

Developing a relationship of trust with indigenous communities and the authorities that represent them is a demanding venture. Some unsuccessful research initiatives in the developing world have been credited to obstacles resulting from inefficient systems of research governance. Barriers that complicate inclusion of indigenous populations in biomedical research will contribute to the existing health disparities.

Here, I discuss these topics from my own field experience and my efforts of implementing metagenomic research in Peru. Chapter 1 presents a case- study of the human gut microbiota of a hunter-gatherer community from the Peruvian Amazon region. The findings challenge some of the early conclusions of the study of human gut microbiome, highlighting the need to diversify the populations examined. Chapter 2 discusses distrust as the main barrier to effectively engage indigenous populations from developing countries in biomedical research. Chapter 3 offers the concept of intermediate research organizations as an alternative to overcome some of the barriers related to the implementation of international research with indigenous populations. The necessary process that researchers must undertake can be streamlined, while enhancing protection for human subjects within a system that lacks clear regulations for research.

Chapter 1

Gut Microbiota of a Peruvian Hunter-Gatherer Community

Introduction

For decades, molecular anthropologists have studied human biology and evolution focusing solely on the *Homo sapiens* genome. However, with the technology of the post-genomic era, we are unveiling deep interrelations between humans and human microbiota and it is becoming clear that humans are super organisms composed of human and microbial cells balancing a mutually beneficial state. This study aims to contribute to the understanding of what it means to be human through the lens of metagenomics. Specifically, this chapter presents a metagenomic study of a Matses community, who are traditional hunter-gatherers from the Peruvian Amazon. I hypothesize that this community's gut microbial ecology will be unique, reflecting Matses behavior and distinctive biological and environmental exposures.

Background

It has long been understood that the human body is colonized by microbes and that these microorganisms have a role in human health. In 1880s, Koch, while studying anthrax and tuberculosis, established a causal relationship between a single pathogenic microorganism and a state of clinical disease (Klaassens, Morrison et al. 2011); around the same time, Theodor Escherich presented his findings on the relationship between the intestinal bacteria of the infant and the physiology of digestion (Shulman, Friedmann et al. 2007). It was becoming clear that microorganisms have an important role in human health and studies in microbiology multiplied. However, the field could hardly move beyond clinical and pathological microbiology, mostly limited by difficulties associated with culturing microorganisms.

The study of human microbiota has been limited by the lack of ability to culture anaerobic microorganisms. Basic methods to culture anaerobic organisms were developed in the late 1960s at the Virginia Polytechnic Institute's anaerobe laboratory (Klaassens, Morrison et al. 2011) and shortly after, in the early 1970s, the first projects aiming to study microbial communities started (Klaassens, Morrison et al. 2011). While culturing methods have improved over the last 50 years, they are insufficient to isolate and characterize most of microorganisms that compose the human microbiota.

Sequencing methods reveal more microbial species than culture-based studies. Suau et al.(1999), using microscopic data, estimated that between 60 and 80 percent of observable bacteria within the human gut cannot be cultivated by existing methods (Suau, Bonnet et al. 1999). To overcome challenges associated to culturing microorganisms, Suau et al.(1999) used comparative sequences of cloned 16S rRNA gene (DNA) to study diversity of the human gut microbiota. Out of the 82 molecular species he obtained using molecular cloning based methods, only 24 percent of them were from previously described microorganisms (Suau, Bonnet et al. 1999).

Culture independent methods, mostly based on 16S data, have become a prolific alternative to study microbial communities. Previous assumptions about human microbiota have been tested and rejected by these methods. For example, the dogma that healthy urine is sterile, or that the highly acidic environment in the stomach only contained *Helicobacter* types, have been rejected with 16S rRNA data: Fouts et al. (2012) concluded that healthy urine microbiome is dominated by Lactobacillales in women and *Corynebacterium* in men (Fouts, Pieper et al. 2012); while Bik et al.(2006) identified 128 phylotypes in the human stomach, independent of the presence or absence of *Helicobacter pylori* (Bik, Eckburg et al. 2006).

With advancements of molecular techniques, largely stimulated by the human genome project (Chial 2008), culture independent alternatives to study human microbiomes have flourished. Next-generation sequencing allows collection of sequence data that is orders of magnitude greater than the data obtained using previous methods, such as Sanger sequencing (Huse, Ye et al. 2012). Although there are still multiple challenges associated to bioinformatic processing of the increasing body of data (Wooley, Godzik et al. 2010) , the study of humans and its inhabitants has moved to a metagenomic level.

Metagenomics refers to a DNA sequencing-based study of an ecosystem (Maccaferri, Biagi et al. 2011), including the ecosystems of human body sites. The human metagenome is heterogeneously distributed across the human body because of different substrates, temperatures, humidity, pH, exposure to oxygen, among other variables that define microenvironments, and in turn, shape human microbiomes. We have a distinctive microbiome profile in the skin (Grice, Kong et al. 2008; Grice, Kong et al. 2009; Fierer, Lauber et al. 2010; Kong, Grice et al. 2010; Grice and Segre 2011; Kong 2011; Blaser, Dominguez-Bello et al. 2012), oral cavity (Dewhirst, Izard et al. 2008; Lazarevic, Whiteson et al. 2009; Wade 2011; Zarco, Vess et al. 2011), upper respiratory tract (Willner, Furlan et al. 2009; Charlson, Bittinger et al. 2011), genital tract (Nelson, Van Der Pol et al. 2010; Price, Liu et al. 2010; Lamont, Sobel et al. 2011; Mirmonsef, Gilbert et al. 2011), etc.

The number of microorganisms that form the human microbiome exceeds the number of human cells by at least ten fold (Gill, Pop et al. 2006; Muegge, Kuczynski et al. 2011). Most microorganisms within the human body inhabit the gastrointestinal tract, and the highest density is found in the distal gut (Sonnenburg, Angenent et al. 2004; Gill, Pop et al. 2006). The human gut microbiome is composed by 10 to 100 trillion members, about 10^{12} microbes per milliliter of luminal content (Sonnenburg, Angenent et al. 2004), representing at least 5,600 bacterial taxa (Dethlefsen, Huse et al. 2008), with a collective genome that might exceed human genome by as much as 100 fold (Sonnenburg, Angenent et al. 2004; Gill, Pop et al. 2006).

The human gut microbiome is built over time. In contrast with a common assumption that babies are born sterile, humans, as any other placental animal, receive their first bacterial inoculums during birth, where the infant is exposed to the vaginal microbiome, mostly dominated by *Lactobacillus* and *Prevotella* (Dominguez-Bello, Costello et al. 2010; Dominguez-Bello, Blaser et al. 2011). This first inoculums take over the neonate's microbiome, and vaginally delivered infants have largely undifferentiated microbiota across their body: skin, mouth and even the meconium display the same bacteria as the mother's vagina at the time of delivery (Dominguez-Bello, Costello et al. 2010). It takes about three years of multiple exposures for the gut microbiome to take the configuration that will prevail as adults (Yatsunenko, Rey et al. 2012).

Adult distal gut microbiome is dominated by two bacterial divisions: Firmicutes and Bacteroidetes. This finding is consistent across human populations (Yatsunenko, Rey et al. 2012). The fact that there are about 70 bacterial divisions, and only two of them consistently dominate the distal human gut microbiome, suggests that these two divisions are somehow specialized to the gut (Gill, Pop et al. 2006).

Observed differences in the microbiome profile might be the result of ongoing selective forces that vary among individuals. Studies have showed significant variation between communities (De Filippo, Cavalieri et al. 2010; Yatsunenko, Rey et al. 2012), within communities (Turnbaugh, Hamady et al. 2009) and even within the same individual, although inter-individual variation appears larger than within the same individual (Durbán, Abellán et al. 2012). There are multiple factors that influence this variation, including the environment (De Filippo, Cavalieri et al. 2010; Huttenhower, Gevers et al. 2012; Yatsunenko, Rey et al. 2012), early exposure to microbial communities (Dominguez-Bello, Costello et al. 2010), host genetics (Khachatryan, Ktsoyan et al. 2008; Hansen, Gulati et al. 2010; Spor, Koren et al. 2011), diet (Herter and Kendall 1910; Backhed, Ding et al. 2004; Duncan, Belenguer et al. 2007; De Filippo, Cavalieri et al. 2010; Arumugam, Raes et al. 2011; Dodd, Mackie et al. 2011;

Dominguez-Bello, Blaser et al. 2011; Fava, Gitau et al. 2012; Kallus and Brandt 2012), among other factors. In addition, bacterial communities tend to self-regulate and stabilize by specific mutualistic relationships (Wooley, Godzik et al. 2010).

Distal gut microbiota provides a wide variety of metabolic functions that the human host have not fully evolved (Sonnenburg, Angenent et al. 2004; Prakash, Tomaro-Duchesneau et al. 2011). Humans depend on microorganisms to harvest energy from nutritional components that otherwise would be indigestible (Sonnenburg, Angenent et al. 2004). Vegetal complex polysaccharides constitute an example of nutrients that, without the gut microbiota, would be inaccessible to humans. Due to the chemical and physical nature of the some dietary components, much remains undigested until it reaches the colon (Flint 2012). Many plant derived components such as cell wall polysaccharides and storage polysaccharides provide a major source of energy for colonic microorganisms; consequently, these substrates will shape the ecological relations in the colonic microbiota (Bik, Eckburg et al. 2006), while enabling humans to absorb the secondary metabolites product of these processes, establishing a mutualistic system.

The immune system is one of the forces that shape the gut microbiome. The structure of the gut microbiota is a direct consequence of the interaction between the microbiota, the mucosal immune system and the mucous layer (Sonnenburg, Angenent et al. 2004). It has been shown that the mucosal barrier is capable of distinguishing and responding to particular microbial species (Sonnenburg, Angenent et al. 2004). Although some bacterial species of the human gut are opportunistic pathogens, the continuous interaction with the host exerts a selective force that favors the health of the host (Prakash, Tomaro-Duchesneau et al. 2011) promoting growth of species that are less pathogenic and that can offer some level of benefit to the host.

Physical forces also contribute to define the gut microbiome. Human intestine is lined with an epithelium that has a rapid and continuous turn over. Between 1 and 3 billion cells per hour are shed from the small intestine and about one tenth of that is shed from the colon (Sonnenburg, Angenent et al. 2004). This constant renewal of gut cells and digestive peristalsis constitute forces that limit and select the establishment of gut microbiota. Sonnenburg et al. (2004) proposes that gut microbiota constitutes a biofilm-like community and that maintenance in such a matrix benefits both the host and the microorganisms because it promotes functions served by the microbiota, such as digestion of luminal contents and strengthening of defenses, while creating a structure that allow bacterial colonization. It has been observed that microbial communities transition from a free-living to a “sessile, matrix-encrusted biofilm state”, that becomes a resilient and stable community with particular transcriptional profiles (Sonnenburg, Angenent et al. 2004). To prevent washout, microbes must have the capacity to develop a polymer based structure that can recognize specific components evolved by microbes to mediate their attachment (Sonnenburg, Angenent et al. 2004). Thus, the observed gut microbiome is the result of a complex balance among multiple selective forces.

Given that the gut microbial profile results from the interplay of multiple behavioral, biological and environmental forces, I hypothesized that remote indigenous communities, who experience unique biological and environmental forces, will harbor microbial profiles that are significantly different to those described from Western and non-Western communities. Here I present a case study of the Matses community anexo

San Mateo, a remote hunter-gatherer community from the deep Peruvian Amazonian jungle. This community has been geographically and socially isolated, and until this day, maintains a traditional life style which includes a diet based on cassava, plantain, fish and game meat. Consumption of dairy and processed food is very scarce and the community does not have potable water or sewers.

Materials And Methods

Sample and data collection

The Matses community anexo San Mateo, is located in the border of the Galvez River, in the Region Loreto, Requena Province, in the Peruvian Amazonian region (Figure 1). Initial contact with the community was through their leaders. A public meeting was conducted to present our project (Figure 2) and ask for community consent. After community approval, plastic polypropylene containers were pre-labeled and distributed house by house. One container was provided for each person living in the house. Emphasis was made on the voluntary nature of participation and only people who decided to bring their sample to the community center the next day were consented.

Figure 1. Geographical location of the Matses community anexo San Mateo. Located on the border between Peru and Brazil, on the margin of the Galvez river.

Figure 2. Public meeting with the community. All community members were invited to a public meeting where we presented the project goals and methods we will use during our research. Pictures taken during the explanation of procedure to collect skin microbiome (cubital fosa). [Pictures taken with informed consent under a protocol approved by the Ethics Committee of the Peruvian National Institute of Health]

Fecal sample collection

Participants were instructed to bring the sample soon after the bowel movement in the provided containers. Upon arrival, fecal samples were subsampled for microbiological analysis and aliquoted for molecular and parasitological analysis. Samples for anaerobic microbiological analysis were kept in an airtight container (Figure 3). With exception of the parasitological samples, all other aliquots and the air tight container were kept on ice while in the field. Samples were kept on ice for four days before arriving to Lima where they were placed at -20 ^{o}C and later transported to US for further processing.

Figure 3. Anaerobic sampling. Picture shows the use of a catalyzer to remove oxygen from chamber. To prevent exposure to oxygen, aliquots were kept in an anoxyc airtight container and then placed on ice inside coolers.

Anthropometry

Height and weight were recorded from all participants (Figure 4). Brachial circumference was recorded in all adult males and waist perimeter was recorded from all adult females. Body Mass Index (BMI) was calculated with the standard formula: $BMI=W/H^2$; where W stands for weight in kilograms and H for height in meters. Interpretation of values was made following the Center for Disease Control (CDC) recommendations.

Figure 4. Anthropometry. Team member recording height and weight of participants. [Pictures taken with informed consent under a protocol approved by the Ethics Committee of the Peruvian National Institute of Health]

Dietary information

A general questionnaire about food frequency consumption was elaborated by a nutritionist collaborating with the project. The questionnaire was prepared and filled in Spanish. Data collection was conducted in Matses with the aid of an interpreter.

Parasite Analysis

Parasite screening was conducted *in-situ* by a licensed medical technologist using an optical microscope and a gas-operated power generator (Figure 5). Freshly passed stool samples were collected by participants in new plastic containers provided by the research team. Using physiological serum (0.85% Sodium chloride) and lugol (10% potassium iodide, 5% iodine) glass slides were prepared for direct analysis. Results were documented individually and later returned to the participants through one-to-one counseling sessions with a physician who was a member of the research team. People found to be parasitized were advised to proceed to treatment with the health post in Angamos. A list of individuals found positive to parasite infection was submitted to the health post.

Figure 5. Parasite screening. Community members had an opportunity to look at their samples through the microscope. [Pictures taken with informed consent under a protocol approved by the Ethics Committee of the Peruvian National Institute of Health]

DNA extraction

Fecal DNA, n=22, was extracted using MoBio PowerSoil® DNA Isolation Kit following manufacturer's instructions. Only modifications to the original protocol are the addition of two heating steps: 10 minutes at 60 ^{o}C before and 10 minutes at 90 ^{o}C after vortexing the samples with Powerbeads®.

DNA amplification

Data from the 16S rRNA gene was used to analyze the Matses gut microbiome. The U341F (CCTACGGGRSGCAGCAG) (Hansen, Tolker-Nielsen et al. 1998) and 806rcbc0 (GGACTACHVGGGTWTCTAAT) (Caporaso, Lauber et al. 2011) primers were used to produce amplicons containing the V3-V4 hypervariable region which allowed taxa identification. Reactions were prepared in triplicates using the

AccuPrime™ Taq DNA Polymerase High Fidelity system according to the manufacturer's recommendations.

Amplicons were verified in a 1X SB, 1% agarose gel. Equimolar proportions of amplicons were labeled and pooled for 454 GS FLX+ pyrosequencing of the total bacterial community.

Data analysis

Raw data obtained from the 454 GS FLX+ pyrosequencer were denoised using the Pyrosequencing pipeline (http://pyro.cme.msu.edu/) to retrieve sequences by barcode, remove primers and provide quality filtering. Table 1 presents the barcode sequence used for each sample. Criteria for inclusion in our analyses required each sequence read to have an exact barcode with exact primer sequences and a quality score over 35 (Huse, Huber et al. 2007).

To address sample heterogeneity, rarefaction was performed to the OTU tables. Rarefaction allows sample standardization by randomly sampling the same number of OTUs from each sample, regardless of the total read count on each sample and then using this information to compare the communities at the same given level of sampling and subsampling (Caporaso, Kuczynski et al. 2010).

The compiled dataset was analyzed with the software package Quantitative Insights Into Microbial Ecology (QIIME; http://qiime.sourceforge.net) (Caporaso, Kuczynski et al. 2010) using default settings . The same software was used to infer taxonomic assignment, analyze alpha and beta diversity and to provide rarefaction curve analysis. Frequency of phyla were further characterized using hierarchical analysis using the hclust script in R (Ihaka and Gentleman 1996).

Description	BarcodeSequence	LinkerPrimerSequence	ReversePrimer
Ex01_EX	ACTAGCAGTA	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
WSM_WA	ACGCGATCGA	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM24_SM	TCTCTATGCG	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM43_SM	TACTCTCGTG	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM11_SM	ATCAGACACG	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM30_SM	CGAGAGATAC	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM34_SM	TCTACGTAGC	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM42_SM	TACGAGTATG	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM44_SM	TAGAGACGAG	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM33_SM	CGTCTAGTAC	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM10_SM	AGCACTGTAG	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM23_SM	CTCGCGTGTC	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM03_SM	AGACGCACTC	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM37_SM	TGTACTACTC	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM40_SM	CGTAGACTAG	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM20_SM	CGTGTCTCTA	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM32_SM	TCACGTACTA	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM02_SM	ACGCTCGACA	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM01_SM	ACGAGTGCGT	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM31_SM	ATACGACGTA	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM18_SM	ATATCGCGAG	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM39_SM	ACGACTACAG	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM29_SM	CATAGTAGTG	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT
SM28_SM	TGATACGTCT	CCTACGGGRSGCAGCAG	GGACTACHVGGGTWTCTAAT

Table 1. Barcode and primer sequences. Unique barcode was used for each individual. Barcodes were incorporated in the final sequence and were used to identify and grouped the data by individual. Linker and Reverse primers were common to all samples.

Our data was compared to a data set from other non-Western populations. The data set with the MG-rast ID ‘qiime:850’ (Yatsunenko, Rey et al. 2012) contains 16S rRNA data from gut microbiome from human populations from Malawi, Venezuela and USA. Raw data was downloaded and processed in parallel with our samples to normalize data sets and taxonomic assignments across all samples.

Statistical significance of observed differences was tested using parametric analysis. T-tests and one way ANOVA were conducted using SPSS 19.0 (IBM), assuming normal distribution of frequencies and similar variances across populations (Levene's test p-value > 0.05).

Results

Pyrosequencing data of the 16S rRNA V3-V4 amplicon was obtained from fecal samples from 22 individuals (9 male and 13 female, between 2 and 52 years old) from the Matses community anexo San Mateo (Figure 6). As with many small indigenous communities, members are mostly derived from a few original families, resulting in individuals with certain level of consanguinity. This sample contained related and cohabiting individuals. Based on BMI calculation, there is no incidence of obesity in this Matses community. With exception of two (9%) slightly overweight and one (4.5%) underweight individual, individuals on this sample fall within the healthy weight range (Table 2).

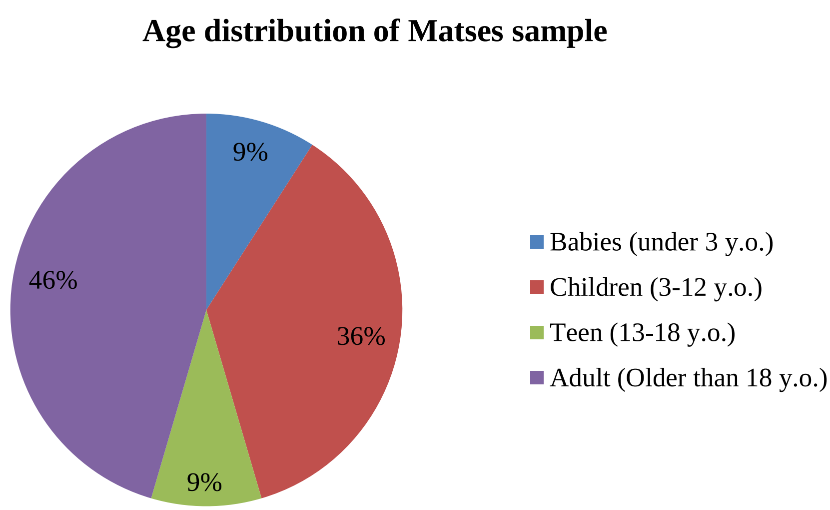


Figure 6: Age distribution of samples obtained from the Matses community anexo San Mateo.

Sample ID	Age	BMI	According to BMI
SM24_SM	2	17.84651993	Healthy weight
SM43_SM	2	15.14960232	Healthy weight
SM11_SM	4	15.7827664	Healthy weight
SM30_SM	4	18.26220684	Healthy weight
SM34_SM	4	15.61849229	Healthy weight
SM42_SM	4	16.73193307	Healthy weight
SM44_SM	4	19.83379501	Healthy weight
SM33_SM	5	16.44608035	Healthy weight
SM10_SM	6	17.81506268	Healthy weight
SM23_SM	7	15.66293367	Healthy weight
SM03_SM	10	15.77251399	Healthy weight
SM37_SM	12	19.83733386	Healthy weight
SM40_SM	18	22.7189744	Healthy weight
SM20_SM	20	21.27062971	Healthy weight
SM32_SM	21	22.14360042	Healthy weight
SM02_SM	25	23.95449447	Healthy weight
SM01_SM	30	N/A	N/A
SM31_SM	30	22.60026298	Healthy weight
SM18_SM	36	26.44282114	Overweight
SM39_SM	40	28.9340689	Overweight
SM29_SM	50	17.89802289	Underweight
SM28_SM	52	19.46582164	Healthy weight

Table 2: Ages and body mass index (BMI) of the participants.

Obtaining diet information through questionnaires proved to be far more challenging than expected. Although we had a loose instrument (a list of food that was constructed by a nutritionist collaborator of the project) based on information we obtained from the Matses leader, Matses diet is very irregular and individual’s answers were consistently vague: “*si lo tengo, lo como*” (If I have it, I eat it), “*a veces*” (sometimes), “ *Cuando encuentro*” (when we find it), and alike.

Dietary data collected from the Matses community anexo San Mateo is presented on table 3. Given the challenges to establish a quantifiable dietary pattern, we use qualifiers to describe frequency. The table also shows the nutritional content of the most common food in their diet. Their diet is based on carbohydrates; cassava and plantain are the staple food in this community. Based on their report, their consumption of protein is below that recommended 0.8 g/kg of body mass a day (Leidy, Mattes et al. 2012) and fat in their diet is mostly derived from fruits (aguaje and unguirahui) complemented by fish and game meat when available. As seen in figure 7, Matses cooking methods tend to drain fat from the meat and fish, consequently, their consumption of animal fat is far below what is observed in Western populations.

<table>
<tr><th></th><th></th><th></th><th></th><th></th><th></th><th colspan="3">Nutritional content (per 100 g)</th></tr>
<tr><th></th><th>Matses name</th><th>Common name</th><th>Scientific name</th><th>Frequency</th><th>Mode of consumption</th><th>Protein</th><th>Fat</th><th>Carbohydrates</th></tr>
<tr><td rowspan="13">Staple food, main source of calories</td><td>Pachid ushu</td><td rowspan="3">Varieties of manioc</td><td rowspan="3">Manihot sculenta</td><td rowspan="3">Everyday / anytime</td><td>Boiled</td><td>0.48</td><td>0.1</td><td>31</td></tr>
<tr><td>Pachid piu</td><td>Drink (Masato)</td><td>0.2</td><td>0.1</td><td>8.9</td></tr>
<tr><td>Pachid taui chëshë</td><td>Fermented and toasted (farina)</td><td colspan="3">No data available. It is estimated that 45% of protein and sucrose is lost during fermentation. Starch digestivibility is increased by fermentation.</td></tr>
<tr><td>mani</td><td rowspan="10">Varieties of plantain/banana</td><td rowspan="10">Musa paradisiaca</td><td rowspan="10">Everyday /anytime</td><td rowspan="3">Raw (avergae of varieties)</td><td rowspan="3">1.2</td><td rowspan="3">0.27</td><td rowspan="3">25</td></tr>
<tr><td>Mani bënë</td></tr>
<tr><td>Manimbo</td></tr>
<tr><td>Mani chëshë</td><td rowspan="3">Chapo (main drink, accompanies all meals)</td><td rowspan="3" colspan="3">Approximately a third of the nutritional content of raw banana (volumetric dilution)</td></tr>
<tr><td>Mani ushu</td></tr>
<tr><td>Mani chotac</td></tr>
<tr><td>Mani masquë</td><td rowspan="2">Boiled</td><td rowspan="2">0.7</td><td rowspan="2">0.3</td><td rowspan="2">36.8</td></tr>
<tr><td>Mani macho</td></tr>
<tr><td>Mani piu</td><td rowspan="2">grilled</td><td rowspan="2">1.5</td><td rowspan="2">0.2</td><td rowspan="2">68</td></tr>
<tr><td>Mani tadan</td></tr>
<tr><td rowspan="5">Fruits</td><td>Aguaje (*)</td><td>Moriche or Ité Palm</td><td>Mauritia flexuosa</td><td rowspan="5">Few portions a day</td><td>Raw</td><td>2.3</td><td>25.1</td><td>18.1</td></tr>
<tr><td>Canchi</td><td>Pinapple</td><td>Ananas comosus</td><td>Raw</td><td>0.4</td><td>0.2</td><td>9.8</td></tr>
<tr><td>Pia bata</td><td>Sugar cane</td><td>Saccharum sp</td><td>Raw</td><td>0.3</td><td>0.1</td><td>20.5</td></tr>
<tr><td>Ungurahui (*)</td><td>Patawa, sehe, hungurahua or mingucha</td><td>Oenocarpus bataua</td><td>Raw</td><td>2.8</td><td>21.1&</td><td>33.7</td></tr>
<tr><td>Uvilla (*)</td><td>Uvilla</td><td>Pourouma expansa</td><td>Raw</td><td>0.3</td><td>0.3</td><td>16.7</td></tr>
</table>

	Dectad	Papaya	*Carica papaya*		Raw	0.4	0.1	8.2
	Pupu	Cocona	*Solanum sessiflorum*		Raw	0.9	0.7	9.2
	Titado piu Titado Ushu Titado Sequid	Pijuayo	*Bactris gasipaes /Bactris sp.*		Boiled (data only available for the raw fruit)	2.8	3.2	41
Other crops	Cadi piu Cadi chëshë	sweet potato	*Ipomoea batata*	Couple times a month	Boiled, grilled	1.6	0.2	26.1
	Piacbo Piacbo bëdi	Maiz	*Zea mays*	Monthly	Bioled, grilled	3.3	0.8	27.8
	Chotacquëna bëaid	Guaba, orange, mangoes. Lemon, grapefruit, rice, cacao, etc. Any crop that is brought from outside the community	various	Sometimes, when available	Various	NA	NA	NA
Mammals	Poshto	woolly monkeys	*Lagothrix lagotricha*	Few times a month	Grilled, smoked, boiled	NA	NA	NA
	Achu	Howler monkey	*Alouatta seniculus*			NA	NA	NA
	Shuinte	Sloth	*Bradypus sp*			NA	NA	NA
	Shëshëid	Spidermonkey	*Ateles sp*			NA	NA	NA

	Shëcten	Collared peccary	*Tayassu tajacu*			85.6	NA	NA
	Sh ëctenamë	White-lipped peccary	*Tayassu pecari*			84.6	NA	NA
Reptiles	Piush	Yellow-footed tortoise	*Geochelone denticulata*		Grilled, smoked, boiled	84.4	NA	NA
	Zeta	Yellow-spotted river turtle	*Podocnemis unifilis*			NA	NA	NA
Birds	Cushu	trinidad Piping Guan	*Aburria pipile*		Grilled, smoked, boiled	NA	NA	NA
	Mando	Grey-winged trumpeter	*Psophia sp*			NA	NA	NA
	Uesnid	Razor-billed Curassow	*Mitu tuberosa*			NA	NA	NA
Fish	Sabalo (*)	Sabalo	*Prochilodus lineatus*	Daily/Few times a week	boiled, grilled or cooked inside leaves (Patarashca)	NA	NA	NA
	Maparate (*)	Maparate	*Hypophthalmus marginatus*			15.1	0.9	NA
	Lisa (*)	Leaping mullet	*Liza saliens*			20.8	3.3	0
	Tucunare (*)	tucanare peacock bass	*Cichla monocullus*			NA	NA	NA
	Boquichico (*)	Black prochilodus	*Prochilodus nigricans*			47.9	9.1	0
Eggs		Turtle, alligator		Irregular.	(Average values)	15.1	11.3	2.9

Table 3: Summary of the Matses diet. Nutritional data was collected using a food frequency questionnaire. Most of the time respondents would state their diet depends on availability, challenging data collection. Plantain and manioc are the only constants in their diet (6 or more portions a day). Similarly to other Amazonic groups, fish appears to be their main source of protein. Nutritional content established from Ezeala (1984), Padmaja et.al. 1994, Galvez-Arbaiza et al (1999), Sanchez (2000) and Reyes Garcia et al (2009)

A.

