

ISHOKU DOUGEN – THE MEDICINAL USE OF PLANTS AND CLAYS BY WILD JAPANESE
MACAQUES (*MACACA FUSCATA YAKUI*)

by

CHRISTOPHER DAGG

(Under the Direction of Carolyn Ehardt)

ABSTRACT

There is increasing evidence from behavioral and chemical ecology that non-nutritional resources have a significant effect on the health of wild animal populations, both through deliberate self-medication (zoopharmacognosy) and dietary prophylaxis. This dissertation reports the first systematic investigation of anti-parasitic self-medication in Yakushima Japanese macaques (*Macaca fuscata yakui*).

Adult female macaques of NA2 group on Yakushima were studied for one full seasonal cycle, their health measured through fecal parasitological analysis, and their foraging behavior recorded through focal observation. A set of candidate medicinal plants and clay were selected from literature and preliminary observations, and interactions with these recorded in continuous detail. Statistical analyses were designed to reveal patterns in parasite load, parasitological stimuli for self-meditative behavior and the functional efficacy of those behaviors. Ethnographic interviews were conducted with several practitioners of traditional medicine in Japan, and their knowledge compared with the macaque results.

The macaques were found to be infected with five species of gastro-intestinal parasite, but appear to tolerate the infections with few symptoms. The coarse grass *Miscanthus sinensis* was found to be associated with severe *Streptopharagus pigmentatus* infection, and may act to physically expel this nematode taxon. Geophagy (clay eating) is practiced year-round, but while it may account for the low incidence of diarrhea, it was not found to be directly associated with parasite infection. Instead it appears to function in the detoxification of invertebrate and plant foods. Records of juvenile observation of their parents suggest that these two behaviors may be a cultural tradition in the population. The ingestion of

several other plants seems to incorporate a range of pharmacologically active phytochemicals into the macaque diet. While some are rare and deserve further investigation, others are utilized as food items and any health benefit accrued incidentally to this. Interview data reveal that the traditional pharmacopeia of Japan differs significantly from that available to the macaques, and is proposed to be due to ecological differences between Yakushima and the centers of traditional medicine in Japan. The significance of these results is discussed with regard to conservation medicine, and theories of the emergence of human medicine.

INDEX WORDS: Zoopharmacognosy, macaque, *Macaca fuscata*, Yakushima, geophagy, parasitology, Japan, ethnobotany, conservation medicine, chemical ecology

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B.Sc., University of Edinburgh, Scotland, 1997

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial
Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2009

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DEDICATION

Mook!

ACKNOWLEDGEMENTS

I owe the completion of this dissertation to Angela Hong, without whom I may have never returned. Her support, understanding, love and timely application of internet telephony kept me sane though many a long period in the field, and when faced with the seemingly insurmountable challenges of data analysis and writing.

Perhaps the most long-overdue professional acknowledgement is deserved by Micheal Huffman of Kyoto University, for the encouragement, guidance and constant enthusiasm he provided over the past decade. From my first interest in zoopharmacognosy as an undergraduate to this day, he has consistently gone out of his way to support the process of enquiry, my entry to graduate school, the logistics of research and residence in Japan, and the particular issues of writing up. This is all the more remarkable in that he has never been an official part of my advisory committee, and all his help has truly been above and beyond the call of duty. I am privileged to know him as both colleague and friend.

Heartfelt thanks are also deserved by Laurent Tarnaud, Alexander Hernandez and Alisa Chalmers of Kyoto University for their invaluable support and companionship throughout and following my time in Japan, Shin-ichiro Aiba of Kagoshima University for the kind donation of his climatological data, Professor Hasegawa of Oita University for sharing his parasitological expertise, Sachiko Hayakawa for the kind donation of the fecal sample featured in chapter 3, and the Yakushima Forest Environment Conservation Center, Kagoshima Prefecture and Kirishima-Yaku National Park for permission to conduct research in the conservation area.

I am also sincerely grateful for the cooperation and time of all the traditional pharmacists, herbalists and wholesalers that kindly donated their time to be interviewed as part of my preliminary research in 2003 and during my ethnomedicinal research of 2007, for the work of my translator, Ueda, without whom I would have been forced to impose my terrible Japanese on unsuspecting informants.

I would like to extend my gratitude to all the researchers and students at the Kyoto University Yakushima Field Research Station for their tolerant support and company throughout this field session: Professor Suguira, Professor Yamagiwa, Professor Hanya, Mari Terakawa, Koda Ryosuke, Naoki and Yoshimi Agetsuma, Miki Matsubara. I must also acknowledge the fortunate acquaintance and kindness of Yuko Obata, who found me both my houses on Yakushima.

Finally, and certainly not least, I would like to express my gratitude to the professors who have served on my Advisory Committee: my Major Professor, Carolyn Ehardt, without whom I would not have come to UGA, for her endless patience and support, Brent Berlin for being tolerant of my crazy ideas, Irwin Bernstein for his clarity and rigor of mind, Susan Tanner and Rene Bobe for stepping up to the crease, and also to Alex Brewis who regrettably moved away. Sincere thanks also for the invaluable support of Charlotte Blume, Margie Floyd and LaBau Bryan for keeping me right through the maze of academic and immigration beurocracy.

This study was funded in part by National Science Foundation, Physical Anthropology Dissertation Improvement Award #BCS-0525156 (2006-7 field season) and preliminary research by NSF Ethnographic Research Training Grant #BCS-0091764 (summer 2003).

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CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Introduction

“The plant world is not colored green; it is colored morphine, caffeine, tannin, phenol, terpine,
canavanine, latex, phytoheamagglutin, oxalic acid, saponin, L-dopa etc.”

(Jansen 1978:73)

Primates interact with their ecological surroundings on multiple levels. Their community role as consumers of, and competitors for fruit, seeds and invertebrates has attracted great attention, eliciting their role in trophic webs and as dispersers of seeds (e.g., Lambert and Garber 1998; Otani and Shibata 2000; Tutin *et al.* 1997). Behavioral ecologists have published widely on the complex social behaviors of primate groups, and the adaptation of these behaviors to available ecological niches, including their innovative use of natural materials as tools to exploit otherwise inaccessible resources (e.g., Biro *et al.* 2003; van Schaik, C. P. , Fox *et al.* 2003; Whiten 2000; Wrangham, R.W. 1983). Less visible, but equally fundamental ecological processes occur at the chemical and micro-community level. The interaction between organisms' internal biochemistry and the chemical milieu of the surrounding environment is arguably more significant than that which occurs on a macro scale. Rather than easily measured packets of nutrition, plants are a complex cocktail of poisons and attractants requiring careful discrimination, detoxification and cost/benefit evaluations by the forager.

That some complex chemical interactions occur across multiple trophic levels formed a pioneering perspective on secondary chemical ecology by Jansen (1978), who raised the possibility that secondary compounds ingested in the foraging of herbivores may actually protect them from certain pathogens. Although foraging is still primarily defined by nutritional needs, it is now becoming

increasingly accepted that some aspects of diet selection seem to have evolved in response to disease stress.

The bodies of macro-organisms are themselves host to whole communities of micro-organisms, ranging from the benign to the overtly pathogenic. The relationship between a host and its microscopic inhabitants is complex and depends greatly on the internal physiological conditions - a harmless symbiont often having the potential to become a pathogen due to a change in gastrointestinal flora, blood pH, or nutritional state. However, an almost ubiquitous relationship in internal ecology is parasitism, whence organisms rely on exploiting the resources of their host's body to grow and/or reproduce. Many parasitic infections are well tolerated, while others can cause a range of debilitating or potentially fatal symptoms such as diarrhea, malnutrition and septicemia.

Mammals, including primates, have evolved many mechanisms to reduce the impact of parasitic infections on host fitness. Amongst behavioral strategies to reduce exposure to infectious agents, such as ranging patterns and grooming, and to aid recovery, such as anorexia and depression (Lozano 1998), there has emerged evidence of the exploitation of external chemical resources (from plants, minerals, etc.) to actively combat parasitosis – behavior falling within the broader category of zoopharmacognosy, or self-medication (Huffman 2001). That this phenomenon encompasses the use of invisible chemical ecological interactions and microscopic or internal infectious agents, accounts for its relatively late emergence into mainstream primate ecology. The superficial similarities with human traditional medicine and folklore also resulted in early anecdotal evidence of such behavioral strategies being tarred with accusations of romantic anthropomorphism (Sapolsky 1994). But as evidence of their validity accumulates, the potential significance of this phenomenon is becoming more widely accepted.

This topic has current application in the emerging interdisciplinary field of Conservation Medicine (Koch 1996). Infectious disease is becoming widely recognized by conservation biologists as a significant threat to endangered populations, especially in the face of habitat fragmentation and degradation, and the proximity of humans and their livestock (Epstein *et al.* 2003; IUCN 2003). In addition to direct veterinary intervention, which is time consuming and expensive, ecological mechanisms

of disease control are becoming recognized as valuable tools in conservation and reserve design (Deem *et al.* 2001). A comprehensive knowledge of self-medication, its acquisition, maintenance and essential resources, has the potential to be an invaluable tool in conservation planning and reserve design.

Overview of Dissertation Research

The aim of this dissertation was to investigate the role of self-medication as a functional response to nematodal endoparasite infection in wild Japanese macaques (*Macaca fuscata*), and to compare any putative self-medication to established traditional medicine practices in Japan. Thus far, detailed studies of self-medication are biased in favor of the great apes, although evidence is emerging of surprisingly widespread adoption of such behavior (Engel 2002). Despite the long scholarly tradition of Japanese primatology, existing self-medication research in Japanese macaques has been restricted to geophagy in Arashiyama monkey park, which was convincingly shown to be a response to indigestion caused by provisioning (Wakibara *et al.* 2001). Although there are several plant species of significance in East Asian medicine among the recorded dietary range of Japanese macaques, there has been no direct investigation of their biochemical role, other than nutrition. This research provides the first systematic investigation of phytochemical self-medication in this species, as well as the first explicit test of an anti-parasitic function for geophagy in Japanese macaques.