B.

Figure 7. Matses cooking. A. Kitchen area within a Matses house. B. Cooking of a sloth on open fire.

Parasite screening, by direct microscopic analysis, revealed that 15 individuals (68.18%) were positive for parasite infection. Thirteen of the individuals found infected had only one parasite while two of them presented two different parasites. Table 4 shows the results of the parasite screening.

	Frequency	Percent
Negative	7	31.8
Trichomonas sp.	7	31.8
Giardia lamblia	3	13.6
Entamoeba coli	1	4.5
Blastocystis hominis	1	4.5
Entamoeba coli and *Giardia lamblia*	1	4.5
Entamoeba coli and *Blastocystis hominis*	1	4.5
Endolimax nana	1	4.5
Total	22	100.0

Table 4. Results of parasite screening.

There were no significant differences in the rate of parasite infection in children younger than 12 years old with respect to the rest of the samples. Individuals positive to parasite infection were distributed across all the houses without significant differences.

An independent sample t-test comparing the mean frequency of common gut bacteria genera from individuals positive to parasite infection compared to individuals negative to parasite infection was unable to reject the null, suggesting that these parasites have no major effect on shaping the frequency of common gut bacteria genera. Table 5 presents the results of comparisons of the frequency of the most representative genus.

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	**Sig. (2-tailed)**	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference Lower	Upper
Bacteroides	Equal variances assumed	1.025	.324	.437	20	**.667**	.000	.001	-.002	.002
	Equal variances not assumed			.348	7.545	**.737**	.000	.001	-.002	.003
Faecalibacteri	Equal variances assumed	5.425	.030	1.118	20	**.277**	.077	.069	-.067	.221
	Equal variances not assumed			.892	7.546	**.400**	.077	.087	-.125	.279
Ruminococcus	Equal variances assumed	2.906	.104	-1.070	20	**.297**	-.015	.014	-.043	.014
	Equal variances not assumed			-1.532	16.402	**.145**	-.015	.010	-.035	.006
Clostridium	Equal variances assumed	.195	.663	1.390	20	**.180**	.006	.004	-.003	.015
	Equal variances not assumed			1.336	10.741	**.209**	.006	.005	-.004	.016
Streptococcus	Equal variances assumed	11.261	.003	1.382	20	**.182**	.012	.009	-.006	.031
	Equal variances not assumed			.920	6.025	**.393**	.012	.013	-.020	.045
Lactobacillus	Equal variances assumed	4.457	.048	-1.047	20	**.307**	.000	.001	-.002	.001
	Equal variances not assumed			-1.480	17.222	**.157**	.000	.000	-.002	.000

Table 5. Independent sample t-test to compare microbial profile of individuals found positive or negative during the parasite screening. Some representative phylotypes are examined and no significant differences are detected between the two groups.

A total of 175,155 16S RNA gene sequences fulfilled our criteria for inclusion: a perfect match to the barcode and primers, a QC index over 35 and minimum fragment size of 200 bases. Reads from individual samples were grouped using barcodes (Table 6).

ID	Counts after trimmed	OTUs	OTUs with taxonomic information	Unknowns
Ex01	5794	5794	5794	0
SM01	8003	8003	8002	1
SM02	7015	7015	7013	2
SM03	7274	7274	7269	5
SM10	8280	8280	8276	4
SM11	7493	7493	7492	1
SM18	7702	7702	7702	0
SM20	9026	9026	9025	1
SM23	7996	7996	7995	1
SM24	8525	8525	8525	0
SM28	8578	8578	8577	1
SM29	6892	6892	6888	4
SM30	6867	6867	6867	0
SM31	8629	8629	8628	1
SM32	6975	6975	6974	1
SM33	7280	7280	7279	1
SM34	7104	7104	7104	0
SM37	7352	7352	7347	5
SM39	5114	5114	5113	1
SM40	5723	5723	5721	2
SM42	5839	5839	5839	0
SM43	9423	9423	9416	7
SM44	5595	5595	5591	4
WSM	6676	6676	6676	0

Table 6. Sequencing yield. We obtained an average of 7298 reads per sample with a standard deviation of 1119. The minimum and maximum number of reads per sample was 5114 and 9423, respectively.

From taxonomic assignment using the RDP database, a total of 16 phyla were identified in the gut microbiome of this Matses population. Fourteen out of the 16 phyla belonged to the Bacteria domain (87.5%). One phylum from Archea (6.25%) and one group of unclassified sequences (6.25%) were also noted. The distribution of these phyla among the samples showed wide variability, but consistent with general understanding of human gut microbiome, Firmicutes and Bacteroidetes are the dominant phyla, accounting for 91% of total reads, and between these two, Firmicutes is largely the most represented phyla in this sample (Figure 8).

In contrast with what has been observed in other non-Western populations, our population displays a higher fraction of Firmicutes than Bacteroidetes: 88.4% of Firmicutes (range between 68.7 and 97.5%) and 2.7% of Bacteroidetes (range between 0.2 and 12.9%). By far, the most abundant order is Clostridiales, accounting for 84.7% of total reads (between 64.1% and 96.4%). The most abundant genus is *Faecalibacterium* representing 19.9% of the total reads (between 2.1% and 58.8%).

Seventeen phylotypes were represented with a frequency above 1% of total reads and this included 14 (82.4%) Firmicutes, one Bacteroidetes, one Proteobacteria and one Spirochaetes (5.9% each). Of the most common genera, 10 out of the 17 (58.8%) were present in all of the samples, and these were all Firmicutes. The genus *Prevotella*, considered a marker of non-Western gut microbiome (De Filippo, Cavalieri et al. 2010; Yatsunenko, Rey et al. 2012), was underrepresented in our samples, with a level consistent to the one observed in a sample from US population (Table 7).

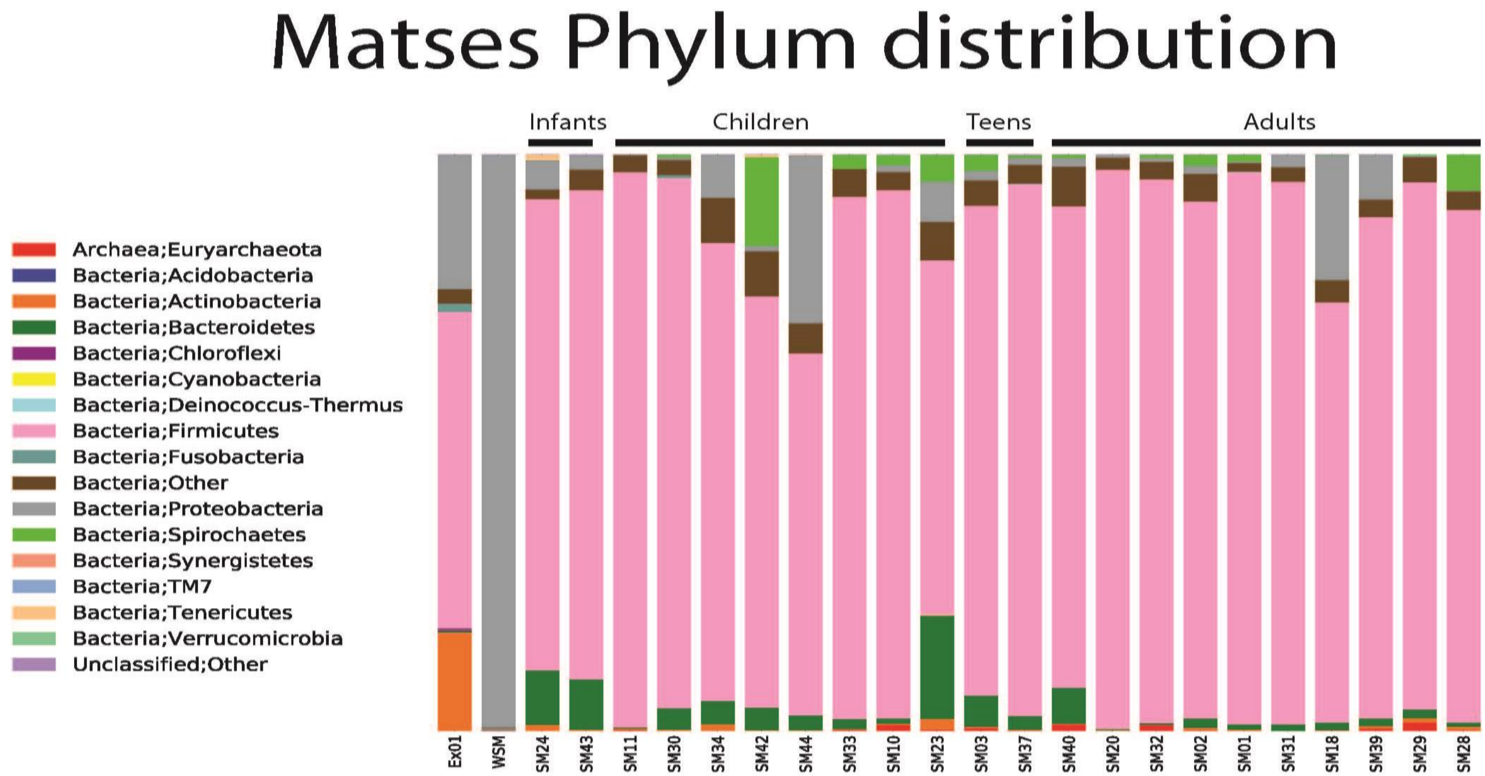


Figure 8. Firmicutes is the dominant phylum in the gut microbiome of our Matses sample. As shown in the figure, Firmicutes (pink bars) is by far the dominant phylum with an average of 88.4% of the total reads (between 68.7 and 97.5% in each of the samples). This observation is consistent regardless of the age of the individual. Ex01 represent a contamination control and WSM a water sample taken from the same community. Although at the phylum level the profile in Ex01 appears similar to the one observed in the samples, further analysis (not shown) demonstrate that genus in the contamination control are distinct to those observed in the samples.

	Matses	Venezuela	USA	Malawi
Bacteroides	0.003837573	0.018574531	0.148836405	0.006355784
Prevotella	**0.018755779**	**0.218622028**	**0.01766608**	**0.281511015**
Faecalibacterium	0.235882413	0.154489823	0.180299078	0.178019892

Table 7. Table of genus frequencies. Matses data is compared with that of Yatsunenko et al. (2012). Within the phylum Bacteroidetes, *Prevotella* is the most frequent genus in the Matses microbiota; however, the level of *Prevotella* observed is much closer to that observed in US than in the other non-Western populations. Note that among these populations, the Matses sample exhibits the highest levels of *Faecalibacterium* and the lowest levels of *Bacteroides.*

Alpha diversity, or diversity within samples, was calculated using QIIME functions. Tables containing Operational Taxonomic Units were rarefied using the default algorithm. Figure 9 shows the rarefaction curves. 19 out of the 22 samples (86.4%) exhibit similar levels of diversity. Contamination controls (Ex01 and WSM) display lower diversity.

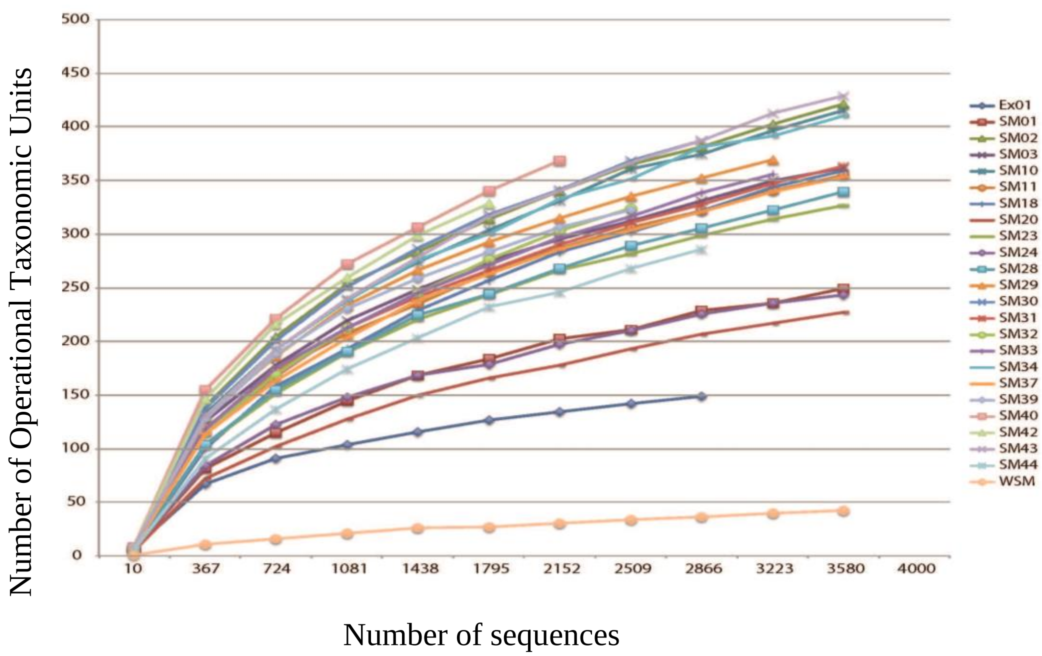


Figure 9. Rarefaction curve of the Matses Samples. Most of samples reveal similar levels of intra-sample diversity. Note that the curves do not plateau even at the 3500 sequences level. These profiles are suggestive of large diversity within these samples.

Data obtained from the Matses community anexo San Mateo was compared with pre-published non-Western populations: Malawi and Venezuela (Yatsunenko, Rey et al. 2012); and a Western sample (US) (Yatsunenko, Rey et al. 2012). At the phylum level, the Matses gut microbiota exhibits obvious differences with respect to the Western and non-Western populations presented by Yatsunenko et al. (2012). Figure 10 presents the distribution of the six main phyla identified in the Matses and compared with samples from Malawi, Venezuela and the US. The profile from the Matses is clearly distinct: the ratio Firmicutes to Bacteroidetes is larger than the observed in the other populations, while Spirochaetes appear as a common trait shared with other non-Western populations.

The statistical significance of the profile, at the phyla level, was tested using one way ANOVA analysis. Given that infant gut microbiome has particular configurations and it takes about three years until it adopts the adult profile (Lauber, Hamady et al. 2009; Dominguez-Bello, Blaser et al. 2011; Yatsunenko, Rey et al. 2012), children under four years old were excluded from this analysis.

Statistically significant differences were detected in the mean frequency of the major phyla across populations. The one-way ANOVA revealed that the frequency of Bacteroidetes, Firmicutes and Spirochaetes was significantly different across the Venezuela, USA, Malawi and Peru-Matses samples (p-value ≤ 0.001) (Table 8). Similarly, the frequency of sequences that by comparison to the database could not be assigned to a specific genus also differs across these populations (p-value 0.001).

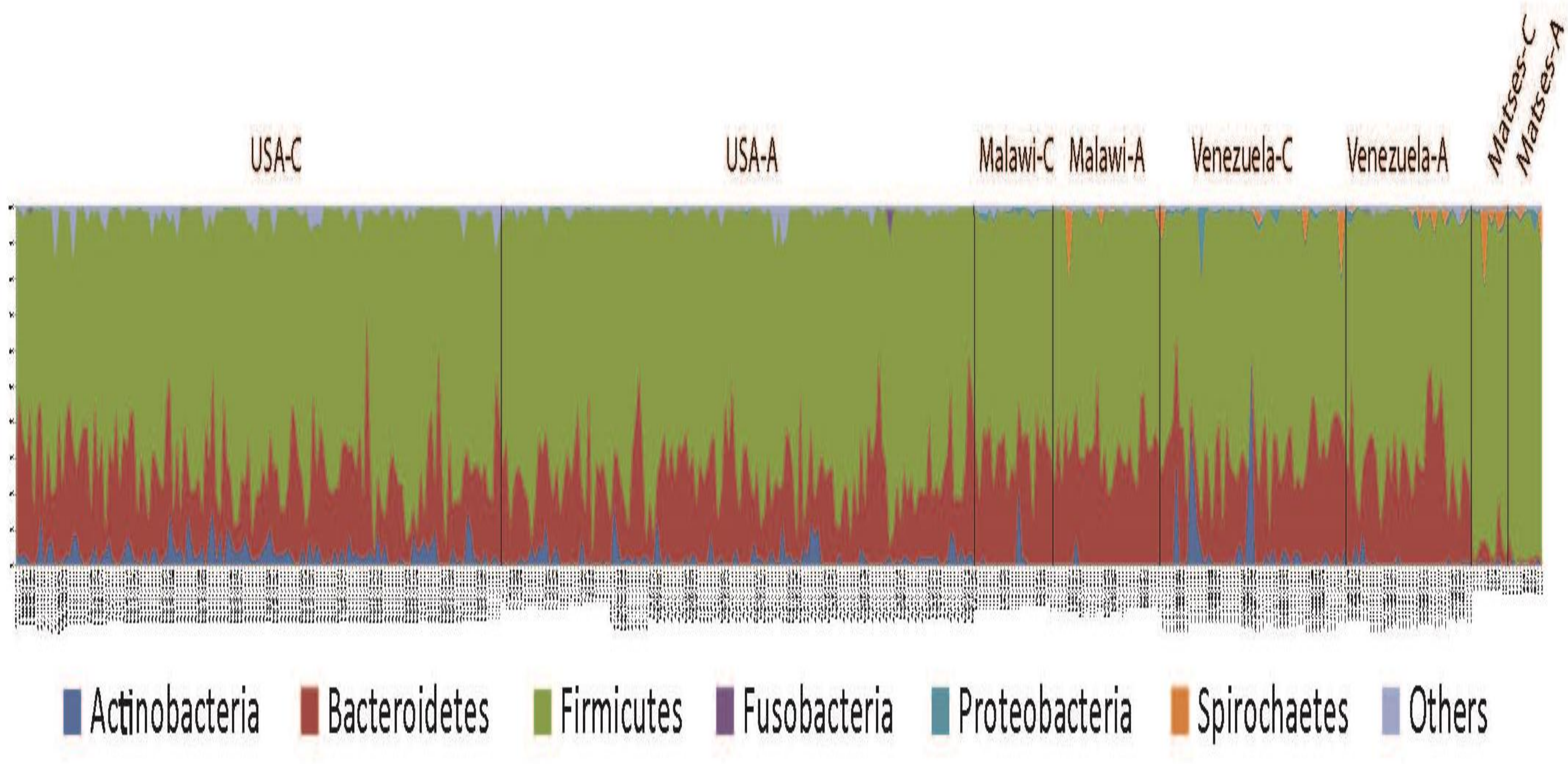


Figure 10: Distribution of most representative phyla across populations. Differences between the proportions of Firmicutes and Bacteroidetes are evident. Firmicutes fraction is larger in the Matses population while the Bacteroidetes one is reduced compared to other non-Western populations. Spirochaetes, as a non-Western trait, is shared by the populations from Malawi, Venezuela and Matses. "-C" and "-A" denote children and adult population, respectively.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Bacteroidetes	Between Groups	1.078	3	.359	29.386	.000
	Within Groups	5.076	415	.012		
	Total	6.154	418			
Firmicutes	Between Groups	1.470	3	.490	33.291	.000
	Within Groups	6.106	415	.015		
	Total	7.576	418			
Spirochaetes	Between Groups	.014	3	.005	16.138	.000
	Within Groups	.119	415	.000		
	Total	.133	418			
ArchaeaUNC	Between Groups	.000	3	.000	.	.
	Within Groups	.000	415	.000		
	Total	.000	418			
BacteriaUNC	Between Groups	.228	3	.076	46.723	.000
	Within Groups	.674	415	.002		
	Total	.901	418			
EukaryotaUNC	Between Groups	.000	3	.000	87.070	.000
	Within Groups	.000	415	.000		
	Total	.000	418			
unclassified	Between Groups	.000	3	.000	5.276	.001
	Within Groups	.000	415	.000		
	Total	.000	418			

Table 8. One way ANOVA to compare means across populations. Analysis reveals statistically significant differences across the USA, Venezuela, Malawi and Matses populations (p-value <0.001)

Furthermore, pairwise comparisons revealed that the Matses population anexo San Mateo's mean frequencies for these phyla are significantly different to those observed in the other three populations. Table 9 presents the results of the ANOVA pairwise comparisons considering Bonferroni correction for multiple observations.

	(I) COUNTRY	(J) COUNTRY	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Bacteroidetes	VENEZUELA	USA	0.0260606	0.01409	0.39	-0.01129	0.063411
		MALAWI	-0.0303032	0.0197	0.748	-0.08253	0.02192
		PERU-SAN MATEO	.2389236*	0.028224	**0.000**	0.164104	0.313743
	USA	VENEZUELA	-0.0260606	0.01409	0.39	-0.06341	0.01129
		MALAWI	-.0563638*	0.016758	**0.005**	-0.10079	-0.01194
		PERU-SAN MATEO	.2128630*	0.026255	**0.000**	0.143262	0.282464
	MALAWI	VENEZUELA	0.0303032	0.0197	0.748	-0.02192	0.082526
		USA	.0563638*	0.016758	**0.005**	0.011939	0.100789
		PERU-SAN MATEO	.2692268*	0.029646	**0.000**	0.190636	0.347818
	PERU-SAN MATEO	VENEZUELA	-.2389236*	0.028224	**0.000**	-0.31374	-0.1641
		USA	-.2128630*	0.026255	**0.000**	-0.28246	-0.14326
		MALAWI	-.2692268*	0.029646	**0.000**	-0.34782	-0.19064
Firmicutes	VENEZUELA	USA	-.0974409*	0.015454	**0.000**	-0.13841	-0.05647
		MALAWI	-0.0060932	0.021608	1	-0.06337	0.051188
		PERU-SAN MATEO	-.2581997*	0.030957	**0.000**	-0.34027	-0.17613
	USA	VENEZUELA	.0974409*	0.015454	**0.000**	0.056473	0.138409
		MALAWI	.0913477*	0.018381	**0.000**	0.04262	0.140075
		PERU-SAN MATEO	-.1607589*	0.028798	**0.000**	-0.2371	-0.08442
	MALAWI	VENEZUELA	0.0060932	0.021608	1	-0.05119	0.063374
		USA	-.0913477*	0.018381	**0.000**	-0.14008	-0.04262
		PERU-SAN MATEO	-.2521066*	0.032518	**0.000**	-0.33831	-0.1659
	PERU-SAN MATEO	VENEZUELA	.2581997*	0.030957	**0.000**	0.176133	0.340266
		USA	.1607589*	0.028798	**0.000**	0.084416	0.237101
		MALAWI	.2521066*	0.032518	**0.000**	0.165904	0.338309
Spirochaetes	VENEZUELA	USA	.0072909*	0.002161	0.005	0.001561	0.013021
		MALAWI	0.0020731	0.003022	1	-0.00594	0.010084
		PERU-SAN MATEO	-.0184334*	0.00433	**0.000**	-0.02991	-0.00696
	USA	VENEZUELA	-.0072909*	0.002161	0.005	-0.01302	-0.00156
		MALAWI	-0.0052178	0.002571	0.258	-0.01203	0.001597
		PERU-SAN MATEO	-.0257243*	0.004028	**0.000**	-0.0364	-0.01505
	MALAWI	VENEZUELA	-0.0020731	0.003022	1	-0.01008	0.005938
		USA	0.0052178	0.002571	0.258	-0.0016	0.012033

		PERU-SAN MATEO	-.0205065*	0.004548	**0.000**	-0.03256	-0.00845
	PERU-SAN MATEO	VENEZUELA	.0184334*	0.00433	**0.000**	0.006956	0.029911
		USA	.0257243*	0.004028	**0.000**	0.015047	0.036401
		MALAWI	.0205065*	0.004548	**0.000**	0.00845	0.032563
BacteriaUNC	VENEZUELA	USA	.0470656*	0.005133	**0.000**	0.033458	0.060673
		MALAWI	-0.0044663	0.007177	1	-0.02349	0.014559
		PERU-SAN MATEO	0.0011882	0.010282	1	-0.02607	0.028446
	USA	VENEZUELA	-.0470656*	0.005133	**0.000**	-0.06067	-0.03346
		MALAWI	-.0515318*	0.006105	**0.000**	-0.06772	-0.03535
		PERU-SAN MATEO	-.0458774*	0.009565	**0.000**	-0.07123	-0.02052
	MALAWI	VENEZUELA	0.0044663	0.007177	1	-0.01456	0.023492
		USA	.0515318*	0.006105	**0.000**	0.035347	0.067716
		PERU-SAN MATEO	0.0056544	0.0108	1	-0.02298	0.034286
	PERU-SAN MATEO	VENEZUELA	-0.0011882	0.010282	1	-0.02845	0.02607
		USA	.0458774*	0.009565	**0.000**	0.020521	0.071234
		MALAWI	-0.0056544	0.0108	1	-0.03429	0.022977
EukaryotaUNC	VENEZUELA	USA	0	6.6E-06	1	-1.8E-05	0.000018
		MALAWI	-0.0000001	9.3E-06	1	-2.5E-05	0.000024
		PERU-SAN MATEO	-.0001974*	1.33E-05	**0.000**	-0.00023	-0.00016
	USA	VENEZUELA	0	6.6E-06	1	-1.8E-05	0.000018
		MALAWI	-0.0000001	7.9E-06	1	-2.1E-05	0.000021
		PERU-SAN MATEO	-.0001974*	1.23E-05	**0.000**	-0.00023	-0.00017
	MALAWI	VENEZUELA	0.0000001	9.3E-06	1	-2.4E-05	0.000025
		USA	0.0000001	7.9E-06	1	-2.1E-05	0.000021
		PERU-SAN MATEO	-.0001973*	1.39E-05	**0.000**	-0.00023	-0.00016
	PERU-SAN MATEO	VENEZUELA	.0001974*	1.33E-05	**0.000**	0.000162	0.000233
		USA	.0001974*	1.23E-05	**0.000**	0.000165	0.00023
		MALAWI	.0001973*	1.39E-05	**0.000**	0.00016	0.000234
	VENEZUELA	USA	0	0	0.652	0	0
		MALAWI	-0.0000001	1E-07	0.747	0	0
		PERU-SAN MATEO	-.0001974*	5.73E-05	**0.017**	-0.00037	-0.00003
	USA	VENEZUELA	0	0	0.652	0	0
		MALAWI	-0.0000001	1E-07	0.655	0	0
		PERU-SAN MATEO	-.0001974*	5.73E-05	**0.017**	-0.00037	-0.00003

	MALAWI	VENEZUELA	0.0000001	1E-07	0.747	0	0
		USA	0.0000001	1E-07	0.655	0	0
		PERU-SAN MATEO	-.0001973*	5.73E-05	**0.017**	-0.00037	-2.9E-05
	PERU-SAN MATEO	VENEZUELA	.0001974*	5.73E-05	0.017	0.00003	0.000365
		USA	.0001974*	5.73E-05	0.017	0.00003	0.000365
		MALAWI	.0001973*	5.73E-05	**0.017**	0.000029	0.000365
unclassified	VENEZUELA	USA	-0.00005	3.75E-05	1	-0.00015	0.000049
		MALAWI	0.0000017	5.24E-05	1	-0.00014	0.000141
		PERU-SAN MATEO	-.0002842*	7.51E-05	**0.001**	-0.00048	-8.5E-05
	USA	VENEZUELA	0.00005	3.75E-05	1	-4.9E-05	0.000149
		MALAWI	0.0000517	4.46E-05	1	-6.6E-05	0.00017
		PERU-SAN MATEO	-.0002342*	6.98E-05	**0.005**	-0.00042	-4.9E-05
	MALAWI	VENEZUELA	-0.0000017	5.24E-05	1	-0.00014	0.000137
		USA	-0.0000517	4.46E-05	1	-0.00017	0.000066
		PERU-SAN MATEO	-.0002859*	7.88E-05	**0.002**	-0.0005	-7.7E-05
	PERU-SAN MATEO	VENEZUELA	.0002842*	7.51E-05	0.001	0.000085	0.000483
		USA	.0002342*	6.98E-05	0.005	0.000049	0.000419
		MALAWI	.0002859*	7.88E-05	**0.002**	0.000077	0.000495

Table 9. One way ANOVA, pair-wise comparisons. The Matses population in study (Labeled Peru-San Mateo) exhibits significant differences with the other three populations, especially with USA and Venezuela. More similarity is observed with the Malawi sample.

Clustering of study subjects using primary component analysis (PCoA) based on Unifrac distances (Lozupone and Knight 2005) revealed that the Matses samples form a separate cluster from the cluster comprising the Venezuela and Malawi samples, reflecting a genetic distance that is smaller within the Matses population than the distance observed between the Matses and the Venezuela or Malawi sample.

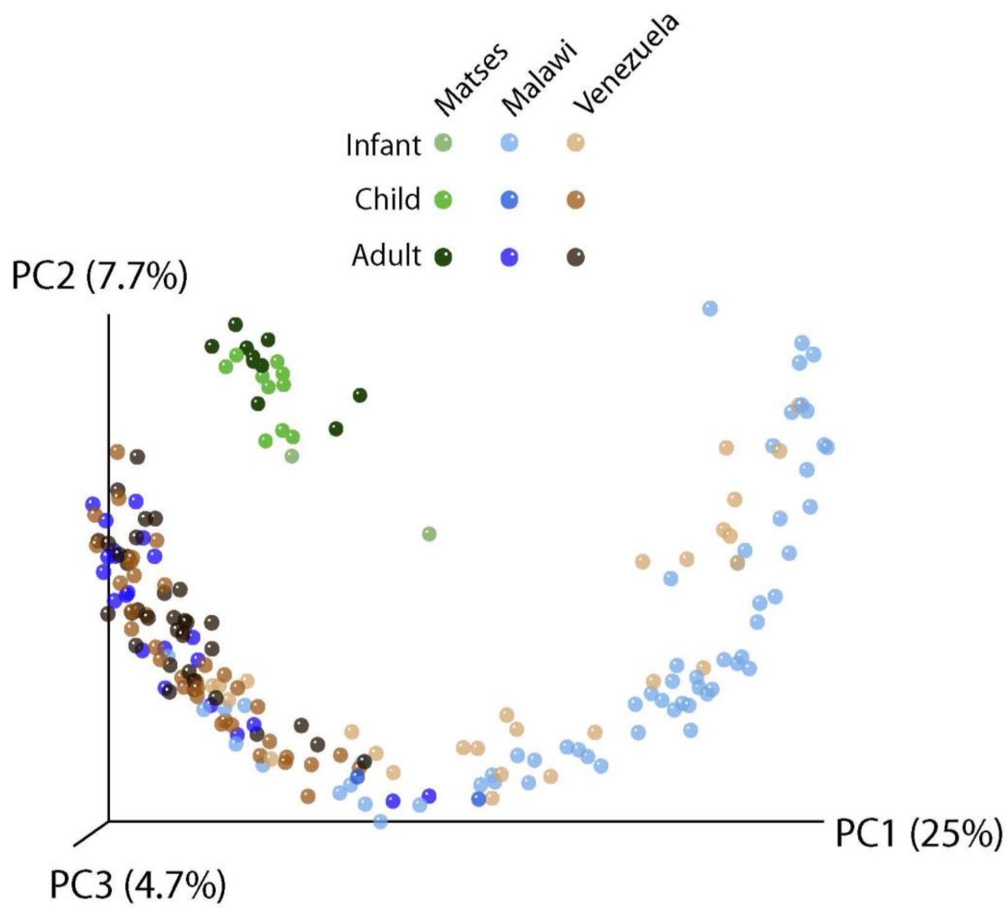


Figure 11. Primary Component Analysis. Clustering shows that based on the Unifrac distances, the Malawi and Venezuela sample are closer to each other than any of them is to the profile of the Matses sample.

Discussion

The gut microbiome from the Matses community anexo San Mateo, exhibits a profile that differs from that previously reported from non-Western communities. Non-Western gut microbiome has been characterized by increased frequency of Bacteroidetes with respect to Firmicutes and the frequency of the genus *Prevotella* rises as a distinguishing trait between Western and non-Western communities (De Filippo, Cavalieri et al. 2010; Yatsunenko, Rey et al. 2012). Our data reveals a gut microbiome largely dominated by Firmicutes with minimal representation of Bacteroidetes and *Prevotella*. We hypothesize that this alternative configuration of non-Western gut microbiome is a consequence of a process of coevolution of the gut microbiota with the human host living in the particular ecosystem offered by this area of the Peruvian jungle. The significant differences between this population and previous reports highlight the importance of including diverse populations to widen our understanding of a healthy human microbiome and the underlying processes that shape the homeostatic state that the microbiota reflects.

The Matses community anexo San Mateo preserves their traditional life style, which includes a natural diet based on local produce, fish and game meat. The community does not have electric power, potable water or a sewer. Their domestic water comes either from the river or from an infiltration in the land that they call "*el chorrito*" (a water trickle resulting from water accumulated in the land during the flooding season). A sample of this water was used as a control for our study (WSM) and, together with the extraction blank, showed bacterial profiles that are not consistent with those observed from the fecal samples, reassuring the accuracy of our results.

The gut microbiome of our population is dominated by Firmicutes. The high levels of Firmicutes and low levels of Bacteroidetes we observe are in disagreement with current understandings of their relationship with human body weight. An excess of Firmicutes has been associated to obesity (Ley, Backhed et al. 2005; Ley, Turnbaugh et al. 2006; Turnbaugh, Ley et al. 2006; Arora and Sharma 2011) . The levels of Firmicutes we observed are consistent with those reported for obese US populations (Ley, Backhed et al. 2005; Turnbaugh, Ley et al. 2006). Yet, our population has no incidence of obesity.

Until now, all reports of non-Western communities have presented lower levels of Firmicutes and higher levels of Bacteroidetes than what we observe in our study. Ley (2006) observed that the relative proportion of Bacteroidetes/Firmicutes is decreased in obese people compared with lean people and this ratio shifts towards a larger fraction of Bacteroidetes/Firmicutes as a response to diet-induced weight loss (Ley, Turnbaugh et al. 2006) . In addition, a hyper caloric diet increases the frequency of Firmicutes (+20%) and a proportional decrease of Bacteroidetes, which results in an increased energy harvest of ~150 Kcal (Jumpertz, Le et al. 2011). It is possible that the increased proportion of Firmicutes is the result of an adaptive phenomenon in the Matses population. Firmicutes' efficient energy harvesting could be favorable in an environment of limited food, such as the one experienced by the Matses, but it would be unfavorable in an environment where food is readily available, as we observe in the sample of Western obese people, which could pose an example of a thrifty microbiome.

The concept of thriftiness is a classic hypothesis in anthropological genetics. The thrifty genotype hypothesis was introduced by James Neel in 1962, who suggested that feast and famine periods during human history favored a thrifty genotype. This thrifty genotype allowed our hunter-gatherer ancestors increased efficiency to store fat to be able to survive during times of scarcity, while this over efficient energy harvesting might be causing disease in environments where food is abundant (Neel 1962). No gene has been found to fit Neel's hypothesis, but the idea of thriftiness is recurrent in human biology and has been recycled in hypotheses such as thrifty phenotype (Hales and Barker 2001; Wells 2003; Wells 2007), the thrifty epigenotype (Stöger 2008), and more recently the thrifty microbiome (Weinstock 2011).

At first glance, the thrifty microbiome hypothesis seems to fit our results: Firmicutes allow for an over-efficient energy harvesting; similar proportions of Firmicutes are observed in the Matses community, where it improves survival when facing food scarcity, and obese individuals from USA, where it might be contributing to the observed metabolic disease. However, analysis at the phylum level could be misleading and there are other factors that might be contributing to the observed profile.

Analysis at the phylum level might lack the resolution to properly characterize the human gut microbiome. Phylum is the second largest taxonomic level, five steps removed from species, and it assimilates significant heterogeneity within it. The statistically significant differences observed at the phylum level are enough to demonstrate the uniqueness of our studied population; however, analysis at the genus level provides stronger elements to discuss the potential mechanisms that underlie the observed profile.

Faecalibacterium sp., belonging to the Firmicutes phylum, is the single genus that dominates the gut microbiome of our population. This genus has been reported to be among the most common microorganisms in human feces (Lopez-Siles, Khan et al. 2012) but in lower proportions that what we detected in our samples (Arumugam, Raes et al. 2011). *Bacteroides spp.*, a genus member of the Bacteroidetes phylum, has been found to be a major component of human gut microbiome (Arumugam, Raes et al. 2011; Yatsunenko, Rey et al. 2012), but is significantly lower in our population.

Faecalibacterium sp. is a clostridial bacteria that has a major role degrading dietary fiber to produce butyrate (Duncan, Holtrop et al. 2004; Benus, van der Werf et al. 2010; Canani, Di Costanzo et al. 2012; De Preter, Arijs et al. 2012). Butyrate is the major source of energy for the cells that line the large intestine (colonocytes) (Fung, Cosgrove et al. 2012), and provides protection against colon cancer (Pryde, Duncan et al. 2006; Fung, Cosgrove et al. 2012). Lower frequency of *Faecalibacterium sp.* has been associated to Chron's disease (Kinross, Roon et al. 2008; Willing, Dicksved et al. 2010) and colon cancer (Kinross, Roon et al. 2008;

Fung, Cosgrove et al. 2012), suggesting an association between *Faecalibacterium sp.* and health.

Bacteroidetes spp., is a bacteroidal bacteria, that has long be recognized as indigenous to the human colon, especially for its role fermenting dietary components that otherwise would be indigestible for humans (Salyers, Vercellotti et al. 1977; Hayashi, Sakamoto et al. 2002). Considering that *in vitro* experiments have demonstrated competition for substrates between *Bacteroides spp.* and *Faecalibacterium sp.* (Lopez-Siles, Khan et al. 2012), it appears plausible that there is a relation between the observed low frequency of *Bacteroides spp.*, and increased frequency of *Faecalibacterium sp*. I hypothesize that *Faecalibacterium sp.* is outcompeting *Bacteroides spp*. in the gut of the Matses population and this could be a consequence of differences in the Matses' lifestyle that might favor *Faecalibacterium sp.* in that population.

Faecalibacterium sp. and *Bacteroides spp.* exhibit differential sensitivity to by-products of digestion. A potential explanation for *Faecalibacterium sp.* successfully outcompeting *Bacteroides spp.* comes from the observation that *Faecalibacterium sp.* is inhibited by bile salts (Lopez-Siles, Khan et al. 2012). *Bacteroides spp.* has shown to be resistant to up to 20-40% concentration of bile salts, a level at which *Faecalibacterium sp.* will be completely inhibited (Lopez-Siles, Khan et al. 2012). Considering that bile salts are directly proportional to fat metabolism, I propose that one of the factors that might have shaped the abundance of *Faecalibacterium sp.*, compared to other populations, is the low concentration of fat in the Matses diet.

Western diet is typically rich in meat and saturated fats while low in complex carbohydrates (Ou, DeLany et al. 2012). It has been found that Western diet is associated with higher concentration of bile acids (Degirolamo, Modica et al. 2011). Considering the inhibition potential of bile acids and bile salts on *Faecalibacterium sp.*, which does not affect *Bacteroides spp.* (Lopez-Siles, Khan et al. 2012), it is plausible that concentration of bile salts is acting as a selective force favoring the growth of *Bacteroides spp.* in the gut of Western populations, allowing *Bacteroides spp.* to outcompete *Faecalibacterium sp*. This hypothesis could be further tested by quantifying bile salts in fecal samples from remote communities to determine the degree of association between concentration of bile salts and frequency of *Faecalibacterium sp.* and *Bacteroides spp*.

Alternative configurations of gut microbiome could also be the result of bacterial horizontal transfer in the human gut. Horizontal transfer is a common mechanism through which bacteria acquires genes from other coexisting bacteria. Numerous examples have shown horizontal transfer in the human microbiome and some of these events have been mapped to the colon (Nikolich, Hong et al. 1994; Shoemaker, Vlamakis et al. 2001; Zaneveld, Turnbaugh et al. 2008; Palmer, Kos et al. 2010; Dunning Hotopp 2011; Wiedenbeck and Cohan 2011). Host-microbiota co-evolution relies on the process of horizontal gene transfer to allow bacteria to adapt to changing environments (Zaneveld, Turnbaugh et al. 2008; van Reenen and

Dicks 2011), facilitating the adaptation of humans, who through their microbiome might also acquire biochemical potential that is not coded in their own genome.

Novel metagenomic approaches will enable the study and characterization of co-evolutionary processes that so far have been limited by the difficulties associated with bacteria culturing methods. Observed concordance between 16S phylotypes and functional gene data (Yatsunenko, Rey et al. 2012) suggests that horizontal gene transfer signal is lost in the noise of the data. However, bacterial horizontal gene transfer is well documented and it remains a hypothesis that needs to be tested when studying remote populations living in previously unstudied environments. Horizontal gene transfer could be explored by studying the relation between functional genes families or biochemical products and specific phylotypes. Another option could be direct isolation and characterization of strains from biological samples, which would determine how individual members of a species are adapting to new environments.

Regardless of the forces that are conditioning co-evolutionary processes, the study of the microbiome of remote populations provides an opportunity to explore microorganisms that might be absent in Western populations. Chlorinated water, antibiotics, germicides, among other elements of the daily Western life are affecting the human microbiome and remote populations might be the only viable alternative to study the indigenous human microbiome (Blaser 2011).

Bacteria are reservoirs of genes with the potential to provide traits that humans lack. Genome sequencing of some microbial communities has identified some previously uncharacterized proteins, suggesting that studies of human microbiome could result in previously unknown gene products (Sonnenburg, Angenent et al. 2004). Discovery of new genes contained in our microbiome has the potential for medical applications (Sonnenburg, Angenent et al. 2004), underscoring the need to expand beyond Western populations to increase our chances of success.

Conclusions

The gut microbiome of the Matses community anexo San Mateo, presents significant differences with respect to other Western and non-Western communities. Observed difference are the result of ongoing selective forces that vary among individuals and communities, such as diet, host genotype, life style and factors such as founder effects and specific mutualistic relationships that can stabilize the composition of these communities (Wooley, Godzik et al. 2010).

Diverse mechanisms are a part of a complex system that produces the observed configuration of the Matses gut microbiome. We suggest that stimuli that are particular to the jungle ecology and the traditional lifestyle of this Matses community are shaping a microbiome that cannot be explained by knowledge based on urban or semi-urban communities. Further studies are warranted to broaden our understanding of the processes involved in establishing alternative configurations of the gut microbiome.

Our study brings to light the need to include remote populations in microbiome research. Besides the particularities of the general profile presented here, studying the gut microbiome of remote communities provides an opportunity to discover new species or strains that might have evolved to confer a different genetic potential to the host. Bacteria and host co-evolve, and a broader study of our commensal or symbiotic relationship will open new opportunities to use these bacteria as markers or even targets for future health related applications.

Chapter 2

Implementing Biomedical Research with Indigenous Peoples: Facing Issues of Trust

Introduction

Traditional indigenous communities, especially those in rural areas, are frequently burdened by significant health disparities. Researchers are often motivated to study indigenous groups to attempt to address these disparities and to gain insights from a human experience distinct than their own. However, engaging indigenous groups in research is frequently contentious. While many have written on the challenges faced by researchers working with indigenous groups, far fewer have provided solutions that are effective broadly.

Each community bears its own history which requires any strategy to take into account their historic particularities. I certainly have seen that with our current study of the Matses. However, building of a body literature, and contextualizing my experience with the Matses leads to one inevitable conclusion. The cornerstone of engagement between communities and researchers is the establishment of trust, a process that can take a great deal of time. Because of the needed time investment, such communities appear doomed to lag behind the general population. This chapter places trust as a forefront objective in community based participatory research, and contextualizes the challenges of trust in a very germane case example, the Matses. Lastly, the chapter proposes a path forward, where trust is a cherished and protected commodity that can be brokered for mutual gains between both the indigenous communities and the researchers.

Trust is one of the basic variables of human interrelations and underlies any individual or collective interaction-dependent enterprise (Blomqvist 1997). Defined as the result of "an assessment of the other party's trustworthiness which informs a preparedness to be vulnerable, that, in genuine cases of trust, leads to a risk-taking act" (Dietz 2011)(p.215), trust is the bridge that can prevent or expose individuals and communities to harm from other individuals or groups.

Public opinion is currently experiencing a crisis of trust in science (Davies and Wolf-Phillips 2006), technology (Chalmers and Nicol 2004) and the biomedical field in general (Ogilvie 2000; McCullough 2002; Armstrong and Trust 2006; Miller 2007; Petersen 2011). There is a generalized perception that scientists, biotechnological companies, medical professionals, and the instances that are supposed to regulate them, are in pursue of their own benefit rather than the best interest of the general public (O'neill 2002), creating a dense atmosphere of untrustworthiness around experts.

Distrust in science and scientists are further supported by the media. Titles like "Gene Therapy Run Amok", "A Lot Of Rules, Too Many Exceptions", "Our Flimsy Surveillance Of Science", "Gene Test Errors Went Unreported", "Protection Of Patients In Research Is Faulted", "Patient's Death In Gene Test Not Reported", or "Can Science Be Supervised?", are often found in newspapers and they attract public attention towards negative perceptions of science, especially genetics (Worton 2001). The addition of these sensationalist headlines to the already obscure power attributed to DNA and genetic information contribute to the fracture between scientists and general public.

Among scholars, there is a general assumption that negative response to science is based on a knowledge deficit (Priest 2001). This hypothesis appears especially attractive when the subject is genetics or any field born to the post-genomic era. The impenetrability of concepts together with popular fiction produce misinformed public who might incubate groundless concerns. A report released by the National Science Foundation (2012) concludes that better knowledge of science and more years of formal education predict favorable attitudes towards science and technology (National Science Foundation 2012). Furthermore, the same report states “A review of numerous surveys from around the world found—**other things being equal**—a weak but consistent relationship between greater knowledge of science and more favorable attitudes toward science” (National Science Foundation 2012:7-29) [emphasis added]. Does this positive relationship between education and attitudes towards science hold in diverse populations? Short answer: No.

Some studies suggest that education is a positive predictor of likelihood to consent to a research study (Wang, Fridinger et al. 2001; Moorman, Skinner et al. 2004; Halbert, Gandy et al. 2006; Sterling, Henderson et al. 2006). However, low education, in these and other studies, also correlates with ethnic minorities, revealing a potential confounding variable in the assessment of the influence of education in attitudes towards scientific research.

Catz et al. (2005) explored the attitudes about genetics in underserved, culturally diverse populations and found that the subgroup that expressed the most concerns about genetic research were the most educated African Americans (Catz, Green et al. 2005), while less educated subgroups, including those who identified as Asian and Latino, only expressed positive feelings about genetics and genetic testing. This observation suggests that when **all things are not equal** (*i.e.* belonging to minority ethnic groups)**,** education is unassociated with a more positive view of science. This conclusion is further supported by an experimental study assessing the attitudes of African American premedical students towards genetic testing and screening. This study found that the implementation of a summer long course, emphasizing in genetic advances, yielded **no** difference in the views of the students towards these developments while the number of students expressing concerns increased (Laskey, Williams et al. 2003).

Mistrust in the biomedical field is not new in culturally diverse populations. Notorious examples are found in the history of African Americans in biomedical research. The Tuskegee syphilis study and the sickle cell screening programs of 1970s are enough to illustrate the scandalous mistreatment to which African Americans were exposed. Although the cases with higher profile involved African Americans, they are not the only ethnic minority that has been vulnerated by researchers. A powerful statement is found in Linda Tuhiwai Smith's writings:

> "(…) the term research is inextricably linked to European imperialism and colonialism. The word itself, 'research', is probably one of the dirtiest words in the indigenous world's vocabulary. When mentioned in many indigenous contexts, it stirs up silence, it conjures up bad memories, it

> raises a smile that is knowing and distrustful. It is so powerful that indigenous people even write poetry about research. The ways in which scientific research is implicated in the worst excesses of colonialism remains a powerful remembered history for many of the world's colonized peoples." (Smith 1999) p.1)

From Smith's point of view as a self-declared colonized person, indigenous populations equate research to hatred and distrust. However this generalization might be too broad; many indigenous groups, principally the ones located in remote locations, have never had experience with researchers, but might have had experiences with outsiders in different contexts.

Often times, indigenous populations do not distinguish between scientific research, journalism, commercial exploration or private visitors (Smith 1999). Given this context, an alternative perspective on indigenous thought emerges. The feelings of distrust, disgust and abhorrence towards research expressed by indigenous peoples and their advocates, are not necessarily related to the research endeavor; instead, those sentiments can be towards the researchers as outsiders where the negativity is based on the history and constant friction between indigenous groups and dominant groups.

Indigenous groups have been long marginalized and isolated by dominant groups. Rather than a phenomenon, this has been the norm in many countries in the world, resulting in the indigenous people's exclusion in most areas of societal development. We can see an example in Peru, where continuous and unresolved conflict between indigenous and dominant population are still tangible.

Peruvian history exhibits constant friction between governing class and indigenous populations. Since colonial times, Peru has experienced a clear socio-racial stratification, where indigenous cultures and identities have been oppressed and undervalued by Spaniards and the "white post-colonial ruling" (Wilson 2000:239) still present in the popular culture.

Although Peruvian populations are mostly admixed, with a significant component of indigenous ancestry, ideas about inferiority of black and indigenous peoples are deeply embedded in Peruvian culture (Golash-Boza 2010). Mestizo population, derived from the admixture of Spaniards with native Peruvians, is the dominant group in Peru. Mestizos distinguish themselves from *"indios"* or "*nativos'*, loaded words in Peruvian society that reflect a tacit but ubiquitously understood racial hierarchy.

Racial hierarchies in Peru result in evident alienation between groups. In a letter from Ricardo Palma, a mestizo scholar and writer, to Peruvian President Nicolas de Pierola in the 1880s, we find a clear illustration of this reality:

> "the majority of the population of Peru is formed by an abject and degradable race that you wanted to dignify. The Indians do not have a sense of *patria*; they are enemies of all white and the men from the coast; for them is the same to be Chilean or Turkish. Educate the Indians, inspire in them patriotism will not be the task of the institution, but the time" (Letter from Ricardo Palma to President Pierola, cited by (Larson 2004)).

Although there are changes in laws and norms that suggest an effort to revaluate ethnic identities in Peru, things remain mostly unchanged. As exemplified by unfortunate declarations of former Peruvian president:

> *"Ya está bueno pero estas personas no tienen corona, estas personas no son ciudadanos de primera clase que puedan decir 400 mil nativos a 28 millones de peruanos 'tu no tienes derecho de venir por aquí', de ninguna manera, eso es un error gravísimo y quien piense de esa manera quiere llevarnos a la irracionalidad y al retroceso primitivo"* (That is enough, these people do not have crowns, these people are not first class citizens that could tell -400,000 natives to 28 million Peruvians- you have no right to be here, no way, that is a huge error and whoever thinks like that wants to drag us to irrationality and to regress to primitivism) (President Garcia declarations to the press, 2009).

Thus, friction between the dominant group and indigenous people is still ongoing.

I conducted my research with the Matses, a hunter-gatherer community located in the deep Peruvian Amazonian region. Due to the geographic location, cultural differences and extreme poverty, the Matses remain a traditional group, mostly isolated from the mestizo culture. Matses, as many other indigenous groups, are not passive victims of socio-political events around them, instead, they are active resisters (Picchi 1998) organized to confront mestizos to preserve their territory and their culture.