The following hypotheses guide the research herein:

H1. Ingestion of candidate plant species is stimulated measurably by helminthic endoparasite load.

H2. The frequency of geophagy in Japanese macaques is stimulated measurably by helminthic endoparasite load.

H3. Observed ingestion of candidate plants constitutes an effective self-medicative response to parasitosis.

H4. Observed ingestion of clays constitutes an effective self-medicative response to parasitosis.

H5. The self-medicative pharmacopoeia of Japanese macaques will be similar to that used by traditional medicine in Japan.

Field Site and Subjects

These hypotheses were tested on *Yaku* Japanese macaques (*Macaca fuscata yakui*), a subspecies endemic to the island of Yakushima (30°20'N, 130°30'E), 60km from the southwestern tip of Kyushu, Japan. This island marks the southernmost limit of the Japanese macaque range. The 55,000 ha island is part of the Kirishima-Yaku National Park, with 11,000 ha also designated as a UNESCO World Heritage Site, primarily on account of the sub-tropical to sub-alpine forest range and ancient Japanese cedar trees. Habitat zones are highly diverse, ranging rapidly from rocky beaches, through steep mountainous forest up to bamboo above 5300 ft. The climate of Yakushima is warm and wet, with rainfall averaging 2600 mm/year at sea level and a mean monthly temperature ranging from 12°C in February to 28°C in August (Min = 2.5°C, Max = 36°C during September 06 – August 07).

The steep coastal forest in the northwest of Yakushima (*Seiburindo*) is home to approximately 20 troops of *Yaku* macaques and is designated a Special Wildlife Protection Area. Entry to the forest requires permission from the Yakushima Forest Environment Conservation Center, Kagoshima Prefecture and Kirishima-Yaku National Park Office. Groups inhabiting this area have been subject to observational research since 1975, when Tamaki Maruhashi first habituated a troop (Maruhashi 1980), and there is a permanent field research station nearby in Nagata village, maintained by Kyoto University. In a departure from the more usual practice in Japanese primatology, provisioning of macaques on Yakushima is strictly prohibited, although many troops remain habituated to observers. These conditions present a valuable opportunity for behavioral observations of Japanese macques in a natural environment, and with their social behavior and foraging patterns unaffected by human intervention (Hill 1999).

The forest in this area consists of primary and secondary, warm-temperate, broad-leafed trees. Dominant species include *Lithocarpus edulis*, *Ardisia seiboldii* and *Castanopsis seiboldii*; some deciduous species, such as *Ficus erecta* and *Mallotus japonica*; and more typically tropical species such as *Ficus microcarpa* (Otani and Shibata 2000). Macro-fauna is limited to *Yaku* macaques, *Yakushika* deer (*Cervus nippon yakushimae*), Japanese weasels (*Mustela itatsi*), and *Tanuki* raccoon dogs (*Nyctereutes procyonoides viverrinus*) which were introduced to the island *ca.* 1994 (Sato *et al.* 2006).

Data Collection and Analyses

In order to detect potentially anti-parasitic self-medicative behavior by *Yaku* macaques, data collection and field methodology focused on both the foraging behavior and parasite ecology of the animals. Preliminary observations were conducted in August 06, during which the Nina- A2 troop (NA-2) was selected, and individuals identified as part of the annual census. This troop consists of approximately 40 macaques, and varies in size due to intergroup transfer by adult males, and the birth of infants. Their home range extends across approximately 71 ha, encompassing a region from sea level to 200m in altitude (**Appendix B1**).

Behavioral observations were conducted on 10 adult female macaques, between September 5th 2006 and August 29th 2007, comprising 191 days of all-day focal follows, and totaling 1373 hours of observation, and a mean of 7.2 hours/day. Daily observation duration varied due to weather conditions, and the time required to locate the focal animal. Each individual was followed for two days each month, when conditions allowed, and an attempt was made for these two days to be concurrent, although this was only achieved on 50 occasions (100 days). During the focal follows, general activity and foraging behavior were recorded as point samples with a 10 minute interval, at which time any plant species and parts being ingested were recorded. Invertebrates and fungi were not identified by taxa but were, instead, subdivided by substrate location (arboreal/terrestrial/on or in fallen wood).

From the previously published range of 135 plant species known to be eaten by *Yaku* macaques (Hanya 2003, 2004; Hanya *et al.* 2003; Hill 1997; Otani and Shibata 2000), a set of candidate plants and materials were selected that were most likely to be involved in self-medicative behavior, allowing more detailed recording of macaque interactions with these items. The plant species *Melia azedarach*, *Rhus succedanea*, *Trema orientalis*, and *Zanthoxylum ailanthoides* were selected as candidates due to their appearance in traditional east-Asian pharmacopoeias (Dash 1991; Duke and Ayensu 1985; Li 2006), published phytochemical and pharmacological analyses (Cheng *et al.* 2005; Cheng *et al.* 2003; Duke 1996; Jassim and Najji 2003; Lee *et al.* 2004; Morita and Shimizu 1966; Tokita *et al.* 2007; Wu *et al.* 2002), and zoopharmacognosy literature (Huffman and Wrangham 1994). Clay and/or soil was also

designated as a candidate item due to the existing literature that suggests several non-exclusive medicinal hypotheses for its ingestion by humans and animals (review in Krishnamani and Mahaney 2000). In addition, *Miscanthus sinensis* leaves and *Lagerstroemia fauriei* bark were included as candidates based on behavior recorded during preliminary observation of NA-2 troop. The method of ingestion by the macaques of both these items was suggestive of a non-nutritional function, and justified their inclusion as candidate medicinal materials following the guidelines of the C.H.I.M.P.P. (Chemo-ethology of Hominoid Interactions with Medicinal Plants and Parasites) group for zoopharmacognosy research (Huffman, 1996).

For all candidate items, interaction by the focal macaque was recorded *ad libitum* until the animal moved out of arm's reach of the material. Recorded variables included: location; proximity time (focal animal within reach of plant, in seconds); the duration of focused manipulation, mastication and ingestion of the material (in seconds); part ingested; number of mouthfuls (when clearly seen); and the occurrence of 'intense observation' by infants and juveniles (Tarnaud and Yamagiwa 2008).

The prevalence and severity of endoparasitic infections were recorded through analyses of fecal samples collected from the focal animals twice daily. Samples were either analyzed on the evening of collection or refrigerated for up to 24hrs. This avoided the use of preservatives, and allowed the observation of live, motile larvae. Quantitative analysis was conducted using a McMasters floatation technique to detect nematodal ova, while qualitative identification of larvae and non-floating ova was carried out through wet-mount examination of a saline-feces suspension. Further samples were desiccated to determine water content, as a measure of diarrheal symptoms. The presence, species and abundance of any adult parasites or undigested leaf matter in the feces was noted, as was the overall consistency of the feces (wet/med/dry). Details of these procedures are described in **Chapter 2**.

Parasitological data were analyzed for patterns of prevalence and fecal abundance relating to climatic and ecological variables, and the age and social rank of individual focal macaques. These same data were then analyzed in comparison with observed plant and clay use. A significant daily association, across the year of observational records, between fecal parasite abundance and the ingestion of candidate

material, was seen as suggestive of a parasitological stimulus for ingestion for that plant or clay (Hypotheses 1 and 2). Records obtained from two successive observation days revealed the short-term dynamics in parasite fecal abundance and symptoms, and a reliable association between reduced parasitosis and plant or clay ingestion was seen as suggestive of an effective antiparasitic function by that material (Hypotheses 3 and 4). Details of these statistical procedures are elucidated in **Chapters 3 and 4**.

Following the field observation period, ethnographic interviews were conducted with practitioners of traditional medicine, both on Yakushima and in the cities of Kyoto and Nara, where there remain centers of traditional, family-owned herbal pharmacies. With the assistance of a translator from Kyoto University, pharmacists were approached, and after signed consent, were asked to identify and discuss the use of the candidate plants central to the macaque analyses. The degree of convergence between macaque plant use and traditional medicine was explored both qualitatively, and by coding responses to allow quantitative analyses (Phillips and Gentry 1993). During the summer of 2003, similar interviews were conducted as part of an ethnographic research training study. These earlier interviews focused solely on the use of clays and other minerals in traditional *Kanpō* medicine in Kyoto and Nara. Details of interview protocols and analyses concerning clays are described in **Chapter 4**, and regarding plants, in **Chapter 5**.

Outline of Subsequent Chapters

Chapters 2-4 of this dissertation consist of pre-publication research articles derived from the 13 months of field observations of macaques on Yakushima, Japan. The first (**Chapter 2**) is the first long-term survey of parasitic infection and health in Yakushima macaques. In addition to the detection of parasite taxa, as previously published by Gotoh (2000), this study used frequent fecal sampling of known individual macaques to provide testable data on the relationship between parasite prevalence, abundance, symptomatology, seasonal climatic cycles, individual age and social rank. This broad approach allows the identification of ecological and epidemiological risk factors in the macaque population, and provides a baseline for the investigation of self-medicative hypotheses.

The second paper (**Chapter 3**) investigates the use of medicinal plants by the Yakushima macaques. Candidate medicinal plants were identified both from ethnomedicinal literature, and from behavioral indications of non-dietary functions. Detailed focal behavioral observations of one social group were undertaken over a full year, and all incidences of putative medicinal behavior were investigated in the light of the parasitological data shown in the first paper. The ingestion and defecation of whole leaves of the grass *Miscanthus sinensis* is presented as an antiparasitic strategy against infection by *Streptopharagus pigmentatus*.