Friction between Matses and mestizos has a long and eventful history. While the Summer Institute of Linguistics presents the Matses group as an isolated group of indigenous peoples non-contacted until 1960s (Fleck 2003) , Verese (2006) argues that Matses are a community of people from the Lower Huallaga river (about 500 Miles south of the area they currently occupy) who runaway from Spanish oppression in the 17th century and hid in the east Amazonian region in voluntary isolation (Varese and Chirif 2006). Regardless of their origin, Matses have lived in the region for many decades in a semi-nomadic state, and considered "brutal savages" by mestizos (Varesse 2006; p. 29).

During1960s Matses-occupied geographic area was attracting national interest due to the possibility to develop ground transportation to facilitate the extraction of natural resources in the area (Varese and Chirif 2006). Socioeconomical development of the country was the priority for President Belaunde Terri who promoted the exploration of the Matses area as a first step towards road building (Varese and Chirif 2006). Faced with the Matses defense of their territory, President Belaunde "personally ordered the Peruvian Air Force to bomb and machine-gun" (Varese 2006, p29) Matses communities in the Yaquerana river. The survivors retreated to the forest. The road was never built, but the event remains in the memory of the indigenous and mestizos involved in the incident and their descent.

From my experience working with the Matses, Matses' distrust and concerns towards researchers appears unrelated to the objectives of our research; instead, their distrust appears primarily motivated by the fact that we are non-community members. Below I present the results of a survey conducted in the Matses community anexo San Mateo in June 2012, when we returned to the community to present the preliminary results of molecular studies initiated in December 2011.

Materials and Methods

Sample and data collection

The Matses community anexo San Mateo, had previously participated in a research project with our group. Members of the community had donated biological samples and participated on public meetings and focus groups as part of our microbiome studies. I developed a questionnaire to explore their attitudes towards their participation in this research.

The questionnaire comprised 12 items with open and close ended questions. Questions aimed to explore their trust in local and regional authorities, their attitudes towards visitors (including researchers), motivation and concerns associated to participation in research and their opinion on sample ownership. The questionnaire was developed and filled in Spanish, but it was presented in the Matses language with the help of an interpreter who was a native Matses speaker and fluent in Spanish. All questions were presented in the same order and participant's volunteered comments were recorded to illustrate their opinions.

Data were collected as a house-to-house survey. A team member visited each house with an interpreter. One adult from each household was invited to participate in the survey and an informed consent was presented and signed prior to data collection. The process of consent was conducted in the Matses language. Given the small sample size (n=12), statistical analysis is limited to descriptive statistics. The small sample size is attributed to the Matses community anexo San Mateo itself being small, with only 12 households total.

Results

While surveys were designed to interview one adult from each household, when surveys took place, the designated adult in most cases engaged in brief discussions with other adult members of the household or other community members as well as the Matses translator. These discussions were in Matses, and by our translator's report, the discussions largely centered on why we were asking specific questions and if we brought something for the community.

Our sample was composed by men and women between 18 and 65 years old. Significant gender differences are observed in the educational level: while six out of seven (85.7%) of females were analphabets, only one out of five males was (20%). Demographic characteristics of our participants are presented in Table 1.

Through an open ended question, we asked participants to identify what authorities represented them. Community members identified with immediate local authorities, rather than regional, national or international authorities. 75% of participants identified the community President as their main authority. Nobody mentioned any authority beyond the regional level (Figure 12).

	n	%
Ages (ranges)		
18-25	2	(16.67)
19-35	1	(8.33)
36-45	5	(41.67)
46-65	4	(33.33)
Gender		
Males	5	(41.67)
Females	7	(58.33)
Education level		
Analphabet	7	(58.33)
Elementary	1	(8.33)
Secondary	3	(25.00)
Post-secondary	1	(8.33)
Marital Status		
Single	0	(0.00)
Concubine	7	(58.33)
Married	2	(16.67)
Divorced	0	(0.00)
Widow(er)	3	(25.00)

Table 10. Demographics of survey participants. This sample represents at least one adult from each of the inhabited homes at the Matses Community anexo San Mateo.

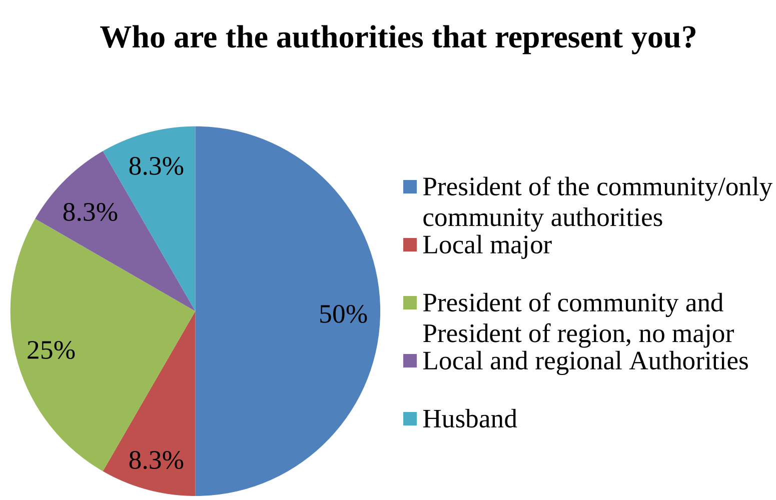


Figure 12. Identification with authorities.

We used an open ended question to explore community's opinion on who should monitor any research activities in the community. Eight participants

(66.7%) considered that visitors' activities in the community should be monitored by the community (community members, community president or community's health promoter) (Figure 13). No answer included any of the political authorities based in Angamos, the closest mestizo colony with jurisdiction in the area.

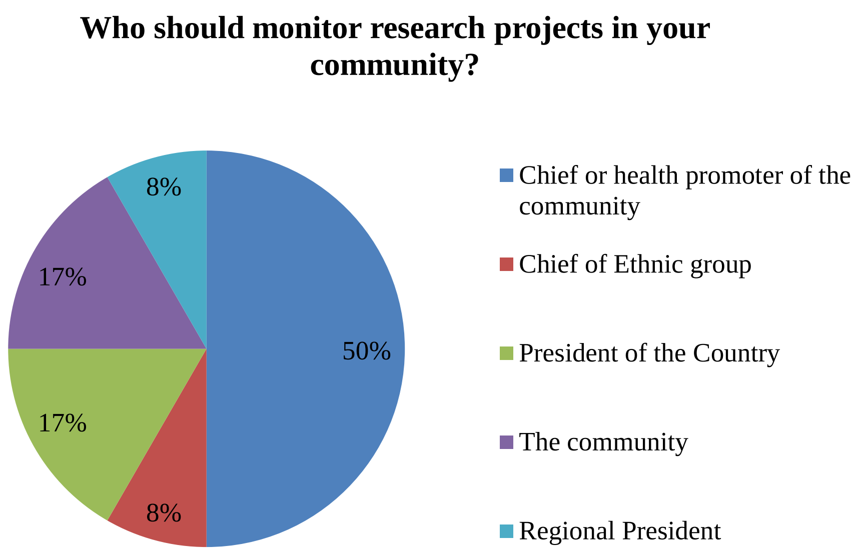


Figure 13. Monitoring of research activities in the community.

Closed ended questions were used to survey participants' attitudes and perceptions towards visitors. Although six participants (50%) do not feel vulnerable and did not express any concern about been hurt by visitors, nine participants (75%) expressed their preference for not having visitors in the community. Participants expressed their understanding that the community might benefit by receiving visitors, stating that visitors could bring medicines, presents or interventions, but still they prefer not to have visitors in the community (Figure 14).

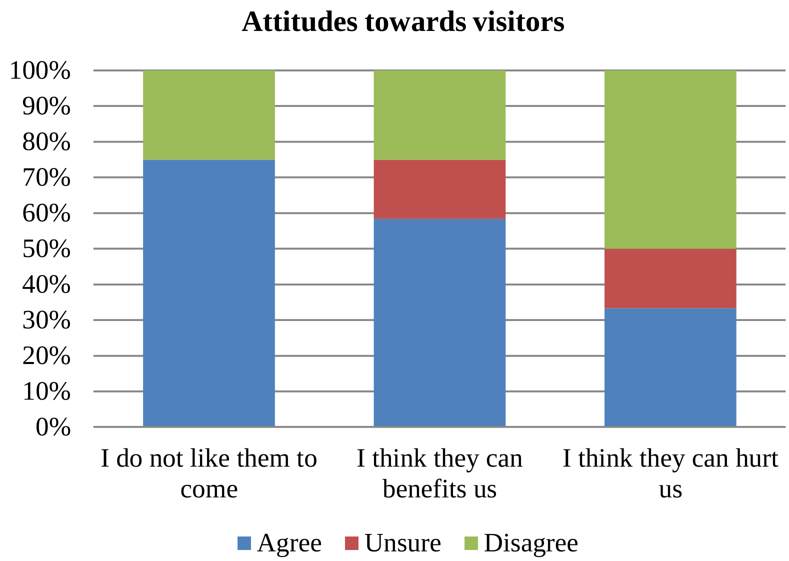


Figure 14. Attitudes towards visitors. Close ended questions were used to explore individual's attitudes towards visitors. Most of participants (75%) expressed their preference for not having visitors in the community.

When asked about their concerns around visitors, including researchers, seven participants (58%) did not express any specific concern. The ones that manifested concerns related to visitors and researchers in their community stated: *"que será lo que estan investigando en la comunidad"* (I wonder what are they really studying in the community), *"asi igual como tu vienes, vienen los que buscan petroleo"* (people searching for oil come the same way as you come), *"no confio en la gente de fuera ni en los investigadores, especialmente cuando no traen nada"* (I do not trust in non-community people nor researchers, especially when they come empty handed), *"los investigadores no cumplen lo que ofrecen, queremos que nos traigan medicinas"* (researchers do not deliver what they offer, we want them to bring us medicines).

Participants mistrust towards visitors appears to be proportional to the distance they perceive from non-community members. Respondents expressed more trust towards national than non-national visitors (Figure 15). A couple of community members explained that they do not trust foreigners because they have more money and can go further away when they do something bad to the community, which may reflect recent experiences with foreign entrepreneur David Nilsson, Australian citizen known as the "Carbon cowboy", who allegedly attempted to strip Matses natives from their rights to their lands and who fled the country once a warrant was issued.

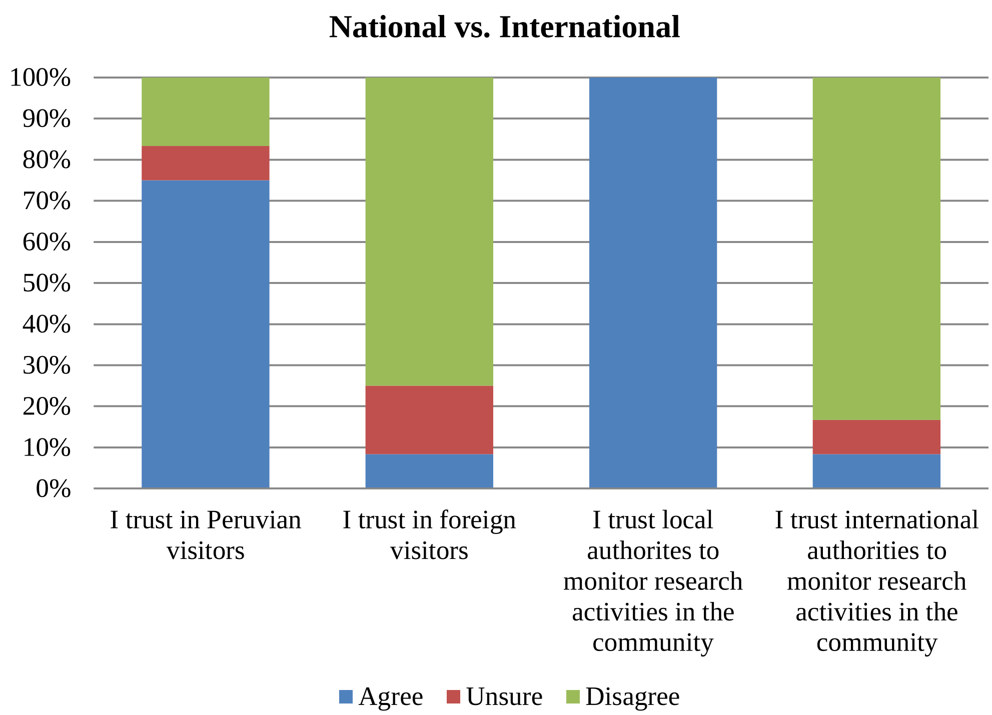


Figure 15. Attitudes towards national and international visitors and authorities. All participants indicated to feel represented by the President of their community and that is the authority they recognized as local authority. Participants expressed higher mistrust towards foreign visitors and authorities.

Similar to what has been observed with other minority groups (Wang, Fridinger et al. 2001; Sterling, Henderson et al. 2006), participants from the Matses community anexo San Mateo are motivated to participate in research that is relevant to their community. Most of respondents are interested in participating in research that will benefit their community, even if the research does not provide individual benefits, highlighting the communitarian identity associated to indigenous groups (Figure 16). Four participants (33%) expressed their willingness to participate in research even if it does not provide any benefit to them individually or as community.

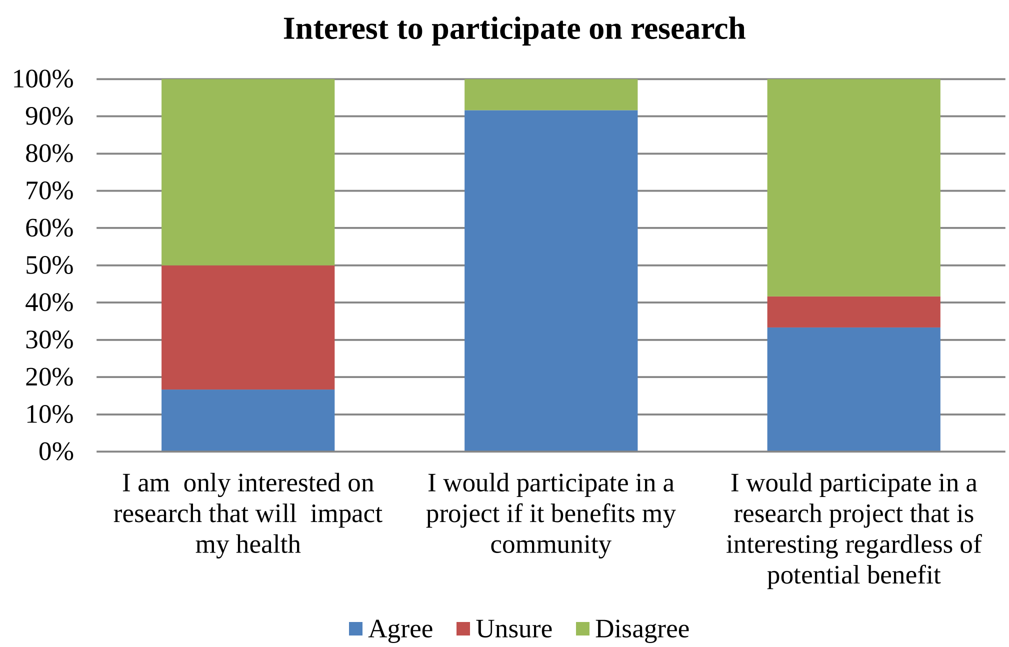


Figure 16. Interest to participate in research. Community members' willingness to participate in research is not conditioned to individual benefits. Benefits for the community appear to motivate participation, emphasizing their communal identity.

I explored their expectation of reciprocity when donating biological samples for research. Six of the participants (50%) denied expecting any compensation for giving biological samples, four (33%) expressed they expect something in return while two participants said they do not know if they would expect something in exchange. This result is consistent with their answers to two open ended questions, which asked at different times during the survey, to explore what the researcher can do to make them more comfortable with participating in research, and what would motivate them to participate in research. The most frequent responses (41%) referred to some form of material compensation (money, clothes, medicines, food, and presents). One third of respondents (33%) expressed that more meetings with the community and the community leader would encourage them to participate; while two members (16%) expressed that there is nothing the researcher could do to encourage their participation.

Sample ownership is one of the most controversial issues around research with human biospecimens. We asked participants, "who owns a sample given for research studies?". Five participants (41%) considered that once donated, a sample belongs to the researcher, while seven participants (58%) expressed that samples are given to the researcher, but the participants maintain ownership of these samples (Figure 17). Six participants (50%) considered that the researchers can do anything with their samples, although their later elaboration on their answers denoted conflicting interpretations of this question. While four people considered it a good idea that the researcher continues further studies on the samples they already gave, two respondents expressed that the researcher can do anything with their samples not because they are authorized but because they are far from the community's reach and nobody can control what the researcher does with the samples once they are collected.

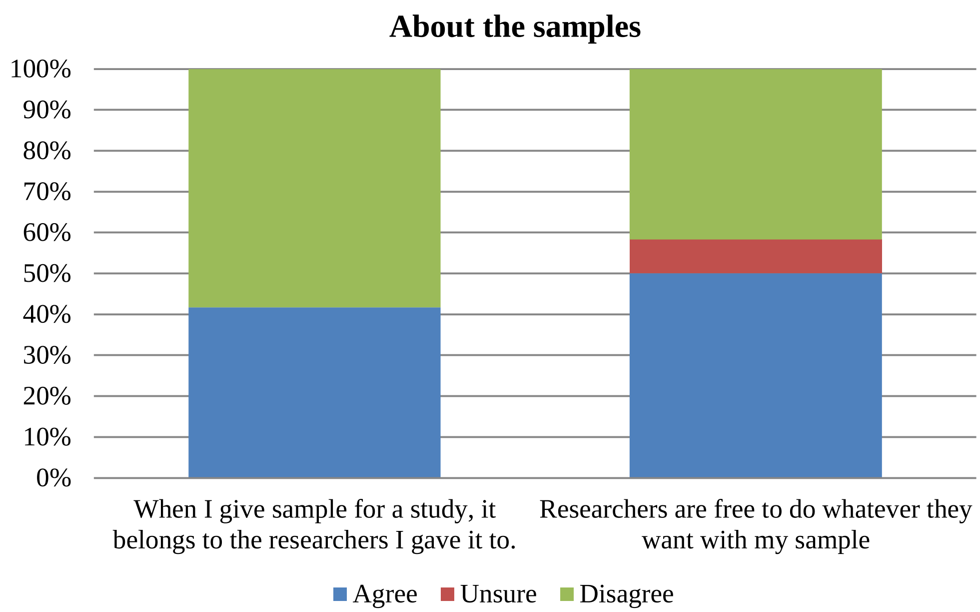


Figure 17. Sample ownership: Opinions about sample ownership are divided. Some concerns about researcher accountability and extent or consent where hinted by participants.

During the public meeting, the research team explained what are microorganisms and the relationship between humans and other micro and macroorganisms. I asked participants about the ownership of their body's microorganisms and in their houses. Eight participants (66%) considered that they own their body's microorganisms while six participants (50%) expressed that they own the microorganisms that live in their houses. Two participants (16%) understood ownership as a matter of responsibility and expressed that microorganisms of the human body belong to health care providers, consequently, health care providers are responsible for keeping microorganisms under control and prevent them from disrupting the health of hosts (Figure 18).

Perception of ownership of microorganisms

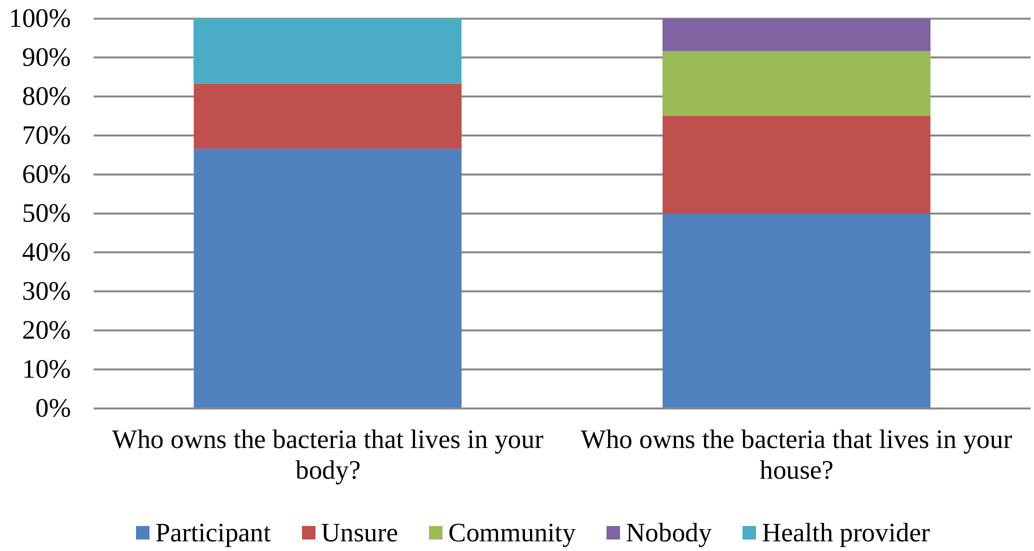


Figure 18. Ownership of microorganisms. Opinions were divided. There were more individuals stating property over their body's microorganisms than asserting property over their houses' microorganisms.

I examined community member's attitudes towards donation of specific biospecimens. As shown in figure 19, most participants are willing to donate non-invasive samples. Particular comments were volunteered with respect to giving blood for a study. Community members were typically opposed to giving blood and mentioned a series of concerns such as: pain, worries about debilitating their health, and the possibility of getting something in their body when the needle goes in. The most surprising of their concerns was about the possibility of getting microchips (*sic*) inserted in their bodies. Matses people fear that the government or some other powerful institutions intends to exterminate them to take control of their lands. The ongoing tale was that there was an intention to use implants for geographic localization of these itinerant population to prepare an effective attack. These comments exhibit knowledge from some current technologies suggesting an outsider origin for these suspicions. Other fears related to what a visitor can do with the samples once they are taken.

There were multiple comments around the belief that the individual is connected to their blood after it leaves the body. Participants expressed their concern that their blood can be used to improve somebody else's health at expense of their own health. Although in the Amazonian region it is common to believe that blood has a monetary value and it is sold to sick people, Matses' belief that they will get sick if somebody gets healthy through their blood is uncommon. Some participants referred to their concerns as facts because they come from warnings presented by a previous local major or from some non-governmental organizations working in the area. Still, some participants expressed that they would give blood, or any invasive sample, if the study

What samples would you be willing to give?

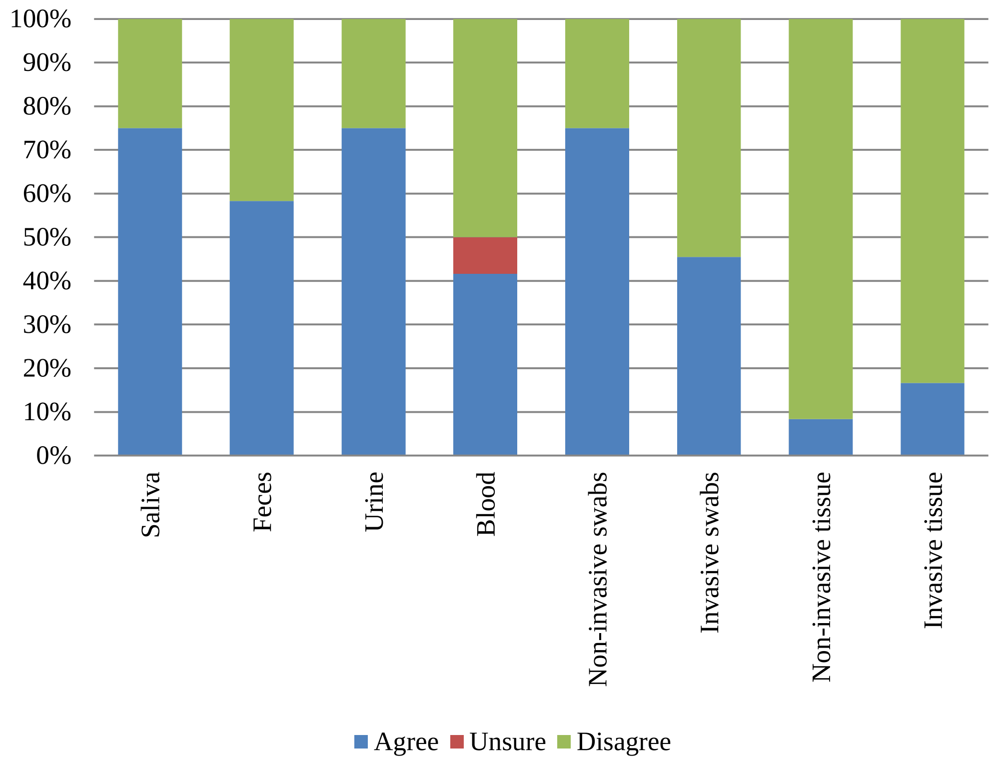


Figure 19. Willingness to donate biological samples. Unless the study will provide any direct benefit for their health, individuals are reluctant to donate invasive samples.

would provide results that could impact their health. Participants expressed that they would be willing to give any sample if they were going to be diagnosed and get appropriate treatment for their untreated illnesses.

To explore potential concerns about privacy, I asked participants about their attitudes towards publishing research results. Participants' voiced some concerns about individual privacy: six participants (50%) **did not** want their names associated to the findings, while ten out of the twelve individuals (83%) **did** want the community's name associated findings (Figure 20).

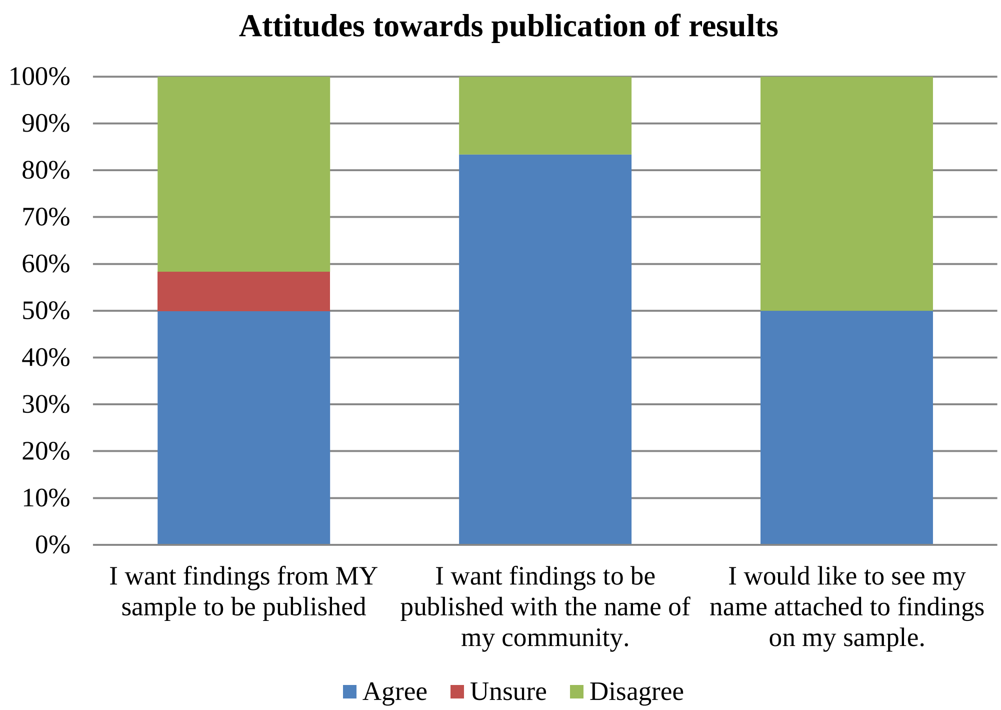


Figure 20. Attitudes towards publication of research results. Individuals' answers suggest some value to the publication of results with the name of the community, while hinting to concerns about individual's privacy.