The final paper (**Chapter 4**) looks specifically at geophagy as a medicative behavior. This phenomenon is treated separately from the plant study due to the multiple non-exclusive hypotheses that exist to account for its occurrence in non-human primate species. One year's data were collected in parallel to the plant study in Chapter 3, and analyzed in relation to parasite dynamics and dietary variations, seasonal and daily. The primary hypothesis tested is that the Yakushima macaques engage in the ingestion of clay and earth to alleviate or counter parasite infection, and its pathological symptoms. In the absence of a clear parasitic stimulus for the behavior, functions relating to the detoxification of diet and alleviation of gastrointestinal distress are explored.

Chapter 5 discusses observations of the candidate plant species that did not display a significantly distinct antiparasitic function in the test population, but remain variously intriguing due to the ingestion of pharmacologically active parts. Ethnographic interviews were carried out with traditional medicine professionals on Yakushima and elsewhere in Japan, their knowledge and use of the candidate plant species analyzed and compared to observed macaque behaviors. The significance of the biochemical properties ingested from these species is discussed with relation to dietary prophylaxis and conservation.

Chapter 6 presents the key findings of the previous sections, how they relate to the existing body of knowledge and address how future research might further develop this area.

As an introduction to the results presented in the subsequent chapters, the remainder of this chapter will provide background on the current state of knowledge regarding zoopharmacognosy. This includes a brief review of the theory of chemical ecology, previous field studies on primate self-

medication, the acquisition and maintenance of such behavior in primate groups, and finally a discussion of practical applications of this topic.

Literature Review

医食同源

Ishoku Dougen

“Food and medicine are of the same origin” (Japanese proverb)

Chemical Ecology

An appreciation of the complexities of chemical ecology, in particular the interaction between plant and herbivore, has been growing significantly over the past 30 years. The nature of some of the subtle dynamics, however, has been noted in much earlier literature, such as Paracelsus (1493-1541), who wrote “*All things are poison and nothing is without poison, only the dose permits something not to be poisonous*” (in Krieger 2001:xxvii). Early 20th century theories were pioneered by entomologists, in particular through the study of butterflies. Dethier (1941) pioneered the appreciation of volatile chemical attractants and deterrents in the choice of food plants by *Papio* spp. larvae. He concluded that the adoption of a novel genus of acceptable food plants was mediated by there being analogous chemical attractants in the essential oils of new taxa, and as such the distribution of *Papio* spp. butterflies is determined largely by the distribution of this chemical family. By 1964, this perspective was consolidated with the newly coined concept of *coevolution*, and the chemical arms-race between plants and herbivores, particularly insects, appreciated as a significant source of biological variation in terrestrial communities (Ehrlich and Raven 1964). It was soon realized that the strategies of this conflict encompassed a second front, since herbivorous insects themselves were prey to mammals and birds. Brower and Brower (1964) first suggested that many insects unpalatable to predators derived 'poisonous properties' from their food plants. Isolation of cardiac glycosides in Monarch butterflies by Parsons (1965) supported the theory that plant toxins may be sequestered in insect tissues and act as a toxic deterrent to bird predation. The ecological irony is striking, since this strategy is of adaptive benefit to the butterfly despite the chemical deterrent itself having originated in the plant, presumably to reduce leaf loss due to such phytophagous

insects. Brower and Glazier (1975) took the research further, to identify that the highest concentration of glycosides tended to be found in the wings, usually the first part in contact with an avian predator. This would allow a captured butterfly to potentially escape, injured but alive, from a bird deterred by the bitter taste of the glycoside. This mechanism allowed an evolutionary mechanism for the strategy without recourse to group or kin selection.

Regarding mammalian herbivores, the prevailing discussion of secondary plant compounds at that time was still focused on simple avoidance and detoxification of harmful or deterrent chemicals (Freeland and Janzen 1974). Soon after, however, a conference on the ecology of arboreal folivores (Montgomery 1978) compelled one of the previous authors to acknowledge the many complexities of plant-mammal chemical ecology, and in so doing, presented a seminal paper that first suggested the possibility of animal self-medication within an established theoretical framework (Jansen 1978). He discussed the difficulty in identifying the adaptive 'target' of a plant secondary chemical (such as fruit tannins once assumed to deter mammalian seed predation, which may in fact reduce fungal spoilage, thus allowing *increased* mammalian ingestion, and further seed dispersal). 'Toxicity' itself is given a relative definition by Janzen (1978), as a situation where the resources needed to process the chemical are inadequate or greater than the nutritional benefit to be gained. Such trade-offs may be common in periods of food scarcity, when a degree of damage is acceptable to gain adequate nutrition. As a logical extension of this analysis, Jansen suggested that mammals may be able to associate positive physiological effects with the ingestion of plant parts, much as they are able to develop specific aversions based on nausea. He cites from older anecdotal records of putative antiparasitic foraging behavior by wild boar (Dharmakumarsinhji 1960) and rhinos (Hubback 1939), and of elephants ingesting a stimulant vine (Hubback 1941).

Investigation of the specific complexities of primate-plant chemical interactions developed over the following years. Glander (1982) considered primate diet in terms of foraging strategy, differentiating between specialist (highly efficient detoxification) and generalist (low dosage of any particular antifeedant) species, representing two equally effective adaptations to the challenges of plant toxins. He also noted that food may not be as evenly distributed as it may seem to a human observer, since the

cost/benefit toxicity of foodstuffs may vary with maturity, season and soil type. Oates *et al.* (1990) tested some of these interactions in a study of colobine monkeys in Tiwai, Sierra Leone. Despite the nutrient-poor soil, and the corresponding high tannin content of leaves, the area supported an unusually high biomass of these herbivorous species. The authors proposed that rather than tannins being a limiting factor in leaf digestibility, as was often previously assumed, the ratio of protein to fiber was more significant, and this was determined by multiple factors of plant diversity, species composition and disturbance. Mowry *et al.* (1996) formed similar conclusions from colobus monkeys in Tana River, finding no significant relationship between plant secondary compounds (phenolics, condensed tannins) and feeding behavior. Again, the protein/fiber ratio seemed more significant, and it may be that the multi-chambered stomachs of these leaf specialists serve as much to denature plant secondary chemicals as they do to digest cellulose. In contrast, a study of sympatric chimpanzee and cercopithecine populations, both ostensibly frugivorous and with simple stomachs, showed dietary niche differentiation in terms of secondary compounds (Wrangham *et al.* 1998). Monkeys had higher overall intakes of tannins, monoterpenoids and triterpenoids than chimpanzees, possibly facilitated by a more generalist (low dosage) foraging strategy. During periods of ripe fruit scarcity the diets of the two groups diverged further, but the relative intake of antifeedants by monkeys compared to chimpanzees was unchanged, the range of acceptable fall-back food items continuing to be determined by their chemical foraging niche. Most subsequent studies focus similarly on alkaloids and phenolics, an abundant group but only encompassing a small degree of the variety of plant secondary chemistry. More recently, Welker *et al.* (2007) accounted for howler monkey leaf selection in terms of sesquiterpenes, the class of phytochemical meticulously avoided by chimpanzees while pith-chewing *Vernonia amygdalina* (Ohigashi *et al.* 1994).

Timothy Johns, meanwhile, applied a chemical ecology perspective to human diet and the origins of medicine. His early work with Andean potato glycoalkaloids and taste evaluation allowed the combination of neurophysiological mechanisms (akin to the previous non-human studies) with cognitive memory and biocultural evolution (Johns and Keen 1986). This study revealed cultural differences in the acceptability of bitter glycosides, and a domestication process that selected a compromise between

palatability and frost tolerance. Johns' book, originally aptly titled 'With Bitter Herbs they Shall Eat It' (Johns 1990), expanded his thesis to explore the role of non-nutrient components in many traditional diets and the origins of human medicine. He suggested that the loss of broad spectrum foraging with the onset of low diversity, starch-based agriculture may have deprived populations of many therapeutic phytochemicals, and necessitated the development of a separate cultural system of plant based medicine. His subsequent works (Johns 1999; Johns *et al.* 2000) concentrated on the medicinal properties of nutrient and non-nutrient components of traditional diets, including the hypocholesterolemic benefits of bitter barks added to Masai meat soups, and the role of exogenous antioxidants, especially in the protection of polyunsaturated fatty acids (PUFA) vital to brain development. He suggests that an appreciation of traditional dietary components may reduce chronic disease incidence in the 'developed world' and ease the impact of integration of indigenous groups into modern economic systems (Johns 1999). Related work, revealing widespread overlap in the concepts of “diet” and “medicine,” has been carried out by Etkin and Ross (1982), who noted that a remarkable 96% of plant species identified as food by the Hausa of Nigeria are also used as medicine, with up to 55% of these being gastrointestinal medicines, depending on the season. This and other similar observations led the authors to call for a more holistic methodological perspective in ethnobotany – focusing on ‘exposure’ to ‘ingestibles’ rather than dosages of medicines or portions of foods (Etkin and Ross 1991). Subsequent studies in other geographical areas support this perspective, with 82% of Korean temple foods having medicinal uses (Kim *et al.* 2006) and non-cultivated vegetables eaten by ethnic Albanians in southern Italy providing a significant source of antioxidants, possibly contributing to the renowned Mediterranean longevity (Pieroni *et al.* 2002). Many widely used spices and condiments in Asian cuisine contain significant anti-tumor agents, and may help combat parasitic and viral infections (Murakami *et al.* 1994; Ohigashi *et al.* 1992). Similarly, the Japanese proverb *Ishoku dougen* literally translates as “food and medicine are of the same origin” (Huffman *et al.* 1998).