Discussion

My experience working with the Matses community anexo San Mateo suggests that remote populations' distrust in research focuses more on the scientists as outsiders, than in the potential risks, findings or benefits derived from the scientific endeavor. Many community members expressed that, although they believe it is more likely to get benefits than to be harmed from the interaction with visitors, they prefer not to have visitors in the community. This detachment from outsiders is also consistent with their perception that they are not represented by political authorities in the region, who for the most part are mestizos; most respondents from the Matses community anexo San Mateo only felt represented by immediate local authorities (*i.e.* president of the community or community's health promoter) who are recognized by Matses organizations but have no power outside their community.

The alienation between Matses people and political authorities is bilateral. Matses representatives are not integrated within the socio-political structure in the region. When we returned to the region to present preliminary results of our molecular studies with the Matses community anexo San Mateo, we were invited to discuss our results at a meeting organized by the regional authorities in Colonia Angamos. We were told that all local authorities would be at the meeting. While all *official* authorities were there, representatives from the health post, police, military base, manager of the region, and mayor, but none of the presidents of the anexos Matses were present. The health of Matses people was in the meeting agenda, with focus on malnutrition and parasitism. We presented the results of the parasite screening conducted previously and our preliminary molecular results which were well received and earned compliments from the authorities at the meeting. There was a discussion that denoted everybody's interests in doing what is best for Matses people; however, Matses people's voice was not represented in the meeting.

Problematic relationships between Matses and outsiders challenge the implementation of community-based participatory research and the development of a relationship of trust. Building a relationship of trust relies on the community's predisposition to trust (Dietz 2011), and Matses are predisposed to distrust outsiders.

Anecdotal evidence suggests that Matses communities remain violent and interactions with visitors can have a volatile nature. In their own words, "When someone's not following the law of the tribe, there's a lot that can happen. They'll gather together and kill dissenters with machetes and spears" (Matses individual cited by (Bodenham 2011)). Considering that the law of the tribe might be obscure to visitors, any type of negotiation can be dangerous: "When someone comes in from the outside and starts putting ideas in people's heads, people aren't going to be OK with it" (Matses individual cited by (Bodenham 2011)). The experience of a health brigade attempting to screen Matses people for Hepatitis a few weeks before our first visit illustrates this issues.

Peruvian governmental agencies are trying to respond to health problems that are prevalent in indigenous communities. A few weeks prior to our visit, a brigade from the Peruvian National Institute of Health tried to implement a screening program based on analyzing a small blood sample collected from each of the members of the community to identify people with hepatitis. For this purpose, they met with all the Apus (president of the ethnic groups and presidents of each anexo). All permits and all guaranties from the national and regional government were presented, and the Apus agreed to bring the information to the communities to present it on assembly before they can accept the proposal. When they arrived for sample collection, some communities received them with arrows and even after public meetings and active engagement with the community, they could only screen around 10% of their target population due to population reticence. Our experience was distinct.

Access of our team to the Matses community anexo San Mateo was greatly facilitated by working with people who has been directly working with them prior to our first contact. I believe that Matses people from anexo San Mateo were more receptive to our project because: a) more than half of our team were people who have had previous contacts with them, b) they saw what we were doing: we offered parasite screening *in situ*; we brought equipment and professionals to provide results and counseling immediately, and c) our sampling method was not invasive (no blood was involved).

A relationship of trust is a continuous feedback process (Mayer, Davis et al. 1995; Bachmann and Inkpen 2011; Dietz 2011) constructed over a predisposition to trust. Knowing the history of friction between Matses and outsiders, we could not rely on Matses people's willingness to trust. We understood the need to provide elements that could evidence our trustworthiness as a start point for our relationship and our best alternative was to procure third party guarantors.

In absence of direct evidence of trustworthiness, a third party guarantor can provide the common ground necessary for dialog (Shapiro 1987; Bachmann and Inkpen 2011). We have met with the Matses' leader, documented the authorization from the Peruvian National Institute of Health, the local health post and the manager of the region prior to entering the community, in an attempt to present authorities as third party guarantors. Although these documents declared accountability of our team and proved to be useful for the interaction with other non-community authorities, they did not make a difference for the Matses people.

Based on data collected during my work with the Matses people, I believe that their assessment of trustworthiness relies more in evidence from direct interaction than any formal institutions. At the time we started implementing our project, there was chaos and confrontations between Matses and outsiders. Besides the historical accounts of abuse and neglect from the government and some visitors, Matses had newer experiences that demonstrated how vulnerable they remain.

Natural resources within indigenous territories increase their vulnerability. Since 1993, the Matses community is the recognized owner of the Matses territory, a 452,735 hectares (1118732.55 acres) that they have inhabited for several decades. This pocket of megadiversity (Harder 2000; Voss and Fleck 2011) harbors petroleum and a huge potential for carbon trading; but, owning that land, by

Peruvian law, does not give them property over the natural resources that are underground. Therefore, minerals and petroleum extraction can be authorized by the Peruvian government without consent from the land owners. The Peruvian government has authorized extraction of natural resources without consultation of the indigenous groups that own lands in different areas of the country, which has resulted in violent confrontations (Orihuela 2012). After several deaths and constant resistance of indigenous groups, the government produced the "Ley de la consulta previa" ("Law of prior consultation") in which indigenous people should be consulted before any extractive activity is implemented in their territory; however, this law is non-binding and does not give indigenous populations the right of veto (Peruvian Law 29785, D.S.001-2012-MC), further supporting indigenous people's distrust in the government.

Matses territory's natural resources have attracted private investors that have tried to take advantage of them. The most recent and notorious case was the Australian David Nilsson, who using subterfuges tried to expropriate Matses' rights to their lands for 200 years offering them millions of dollars in revenue from carbon bonds trading (Bodenham 2011). Nilsson's strategy shares some similarities with our approach: Nilsson used third party guarantors to facilitate his access to the community and he talked about research and building capacity as some of the benefits of their interactions.

It is predicable that the use of "common networks of social relations" (Shapiro 1987) as third party guarantors will enable outsiders to start a relationship with the community. Nilsson hired a conservationist, who has worked in the area previously, to introduce him to the community (Bodenham 2011). Based on his harmless appearance (Figure 21) and his much elaborated lies, Nilsson managed to attract Matses attention; the Matses opened their houses to receive this new friend who had a project to bring large amounts of money to communities under extreme poverty. Nilsson also described his activities as an opportunity for social research and building capacity in the Matses community. During an interview with the Sydney Morning Herald, Nilsson declared: "This could be some kind of anthropology project - tribal people in the modern world (…) I'll get a good model set up and really pump the training into them. Not just pump it into them, but put them under contract and loan them the money to go to university." (Bodenham 2011). Even more, Nilsson was legitimizing his venture by representing himself as affiliated to the World Bank and to United Nations (Bodenham 2011; Wiesse 2012), a relationship proven to be unreal by AIDESEP authorities but assumed as authentic by Matses and some local authorities, further damaging Matses' perception of international institutions.

Although problems between David Nilsson and the Matses started in 2010, they were not publicized until mid-2012 (Figure 22 and 23). Our team did not have knowledge of Nilsson when we met the leader of the Matses in 2011. During our dialog, the leader of the Matses hinted at issues of credibility around our proposal. He told us not to expect the community to trust us just because we say we will do

Figure 21. Visitors in the community. David Nilsson visiting indigenous groups in the Peruvian Amazonian región. Foto: Servicios en Comunicacion intercultural (SERVINDI 2012)

research or because of our offer of educational experiences. The leader explained that our strategy is the same strategy used by petroleum companies trying to gain access to their natural resources.

Any potential predisposition to trust in outsiders was shattered by so many events it the Matses recent history: violent encounters with explorers, the 1960s bombing, covert Petroleum surveys by the government, insulting statements from previous Peruvian presidents, and the stratagems of David Nilsson. The prior offenses are far from forgotten by the Matses. Moreover, after working with native peoples in other areas of Peru, including the highlands, such justifications for distrust sound all too familiar. However, the manifestation of this history marred by betrayal and disrespect clearly shaped the dialogue with the Matses .

Distrust in foreigners, individuals or institutions, appears to be stronger than the observed with compatriots. Matses participants expressed that they do not like visitors in general, but they are especially distrustful of foreigners who have more money and can run away further, which is likely a phrase brought about by their experience with Nilsson.

The lack of trust in foreigners led a suspicion that an appropriate reciprocity would be of the utmost concern when building an environment from trust. While half of our respondents (six out of twelve) denied any expectation of reciprocity, further interaction clearly demonstrated that this expectation exists. Whether it be a diagnosis and treatment to their diseases or monetary compensation, their expectation of tangible benefit was evident at multiple moments of our interaction. However, this expected reciprocity opens the possibility of exploitation.

OLICIA NACIONAL DEL PERU
VISION DE DEFENSA NACIONAL DE CONTROL
DE FRONTERA - VDTPI
PVF - ANGAMOS

MINISTERIO DEL INTERIOR
POLICIA NACIONAL DEL PERU.

NOTIFICACION.

Señor ; Daniel Manquid JIMENEZ HUAMAN. (41)

Domicilio ; Comunidad nativa el Estiron - quebrada Chobayaco.

Mediante el presente documento queda Ud. debidamente NOTIFICADO, para que concurra ante la V-FPM-Maynas - - sito en la calle Morona Nro. 148, para el día lunes 18 Julio --- del 2011 a horas 10,00 de la mañana, a fin de llevarse a cabo la diligencia de reflexión a mérito de la denuncia interpuesta - por la persona de David Jhon NISSON (Nacionalidad Australiana) por presunta coacción del delito por violación de correspondencia contra el patrimonio hurto agrabado, estafa contra el patrimonio extorsión en la modalidad de chantaje, delito informático agrabado, contra la falsa declaración de justicia, falsa declaración administrativa, contra la fe pública, falsificación de documentos privados y falso genérico en agravio del señor David Jhon -- NISSON.

Colonia Angamos, 13 de Julio del 2011.

ENTERADO:

Firma ;
Pos-firma ; Daniel M. Jimenez Huaman
DNI. Nro. ; 05307482
Fecha ; 13/07/11
Hora ; 14:50

Hector F. CARBAJAL PEÑA
SOT2. PNP.
JEFE ACC. PIVCT-ANGAMOS.

Figure 22. Attempts to divide the community. Document showing David Nilsson's attempts to divide the Matses community by making a police report denouncing a community member for embezzlement, fraud, blackmail, forgery of documents and "generic falsification"(AIDESEP 2012).

DLES 11 DE JULIO DEL 2012 www.elcomercio.pe

INDEPENDENCIA Y VERACIDAD

El Comercio

Un programa periodístico denuncia a estafador de comunidades indígenas

Se trata de David Nilsson, un australiano que ha engañado a los yaguas con el pretexto de los bonos de carbono.

Una investigación del programa de televisión australiano "60 minutos" reveló que el empresario David Nilsson –nacido en Australia– ha estafado a comunidades amazónicas de Loreto con el engaño de que ganarán millones de dólares por créditos de carbono.

El periodista Liam Bartlett obtuvo una reseña del contrato firmado por los integrantes de la etnia yagua. En ese documento, la compañía de Nilsson, Amazon Holdings, se adueña de sus bosques por 200 años, obtiene la mitad de las ganancias y establece cortar los árboles nativos para reemplazarlos luego con palmas aceiteras.

"[Los nativos] son unas personas pobres y desesperadas, fáciles de manipular, que no sabían que el abogado que los asesoraba independientemente [Walter Cambero] era también el defensor de Nilsson", señala Bartlett, quien se contactó con Dan Pantone, un científico estadounidense que vive muchos años en el Perú y que le presentó a los indígenas a Nilsson, porque este se habría hecho pasar por un filántropo.

IMPORTANCIA

LOS CRÉDITOS DE CARBONO

También llamados bonos de carbono: son un mecanismo internacional de descontaminación para reducir las emisiones de gases contaminantes en el mundo, que provocan el calentamiento global. Es uno de los tres mecanismos propuestos en el Protocolo de Kyoto. Las grandes empresas compran estos créditos que sirven para luchar contra la deforestación de los bosques. Un bono de carbono representa el derecho a emitir una tonelada de dióxido de carbono.

LA MULA

El programa "60 minutos" ha puesto en evidencia a Nilsson.

Figure 23. Suspicious activities in the Matses community becoming public . Newspaper section reporting the problems with David Nilsson. Problems started in the late 2010 but they did not become public until 2012. We were unaware of these problems when we started contact with the Matses in 2011.

Matses people's economy is precarious. Their agriculture is incipient and it consist of "*abrir chacras*" (delineating plots) were they grow products that are naturally occurring in the area. Plantain, manioc, pinapple, aguaje, sugar cane, ungurahui, uvilla among other local produce are mostly used for their subsistence; the low commercial value of these wild products prevents Matses people from entering the market economy. Without money, Matses have no access to industrial goods. Any industrial product is highly appreciated by the community; a personal size soda or pack of crackers can be exchanged for a whole plantain bunch, revealing the low exchange value of their goods. The hyper-valorization of non-community goods associated with their unmet needs and their disconnection with non-community institutions (political authorities in the region) puts Matses people at high risk of been exploited by outsiders.

Matses communities are quite attractive for researchers and commercial entrepreneurs. While commercial interest focuses on timber and petroleum, those are just two of the many elements that conform Matses' natural richness. By reports of the Chicago Field Museum (2006), Matses territories harbor between 3,000 and 4,000 species of flora, around 300 species of fish, more than 200 species of amphibians and reptiles, around 550 species of birds and this "is one of the areas with highest mammal diversity in the world" (Field museum report 2006, p.143) with at least 65 species of large mammals; a significant number of these species are expected to be new to science (Field museum report 2006). Besides describing these new species and the novel interactions that can be found in these intact ecosystems, scientist have the opportunity to study particular phenomena of co-evolution between these species and human populations and to learn about their biocultural adaptations to infections and disease. Given their clear unmet needs and their minimal experience negotiating with outsiders, Matses' are at risk of establishing unfair agreements with outsiders.

Critics of biomedical research with indigenous communities cite exploitation and neocolonization at the top of the list of bioethical issues associated to biomedical research practices. Although biomedical research is a real risk, as observed in some experimental trials (De Zulueta 2001; Emanuel, Wendler et al. 2004; Lurie and Wolfe 2013) or attempts to establish patents from indigenous knowledge or samples (Longacre 2002), there was no sign of concern for these risks within the Matses. Perhaps the Matses absence in the market economy prevents these ideas from entering their views while allowing the persistence of other concerns, such the general distrust of outsiders.

Biomedical studies with indigenous peoples are challenged by people's believes around biospecimens. Our results suggest that distrust plays a fundamental role shaping participants' attitudes towards biological samples. There is a generalized tale in the Peruvian Amazonian region that human blood, even the small leftovers from clinical testing, have commercial value, that sick people buy blood from healthy people to get better. Some Matses individuals believe this myth, implying that individuals remain connected to their blood even after it is removed from their body.

Community members complained to us about health providers that come and take a lot of blood (one or two tubes). They believe that their blood is sold to improve other people's health at expense of the donor's health. Yet, about half of the participants said they would agree to give blood for my research. Participants expressed a positive attitude towards providing biological samples in general, especially the less invasive samples such as saliva, feces, urine and non-invasive swabs. Invasive swabs and blood had some acceptance (around 50%), while donation of tissue samples, even non-invasive tissues, was rejected. Participants elaborated on different occasions that they would be willing to give any sample if it brings a benefit for their health, specifically if it will provide diagnosis and treatment for any illness they might have.

Matses people expressed interest to participate in research and to provide biological samples **if** the research is relevant to them and, in most of cases, **if** there is some tangible benefit for them. Knowledge is not considered a serious benefit to them, but they emphasized their interest in biomedical research that will address their unattended diseases. These results are surprising. The health brigade before us offered screening and potential treatment for those infected with hepatitis, but most of Matses did not participate. Similarly, while Matses community members wanted us to bring medicines to remove parasites, the health post in Angamos reports that Matses are non-compliant with their treatment against parasites. I find three potential explanations for this discrepancy: a) discrepancies between what authorities and community members perceive as a need in the Matses community; b) Matses people do not trust public programs and c) working with blood, regardless of research or service purposes, will always discourage participation. The first two explanations appear the most substantiated. Given the Matses distrust in the government, Matses people are reluctant to accept any public program that is directed at them without extensive engagement with their local community authorities.

Figure 24 presents a summary of a retrospective analysis of the process of earning Matses people's trust. There is no predisposition to trust in the outsiders, but the incorporation of local people that were known and trusted by the Matses as part of our research team, influenced the community to become more receptive towards us. Only after some trust was earned it was possible to initiate community engagement. Community engagement further developed the relationship of trust, but it was only achievable over a pre-existing structure (trust) provided by the local team members.

In the view of many indigenous communities, such as the Matses, researchers are part of the dominant group with discredited trustworthiness. Researchers cannot expect to solve this broader societal issue. Moreover, indigenous communities cannot expect to overcome their health disparities without participating in the research the attempts to reveal and respond to health disparities. Thus, there is a clear need to develop a mechanism to facilitate the interaction between indigenous isolated groups and researchers in a partnership in which both share responsibilities, risks and benefits.

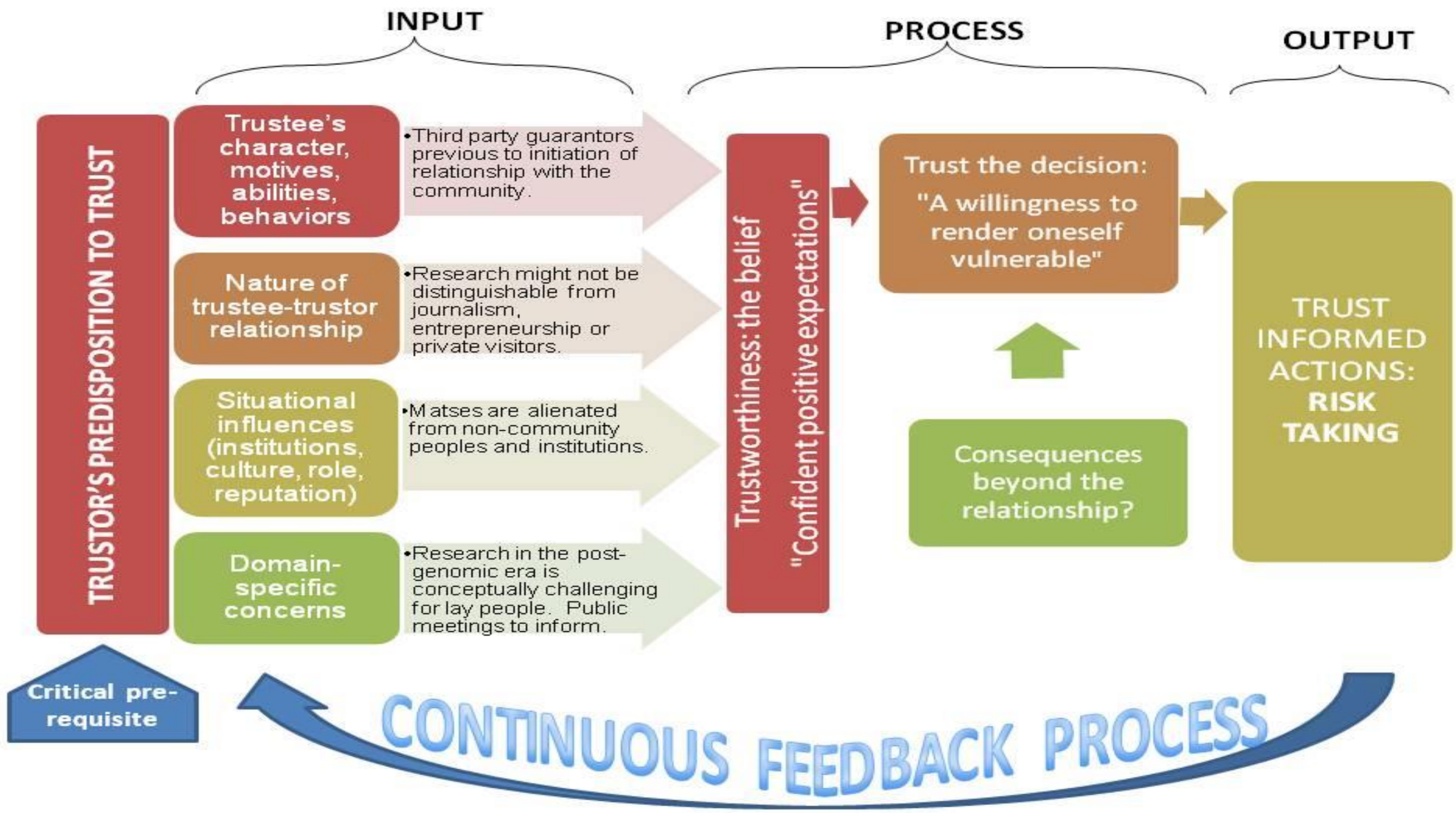


Figure 24. The trust process (adapted from (Dietz 2011) and (Mayer, Davis et al. 1995). Matses are predisposed to distrust. Distrust acts as a filter limiting community engagement's impact in the process of building a relationship of trust. Actions taken to initiate a relationship with the Matses community are in black letters. If distrust is not addressed at the beginning, there is no opportunity for real engagement of communities

Conclusion

People's perceptions and attitudes towards research are shaped by cognitive, ethical, environmental, regulatory, communication and socioeconomic factors. The scientific community tends to assume a model of deficit of knowledge as the primary explanation for people's disinterest to science and scientific research (Priest 2001). Based on my experience working with the Matses community and the responses to the survey here presented, I argue that distrust and alienation from non-community persons and institutions are the main obstacles to include indigenous communities in research.

Without trust, or at least a predisposition to trust, most of researcher's efforts to develop a relationship with the community are impaired. Assuming lack of knowledge as the underlying cause of indifference or negative perception of science, researchers could provide educational activities to counteract this obstacle. Without a relationship of trust, community engagement falls short from reaching its goals. Outsider's proposals or ideas are sterile if the community does not trust and reject the individuals who would implement these ideas.

Indigenous peoples are interested on participating in research under appropriate conditions. Matses will participate in research if the project involves people they trust, if the topics are meaningful for them and if some tangible benefit is secured for the community. Matses conditions of participation are reasonable although complicated to implement.

Building a relationship of trust with indigenous communities is costly and requires a greater time investment than working with the general population, which might be incompatible with the overall research enterprise in which efficient knowledge production is a measure of success.

Chapter 3

Research In Wonderland: Including Indigenous Populations in International Biomedical Research

Introduction

A deep understanding of biological phenomena is crucial to design interventions to improve human health. Some developments, such as antibiotics, are based on biological principles that are common to any living population; therefore, they can be widely applied under similar conditions. In contrast, medical interventions generated in the post-genomic era, such as molecular diagnosis or pharmacogenomic therapies, are affected by an individual's genotypes and his or her lifestyle; consequently, any development will most likely benefit the populations whose data were included in the research (Licinio 2001) while the benefit for other groups may be delayed or even nonexistent. The scientific goals of engaging indigenous communities in research are obvious, but including these communities in research entails a series of ethical, legal, and social issues that complicate the research enterprise.

Some large promising scientific endeavors have failed because of issues related to governance of samples and data as well as the distribution of risks and benefits across the different stakeholders in the research enterprise (Winickoff and Winickoff 2003; Winickoff and Neumann 2005). Chances of success are even lower if the goal is to conduct genomic studies with indigenous populations in the context of international collaborations.

Distrust is at the root of the issues associated to including indigenous peoples in genomic research. In dominant societies, researchers face the public's mistrusts of science (Davies and Wolf-Phillips 2006), technology (Ogilvie 2000; Chalmers and Nicol 2004), and the biomedical field in general (McCullough 2002; Armstrong and Trust 2006; Miller 2007; Petersen 2011), while indigenous peoples' distrust in research is often related to the researchers as outsiders, as representatives of a dominant group with whom they have a long history of social tension. Another layer of complication is added when the researchers are from developed countries: fears of imperialism and neocolonialism motivate distrust from authorities that often result in difficult to enforce rules and regulations aiming to protect their vulnerable populations and their physical and intellectual property. However, these distrust-inspired strategies often result in obstacles for science without any of the intended protection for the indigenous populations. There is a need to articulate a protectionist, and not paternalist, framework that facilitates inclusion of these populations in research or they would continue to be either exploited or excluded from research ventures reinforcing their marginalization.

Developing a relationship of trust must be at the base of any framework aiming to include indigenous populations in genomics or any other research initiative. However, developing a relationship of trust with indigenous populations and their authorities might be close to unachievable in the timeframe of a funded research project, which rarely extends longer than three to five years, and is in perpetual risk of losing funding if not producing tangible results, such as scientific publications.

I propose an intermediate research organization that facilitates the inclusion of indigenous populations in biomedical research. This type of organization will be especially useful when target populations are from developing countries. Developing countries, such as Peru, have disarticulated systems of research governance that become a potential minefield for researchers without offering any real protection to human research subjects. My model is an outgrowth of the biotrust model proposed by Gottlieb (1998). The original model is limited to sample and data management (Winickoff and Winickoff 2003; Winickoff and Neumann 2005), which confines populations involved to have a passive role in the research enterprise. Working with populations from developing countries should aim to a higher goal: research collaborations involving populations from the developing world are opportunities for capacity building in the host countries.

International research collaborations can have a meaningful impact in developing countries. Interactions between local and foreign researchers provide an opportunity for technological transfer, posing innovative research challenges structures of research governance that will stimulate the development of clearer norms to guide research activities. Partnering with communities can have a synergistic effect, improving feasibility of research while ensuring that participants benefit from these activities. This chapter argues for a non-profit, non-governmental, nucleus that facilitates interactions between all stakeholders in the research enterprise which would promote scientific research while maximizing protection and benefits for vulnerable populations involved in the process.

Diversifying human populations in biomedical research

In 1996, the National Science Foundation and the National Institutes of Health promoted the organization of the Committee on Human Genome Diversity to address issues related to the Human Genome Diversity Project and its goals to collect and study samples from indigenous populations around the world (National Research Council

1997). The scientific merit of the project was indisputable, but numerous ethical, legal, and human rights challenges were identified.