Gerber *et al.* (1999) returned to the theoretical perspective of Paracelsus in exploring the complex dynamics that allow the aforementioned overlap between food and medicine. They explore the “nutrient-

toxin continuum” wherein several different cost-benefit functions may determine the relationship between dosage and potential fitness benefit, including *hormetic* substances that are beneficial at low dosages but toxic at greater concentrations. The authors speculate that natural selection would be expected to optimize dosage according to the cost-benefit dynamic, through innate mechanisms, ontogenic processes that allow learning based on exposure, and culturally maintained knowledge. While many plant antifeedants taste unpleasantly 'bitter' to us, the specificity and strength of aversions or preferences in humans and other species are subject to coevolutionary selection and ontogenic modification. They have been shown to vary between species' dietary strategies (Laska and Salazar 2004; Laska *et al.* 2003), particularly the degree of herbivory (Glendinning 1994; Nolte *et al.* 1994). Within taxa, ontogenic effects have been attributed to local differences in food chemistry (Hladik 1993; Simmen 1994) and with learned pharmacological benefits, such as the stimulant effect of coffee (Mattes and Beauchamp 2000).

6-*n*-propylthiouracyl (PROP) has been used widely as an experimental representative of 'bitter' substances (Chang *et al.* 2006; Mattes and Beauchamp 2000; McAnally *et al.* 2007; Prutkin *et al.* 2000; Verma *et al.* 2006) and remains the only recorded example of true taste polymorphism in humans (Drewnowski 2001). There is, however, a wide range of chemical groups that elicit the bitter response, both organic and inorganic. These have been categorized into the families of amines, ureas/thioureas, amino-acids, alkaloids, acetylated sugars, isohumulones, phenols, carbamates and ionic salts (Delwiche *et al.* 2001). Bitter perceptive sensitivity to one of these families does not necessarily predict sensitivity to another. Delwiche *et al.* (2001) identified at least four groups of bitter chemicals within which sensitivity tended to correlate strongly. Further evidence of 80-100 distinct protein receptors for 'bitter', and taste cell specificity for particular bitter compounds (Brown 2001), suggests that there may be many distinct bitter neuro-sensory pathways, able to distinguish between chemical families, and thus providing a highly sensitive and flexible sensory evaluation of ingested items.

Self-Medication: Phytotherapy

By far the most intensively studied case of primate pharmacological self-medication is that of chimpanzees and their use of the plant *Vernonia amygdalina*, known in English as ‘bitter leaf,’ and aptly so due to its strong unpleasant taste (Koshimizu *et al.* 1993). In 1987, Huffman and Seifu observed an apparently sick female chimpanzee at Mahale, Tanzania, suck the pith of young shoots of this plant (Huffman and Seifu 1989). *Vernonia* species occur throughout tropical sub-Saharan Africa, and pith-chewing of this genus has since been observed in chimpanzees at Gombe National Park in Tanzania, and in the Hilali Reserve and Kahuzi-Beiga National Park in the Democratic Republic of Congo (DRC) (Huffman 1997). Chemical analysis has revealed that the plant contains the sesquiterpine lactones *vernodalol* and *vernolide*, as well as the steroid glucoside *vernoneside B₁* (Koshimizu *et al.* 1994). All of these constituents show egg-laying inhibition and reduction in adult movement of *Schistosoma* parasites (Ohigashi *et al.* 1994). The choice of ingested part is very specific, the young shoots being meticulously peeled of bark and leaves to expose the pith, from which the juice is sucked (Huffman and Seifu 1989). Interestingly, the pith contains negligible amounts of the more toxic sesquiterpine lactones, while retaining an effective dose of the steroid glucoside component (Koshimizu *et al.* 1994). In one case of detailed observation, the fecal abundance of nematodal *Oesophagostomum* sp. was noted to drop from 130epg to 15epg in 20 hours after ingestion of *Vernonia* pith (Huffman *et al.* 1993). Although the plant is available throughout the year, observations of bitter pith chewing are highly seasonal, mainly occurring in the rainy season during November - January. This time corresponds to the greatest infection rates of chimpanzees by *Oesophagostomum* sp., further supporting the situation specific, therapeutic nature of pith chewing (Huffman *et al.* 1997). Analogous behavior has been observed in the Tai National Park in the Ivory Coast, where chimpanzees chew bitter pith from *Paliosota hirsuta* and *Eremospath macrocarpa*, although no data on parasite load or phytochemical activities have yet been published (Huffman 1997). At Kahuzi-Beiga National Park, gorillas have also been recorded ingesting pith and stems of several *Vernonia* species, but again, no parasitic or phytochemical data have been published to evaluate this behavior as potential self-medication (Yamagiwa and Basabose 2006).

Another well-published self-medicative phenomenon illustrates that the physical properties of plant materials may be utilized similarly to secondary chemicals. Wrangham and Nishida (1983) recorded an unusual feeding behavior in the chimpanzees at Gombe National Park and in the Mahale Mountains. Unlike the usual rapid and continuous ingestion of leaves during feeding, leaves of *Aspilia* spp. were eaten singly and slowly, without chewing. They were often rolled around and folded within the mouth before swallowing, and fecal examination revealed entire leaves with little damage and no evidence of chewing. At Gombe, the feeding appeared to be restricted to the first hour after dawn on empty stomachs, even if there were no leaves near to the nesting site, and the behavior was most frequent at the onset of the rainy season (Wrangham and Nishida 1983), suggesting a relationship between the behavior and the risk of parasite infection. Equivalent behavior has been observed in the ingestion of 30 plant species, including *Rubia cordifolia*, *Trema orientalis* and *Lippia plicata* in several groups of chimpanzees (Huffman 1997), and *Manniophyton fulvum* by bonobos (Dupain *et al.* 2002). Inter-population variance in species preference seems not to be determined by ecological availability, but rather constitute local traditions (Dupain *et al.* 2002).

Chimpanzees at Kahuzi-Biega National Park were found to be swallowing leaves of *Commelina ceciliae* and *C. difusa* (Basabose 2002). Entire leaves were found in only 2.19% of fecal samples, indicating that it is a relatively rare behavior, but were observed in 42 of 78 months in this study. More recently, 4% of fecal samples collected from chimpanzees (*Pan troglodytes vellerosus*) in Nigeria have been found to contain whole unchewed leaves of *Desmodium gangeticum*, and 2% an unidentified coarse grass species (Fowler *et al.* 2007). These samples were only found during the rainy season, and on two occasions, whole adult *Oesophagostomum stephanostomum* worms were found adhered to the surface of the leaves. The occurrence during the period of greater infection risk, and the physical association of expelled worms are highly suggestive of an antiparasitic function.

Initial hypotheses on the therapeutic mechanism of leaf-swallowing concerned buccal administration of phytochemicals, based on ethnomedicinal data, but these analyses proved equivocal, as the active constituents were either too variable or failed to display adequate activity (Huffman and

Wrangham 1994; Messner and Wrangham 1996; Page *et al.* 1997). All swallowed leaves, however, shared a physical characteristic of rough, bristly leaves (Huffman 2001). The presence of whole intestinal worms, particularly *Oesophagostomum* sp., adhered to the leaves found in the feces, suggested the currently accepted hypothesis, which posits that the roughness of the whole leaves increases gut motility and concurrently encourages the emergence of encysted L4 larvae from the proximal hindgut, whereupon larvae and adults are rapidly expelled in the feces (Fowler *et al.* 2007; Huffman and Caton 2001). The frequent utilization of this behavior during periods of high infection risk may effectively reduce worm burdens for the forthcoming season, indicating a tangible health benefit and an adaptive coevolutionary strategy by the chimpanzees to a health stressor to which they have little immunological defense.

A review of wild and sanctuary-living apes found that whereas *Oesophagostomum* infection often causes severe morbidity and mortality in captive animals (during the 1960's up to 80% of captured infant gorillas succumbed to oesophagostomiasis (Cousins and Huffman 2002)), wild populations, while similarly infected, show few debilitating symptoms (Krief *et al.* 2008). The authors suggest that access to suitable self-medicating materials in the wild, such as rough, *Aspilia*-like leaves, effectively mediate worm loads and reduce pathogenic effects. The proximal stimuli for the behavior (symptoms of parasitosis) may not be specific, however, and in some cases may be maladaptive. Wrangham (1995) recorded the use of leaf-swallowing by Kibale chimpanzees in association with tapeworm (*Bertiella stuederi*) infection. In this case, the expulsion of protoglottids in feces is part of the tapeworm's reproductive strategy and may in fact increase environmental contamination and infection risk.

A similar explanation has been proposed for the ingestion and defecation of large seeds by tamarins in Panama (Garber and Kitron 1997). Given their small body size, it seemed unusual that these tamarins pass large numbers of seeds measuring 11mm across, and the authors suggested that the seeds may function to dislodge or damage spiny-headed worms (*Prosthenorchis spp*) and their nodules. However, no evidence was offered of statistical association between parasite load and seed-swallowing, or of any effective therapeutic effect, and so this hypothesis remains putative.

The Awash River valley in Ethiopia is populated by anubis and hamadryas baboons which show a marked difference in their feeding preferences above and below the Awash Falls (Phillips-Conroy 1986). The tree *Balanites aegyptiaca* is found in both areas, but its leaves and fruit have only been observed to be eaten by the baboon group in the canyon below the falls. This part of the river flows more slowly than the upper areas, allowing inhabitation by *Biomphalaria* snails which serve as intermediate hosts for the *Schistosoma* trematode. The fruit of *Balanites* spp. contain a toxic saponin, diosgenin, known locally as a molluscicide. The saponin has been demonstrated to be toxic to *Schistosoma* and may affect the maturation of adult worms in the baboons (Phillips-Conroy 1986). This prophylactic hypothesis, however, has not been confirmed through direct epidemiological studies, and subsequent studies of diosgenin activity in mice actually demonstrated that ingestion of the saponin exacerbated the schistosomal infection (Lozano 1998).

Sifakas (*Propithecus verreauxi verreauxi*) all consume a similar yearly amount of dietary tannins from their most common plant foods, and the monthly intake varies with fluctuations in the tannin expressed in the plants. Periparturient females, however, show a significantly greater increase in tannin ingestion from the pregnancy season to the birth season, largely through short feeding bouts on rarely consumed plant species (Carrai *et al.* 2003). This behavior is initially puzzling as tannins have a well known inhibitory effect on digestive absorption of proteins (Robbins *et al.* 1987), which are in high demand during this period in females. A self-medication hypothesis has been proposed, however, based on the observation that physiological prioritizing of proteins to the reproductive organs causes increased immunological susceptibility to parasite infection (Houdijk *et al.* 2001). The dietary tannins may be effective as a prophylactic anti-parasitic agent with a direct fitness effect on the health and survival of offspring. The study population was notable in that it habitually produced a litter each year compared to the two-year cycle of other sifaka populations, but this hypothesis awaits comparative studies for verification of the beneficial effect (Carrai *et al.* 2003).

While parasite control has attracted the most attention from self-medication studies, more subtle effects have been proposed by Strier (1993) in her observations of muriquis (*Brachyteles arachnoides*) in

Brazil. This species exhibits strongly seasonal reproduction, concurrent with two observed anomalies in their feeding behavior. The first is the intensive ingestion of *Apuleia leiocarpa* and *Platypodium elegans* leaves, differing from the usual casual consumption of leaves as the animals forage. These leaves are antimicrobial and unusually low in tannins, potentially providing an important protein source for the forthcoming mating season. More intriguing, however, are the monkeys' forays to the far periphery of their range to feed just briefly on the fruit of *Enterolobium contortisiliquum* [Leguminosae]. These fruit contain high levels of stigmasterol, a precursor of progesterone, and may play a role in regulating and synchronizing reproductive behavior in the group. Subsequent studies in baboons have confirmed that dietary progesterone (in this case from the African black plum, *Vitex doniana*) can significantly affect reproductive cycles (Higham *et al.* 2007), while studies of multiple species suggest that in some social situations, reproductive synchrony may be adaptive through predator satiation (Findlay and Cooke 1982; Ims 1990), predator avoidance (Wissel and Brandl 1988) or group rearing of offspring (Radespiel and Zimmermann 2001). In contrast, however, full synchrony may be maladaptive in some situations through increased inter-female competition for male mates (Radespiel and Zimmermann 2001), and Schank (2001) suggests that reports of estrus synchrony in primates may simply be a methodological artifact.

Self-Medication: Fur-Rubbing

In addition to ingestion of medicinal materials, there is evidence in primates of the external application of volatile plant materials to skin and fur. This has precedent in bird 'anting' behavior, initially coined to describe behavior wherein live ants are rubbed through feathers, spreading formic acid secretions across the skin (Rothschild and Clay 1952), or where ants are allowed to crawl across the bird while it rests on an ant-mound. The definition of 'anting' has since been expanded to include the use of any invertebrate or pungent substance in this manner (Clayton and Vernon 1993; VanderWerf 2005). Early hypotheses of anti-parasitic functions for anting (Ehrlich, P. R. *et al.* 1986) have been challenged by assays of ant defensive chemicals (Revis and Waller 2004) which instead found them only effective against fungal or bacterial infection. Common Grackles (*Quiscalus quiscula*), however, have been

observed rubbing their plumage with marigold flowers, which contain pyrethrum and sytosterol, both of which are high effective inhibitors of mite oviposition (Dennis 1985). The same species has also been recorded ‘anting’ with lime peel (Clayton and Vernon 1993) which was attributed by the author to its activity against lice, but could equally function as an antibacterial or antifungal. In a related field, Clark and Mason (1985) found that European starlings choose a specific set of aromatic plants to line their nest each season. While not directly applied to feathers, the plants, such as yarrow (*Achillea millefolium*), tended to be high in mono- and sesquiterpines, which are effective at reducing fowl mite and bacterial infection in the nest and chicks. Thus it would seem that no single hypothesis can account for all occurrence of avian ‘anting’, and the same undoubtedly applies to primates.

Baker (1996) conducted research on capuchins (*Cebus* sp.) and spider monkeys (*Ateles geoffroyi*), and observed fur-rubbing with citrus fruit, seed pods and vine leaves (*Piper* sp. and *Clematis* sp.). Fur rubbing behavior generally consists of partially masticating or bruising material, and then applying the saliva/juice over the entire body using feet, hands and tails. Fur rubbing behavior may be solitary or social, with some capuchin rubbing bouts resulting in “a mass of wet, drooling monkeys with bits of citrus and juice or broken leaves sticking to their fur, squirming and rolling over around each other” (Baker 1996:266). Rodriguez and Wrangham (1993) list plant parts of eight genera used by capuchins, some of which contain volatile terpenes (e.g., *Eugena*, *Protium*, *Virola*) and others containing bioactive alkaloids (*Piper*, *Virola*). The species tend to be pungent and are used in ethnomedicinal preparations for a range of skin conditions, including insect bites and minor wounds (Schultes and Raffauf 1990). Pharmacological testing further reveals that *Piper*, *Citrus* and *Clematis* exhibit insecticidal and antiseptic properties (Baker 1996). Capuchins display fur-rubbing more frequently in the wet season, when they are susceptible to fungal and bacterial skin infections, and this appears to be the strongest functional stimulus for the behavior (Baker 1996). Meunier *et al.* (2008) conducted an experiment with a group of captive capuchins which fur-rub with onions. When the timing of the behavior was analyzed, the results suggested that individual rubbing was stimulated by conspecific behavior, such that there was socially facilitated synchronization. The authors propose that such collective behavior may increase its efficacy

against skin infections or ectoparasites by producing a group-wide barrier to cross-infection (Meunier *et al.* 2008).

More recently, evidence has emerged of fur-rubbing in great apes. Bornean orangutans have been observed masticating and applying *Commelina* spp leaves to limbs and joints (Morrogh-Bernard 2008). In this case the plant is used traditionally by humans to relieve muscle and bone pain, and is hypothesized to function as an anti-inflammatory for the orangutans. In contrast, superficially similar fur-rubbing behavior in spider monkeys (*Ateles geoffroyi*) with *Citrus* and *Zanthoxylum* leaves, was directed only at the sternal and axillary regions of the body, performed mostly by males, and is thought to be involved in scent marking rather than for any pharmacological activity (Campbell 2000). Finally, the source of primate fur-rubbing materials is not restricted to plants, as is demonstrated by wedge-capped capuchins in Venezuela, who anoint themselves with millipede excretions during the rainy season, more akin to the original 'anting' behavior in birds (Rothschild and Clay 1952). The benzoquinone excretions are themselves irritant, and as such are unlikely to function to relieve skin conditions, but they are also strongly repellent to insects such as mosquitoes, which are most abundant in the rainy season (Valderrama *et al.* 2000).

Self-Medication: Geophagy

The practice of geophagy (deliberate ingestion of soil) is widespread across the mammalian and avian orders, including human (Wiley and Katz 1998) and non-human primates (Ferrari *et al.* 2008; Krishnamani and Mahaney 2000). Despite being recorded as a therapeutic agent as early as 10 BCE, the phenomenon was, until recently, considered a pathological behavior, requiring intervention (Danford 1982). Several primatologists and anthropologists have since proposed functional hypotheses for the expression of geophagy. These hypotheses are recognized to be non-exclusive and the role of each may vary between species and populations due to the differing adaptive value of the practice under various ecological conditions. Indeed, the various published evidence of the phenomenon in non-human primates has supported all of the possible hypotheses, often finding evidence for several in the same study (reviews in Krishnamani and Mahaney 2000; Wilson 2003).

Several self-medicative functions have been proposed based upon the ability of the minerals kaolinite and halloysite to adsorb intestinal insults, such as plant toxins and infectious diarrheal enterotoxins, first suggested by Hladik (1977) and Oates (1978). Such a function would be adaptive in allowing the exploitation of marginally toxic food plants, or reducing morbidity and mortality from diarrheal conditions. Vermeer and Ferrel (1985) documented the use and trade of *Eko*, a human medicinal clay in Nigeria, noting the predominance of kaolinite and its use for diarrheal conditions. The authors noted a high frequency of use amongst pregnant women, which is compatible with the protective hypothesis for 'morning sickness' discussed below (also Wiley and Katz 1998). Johns (1986) noted the use of clays in the detoxification process of Andean potatoes, effectively adsorbing toxic glycosides in a range of simulated gastric conditions, and thus providing a biocultural key to exploiting this staple food plant. Mahaney *et al.* (1995) suggested a similar function for gorilla geophagy in the Virunga Mountains. In these animals, the ingestion of halloysite-rich clay was closely associated with a seasonal dietary shift to *Lobelia* and bamboo species, known to contain toxic alkaloids and hydrogen cyanide (HCN). The relatively meager levels of dietary minerals in the clay suggested that the primary function was the detoxification of these antifeedants. Gilardi *et al.* (1999) tested a range of hypotheses for geophagy in Amazonian parrots, including acid-buffering, mechanical digestion aid and mineral supplementation, but found experimental and ecological support only for a dietary detoxification function, increasing the bird's dietary breadth in the face of many species of chemically protected seeds. Similarly, in red-handed howler monkeys, geophagy was observed to be seasonally associated with increased ingestion of mature leaves, also suggesting a protective function against plant secondary compounds (de Souza *et al.* 2002). These ecological observations were subsequently supported by experimental evidence. Dominy *et al.* (2004) found that kaolin was able to reduce the bioavailability of quinine (an alkaloid), tannic acid and condensed tannins by at least 30% under *in vitro* intestinal conditions.

Another common cause of diarrhea in wild primates is parasitosis, and some geophagy may have a protective effect against the debilitating symptoms of these infections. Knezevich (1998) observed that rhesus macaques (*Macaca mulatta*) on Cayo Santiago displayed a very low incidence of diarrhea, despite

the high prevalence of nematodal parasite infection. The author suggested that habitual geophagy ameliorated the pathogenic effects of the parasites. A similar suggestion was made by Bicca-Marques and Calegari-Marques (1994) in black howler monkeys (*Alouatta caraya*). Although this effect may well be valid, Knezevich mentions that the incidence of geophagy was temporally associated with chow provisioning, and so a digestive benefit cannot be discounted as a primary function in this case.