The practice of collecting and storing biological samples is common in the biomedical sciences. However, the Human Genome Diversity Project was designed as a multinational and multicultural initiative which posed particular challenges. Around the same time, the California Supreme Court had ruled that and individual cannot "assert ownership interest in his own cells" (National Research Council 1997: 67), raising serious concerns about patenting, potential exploitation, and fair share of benefits between researchers and human research subjects. The Committee on Human Genome Diversity recognized this problem and stated:

> "A more-sophisticated and more-complicated approach would be to form an international organization to serve as a trustee and fund-holder for all the sampled populations. Patents would be issued in the name of this trustee organization, which would license anyone who signs an agreement to share a portion of the net proceeds from products made from any patented gene, gene sequences, or cell line with the trustee organization. The trustee organization, in turn, would be required to ensure that the revenue benefited the participating populations, which would be represented in the trustee organization. Such an organization not only could ensure that financial fairness is observed in genetic diversity research, but also could develop, monitor, and enforce universal rules for protocol review and informed consent in such research." (National Research Council 1997:67).

This idea of using a trustee organization to manage samples for genomic research has later been discussed and promoted as an alternative for a new social contract in the genomic era (Winickoff and Neumann 2005) and especially to address issues related to accessing biological samples from the developing world (Emerson, Singer et al. 2011). However, this trust has not yet been applied to any population in the developing world. Built from experiences working indigenous communities in Peru, this chapter discusses a model to facilitate inclusion of indigenous populations from developing countries in international collaborative research in a way that streamlines the research activities while maximizes protections for individuals and communities.

Overcoming issues of trust

The relationship between indigenous peoples and outsiders in developing countries, including non-immediate local governments, is analogous to the relationship between developing and developed countries; there is a clear asymmetry of power accompanied by an unfortunate history of friction between powerful and powerless groups. An international collaboration aiming to engage indigenous communities from developing countries occurs in the mist of these frictions.

Indigenous peoples are predisposed to distrust non-community members. In absence of trustor's openness or predisposition to trust, a prerequisite to initiate a relationship of trust (Mayer, Davis et al. 1995; Dietz 2011), scientists' possibilities to establish a partnership with communities is limited. My experience working with one indigenous group from the Peruvian Amazonian region suggests that even if they believe that they could benefit from interactions with visitors, they prefer not to have visitors in their community. My efforts to engage this community benefited from teaming with people who had already established a relationship with the community.

Continuous positive interactions between a research team and an indigenous community will result in a relationship of trust; however, continuous interactions with remote communities will demand significant time and resources, which pragmatically, is impractical for many research teams. Working with groups or people who already have a rapport with the community will accelerate development of a relationship between communities and researchers that are foreign to them. I was able to engage the community by working with people who had work or lived with them in the recent past. These persons acted as third-party warrantors and helped introducing the team and the project to local authorities and the community streamlining the process of engagement. However, finding suitable collaborators and establishing research partnerships in developing countries can take years and working with private local collaborators does not warrant one will have sufficient guidance to properly engage indigenous communities and authorities.

Some developing countries, like Peru, lack clear policies to oversee research with human populations. Although some structures and policies might be in place, processes are obscure and information is almost inaccessible for people out of the circles that were involved in the development of guidelines. I observed just that: Peruvian authorities require ethical evaluation by a local research ethics committee for any research project involving human subjects; however, most universities in Peru do not have research ethics committees and ethical review is rarely requested for observational studies. Considering that defense of a research project is a requirement to obtain the professional license in many health sciences in Peru, we can infer that most of the research in Peru is conducted without any ethical review. This fact is known and tolerated by Peruvian authorities; however, this level of tolerance is not offered for foreign researchers.

Peruvian authorities only recognize ethical reviews of local ethics committees. There are different types of ethics committees in Peru: associated with hospitals, universities, or private committees that can potentially evaluate any research project. The research ethics committees that review therapeutic studies need to be registered with the *Instituto Nacional de Salud* (INS, Peruvian National Institute of Health) for the INS to recognize them as legitimate, while the ones that evaluate only observational studies do not have any type of accreditation. As in many other developing countries, some ethics committees are composed by people who lack training in ethics (Hyder, Wali et al. 2004), which limits their capacity to properly evaluate and monitor research projects. Gilman and Garcia (2004) sustain that "Institutional Review Boards (IRBs) tend to operate on the presumption that investigators are intent on exploiting disadvantaged and poorly educated subjects" (Gilman and Garcia 2004:248). Under the assumption of researcher's intentions of exploitation, authorities put in place roadblocks that limit potential research with vulnerable populations without improving protective measures for these vulnerable populations.

Most of the efforts to strengthen ethics committees in Peru have focused on protecting participants in clinical trials who are becoming increasingly common in the country. Unfortunately, these trials, often sponsored by international pharmaceuticals, have raised concerns of exploitation of Peruvian participants, a grounded suspicion that has extended to any international research initiative in the country, even more if it involves indigenous populations.

Obtaining ethical approval for studies in Peru, and other developing countries, is a complex process. Gilman and Garcia (2004), from their experience working in Peru, claim that it is a long and unreasonably complicated procedure that constitutes an barrier raised from "trivial, misplaced or simply invalid concerns" (Gilman and Garcia 2004) p.248) pushing research back months or even years in a bureaucratic process that rarely yields any improvement on subject protection (Gilman and Garcia 2004). This process of review is especially difficult for studies sponsored by institutions from developed countries (Gilman and Garcia 2004), impacting negatively the potential of international collaborations. In my experience, it took four months to get the approval for the research protocol, but it took me two years to develop the relationships that allowed me to navigate the process and get started on the project.

In brief, there are multiple intricate layers of distrust that a scientist has to get through to include indigenous peoples from developing countries in observational biomedical research. Overcoming the presumption of guilt from local authorities (Gilman and Garcia 2004) and engaging suitable local collaborators is just the first step to initiate a partnership with communities that leads to the implementation of the research project. Consequently, unless there is enough time and funds, scientists might be forced to reformulate, resource to less than ideal methods, or be discouraged and exclude these vulnerable populations from research, which in the long run might contribute to the disparities experienced by these communities.

All strategic alliances must be grounded in trust. Cullen (2000) distinguishes a rational and an emotional component of trust (Cullen, Johnson et al. 2000). The rational component, or credibility trust, is based on the rational examination of the partner's capacity and intent to meet his promises and acquired responsibilities in the alliance, collecting evidence later used to decide to place or to withdraw trust. (Cullen, Johnson et al. 2000). In short, credibility trust is the rational evaluation of a partner's trustworthiness and is operationalized by questions such as: can the partner achieve what he is committing to? Do they have the skills, capacity, and resources? Trustworthy and untrustworthy actions encourage or discourage trust, respectively. When untrustworthy actions are generalized, potential trustors avoid placing trust as the only alternative to misplacing trust (O'neill 2005). An emotional component complements the rational component of trust.

Benevolent trust is the emotional component of trust (Cullen, Johnson et al. 2000). Benevolent trust relates to beliefs about partner's caring commitment in the relationship: would this partner harm the relationship? Would this partner protect the alliance?. The contribution of each of the components of trust depends on the type of relationship. While professional relations or alliances with authorities rely heavily on the rational component, developing a relationship of trust with vulnerable populations relies on a deeper emotional connection.

Establishing a relationship of trust with indigenous communities requires direct interactions. Dietz (2011) states that different types or models of trust (contract-trust, competence-trust, goodwill trust, etc.) have a common universal feedback process: a constant assessment of other party trustworthiness leading or discouraging a risk-taking act (Dietz 2011). Baier (1986) suggests that placing trust relies an assumption of goodwill from the person or institution in which we trust:

> "Where one depends on another's good will, one is necessarily vulnerable to the limits of that good will. One leaves others an opportunity to harm one when one trusts, and also shows one's confidence that they will not take it. Reasonable trust will require good grounds for such confidence in another's good will, or at least the absence of good grounds for expecting their ill will or indifference. Trust then, on this first approximation, is accepted vulnerability to another's possible but not expected ill will (or lack of good will) toward one." (Baier 1986:235)

Indigenous communities have a surplus of evidence of outsiders' untrustworthiness; the challenge for researchers is to provide evidence of trustworthiness as a foundation of any relationship between community and non-community members.

Bachmann (2011), suggests that institutional-based, rather than individual-based trust, is a viable model to repair distrust (Bachmann and Inkpen 2011). Relying on direct face-to face interactions, individual researchers or small teams are able to develop or repair relationships of trust with specific communities. However, this mechanism is personal and will limit the relationship to particular individuals or groups. This micro-level trust (Bachmann and Inkpen 2011), has limited impact on the purpose of including diverse communities in biomedical research; moreover, it does not offer any streamlined alternative to a researcher interested in starting a collaboration with a community. Macro-level trust, or institutional trust (Bachmann and Inkpen 2011), can provide a robust structure that can facilitate the inclusion of diverse communities in research.

Interpersonal trust requires constant direct interactions that, in the case of remote communities, translate into prohibitive budgets leaving as alternatives “safari research” or plain exclusion of those populations. Alternatively, relationships framed in an institutional-based trust could provide a scaffold upon which to build new relationships between communities and researchers, by establishing agreements and safeguards that propitiate trust through a system of professional accountability. An institution that is embedded in local culture and is knowledgeable and compliant of local regulations will constitute a valuable resource for communities and researchers interested in research collaborations. A trusted institution can act as a warrantor providing middle ground for efficient negotiation with all stakeholders of the research enterprise and their own experience will constitute guaranty for authorities and other groups involved in research. Additionally working at institutional level, can facilitate interactions with authorities and contribute to the implementation of systems of accountability that result in proper protection to human research subjects without postponing scientific development.

Distrust is evident from potential participants as well as from national, regional, and local authorities who strive to protect them. Indigenous communities are alienated from the majority and base their distrust on a sense of exploitation, abuse, and impunity around non-community members. Authorities' uneasiness is caused by their lack of norms, laws, and any structure of accountability, resulting in loose, if any, capacity for research oversight.

Problems derived from research are often the catalysts for the development of research oversight. In the last four years, three different laws related to biomedical research with human subjects have been proposed in the Peruvian congress, but to this day, none have been approved, and the system is becoming rather complicated by the rise of spontaneous regulations which are unsuccessful in their attempts to protect human research subjects.

Working with vulnerable populations from the developing world: Ethics of protection

Working with indigenous populations from the developing world means working with vulnerable communities. The Council for International (CIOMS) defines vulnerable persons as "those who are relatively (or absolutely) incapable of protecting their own interests. More formally, they may have insufficient power, intelligence, education, resources, strength, or other needed attributes to protect their own interests"

(CIOMS 2002:64). CIOMS requires that any research involving these individuals must guarantee protection to their rights and welfare. However, most developing countries lack structures and guidelines to provide these protections (Barboza, Minaya et al. 2010).

At the international level, ethical principles that emphasize human rights have been the focus of declarations such as those presented by UNESCO or United Nations that have had little, if any, impact on developing protections for vulnerable populations from independent countries. Instead, independent communities, civil societies, and non-governmental organizations have promoted solutions to protect populations at the local level (Kottow 2012).

Principalist ethics, based on egalitarian principles, is insufficient to address the issues resulting from working with populations in some sort disadvantage (Schramm 2005). Focusing mostly on the issues related to disparities observed in Latin America, Kottow and Schramm (2001) suggested a moral framework based on ethics of protection as an alternative to address conflicts and moral dilemmas in Latin America (Schramm 2005).

Bioethics of protection focus on protecting marginalized individuals and communities who lack of means to guarantee their inclusion and participation in the general society (Schramm 2005). According to Kottow (2012),

> "Protection goes beyond the ethics of care that is committed to proximal aid within familial and neighborly relations, for protection is equally due to the marginalized, the disempowered, and the distant destitute. Poverty not being a natural condition, has historical roots of dominance, colonialism, exploitation which need to be repaired." (Kottow 2012:45).

In other words, the aim of ethics of protection is to “reach those who are, as matters stand, too radically excluded to claim human rights, the voiceless and disempowered who are beyond the reach of helping hands” (Kottow 2012:46). Most indigenous communities from developing countries fit this description.

Including socially excluded populations in community-engaged research requires meaningful partnerships between researchers and community members. Establishing a just partnership requires both parties to be able to negotiate the terms of the society. Although some indigenous populations have the necessary capital to look after their own interests, numerous indigenous groups lack of these resources. Researchers working with vulnerable indigenous groups ought to consider the moral imperative to protect these populations, at least until they are empowered enough to be able to protect themselves (Kottow 2012).

An ethics of care centers on interpersonal emotional attachment (Bennett, Callanan et al. 2006), “caring relations and their associated concerns of trust and mutual responsiveness” (Held 2005:158). The ethics of protection goes beyond interpersonal relationships and involves the individual and a “political attitude of sustaining and empowering the weak.” (Kottow 2012:50). Thus, while the ethics of care might be appropriate for micro-level relationships, the ethics of protection aims for the macro-level aiming to involve all instances of societal organization in addressing the issues affecting marginalized people.

The ethics of protection should not be confused with paternalism. Although both look to improve people’s lives, paternalism proposes to improve people’s lives **for them**, while protectionism aims to support their independence.

> “Protection aims to help develop personal and collective autonomy, and should not be confused with paternalism which supplants and often disregards autonomy, especially in its unjustified authoritarian form.”(Kottow 2012:45)

Paternalism strips individuals from their capacity to decide, consent and act freely; from my experience, the embryonic structures that confine research, and any other matters involving indigenous populations in developing countries, lean towards a paternalistic rather than protectionist model, where authorities made decisions *for* them and *without* them.

The advocates and authorities, with jurisdiction over indigenous peoples and territories, are at risk to adopt paternalistic paradigms. Legal paternalism is the theoretical principle that aims to prevent “harm to the person being prohibited from acting” (Dworkin 2005:305). Discouraging researchers from approaching indigenous peoples to engage them in research is a paternalistic posture. Indigenous peoples should have the opportunity to accept or refuse participation on their own; their autonomy should be respected and encouraged if we are to promote their development. Still, the misbalance of power between researchers and indigenous peoples requires norms and sensible limits to efficiently protect vulnerable populations in this context.

History shows that protection of human research subjects requires independent oversight. As the Final Report of the Tuskegee Syphilis Study Legacy Committee concluded: “Society can no longer afford to leave the balancing of individual rights against scientific progress to the scientific community alone” (Committee 1996). However, systems of research governance should be carefully designed to ensure that efforts to protect communities do not become roadblocks for science. Sharing power through partnerships within a system that ensures professional accountability and communication provides a viable option to facilitate participation of vulnerable populations in research.

Research governance should be based on intelligent forms of accountability (O'Neill 2004), meaningful indicators of what and how research is been performed rather that “stupid accountability” (O'Neill 2004) based on audits or managerial indicators. Intelligent accountability implies expert oversight. Professionals and experts can identify real risks and assess the appropriateness of a research protocol (benefits offered, the informed consent process, and the entire process of community engagement). Without proper understanding of project goals and methods, it would be impossible to establish congruent indicators of accountability to properly monitor research activities.

A misbalance of power often characterizes interactions between researchers and research subjects. Accountability can limit researcher’s power (Kottow 2012). However, a system of accountability needs to balance the limitations and the licenses given to the researchers:

> “No professional can function properly without discretionary latitude. The more discretionary latitude we permit our professionals, the more vulnerable we become. Yet to limit that latitude is to limit the capacity for good as much as it may limit the capacity for harm. (Pellegrino, Veatch et al. 1991:74)”.

Thus, accountability must be a constitutive part of research governance but must balance both scientific advance and human subjects protections, a task that needs to be independent from the research team, and it could be facilitated through an intermediate research organization that understands and advocates for both and has no conflict of interest, thus is able to offer objective evaluations.

Before accessing indigenous populations in developing countries, researchers must address issues of trust with authorities. Regulations and structures of oversight intend to address untrustworthiness. It has been suggested that “a new public culture based on accountability and transparency” (O'Neill 2004:269), operationalized as an external sturdy legal system, is necessary to counteract the failure of trusting social relations which in some cases has enabled corruption to thrive (O'Neill 2004). For some, trust has become futile and the alternative is to establish a system of accountability and compliance (O'Neill 2004), what has been labeled as the ‘audit culture.’

The audit culture, also known as ‘audit society’ (Power 1999) or ‘managerial accountability’ (O'Neill 2004; Blackmore 2009) , propose to use audits to create accountability through objective and quantifiable judgments on functions previously conducted. This is a type of second-order management that uses targets and scores to measure performance and establishes sanctions to non-compliant parties It is a role that is often performed by auditors who might or might not be knowledgeable on the subject of the primary services or obligations. Power (1999) and O’Neil (2004), define audit culture is an unintelligent form of accountability (Power 1999; O'Neill 2004).

Audit-derived accountability discourages trust (Power 1999; O'Neill 2004). Rather than providing evidence of professional trustworthiness, audit-derived accountability establishes standards that are uninformative of the quality of the activities or services offered (O'Neill 2004). Knowing the time spent in a medical appointment does not inform us about the quality of the appointment; similarly, the time invested recruiting human research subjects does not correlate with the quality of the informed consent. Without knowledgeable evaluation of real evidence of the professional practice, how can we build trust in the professionals? Building tighter systems of governance and oversight, with detailed rules and measurable indicators, as advocated by the audit culture, might result in bureaucratic complications without any, or even negative, impact in the development of trust. Furthermore, the need for complex systems of oversight might be seen as evidence of untrustworthiness (O'neill 2005), discouraging relationships of trust.

It is impossible to establish a meaningful system of accountability without deep understanding of a subject matter. The involvement of the experts is crucial to identify real indicators to evaluate a given enterprise. Proper accountability relies on knowledgeable judgment; a superficial understanding will not be sufficient to identify accurate monitoring standards or investigate malpractices (O'Neill 2004). Accordingly, a system of accountability cannot be improvised. Accountability requires careful planning and a qualified team to support and conduct the process.

Developing countries are in process of creating systems of accountability to protect human research subjects. As in the rest of the world, scientific development outpaces the development of guidelines and regulations and, in some cases, these norms are improvised, resulting in complex, and sometimes incompatible layers of oversight.

In research, accountability is operationalized through ethical reviews and monitoring. The process of ethical review is a mechanism aimed to address ethical issues and to protect human research subject before initiating a research project. In developed countries, most research institutions have research ethics committees that monitor research activities with human subjects. In the USA, the Federal Policy for the

Protection of Human Research Subjects, generally known as the Common Rule, requires that Internal Review Boards, or IRBs, examine and monitor research protocols to ensure protection of participants prior and during implementation of research projects. In developing countries, the mechanisms for ethical review are unclear, weak or sometimes nonexistent (WHO 2002). Moreover, some ethics committees in the developing world lack funding and training in ethics (Hyder, Wali et al. 2004) resulting in subjective and inappropriate ethical reviews that challenge research. Developing collaborations with local researchers is a necessary, although not sufficient, condition to navigate these often disjointed systems of research governance.

International research collaborations

Developing international research collaborations with the developing world is a demanding venture. From the often protectionist approach of national authorities to earning trust of potential participants, there are multiple challenges that jeopardize the implementation of scientific projects in the developing world. These issues are further problematic if the communities or their representatives have previous negative experiences with foreigners. Additional barriers appear if the topic of research is highly specialized, becoming almost incomprehensible for people outside the field. The risks and concerns associated to including indigenous populations from developing countries in biomedical research have been largely discussed. What is often neglected in the discussion is that these projects facilitate the technological transfer and provide funding to explore questions that otherwise would remain unexplored.

Biomedical research in developing countries is mostly funded by developed nations. Most of the world's research budget comes from developed countries that dedicate a substantial amount of their gross domestic product (GPD) to research (Figure 25A). Interestingly, some developing countries, such as Peru, Venezuela, Nigeria, or Cameroon, have a significant number of scientific and technical journal articles, while having minimal investment in research (Figure 25). International collaborations might be the mechanism that allows this phenomenon by encouraging participation of local scientists, building capacity, and sharing resources across the borders. However, the number of publications in science and technology is an indicator of professional development of a segment of the population only and it does not inform us about the type of research or who benefits from these studies. Research in developing countries should include goals that benefit the host country; especially in the biomedical sciences, where research can help reduce the global health disparities.

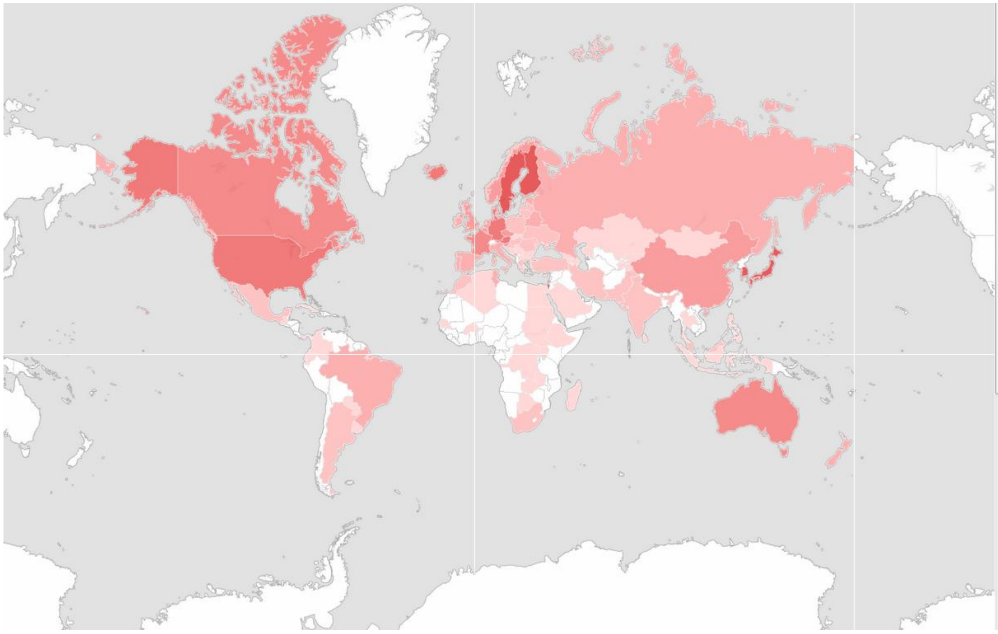
Most biomedical research is oriented to address health issues of the developed world. The 10/90 disequilibrium, or 10/90 gap, refers to the disparity on allocation of the world research budget: the developing world carries 90% of the global burden of disease, and only 10% of the world research budget is dedicated to study these diseases (Tan-Torres Edejer 1999; Doyal 2004; Osei-Twum and Wasan 2012; Rashid, Khandaker et al. 2012). A level of disequilibrium is expect given any country is likely to favor funding of research that more directly impacts their citizens. However, approaches to encourage research in developing countries will help reduce this disparity gap. The developing world offers unique natural phenomena that are often engaging to scientist, but the restrictions, risks, and ambiguities of working with vulnerable populations can be deeply discouraging for research. Thus, the development of structures that nurture international collaboration will provide an opportunity to advance science, while providing meaningful benefits to both the developed and developing world.

Fair share of benefits and property rights

Participation of indigenous peoples in research has often been labeled as exploitative by indigenous peoples' organizations and advocates. According to Emanuel (2004) "A exploits B when B receives an unfair level of benefits or unfair burden of risks as a result of interacting with A" (Emanuel, Wendler et al. 2004). In a research project, researchers will benefit from publications, grant money, and career development; while often times, there is no meaningful benefit for participants.

Most of the time, research in developing nations is curiosity-driven. Research with indigenous peoples rarely responds to communities' needs or interests, sometimes considered "undue exploitation of vulnerable populations" (Bhutta 2002:116) aiming "to add to the body of scientific knowledge without focusing on the needs of a specific community" (Arbour and Cook 2006:155). Ethically acceptable research should depart from *using indigenous peoples for research* and commit to *doing research with indigenous peoples*, but the later might be exceedingly difficult to attain.

Implementing research with indigenous populations requires special considerations of the specific needs and circumstances of these communities. It is widely accepted that to address communities' vulnerabilities it is necessary to engage



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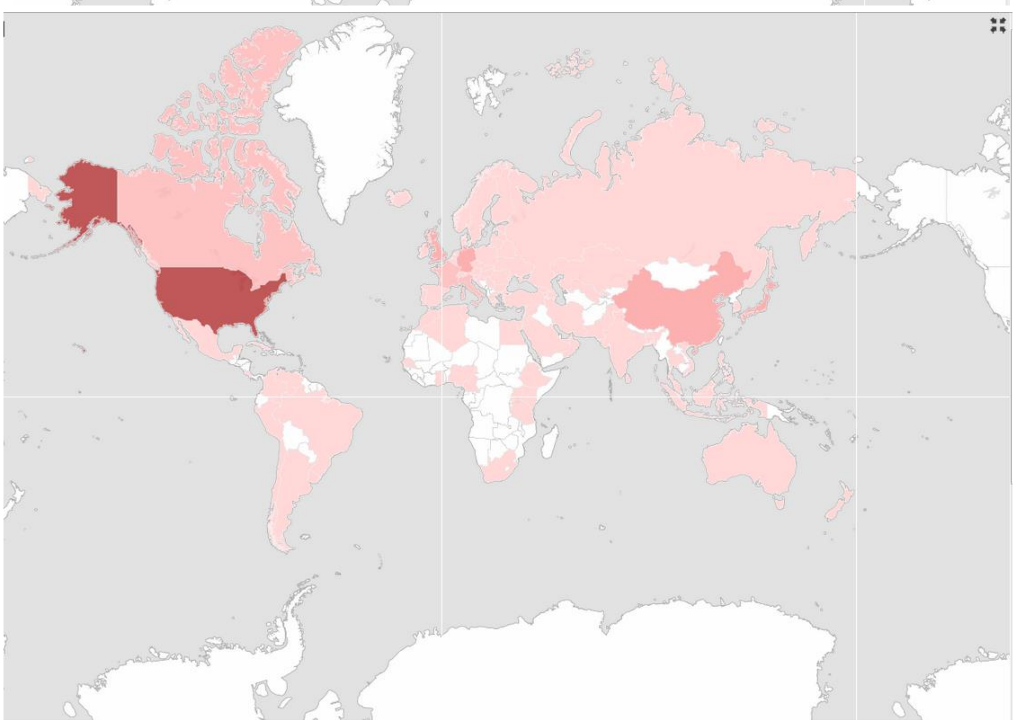

B.

Figure 25 Snapshot of research in the world (The World Bank 2012). Darker colors represent larger numbers. (a) Most of research funding comes from developed nations, but publication of scientific findings is spread beyond those borders (b), including developing countries which investment in research and technology are minimal.

the communities in the research (Foster, Sharp et al. 1999). The main goals of community engagement are: to ensure relevance of research (Tindana, Singh et al. 2007), to avoid exploitation (Emanuel, Wendler et al. 2004; Diversity 2011), and to enhance community protection (Foster, Sharp et al. 1999; Tindana, Singh et al. 2007). Accomplishing these goals requires resources and careful planning which in absence of proper local infrastructure might be beyond reach.