Davies and Baillie (1988) found evidence in red leaf monkeys to suggest the alleviation of digestive disorders through geophagy, such as acid indigestion. Kaolin has been found to be an effective veterinary antacid (Krishnamani and Mahaney 2000), similar to the pharmaceutical preparation Kaopectate™, and several studies supporting this function have found kaolinite (Wakibara *et al.* 2001) or the closely related metahalloysite (Mahaney *et al.* 1997) to be prevalent in the sampled clay .

An intriguing, and seemingly contradictory effect of geophagy has recently been recorded in Kibale chimpanzees by Klein *et al.* (2008). These animals engaged in geophagy often just before and after the ingestion of the antimalarial plant *Trichilia rubescens*. When tested under simulated gastric conditions, the kaolin-rich clay appeared to *enhance* the properties of the leaves against *Plasmodium falciparum*, rather than bind the active secondary chemicals, as might have been assumed previously. The mechanism by which the adsorptive clay acts differentially on toxins versus bioactive compounds is unclear at this time.

Many authors include mineral supplementation as a possible function of geophagy (Davies and Baillie 1988; Heymann and Hartmann 1991; Inoue 1987). Material ingested from termite mounds (Mahaney *et al.* 1996) and ant-hills (Heymann and Hartmann 1991) are often associated with this hypothesis as the process of soil evaporation on the mound surfaces leads to relatively high concentrations of mineral salts, such as iron, sodium, calcium, magnesium, potassium and phosphorus.

The perceptive reader will notice that only few of the field studies discussed above include complete empirical support of their proposed self-medicative function. Rarely can field observations account for the complex variables of pharmacodynamics: factors such as dosage, detoxification,

absorption, and the actions of concurrently present drugs can all influence the efficacy of a putative medicinal substance. In addition, the question of how the animal learned such behavior is still under debate. These present shortcomings have laid the subject of zoopharmacognosy open to charges of romanticism and anecdotal evidence – that based on a small number of isolated observations, conclusions are drawn that animals have some innate “knowledge” about natural medicine (Sapolsky 1994). The pioneers of zoopharmacognosy studies, however, have taken pains to rebut such accusations (Wrangham *et al.* 1994), and more recently, a large multidisciplinary effort has begun to address the thorny questions surrounding the innovation, acquisition and maintenance of self-medication in primate societies.

Acquisition and Maintenance

The question of whether self-meditative behavior in non-human primates is a genetically mediated trait or a ‘culturally’ acquired and transmitted phenomenon remains equivocal, and indeed the two models may not be exclusive.

a. Neurophysiology

One mechanism to account for ingestive self-medication may be found in the neurophysiological processes of ‘taste mediated aversions.’ An ultimate explanation suggested for the development of such aversions is the selective advantage of being able to detect and avoid toxins in food items prior to ingestion. This aversion mechanism may be innate or acquired (Bernstein 1991). Innate aversions require no prior experience with the substance, but can be reversed through training. Innate aversions are often associated with sour material (often taken to indicate spoilage) or bitter substances (Glendinning 1994). Recognizably stereotypical ‘gustofacial’ expressions in response to bitter taste have been recorded in human neonates, before any experience with food (Steiner 1974, 1997), as well as in nonhuman primates (Hladik *et al.* 2002; Steiner and Glaser 1984). The response to bitter is characterized in humans by a suite of behavioral and physiological reflexes such as tongue retraction, gaping, increased latency to swallow, lowered heart rate and nausea – all reactions that tend to purge material from the oral cavity or stomach before they are allowed into the lower digestive tract (Glendinning 1994). Evidence has recently emerged

in rodent studies of a secondary set of 'bitter' receptors located in the intestines that cause delayed gastric emptying and additive reinforcement to taste aversions (Glendinning *et al.* 2008). Acquired aversions are based on firsthand experience, and often associated with ingestion of a foodstuff prior to illness or discomfort. This response is unusual amongst learned reflexes in the rapidity of its acquisition (often only a single incident) and in the tolerance of delays of up to 12 hours between the stimulus (ingestion) and the effect (illness) (Schafe *et al.* 1995).

Furthermore, and most relevant to the discussion of self-medication, there is evidence that taste aversions can vary according to the health state of an individual organism. This might allow the ingestion of a normally unpalatable substance under circumstances in which it would be therapeutic. Evidence of such adaptive plasticity in taste includes Gombe chimpanzees' acceptance of tetracycline-laced bananas when sick, but rejection of them when healthy or recovering (Goodall, pers. comm., cited in Huffman and Seifu 1989). Similarly, there is a tradition in Japanese *Kanpo* medicine wherein a patient is asked to taste a range of medicines and the least 'bad tasting' of the selection is then chosen as the most effective remedy for that condition. Later, when the medicine again becomes unpalatably bitter, it is seen as a sign that the herb has done its job and may be discontinued (Koshimizu *et al.* 1994). A model of 'dual-aversion,' first to the normal dietary range (allowing medication) and then to the medicine (stimulating a return to the normal diet) has been suggested for this phenomenon, but the initial aversion would need to be strong enough to cause the shift, yet weak enough for it to be subsequently forgotten (Lozano 1998). An analogous mechanism of physiologically *enhanced* aversions has been well documented in the taste changes and nausea ('morning sickness') during human pregnancy. This is thought to be an adaptive mechanism to protect the chemosensitive fetus from teratogens, and the mother from infection, whilst maintaining adequate nutrition (Flaxman and Sherman 2008; Sherman and Flaxman 2001). In support of this hypothesis, aversions tend to be enhanced for foodstuffs either rich in bitter phytochemicals (such as coffee), or prone to bacterial spoilage (meat and eggs), and the phenomenon is associated with decreased risk of miscarriage (Czeizel *et al.* 2006).

The concept of the reverse mechanism - specific *preferences* existing for certain medicinal substances in response to physiological illness - is appealing in the face of observational records of apparent self-medication. That taste hedonics (subjective pleasantness) in primates can be modified through positive association is amply illustrated by human likings for certain culturally valued, bitter food items such as coffee, yerba mate or beer hops (Mattes and Beauchamp 2000). All of these flavors tend to be unpalatable in children but are modified, by association with a positive pharmacological effect, into a positive taste preference, or at least a reduction in their aversiveness within certain dosages (Vitazkova *et al.* 2001). A category of *Familiar-positive* was previously suggested by Zahorik (1977) for tastes that are associated with recovery from illness, distinct from the more common *Familiar-safe*. The author found rats to prefer 'recovery' flavors over familiar flavors under controlled experimental situations. Hart (2005) posited an evolutionary model of the origins of medicine in humans and non-human animals, wherein individuals suffering from acute disease symptoms are innately attracted to certain taste 'markers' in plants (i.e., bitterness, astringency) that may indicate a non-specific therapeutic efficacy, but discounted the role of acquired associations between ingestion of a particular material and its therapeutic effect. His objection lay in the long delay between application and reinforcing benefit, and in the initial dietary barrier of unpalatable taste. Recent experimental research in sheep successfully produced conditioned associations between induced states of illness, and the correct choice of curative medicine (Villalba and Provenza 2007; Villalba *et al.* 2006) – essentially 'teaching' them to self-medicate. Animals which were exposed to the same materials but without temporal association, however, did not learn to self-medicate. This evidence is highly suggestive of specific acquired taste preferences, allowing greater accuracy of medicine choice.

b. Cultural Tradition

The other major explanatory branch is found in culturally maintained behaviors, although some debate still exists as to what constitutes a cultural trait in non-humans, the full breadth of which is beyond the scope of this review. Early studies in Japanese macaques (Imanishi 1952) define it simply as behavior

transmitted from parent to offspring, reasoning that differences in conspecific behaviors could thus be accounted for by culture. Some (e.g., Cavalli-Sforza and Feldman 1981) have since refined the definition as all non-genetic transfer of information, while more recent authors are more specific, additionally requiring intra-group consistency and inter-group variability which cannot be accounted for solely by environmental variation (Boesch *et al.* 1994; Boesch and Tomasello 1998). Subsequently, definitions have additionally required that a cultural trait is dependent on social means for its diffusion and maintenance (Perry and Manson 2003). A model of the social transmission and maintenance of a cultural trait is outlined by Huffman and Hirata (2003) wherein a novel behavior is *innovated, transmitted* throughout the group according to spatial and social interaction, and becomes true *tradition* when transmitted vertically from parent to offspring. The authors note that, contrary to earlier models where social transmission was assumed to accelerate through a group with increasing acceptance (Cavalli-Sforza and Feldman 1981; Whiten 2000), large group sizes may in fact inhibit social transmission through buffering by the presence of many naïve bystanders. In addition, the 'conformity bias' often detected in large groups of humans and non-humans may impede the adoption of a novel, minority behavior, independent of its benefit (Henrich and McElreath 2003; Kendal *et al.* 2004; Whiten *et al.* 2005).

Biro *et al.* (2003) found nut-cracking behavior in wild chimpanzees to satisfy all the criteria of a cultural tradition, noting that inter-population variability in technique and nut-choice could not be accounted for by ecological determinants. The authors suggested a mechanism of social learning through local enhancement (facilitating the presence of the required materials and tools) and emulative learning on observation of the reward. Other studies have recorded group-specific behavioral traits and significant inter-group behavioral variability in capuchins (*Cebus* spp.) (Chapman and Fedigan 1990; O'Malley and Fedigan 2005), and orangutans (*Pongo* spp.) (Fox *et al.* 2004; van Schaik, C.P., Ancrenaz *et al.* 2003). In Japanese macaques, several cases of innovative, culturally maintained behavior have been recorded. Perhaps the most influential and well-known case is the innovation of sweet-potato washing (SPW) and 'placer-mining' on the island of Koshima by a young macaque, *Imo* (Kawai 1965). SPW, wherein sand is washed from provisioned sweet-potatoes in seawater, was first observed in 1953, subsequently spread via

lineages and playmates throughout the group, and later developed into a habit of 'seasoning' the potatoes with salt-water during eating. The 'placer-mining' technique allowed the separation of sand and wheat by throwing them into fresh water, and retrieving the floating grains (Kawai 1965). Although all the monkeys present at the emergence of these behaviors have long since died, the group is still employing both techniques (Matsuzawa 2003).