Ensuring relevance of research and avoiding exploitation

Most of the time, research with indigenous communities neglects incorporating the needs of the specific community. In an *ideal* world,

> "The research *must* reflect the needs of the community and must be considered, by the community and researcher, an appropriate research problem to explore.(…) The community needs to be involved with the development of the research from the time the research question is asked." (Bhutta 2002:154)

In the *real* world, funding agencies' programmatic priorities shape research goals.

Research priorities of funding agencies encourage and discourage certain lines of research. Understandably, developed countries' research budget prioritizes areas of inquiry that will benefit their needs and interests; however, this should not be an obstacle to incorporate benefits to diverse populations participating in the research.

Although monetary benefits are the most obvious, there are options to provide non-monetary benefits that can positively impact host populations. Other forms of acceptable sharing of benefits, proposed by the Nagoya Protocol on access to genetic resources and the fair and equitable sharing of benefits arising from their utilization are listed in Table 11.

What constitutes an appropriate compensation or reciprocity is group dependent. A researcher that is unfamiliar with the specific target group might not have enough

Monetary benefits	▪ Access fees/fee per sample collected or otherwise acquired ▪ Up-front payments ▪ Milestone payments ▪ Payment of royalties ▪ License fees in case of commercialization ▪ Special fees to be paid to trust funds supporting conservation and sustainable use of biodiversity ▪ Salaries and preferential terms where mutually agreed ▪ Research funding ▪ Joint ventures ▪ Joint ownership of relevant intellectual property rights.
Non-monetary benefits	▪ Sharing of research and development results ▪ Collaboration, cooperation and contribution in scientific research and development programs, particularly biotechnological research activities, ▪ Participation in product development ▪ Collaboration, cooperation and contribution in education and training ▪ Admittance to ex situ facilities of genetic resources and to databases ▪ Transfer, from research teams to host population, of knowledge and technology under fair and most favorable terms emphasizing biotechnology that is relevant to the conservation and sustainable utilization of biological diversity ▪ Strengthening capacities for technology transfer ▪ Institutional capacity-building ▪ Human and material resources to strengthen the capacities for the administration and enforcement of access regulations ▪ Training related to genetic resources with the full participation of countries providing genetic resources, and where possible, in such countries ▪ Access to scientific information relevant to conservation and sustainable use of biological diversity, including biological inventories and taxonomic studies ▪ Contributions to the local economy ▪ Research directed towards priority needs, such as health and food security, taking into account domestic uses of genetic resources in the Party providing genetic resources ▪ Institutional and professional relationships that can arise from an access and benefit-sharing agreement and subsequent collaborative activities ▪ Food and livelihood security benefits ▪ Social recognition ▪ Joint ownership of relevant intellectual property rights.

Table 11. A fair share of benefits between researchers and host communities. There are monetary and non-monetary alternatives to promote a just share of benefits. Note that benefits for host populations are not limited to benefits for actual participants; knowledge and technology transfer and acceptable options to indirectly benefit host populations by building capacity to further their potential to address research priorities independently (Diversity 2011).

information to include proper benefit sharing in the research protocol, highlighting the relevance of partnering with community members, researchers that are knowledgeable about the community or both, from protocol's inception.

Property rights

Some research projects with human subjects are producing profit for researchers, universities and companies (Emerson, Singer et al. 2011) . Property rights are claimed in the form of patents, or even commercialization of biological samples and their attached data (Winickoff 2003; Winickoff and Neumann 2005). These economic benefits rarely translate to benefits to the source populations.

Biobanking, a systematic collection of biological samples, has been a common practice for decades, but this secondary use of biological samples has taken a central role in biomedical research in the post-genomic era (Cambon-Thomsen 2004). Biotechnological developments allow us to collect increasing amounts of molecular data which, in order to detect linkage with phenotypic information, require large cohorts that demand sample sharing. However, this management of samples has raised numerous questions about ownership of these samples and data.

What warranties are offered to participants? Who owns and who can decide over biobanked samples and data are some of the contentious questions associated to biobanking. Protecting individual's privacy and confidentiality is often operationalized by de-identifying the samples; however, if samples are completely anonymized participants' rights to withdraw from the study or the biobank are stripped. Human research subjects' biological samples are considered donations and as such, they *become property* of the investigator responsible of the project, who decides how to use the samples, and in some cases the samples are sold to private companies (Winickoff and Neumann 2005). It is unclear if there can be a process of consent that can properly *inform* individuals participating in biobanking, after all, these are samples collected for undefined studies. Thus, there is a plethora of ethical issues associated to sample banking and some of them could be addressed through a model that departs from private ownership of samples: a biotrust model.

The biotrust model was conceived following the organization of a charitable trust.

> "The Biotrust Model consist of a legal structure for handling the property rights and management of donates genetic and informational resources, and social structure aimed at bolstering community participation, representation and trust in genomic governance- necessary conditions for sustainable collaborations." (Winickoff and Neumann 2005),p.10)

Thus, in the biotrust model the sample receptor acts as a trustee and not as a broker. The trustee assumes the responsibility of keeping and using the samples –and attached data- for the benefit of donor's community or the general public, avoiding potential conflict of interest and, more importantly, the trustee will mediate relationships between the donors and the researchers that wish to use the samples banked (Winickoff and Neumann 2005)

The biotrust model offers an alternative to avoid exploitation of donors. Under this model, storage and usage of samples do not overlap: the trustee is a middle ground between researchers and donors and researchers do not hold ownership rights over samples (Winickoff and Neumann 2005). Instead, the trustee assumes the responsibility to protect donors' wishes and facilitate the communication between researchers and donors. It has been even proposed that a biotrust could allow commercial use of the samples (Winickoff and Neumann 2005), but in this case, the benefits will be likely to reach the community, while in the regular biobank model, any benefit derived from the samples resulted in direct benefit for the researcher holding property rights over the samples.

The biotrust model offers an ideal alternative to include indigenous peoples in research. Trustees would keep samples and mediate research projects with research teams and might be better equipped to negotiate benefits for the community following donors' guidelines. The type of relationship modeled by charitable trusts is consistent with the interactions that would support community-engaged research.

Implementing metagenomic research with indigenous populations in Peru

Building a team

Traditional models of research are research-centered. As shown in Table 12, traditional research does not engage the community in any capacity besides being the source of samples and data. We aimed to conduct community-based research and recognized that national collaborators, who have experience working with indigenous peoples and have an ongoing relationship with the communities of interest, are a key element to this end. Through a newly developed relationship with the Center for Intercultural Health (CENSI) at the Peruvian National Institute of Health, I identified and connected with two health professionals who work actively with indigenous peoples. Their early involvement in protocol development was crucial to the selection of the participating communities and to appropriately budget time and resources for community engagement and our scientific goals. Additional members were recruited later on based on the specific needs derived from our protocol.

	Traditional	Community engaged	Community-based participatory research
Research aims	Respond to researcher interests and/or funding priorities.	Dialog with community to adapt researcher's interest to community's needs or interests.	Meaningful partnership with community to identify research topics that are priority in the community.
The research plan	Guided by scientific knowledge, the scientific method and viable options. Researchers keep control.	Research plan is discussed with the community and modifications are made to ensure it is culturally appropriate. Researchers have control in consultation with community.	Community participates in the research design. Real partnership, control is shared between researchers and community.
Development of instruments	Preference for previously validated instruments.	Instruments are adapted to be acceptable to the community.	Instruments are adapted or developed with active participation of the community.
Data collection	Lead by non-community members (researchers).	Community members are involved in data collection (planning, implementation or both).	Focus on capacity building. Community members participate in data collection to the extent possible.
Data analysis and interpretation	Researcher controls how data is analyzed and interpreted.	Results are presented to the community to collect potential comments or concerns around the findings that should be taken into consideration when the data is presented.	Community and researchers work together to analyze and interpret results.
Result dissemination	Disseminate within academic community.	Dissemination occurs in both general public and scientific community.	Research team (including community members) decided the venues for dissemination of results (includes scientific journals and general access publications or any other venue considered appropriated).
Net gain of skills, career, personal and professional development	Researchers.	Community members may gain some skills from the Researchers.	Researchers and community.

Table 12. Comparing distinct models of research with respect to level of community participation. Community-based participatory research is the ideal model

to work with indigenous peoples, but its implementation is time and resource intensive limiting its application.

Obtaining ethical approval of the project

Peru lacks clear regulations or policies to govern research with human subjects (Barboza, Minaya et al. 2010). From identifying the local institutions and the regulations that govern research at the local, regional and national level, to obtaining appropriate approval, there is a disorganized and subjective system almost inaccessible to outsiders. Figure 26 presents the different authorities that have some level of jurisdiction over a biomedical research project in Peru. One of the challenges that the Peruvian system has to develop clearer policies is the fractioning between their instances and their continuous struggle to establish hierarchy one over another.

Since 2010, there has been an initiative to create a National Council of Research Ethics in Peru. This institution would be the central resource to create and monitor policies that govern biomedical research in the country (Barboza, Minaya et al. 2010). This organization would also promote the development of proper committees for ethical review and establish the standards of accountability that will legitimize their practices. Unfortunately, this proposal was ill received, by two other players of bioethics in Peru: the National Council of Bioethics (created in 2001) and the National Network of Research Ethics Committees (created in 2004); both considered the proposal redundant and unnecessary. As I have witnessed in more than one occasion, the possibility of dialog between groups is minimal and their discussions are more focused on reciprocal aggression than in constructing a system of governance for biomedical research. Consequently, three years have passed, and the country is still without a solid structure to protect human research subjects.

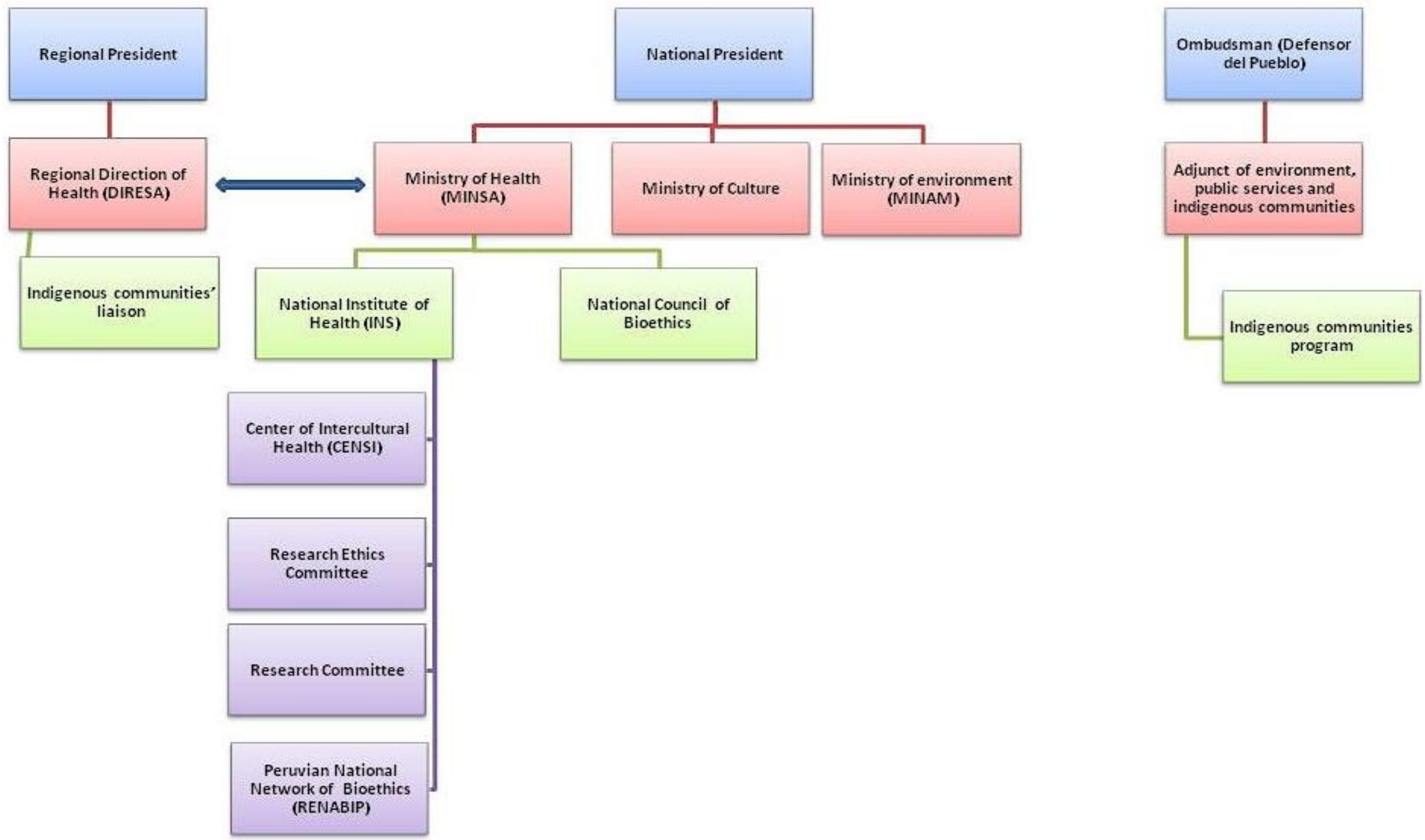

Figure 26. Authorities with jurisdiction over research projects with indigenous peoples in Peru. The mechanism for governance is unclear and the information provided by each of them might be incongruent from one to another.

In absence of a clear legal framework, ethical reviews in Peru are conducted in the light of legally binding documents, not necessarily to the level of a law, which indirectly address aspects related to research with human subjects. In addition, many academic institutions do not have ethics review processes, nor do they offer any type of training in research ethics to their researchers. Consequently, Peruvian researchers often conduct research without any review and are unaware of regulations. Moreover, among the existing ethics committees in Peru, 44.4% do not have any member trained in Bioethics (Minaya 2012) which poses questions about the ethical review these committees perform. Aiming for the highest possible standards, my project was reviewed by the research ethics committee from the Peruvian National Institute of Health (INS).

Before the Research Ethics Committee at INS reviews a protocol, a scientific committee either at INS or at a local collaborating institution must review it. The present project was a collaborative effort with the school of Human Nutrition and Dietetics at Universidad Cientifica del Sur (UCSUR). Consequently, my project had to go through different instances of review before it could reach the ethics committee. Reviews at UCSUR , by the Dean of the School and the Vice-president of Research's Office, were concurrent and their report was consolidated. Once the modifications were implemented, the protocol was sent to INS for ethical review.

Official communication in Peru requires physical documents. Initial communications are to be hand-delivered at *Mesa de partes*, an office whose sole purpose is to receive and derive documents to the corresponding staff. In governmental and some non-governmental institutions, each document is assigned a reference number that should be used for further communications with the relevant institution. To catalyze the process, a person was made available to collect the documents from UCSUR and hand delivers them to INS' Mesa de Partes. Although telephone and email became an acceptable form of communication later in the process, the need to deliver and collect paper documents at INS was a constant. Figure 27 shows the process followed to obtain ethical review. It took four months from the time the protocol was submitted to UCSUR until I could obtain approval to initiate the project. It is my understanding that average process can take longer, even if it does not involve international collaboration.

Engaging regional authorities

Through the ongoing relationship between local collaborators and the Matses ethnic group, we arranged a meeting with the Matses leader that represents the fourteen Matses communities established along the rivers Galvez, Yavari, and Chobayacu. During this meeting we presented the project and discussed potential concerns about it. The only concern raised by the Matses leader was related to trust: the community might not want to participate or work with me because they did not know me and my approach was similar to previous approaches from oil companies. The leader doubted that public meetings with me would be of any help, but he offered to present our project to the fourteen community leaders in an upcoming Matses meeting. We provided a summary of our project to be used on the meeting. He authorized us to enter the communities and talk to the community leaders; however, he emphasized that each community is independent and their assembly should decide their participation.

Meeting with the Matses leader provided me with valuable information about the Matses communities, including details about their lifestyle and how it is changing, as well as their kinship systems and social organization. Collaborators' accounts, whose experience with the community related to topics that differ from mine, could have not provide the information I gathered from the Matses leader that allowed me to better prepare to implement our project.

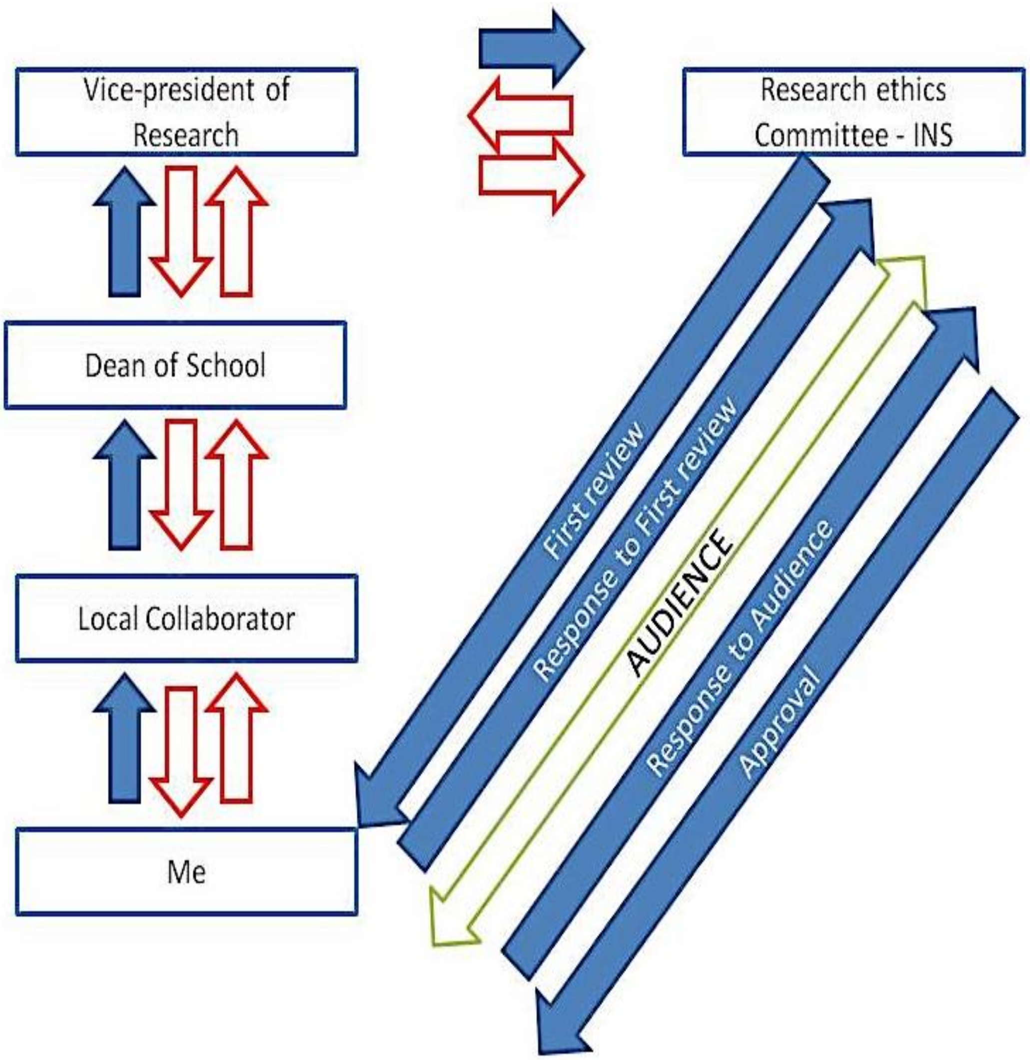

Figure 27. Ethics review process. Solid arrows represent the process followed to obtain approval of my protocol. The outlined arrows represent what the 'normal' process should be, all in hard copy, which would have delayed significantly the project. The active communication between me and INS was the result of my proactive presence in their offices. The audience (represented by the green arrow) is not part of the regular review process, it was provided at my request with the purpose to smooth the communication process and abbreviate the processing time.

Once approval from ethical review was obtained, we proceeded to travel to Iquitos city, Region Loreto, where a team member presented our plan to the liaison of indigenous communities at the Regional Direction of Health (DIRESA). It was not until then that I learned that DIRESAs have dual governance where they coordinate services through MINSA, while –administratively- they are dependences of the Regional Governments. Regional Governments are autonomous and it is possible to have regional laws where national law is inexistent. For example, one out of the 25 autonomous regions in Peru, the Cusco region, has regional laws to protect cultural and natural patrimony from biopiracy (O.R. Nº048-2008-CR/GR), requiring prior informed consent from indigenous communities, promoting benefit-sharing with communities, and limiting the potential patenting of genetic resources (iied 2009). None of these points are clearly stipulated on any national Peruvian law.

The existence of regional laws is fairly recent in Peru, and most professionals or lay people are unaware of them. The discrepancies between national and regional law represent another risk for researchers attempting to engage indigenous communities. Given general unawareness of national and regional regulations, it is possible that an outsider can transgress local norms that appear invisible to them, and when those norms are legally binding, it can result in major offenses of which the researcher might remain unaware. The best alternative is to connect with people who are knowledgeable on these topics and who can provide guidance in the national and regional legal structures, such that they can be included in the planning and implementation of any research project.

Although Matses do not identify with mestizo authorities, mestizo authorities have jurisdiction over the Matses reservation. The closest mestizo town is the Angamos

Colony, where the military, police, and medical posts that serve the Matses reservation, in addition to the *gerente* (manager) of the sub-region Yaquerana, are located. Prior to entering the Matses area, we presented our project to the manager, who reported our presence to the Regional office in Iquitos, and to the health care personnel at the health post. Our initiative was supported and we proceeded to prepare to enter the Galvez River.

Engaging communities

Engaging communities in research can be more challenging than engaging authorities. Professional credentials and networks are often perceived as evidence of professional trustworthiness. These professional ties provide mechanisms to hold scientists accountable and encouraging some level of trust from authorities; however, this effect might not extend to indigenous communities.

Upon arrival to the Matses community anexo San Mateo, we requested to speak with the community leader. We learned about some preconceptions related to researchers and some concerns related to our project. Matses people were concerned that we were working with the petroleum companies, and that our research might provide the entrepreneurs with the knowledge and tools needed to exterminate them and take possession of their lands. Furthermore, we heard they had been warned about potential intervention to implant sub-dermic microchips that would reveal their geographical movements for a future attack. Thus, we decided to reformulate our health screening services and avoid any blood test. Our service to the community was limited to parasite screening.

After discussion with the immediate authorities, community president and his team, they agreed to allow us to present our project to the general assembly. We agreed that we would respect the community decision and after their approval we would also seek individual consent. We presented the project at the community assembly and demonstrated the sample collection process. We explained that blood would not be used for our study and allowed them to verify that our swabs were inoffensive, which dissipated many of their concerns. All interactions were in Matses, and to present our project we worked through one of the interpreters who is a clinical laboratory technician with a good understanding of our research goals.

Incorporating community research interests

Throughout the whole intervention the immediate local authorities accompanied our team. These interactions during project implementation encouraged immediate local authorities to propose their own research goal: the community was concerned about contamination in their water and requested we analyzed samples of their source of drinking water. Although environmental samples were not part of our project, we agreed to perform microbial analysis of their water and proceeded to develop a written agreement between the president of the community and myself, documenting our commitment to present the results of the water analysis in a future visit. Our concerns about the need of additional permits to collect environmental samples were relieved by understanding that Matses were sovereign and the samples provided (collected by the community president), as private property, do not require the type of permit that would be needed from environmental samples collected outside the reservation.

During our meeting with the community, we committed to return the results of parasite screening the day of the intervention, report the cases of parasitism to the local health post in Angamos, return with preliminary results, provide copies of our posters for the community, and conduct community consultation prior any publication using the name of the community. Our agreement was fulfilled six months after the first intervention and the assembly authorized us to use the name of the community in the dissemination of the results.

Providing benefits for the community

The scientific goal of my project focused on characterizing microbial communities living in and on the human body. Given the nature of the samples required for research analysis and considering that malnutrition and parasitism are main problems of public health in Peru, we design our intervention to provide a health screening for the communities participating in our study. We had decided to offer some demonstration of reciprocity to the whole community to avoid undue influences as well as potential frictions consequence of individual benefits for participants only. Capitalizing in the health care providers recruited for our team we were able to incorporate this activity within our fieldwork plan. The addition of community service as part of our protocol was well received during the ethics review and our efforts to provide some meaningful benefit to the community was later replicated in some other study involving indigenous peoples in the country. Because of the interdisciplinary nature of our team, we were able to provide this service free for the community and with minimal hassle to the study budget. We recognized that our project was unlikely to produce any information directly relevant for the community and there was no real benefit for the participants from indigenous communities; the scientific goals of the project remained unchanged but we actively avoided exploitation by maximizing the benefit for community members who generously had accepted to participate in our study.

Engagement of the Matses, within the budgeted time and funding, would not have been possible without the appropriate local collaborators. The support of a local institution (UCSUR) that had positive relations with national authorities (INS) facilitated our interactions and provided guidance to obtain ethical approval; team members who were familiar with the area and had an established relationship with the community streamlined the engagement of local authorities and the community. It has been reported that some projects in Peru never get done because of the complicated system to get proper authorizations (Gilman and Garcia 2004) highlighting the inefficiency of current research governance and there multiple anecdotal accounts of teams performing less than ideal research *in* indigenous communities suggests that these complex systems are unable to protect these populations.

Time and resources used in finding optimal local collaborators and obtaining appropriate approvals might endanger the scientific goals of international research collaborations. An intermediate research organization could offer scientists the structure necessary to implement biomedical research with indigenous populations in developing countries. This model aims to facilitate the advancement of science in partnership with populations that are traditionally marginalized, promoting the highest ethical standards and engaging local authorities in a way that induce continuous evolution of sensible norms and guidelines that effectively protect these vulnerable populations while providing a clear path for the research enterprise.

An Intermediate research organization

Working through intermediary organizations is an alternative to overcome some of the issues associated to implementing international collaborations for biomedical research with indigenous communities. The organization I am proposing is a non-profit, issue-focused intermediate organization, aiming to build coalitions among different agencies or groups united by a common interest (Sherman 2002). In this case, the focus is to promote the inclusion of diverse populations in biomedical research.

Intermediate organizations are widely used to implement policies between levels that otherwise would have difficulties partnering (Shea 2011). The main role of an intermediary institution is not to provide services as such; instead, these institutions facilitate the exchange between agencies, institutions, or providers and their mission is to help them do what they do, but better (Brown, Davis-Richardson et al. 2011):

> "The intermediaries […] often acted as bridge builders between sectors. As such, they speak the language of the community and the language of government, foundations, and businesses and thus enable understanding, cooperation, and coordination that is otherwise impossible or very difficult to generate. In addition, intermediaries transfer innovation and knowledge from one sector to another." (Brown, Davis-Richardson et al. 2011:25).