More recently, another unusual behavior is providing a valuable insight into cultural behavior in Japanese macaques. Stone handling is a form of solitary object-play found in several populations of provisioned Japanese macaques (Huffman 1984; Leca *et al.* 2007). The occurrence of the behavior seems to correspond with provisioning frequency, with no stone play having been recorded in non-provisioned troops (Leca *et al.* 2008). It is significant that all three of these Japanese macaque behaviors are associated with provisioning, since this has been shown to alter both social structure and activity budgets (Hill 1999; Huffman and Hirata 2003). Some of the authors suggest that stone-play may reveal the influence of social and environmental conditions on the emergence of human stone tool use (Huffman and Quiatt 1986; Leca *et al.* 2008).

Recent experimental studies suggest a dual role for imitative learning and local enhancement in social transmission of a behavior. Hopper *et al.* (2007) introduced two alternative techniques (“lift” and “poke”) to gain a puzzle reward to two isolated populations of chimpanzees, plus a non-trained control. “Lift” was only adopted by the accordingly ‘seeded’ population, conforming with the intergroup variability required by Boesch (1994), but that group, like their counterparts, subsequently adopted “poke” as the dominant tradition. Mechanical 'ghost' demonstration of the puzzle without a chimpanzee model did not evoke learning of either technique, illustrating the importance of an animal model to provide the required social focus for learning. The authors suggested that adoption of a novel technique is relative to two thresholds of cognitive difficulty: below the lower threshold, most animals will be able to discover the skill themselves, given the relevant tools and focus (local enhancement and emulation, “poke”); between the two levels, imitative learning is normally required to acquire the skill from an expert model (“lift”); above the upper threshold, the technique is unlikely to be acquired, even after

observation. This model of difficulty:cognition relativity may account for some of the seemingly variable results in other literature (e.g., Bugnyar and Huber 1997; Call *et al.* 2005; Whiten *et al.* 2005).

Actual teaching is rarely attributed to non-human primates. Teaching requires an individual *A* to modify her behavior in the presence of naïve *B*, at a cost to herself, but providing guidance to *B*, such that *B* acquires a skill more quickly (Caro and Hauser 1992). Unlike its human analogue, this does not necessarily require intentionality. Suggestive, but not definitive evidence of teaching can be drawn from recorded infant *encouragement* in yellow baboons (Altmann 1980) and *discouragement* in wild chimpanzees (Nishida 1983; Wrangham 1977), as well as from experimental studies in captive chacma baboons (Fletemeyer 1978). Intriguing anecdotes also exist of sign-language 'moulding' as an instructional technique between captive chimpanzees (Gardner *et al.* 1989) and rare observations of maternal assistance in nut-cracking (Boesch 1991). In all these examples, the 'teacher' modifies their behavior in the presence of their 'pupil' but both the recognition of naïvety and a cost to the teacher are more difficult to measure. Recent evidence suggests that teaching is more prevalent in non-primate taxa such as ants, bees, meercats and other carnivores, species within which neither imitative social learning nor particular cognitive prowess exist (Thornton and Raihani 2008). It suggests that processes of teaching may evolve independently of social learning, and rely on selection through inclusive fitness, be it kin selection or the benefit afforded the teacher by the proximity of a skilled pupil, irrespective of relatedness (Hoppitt *et al.* 2008).

Inasmuch as self-medication can be seen as a culturally shared set of behaviors, there still remains considerable contention as to the circumstances allowing for its initial emergence. In the case of non-human primate self-medication there is experimental evidence to suggest that such behavior is based within a species-level associative predisposition, but subject to cultural acquisition and transmission. In an experimental study with chimpanzees by Huffman and Hirata (Huffman and Hirata 2004), rough hairy-surfaced leaves similar to those swallowed therapeutically in the wild (see above), were introduced to eleven naïve, captive animals. Two of the group spontaneously displayed rolling-swallowing behavior akin to the wild self-medication, possibly in response to the rough-surface stimulus, while 75% of those

who observed these two models subsequently adopted the trait. The four who consistently abstained from leaf-swallowing were noted to never closely observe the behavior. This dual mechanism of acquisition and maintenance allows a role for both investigatory strands discussed above, the rough hairy leaves providing a sensory 'marker' of plant function (Hart 2005), while the specific behavior was reinforced socially.

The chimpanzee diet is very conservative (Nishida 1987), however, so initial contact with a pharmacologically active medicinal plant is likely to have required unusual circumstances. Huffman (1997) suggests that initial experience with novel medicinals may have occurred during times of extreme food scarcity, during which animals would be increasingly vulnerable to infectious diseases. Marginally toxic plants would be sampled out of a necessity to expand their dietary range (Jansen 1978) and subsequent health improvements may have been associated with the new tastes, akin to Zahorik's (1977) model. In this situation, physiologically adaptable taste preferences and aversions may have contributed to an innate predisposition to certain classes of functional medicinal materials, while social learning and reinforcement henceforth helped maintain the skills in the population and modify their application to locally relevant stressors.

Practical Applications

This perspective on primate health has profound implications in the areas of conservation, veterinary care and drug prospecting.

a. Conservation Medicine

Conservation medicine is a recently defined field (Koch 1996) that approaches medicine from a holistic, ecological perspective. It has been adopted as a primary objective by the Wildlife Trust (Aguirre *et al.* 2002), the Center For Conservation Medicine (Meffe 1999), and was listed as a key emerging issue at the 5th World Parks Conference (IUCN 2003). At the heart of the discipline is the recognition that health is a state involving not just human populations, but domestic animals, wild animals and the

functioning of the ecosystem as a whole. Conservation medicine considers the health of threatened populations to be fundamental to their survival (Deem *et al.* 2001), and frequently focuses on the effects of anthropogenic habitat alteration and interspecies contact as risk factors and influences on local disease ecology (Cunningham *et al.* 2003; Daszak *et al.* 2000; Harvell *et al.* 2002). An essential complement to risk assessment in this field would be to identify the mechanisms and resources by which wild populations protect themselves from disease. This ability could then be actively incorporated into conservation plans and reserve design to ensure that threatened populations have a self-sustainable resilience to health stressors. For example, if a particular plant is being effectively utilized to control parasite load, the removal of access to that resource may be of significant detriment to the survival of that population, and so should be included as a vital resource in designing any protected area, along with and equal to the nutritional requirements.

b. Sustainable Veterinary Medicine

Captive and domesticated animals often require intensive veterinary care. The collaboration of zoopharmacognosy with ethno-veterinary research (e.g., Bøgh *et al.* 1996) may allow a reduction in expensive and stressful intervention, especially in the face of emerging drug resistance in many parasite taxa (Geerts *et al.* 1997; Jackson 1993). As well as facilitating directed prospecting for novel drug treatments, behavioral and chemo-ecological studies of primate self-medication may reveal strategies of parasite control that inhibit the development of chemoresistance. For instance, the previously discussed cases of bitter-pith chewing and leaf swallowing in chimpanzees demonstrate the use of both complex chemotherapy and combination treatment (Huffman *et al.* 1998).

One application of this strategy is in zoo-captive populations of wild, often-threatened species. Such populations may play a significant role in conservation through captive breeding programs, but are often limited by poor health and stress. The introduction of medicinal plants into animal enclosures was first developed at Apenheul Zoo (Holland), largely consisting of European aromatic herbs, as an enclosure enrichment measure (Van Asseldonk and Haas 1997, 2006). The idea has since been implemented in

several other zoos, especially in primate enclosures (e.g., Eicher 1996) and is often targeted at stress reduction or digestive actions. These measures can benefit captive breeding programs in several ways : 1) direct medicinal effects of the plants may reduce the need for veterinary care, reducing costs and stress to the animals; 2) aromatic and unusual plants may engage the animals' interest, providing effective habitat enrichment, and thus a reduction in boredom, stress and the detrimental health effects they may cause (Vermeer 1995); 3) public education is enhanced due to interest in herbal medicine and the more 'natural' behavior and environment of the animals (Davey *et al.* 2005); 4) incorporation of medicative materials in reintroduction programs may maintain relevant behavioral traditions and increase survival in the wild (Krief *et al.* 2008); and 5) in addition to medicinal actions, aromatic and medicinal plants are often rich in vitamins and trace minerals and effectively supplement the standard diet (Vermeer 1995).