In the context of the research enterprise, an intermediate research organization will enable the communication among all the stakeholders (Figure 28). This organization will be able to assist scientists in navigating evolving systems, by introducing them to new regulations and requirements from national and local authorities, as well as by smoothing the process of engaging indigenous peoples. At the same time, the organization will centralize information that might become useful to the development of public programs and to promote research that addresses the needs and interests of communities. Furthermore, involvement in multiple research projects will provide information about research practices that could be used to spark dialog with local authorities and could contribute to policy development.

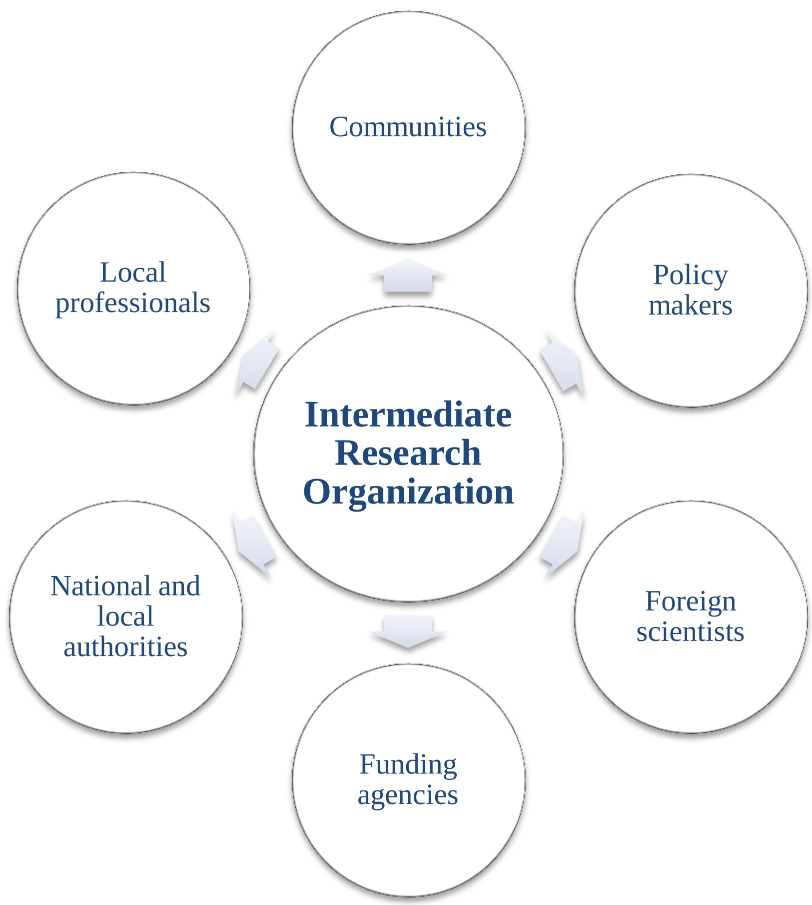

Figure 28. Interactions among stakeholders in the research enterprise under the Intermediate Research Organization model. The organization serves as resource allowing for seamless flow of information across them.

Building an intermediate research organization

The intermediate research organization here proposed is fundamentally distinct to contract research organizations. While contract research organizations are basically for-profit organizations that provide services for pre-clinical and clinical trials, the intermediate research organization is a non-profit organization that collaborates with researchers and communities to facilitate meaningful partnerships to achieve scientific goals while protecting the interests of the vulnerable populations involved. In contrast with contract research organizations, the intermediate research organization will focus on promoting research that is relevant to the host populations aiming to incorporate goals that address their needs, while contract research organizations' services are focused on industrial targets that are mostly relevant to the developed world. Unless a trial will directly benefit the communities in study, this intermediate research organization will purposely avoid participation, and even discourage any clinical trial in vulnerable populations, to prevent the main negative trait associated to biomedical research with indigenous populations: exploitation.

This organization is inspired on the biotrust model (Winickoff 2003; Winickoff and Neumann 2005) but it is not limited to governance of biobanking activities. This model is grounded on three main goals: inclusion of indigenous populations in biomedical research (researcher initiated projects), promotion of research to address needs and interests of vulnerable populations (community initiated research) and community-engaged biobanking (biotrust) (Figure 29).

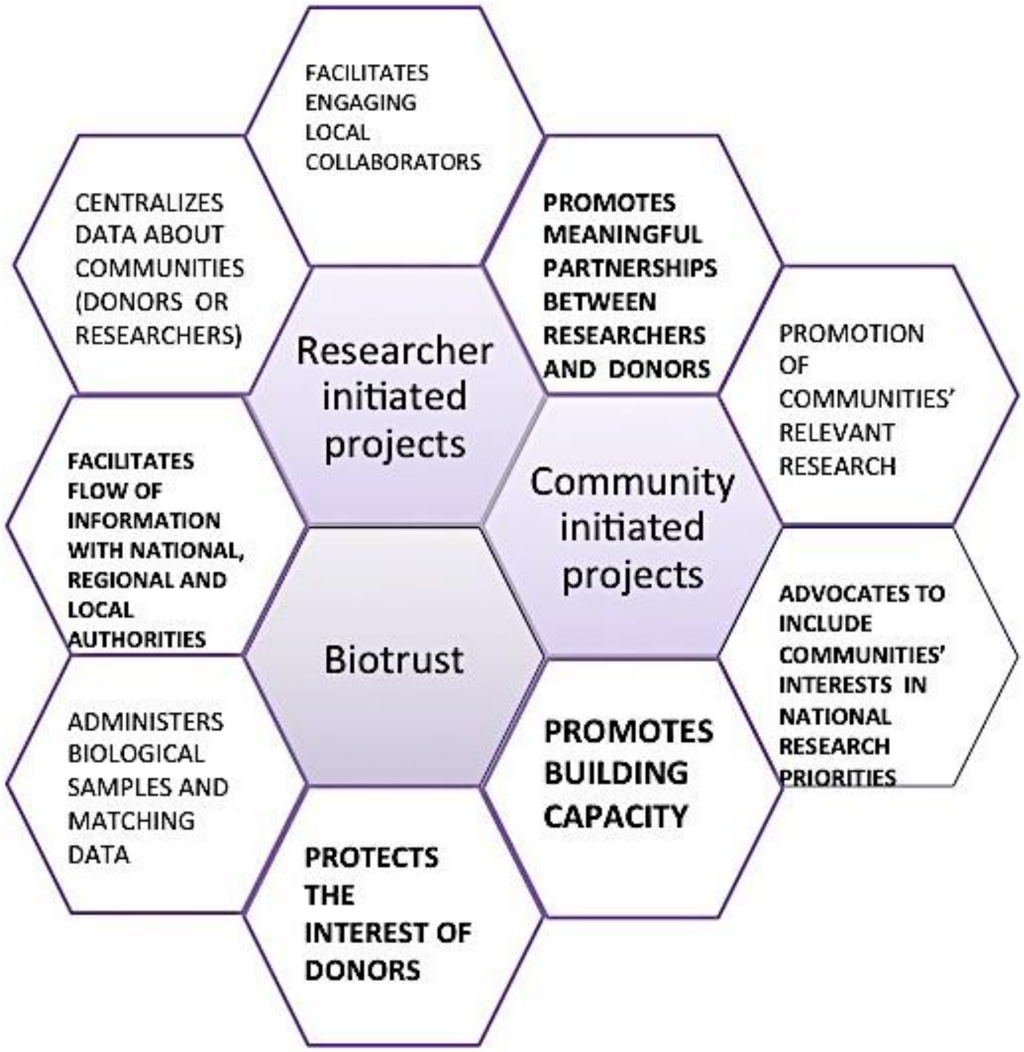

Figure 29. Goals of the Intermediate Research Organization. At the core the three main domains of the organization: Research initiated projects (inclusion of indigenous populations in biomedical research), community initiated projects (promotion of research to address needs and interests of communities) and managing a biotrust(community-engaged biobanking). Examples of functions that support these domains are presented around them.

Researcher initiated projects

Curiosity-driven research with indigenous populations is often considered exploitative because it does not address the needs or interests of host communities (Bhutta 2002). However, curiosity-driven research with indigenous peoples does not need to be exploitative. Even if the goals of a research project are not relevant to the community, a project can be designed to provide some type of meaningful benefit for the community.

Relevance of a research project and defining reasonable benefits varies across communities. Intermediate research organizations could centralize information and provide communication channels to improve projects' relevance, identify culturally defined risks (Anderson and Metcalfe 2008) and benefits that researchers could use to prepare a suitable proposal for the community, providing a sensible starting point for the actual community engagement.

Taking Peru as an example, there is no official census of indigenous groups and many of them are disperse and do not have an evident social structure. Verified knowledge about communities will help researchers to evaluate feasibility of research projects while this information could also be useful to indigenous peoples' advocates that aim to develop programs and services for these communities. Ideally, an intermediate research organization will become a resource to connect people, knowledge and samples for improvement of the general society, but adequately protecting and empowering indigenous groups involved in these initiatives.

Researchers and communities can negotiate research agreements that fit to their specific circumstance; however, an intermediate research organization can facilitate the dialog acting as third party warrantor during the transaction. The intermediate research organization can provide professional expertise to advise community member s and cultural interpreters for researchers to even the ground for a meaningful community engagement.

Community engagement is grounded in a relationship of trust. Indigenous peoples are predisposed to distrust outsiders but, in absence of direct experience between researchers and communities, people that have established relationships with communities can provide the middle ground to start the dialog. Engaging local collaborators to facilitate community engagement is a common practice but not all local professionals would be able to fulfill this role. Anecdotal information suggests that some local collaborators might be willing to exploit indigenous peoples in exchange for career development. An established nucleus (the intermediate research organization) can facilitate collaborations with quality collaborators, and working through such an institution will provide a layer of professional accountability that can further protect human research participants.

Improving accountability through an intermediate research organization

Countries without a system of research governance have no mechanism to protect human research subjects. In Peru, there is no mechanism to hold researchers accountable and as long as the study is not related to clinical trials, unregistered and unaccredited research ethics committees can supervise the studies. Often times, authorities' evaluations are subjective and they express more concern about exploitation by foreign researchers than by local researchers. However, local researchers have more opportunity and less training in research ethics, a dangerous combination that puts at risk vulnerable populations. Rather than limiting the actions of foreign or local researchers, I propose to frame research activities and interaction between international and local researchers through an institution that can provide oversight and accountability. This layer of regulation is especially important when the research is performed with indigenous peoples.

An intermediate research organization can provide research oversight and facilitate intelligent forms of accountability. As O'Neil (2004) emphasized, intelligent forms of accountability are derived from expert opinion and not from audit culture (O'Neill 2004). An intermediate research organization will be composed by a board of trustees and have advisory committees in ethics, biological and social sciences, that can provide expert reviews of research projects. These expert reviews will be able to properly assess research activities and professional conduct. Given the close relation that this organization would have with authorities, researchers can be hold accountable and regulations can be enforced. An important point to make is that embryonic governance structures, like the ones observed in Peru, composed by disjointed regulations from distinct governmental institutions can be a minefield for researchers new in the area. By partnering with intermediate research organizations researchers can be properly guided through rules and regulations, avoiding that researcher's unawareness results in serious transgressions that could jeopardize research enterprises. In the case of Peru, there are multiple institutions that would have some level of jurisdiction over research projects involving indigenous communities. Figure 30 presents different governmental organization who could claim authority over research endeavors. The newly created ministry of social inclusion focuses on addressing issues of marginalized populations, such as indigenous communities and its role in research is still unknown. All intermediate research organizations must be registered with all this instances that should monitor the work of the organization within each of their areas. There should be a registration process for individual projects within the ministries that are relevant to the project to provide with some levels accountability that does not exist in the researcher to researcher relationship. In addition to these national level authorities, there are local organizations, such as the FENAMAD (National Federation of Madre de Dios) or AIDESEP (Interethnic Association for the Development of the Peruvian Rainforest), or regional authorities such as DIRESAS, where projects should be registered as well. The idea of registering research projects in all this instance should not be seen as a mere bureaucratic complication, instead, it is an safeguard for indigenous peoples who will have mechanisms to present any concern or complain about researchers.

Community initiated research

The role of intermediate research organizations is multidirectional. Although it is expected that most of initial projects are researcher initiated (From researcher to the community), the organization will also promote research that is initiated from the community. Engaging communities in research should empower them to actively engage researchers to address their needs and interests.

Community-researcher partnerships should be a bidirectional relationship where communities input can be implemented in researcher's projects but it could also generate new initiatives from the community. This intermediate research organization can partner with communities to identify researchers that can address their goals and

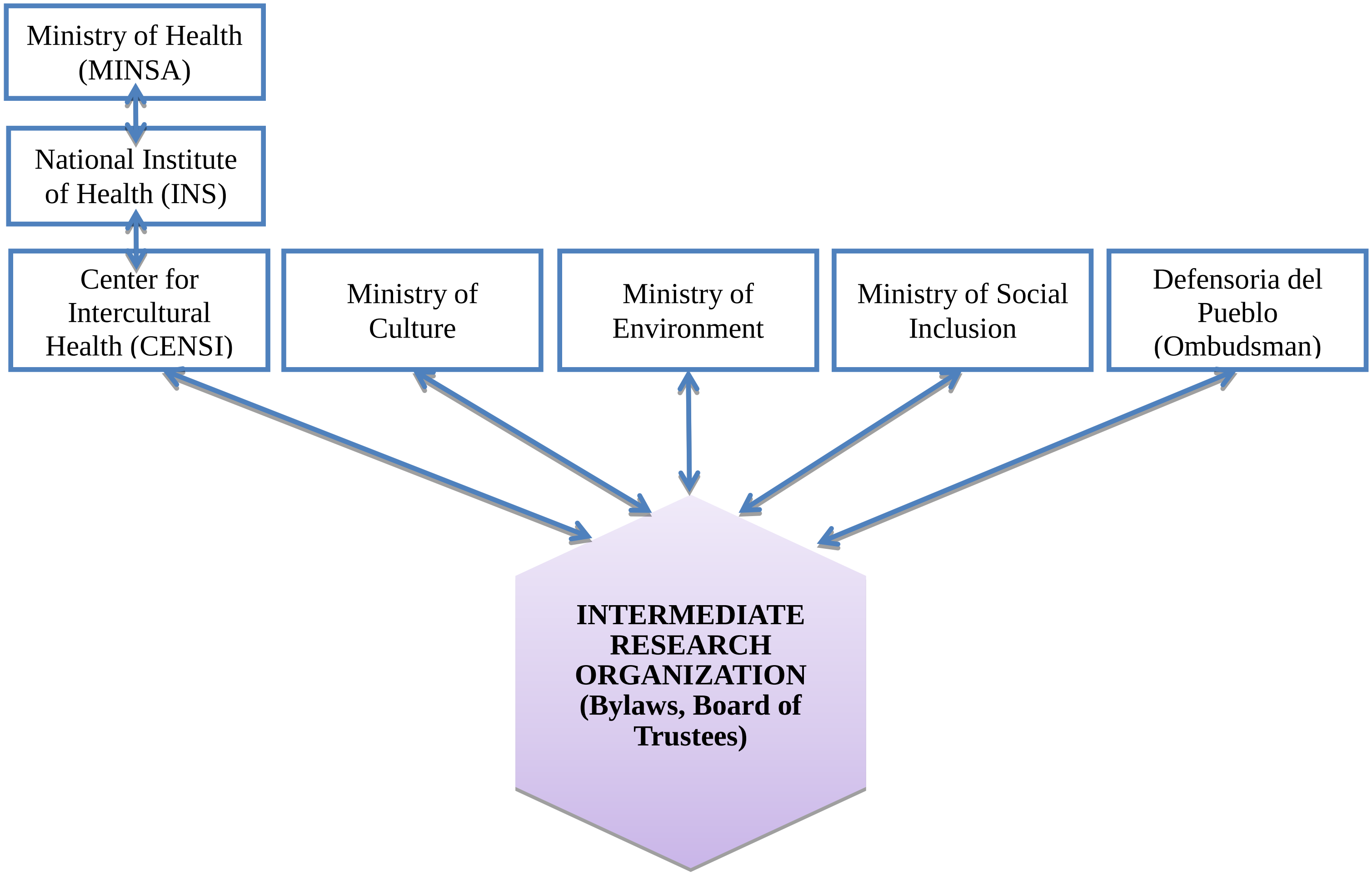

Figure 30. Structuring an Intermediate Research Organization. The board of Trustees would have a core of three members from different backgrounds: a lawyer, a biomedical scientist and a social scientist. These members will have a staggered period of service to ensure continuity.

concerns. The knowledge gain about communities' needs, interests and initiatives will constitute evidence the intermediate organization can use to engage authorities to support the incorporation of these goals in national priorities and identify potential funding opportunities. In other words, the intermediate research organization would facilitate the research enterprise as a whole.

Managing biological materials

Partnership between the intermediate research organization and indigenous communities includes the preservation and administration of their biological samples. One of the biggest challenges associated with access and storage of human biological samples is the establishment and preservation of a relationship of trust with donors (Winickoff and Neumann 2005) . A biotrust model offers an alternative mode of governance of samples that facilitates secondary use within the guidelines defined by the donors.

It is common practice to keep the biological samples of each research study. Unless samples are collected with consent to be stored, they should be destroyed after the research is completed (Godard, Schmidtke et al. 2003). From my experience, research participants consider that scientist can do as they please with their samples not because they are consenting to it, but because once the sample is provided, they lose any control over what can be done on them. In fact, it has been argued that it is impossible to consent to studies unspecified at the time of sample collection (Cambon-Thomsen 2004), suggesting that proper consent for biobanking is unachievable. On the other hand, it has been showed that research participants do not wish to be recontacted for individual consent every time a new research project is using their samples

(Cambon-Thomsen 2004). A viable middle ground can be shaped within the biotrust model.

In the biotrust model, the recipient of samples (the trustee) assumes the responsibility to ensure protection of the contribution and the contributor (Winickoff 2003). Ownership is not transferred; the trustee only administers to maximize benefits for the donors and for the cause for which the samples were provided. A biotrust will be better equipped to monitor what is done with the samples and even negotiate potential benefits for the communities when the samples are used for new endeavors. Through continuous engagement with the community, the biotrust can keep the community informed and adjust to the usage of materials according to community wishes.

The concept of an intermediate research organization here presented is originated from the retrospective analysis of my experience implementing metagenomics research with indigenous populations in Peru. Although still in embryonic state, this model could enable researchers and communities to partner in research projects through a peer-regulated system that, in absence of formal research governance, can provide some level of protection for indigenous peoples while removing roadblocks for scientific advancement. Figure 31 presents a scenario of how the intermediate research organization can streamline the process for researchers while involving the necessary instances to protect indigenous peoples involved in research.

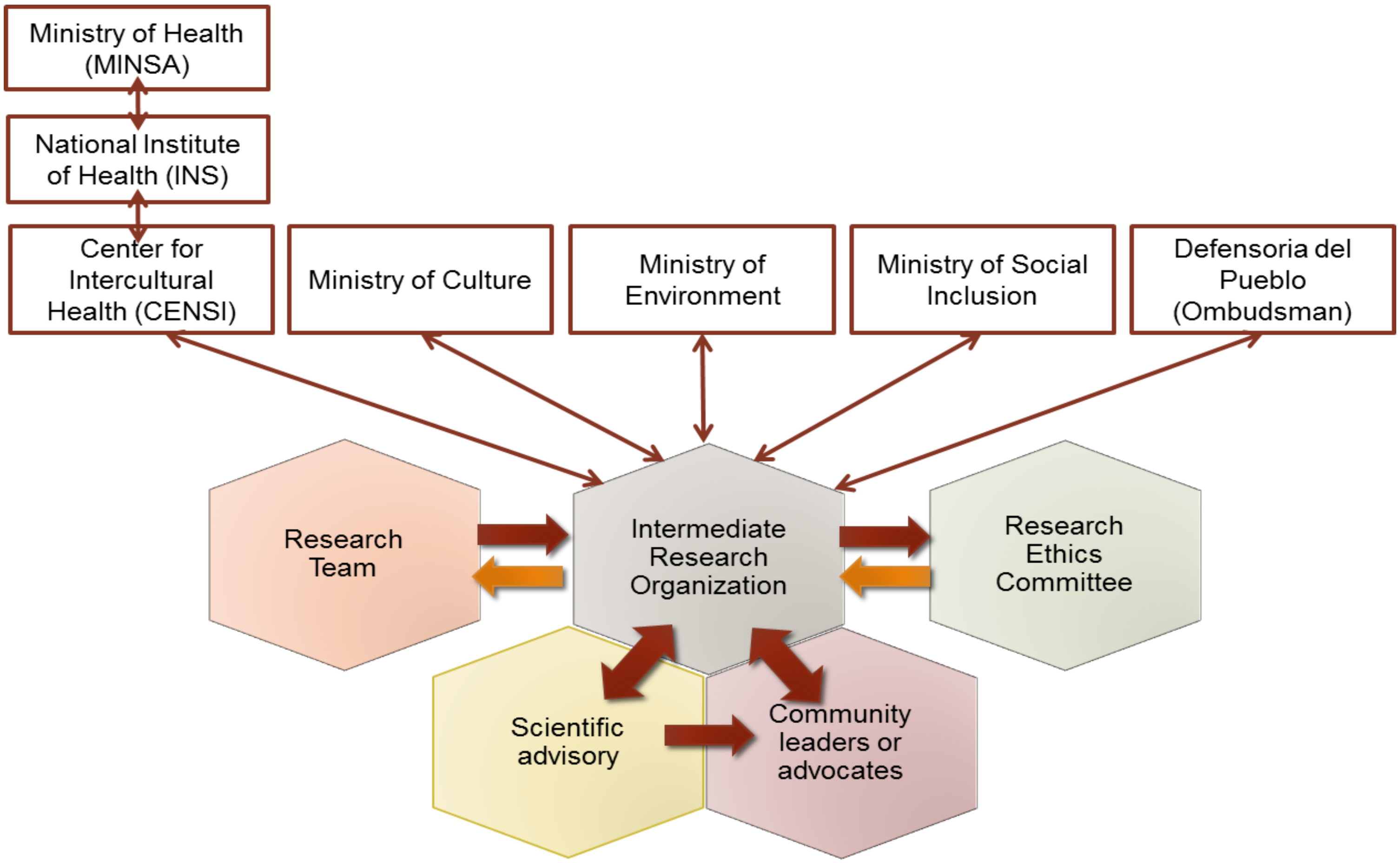

Figure 31. Researcher initiated research. Blue arrows represent the processes in which the intermediate research organization can guide researchers. The thick ones are active constructive processes while the thin ones are focused on compliance and accountability The red arrow represents approvals and authorizations that go back to researchers without further layers of complication.

Conclusion

Excluding indigenous peoples from biomedical research reinforces existing disparities. Researchers' efforts to include these groups might be counteracted by the multiple layers of complications experienced when trying to engage indigenous communities from developing nations. Obstacles to include indigenous populations in research are observed at the community and at the authority level.

Researchers attempting to include indigenous populations from the developing world face distrust from community members but also from national, regional and local authorities. Addressing these issues of trust demands time and funding that might not be available to average researchers. Norms and structures of research governance are been created in the developing world, but these developments are disarticulated and constitute roadblock to science without accomplishing any constructive goal. This reality in developing nations forces researchers to decide between attempting to navigate the system, proceed with less than optimal protocols or abandon the research initiative, resulting in further marginalization of vulnerable populations.

All parties can benefit from coordinated research activities with indigenous populations. Building in research experience, I discuss the need of an intermediate research organization that can catalyze the dialog among stakeholders in the research enterprise. Although it might seem an unnecessary layer of bureaucracy, I argue that in absence of clear norms and guidelines, there is a need for a local mechanism that can enhance researchers' capacity to partner with indigenous populations for research. It should be obvious from figure 31 that individual research groups that aspire to collaborate with vulnerable population are heavily burdened by the complexity of establishing such collaborations and satisfying all potential regulatory restrictions, and such complexity further marginalize the vulnerable populations that these regulations aim to protect. In contrast, the Intermediate Research Organization would enhance researcher's accountability, promote active engagement of communities and coordinate across researchers, communities and authorities. This niche can be fulfilled through non-governmental, non-profit intermediate research organizations which main goal is to facilitate the inclusion of indigenous communities in research.

At is core, an intermediate research organization is a resource to facilitate research while protecting the interests of the communities involved. It is constructed using the concept of a biotrust, in which the intermediate research organization does not claim property over the communities' samples or data, instead, the intermediate research organization acts as a trustee promoting benefits for the donors and for the general public in the context of biomedical research.

Epilogue

Most of curiosity-driven research does not produce any immediate benefit. Basic science is a necessary, but insufficient, step to produce biotechnological development. Development of medical applications depends directly on the industry's investment, often focusing on products designed to meet the needs of privileged communities. These products might not be accessible to all populations; more specifically, biotechnological applications might not be available to lower income and underinsured individuals.

Developed societies are more likely to benefit from research and, based on this premise, biomedical research involving indigenous populations from developing nations can be exploitative. However, direct access to the applications derived from international research is not the only benefit that can be offered to indigenous populations from developing countries participating in research.

Implementing basic research in the developing world represents an opportunity for capacity building and technological transfer. International research collaborations are unlikely to solve the health issues of populations in developing countries, but technological transfer provides an opportunity to catalyze development of national lines of research that will impact the health needs of these populations. While in the past researchers from developing countries were limited by their lack of access to machines and reagents, current scientific scenario is filled with opportunities for outsourcing and collaborations that provide an opportunity for deep and thorough studies, even in absence of an expensive laboratory. However, these opportunities will only be available to scientists who acquire the skills and knowledge that becomes available through active interactions with scientist from developed countries.

It is arguable that technological transfer and building scientific capacity does not necessarily benefit indigenous communities, suggesting the need of another form of direct compensation for these communities. Some projects can include some aims that are relevant and directly benefit the community, such as our addition of a parasite screening as a service for the community. Other projects might need to consider other types of reciprocity. Individual material compensation is a problematic alternative; the exchange of goods for samples might result in some form of coercion in communities that are under extreme poverty. Community material compensation might raise expectations that are unreasonable within the budget of a research project; we had a community that asked for a chapel as compensation. The best approach is to work with the community and their representatives to determine reasonable options to provide effective benefit for the participants.

Establishing an intermediate research organization will be challenged by two key elements: 1) sustaining funding and 2) the distribution of power among the different stake holders in the research enterprise. While these issues might have different variants in the particular realities of each country, they will be critical for the success of this framework. In Peru, I envision the startup funding coming from a non-governmental institution, such as a university, followed by indirect costs from research projects. A more complicated matter is to ensure the right balance in the distribution of power, particularly to provide enough opportunity to have meaningful participation and representation of indigenous populations. More research is needed before a viable answer can be provided.

The bottom line is that scientists will always advocate for science. Unless there is a system that guides the research process with indigenous communities, these populations will be either excluded or at risk of unjust treatment in research endeavors. Intermediate research organizations can provide a middle ground for fair negotiations in an environment of trust and verified information. This neutral approach will facilitate the process for all players, which is especially useful in countries where a solid structure for research governance is still far in the horizon.

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