A current illustration of the potential of medicinal enclosure enrichment can be found in the prevalence of fibrosing cardiomyopathy in captive gorillas. This condition is responsible for 41% of adult gorilla deaths in captivity (Dybas 2007), but there have been no reported fatalities in wild populations. A significant contribution may be the absence of *Aframomum melegueta* (“Grains of Paradise”) in their zoo diet. The plant, a member of the ginger family, is a staple food item of western lowland gorillas, and a highly potent anti-inflammatory, antibacterial and antiviral used in traditional Nigerian medicine (Cousins and Huffman 2002; Popovich and Dierenfeld 1997; Umukoro and Ashorobi 2008), and may protect against the inflammatory myocarditis known to be contributing factors in cardiomyopathy(Dybas 2007).

c. Drug Prospecting

The multidisciplinary study of non-human self-medication has great potential to provide leads to future sources of medicine to control and treat many conditions, especially in the face of emerging diseases and increasing multiple drug resistance (Huffman 2003). Rapid habitat destruction or degradation, and the low ‘hit rate’ of random broad spectrum phytochemical sampling have stimulated natural-product chemists to adopt a ‘biorational’ approach, drawing from other disciplines to focus their attentions on the most likely candidates for pharmacological development (Berry *et al.* 1995). This

avenue of inquiry is nothing new. There are many reports throughout history of human societies acquiring ethnomedicinal materials and techniques from the observation of animal behavior. One of the most well known is the popular tale of the discovery of the stimulant effect of coffee by Ethiopian goat herders after their flock grazed on wild coffee berries (Huffman 2003). Harrison (1969) reports numerous folk-stories about gorilla, porcupine and bush-pig digging and ingesting the root of *Tabernathe iboga* (Ibogaine), before going into a “wild frenzy.” He suggests that this may have been the origin of the ritualized use of this highly psychoactive stimulant in Gabon. The use of the Chota-Chand root as a snake anti-venom in the Himalayas is said to have derived from observations of mongoose feeding on the plant before combating a cobra (Balick and Cox 1996). Huffman (2003) retells the origin of the Tongwe medicine *mulengelele*, now a widespread treatment for diarrhea, syphilis and gonorrhea. An interviewed healer's grandfather followed a porcupine which was suffering from similar bloody diarrhea as were members of his village. He saw the animal chew on a plant previously shunned as a poison, and subsequently recover. After cautious testing, a preparation was made from the plant that was found effective against the villagers' ailment. Similarly, the Navajo of the southwest USA attribute the discovery of the topical anesthetic and antibacterial Osha root (*Ligusticum porteri*) to its use by black, brown and Kodiak bears, who dig up the root, chew it vigorously, and then rub it into their fur (Engel 2002; Grisanzio 1992).

Conclusions

Primate self-medication, or ‘zoopharmacognosy,’ is a complex and relatively recently discovered phenomenon that emerged from chemical ecology theory. It is becoming increasingly clear that medicine, long held as the sole domain of humans, has close parallels in the behavior of many vertebrate taxa. Such medicative behavior involves the ingestion or application of plant and inorganic materials for both their chemical and physical properties. The most comprehensive studies focus on an antiparasitic function, but such behavior may also include control of reproduction, insect deterrence and the treatment of skin conditions, inflammation, and various digestive disorders. These behaviors may be acquired and maintained through both neurophysiological mechanisms and cultural tradition. The study of self-medication has significance for the understanding of chemical ecology, biological conservation, veterinary and human medicine. This dissertation represents the first systematic study of potentially self-medificative behavior in Japanese macaques. Longitudinal parasitological data are analyzed and presented, and behavioral ecology scrutinized for both plant and mineral based therapeutic phenomena.

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CHAPTER 2

THE PARASITE ECOLOGY OF JAPANESE MACAQUES (*MACACA FUSCATA YAKUI*) ON
YAKUSHIMA¹

¹ Dagg, C. To be submitted to *International Journal of Primatology*.

Abstract

I examined the intestinal nematodes of wild, adult female *Yaku* Japanese macaques (*Macaca fuscata yakui*) over one year on the island of Yakushima, Japan. Data derived from fecal analyses were used to test the role of social rank and age on parasite distribution, and the effect of seasonality and foraging patterns on egg deposition. I identified five nematode taxa: *Oesophagostomum aculeatum*, *Streptopharagus pigmentatus*, *Strongyloides fuelleborni*, *Trichuris sp.* and *Gongylonema sp.* Prevalence ranged from 45% for *Trichuris* to 100% for *Oesophagostomum aculeatum* and *Streptopharagus pigmentatus*. Rainfall seasonality was not distinct on Yakushima and this may limit the influence of climatic factors on parasitic ova abundance. There is, however, some evidence that parasite fecundity is adaptively synchronized with ecological variables that might aid their transmission from host to host, such as tolerable temperature ranges and intermediate host abundance. *Oesophagostomum aculeatum* infection risk and intensity may have a complex relationship with social rank, seeming to most severely affect those at the high and low extremes of the dominance hierarchy. *Strongyloides fuelleborni* infection appears more frequently in younger females, while fecal ova counts of *Streptopharagus pigmentatus* are more abundant in older females. Behavioral observations and fecal consistency suggest that, under normal conditions, intestinal parasites are well tolerated by the *Yaku* macaque population.

Introduction

Intestinal parasites are recognized as a significant factor in the distribution, density and evolution of mammalian populations (Gulland 1992, Stuart and Strier 1995). Severe parasitosis can impact host survival and reproduction through hemorrhage, tissue damage, developmental abnormalities and chronic diarrhea (Schmidt and Roberts 1985). Although less severe infections are more common, they can still affect host fitness through impairment of digestion, predator evasion and competition for food and mates (Coop and Holmes 1996, Hudson *et al.* 1992). There is increasing recognition of the particular role of parasitic infections in the health and survival of endangered populations, especially in non-human primates (Chapman *et al.* 2006, Gillespie *et al.* 2005). Many primate species are particularly susceptible to intestinal parasites because they live in large close-knit social groups, often sleeping in habitual locations, thus providing a high risk of infection during social grooming and from contamination of foodstuffs (Hart 1990, Stoner 1996). Many primate populations are also in close proximity to humans, including tourists, hunters and farmers, increasing the risk of interspecific disease transfer (Cunningham *et al.* 2003). Such contact is recognized both as a significant source of novel emerging diseases in humans and as a disease risk to endangered primates (Daszak *et al.* 2000). Such considerations are central to the application of conservation medicine, an emerging multidisciplinary field investigating the effects of disease on threatened species, both as short-term challenges and through long term coevolution (Aguirre *et al.* 2002). This field is of increasing importance in the face of habitat alteration, degradation and climate change, and was listed as a key emerging issue at the 5th World Parks Conference (IUCN 2003).

Despite well-established parasitological research elucidating the rules underlying parasite aggregation in host populations and their effect on host population regulation and trophic interactions (Anderson and May 1979, Freeland 1983, Lafferty and Kuris 2002), studies exploring the behavioral and ecological interaction of parasites and primates in field situations are rare. Few threatened primate populations have been subject to longitudinal parasite surveys, which are vital for the comprehensive detection of parasitic taxa and the identification of seasonally driven variables affecting parasite dynamics and infection risk.

A near-universally observed pattern in parasite ecology is an aggregated distribution within a host population; that is, a relatively few host individuals will harbor a large proportion of the parasite population (Poulin 2007). Factors mediating this distribution are thought to include host age, gender, social rank and behavior (reviewed in Shaw *et al.* 1998). Some studies have found correlates between social rank and parasitism, such as the greater fecal abundance of nematode ova in high-ranking male yellow baboons, *Papio cynocephalus* (Hausfater and Watson 1976), while others have recorded the opposite (e.g., goats, Ungerfeld and Correa 2007) or have found no evidence of such a relationship (Knezevich 1998, Müller-Graf *et al.* 1996). The interaction between rank and parasitism is complex, and may be mediated by psychosocial stress, foraging patterns and social contact (Nunn and Altizer 2006). Further investigation of this dynamic is clearly required to illuminate the mechanisms involved. Host age may also play a role in parasite distribution, including the accumulation of acquired immunity (Cattadori *et al.* 2005), although this also has not been thoroughly tested in primate field studies and may be complicated by cohort effects, age dependant exposure (Cornell *et al.* 2008), or parasite strain diversity (Galvani 2005). Intraindividual variation in behavior may also affect the intensity of infection across the year. Social interaction may provide greater transmission opportunities (Stuart and Strier 1995), while the ingestion of certain plant parts may rapidly reduce parasite load or activity (Huffman 2001).

Seasonal changes are perhaps the most ubiquitous and predictable external source of variation in ecological systems, and this relationship has been clearly documented in disease ecology, including host-parasite dynamics (Altizer *et al.* 2006). The identification of causal seasonal mechanisms in parasite ecology is often difficult due to the complex multifactoral relationship between host ecology, environmental factors and parasite infection. Parasite survival may be affected by seasonal variations in host immuno-competence due to nutritional health (Cornell *et al.* 2008) or parturition (Cattadori *et al.* 2005, Tembely *et al.* 1998), environmental temperature (Cornell *et al.* 2008), seasonal breeding, or social aggregation densities (Hosseini *et al.* 2004). Studies of ruminants have concluded that the most likely determinant of seasonal fecundity in nematodes is hypobiosis (arrested larval development in the host)

during adverse environmental conditions (Eysker 1993, Langrová *et al.* 2008, Stien *et al.* 2002). Similar analyses have yet to be carried out in primate populations.

Japanese macaques (*Macaca fuscata*) have been extensively researched in provisioned and non-provisioned groups (e.g., Fedigan and Asquith 1991) and their social structure well elucidated. As well as being easily identified, the matrilineal kin-groups within Japanese macaque troops are generally stable, non-dispersing and consist of linear hierarchies (Yamagiwa and Hill 1998). Short-duration parasitological surveys have previously been carried out on Japanese macaques found on the island of Yakushima. These studies found most (96%) to be infected with at least one of several pathogenic helminths, including *Strongyloides sp.*, *Streptopharagus sp.*, *Trichuris sp.*, *Oesophagostomum aculeatum* and *Gongylonema pulchrum* (Gotoh 2000, Hernandez *et al.* 2009). The warm and wet climatic conditions on Yakushima are highly amenable to the survival and transmission of intestinal parasite infective stages, affording them great potential as a source of mortality and morbidity in this macaque population. Several well-studied Yakushima macaque troops suffered mass mortality between 1998-1999 (33-100% of troop populations, mostly adult females and infants) due in part to a poor fruiting season, but potentially exacerbated by an unidentified pathogen (Hanya *et al.* 2004).

This paper describes the results of a one-year survey of parasite prevalence and fecal abundance in adult female *Yaku* macaques, and uses these data to test whether distribution of parasite infection intensity is related to social rank or age. In addition, longitudinal behavioral and parasitological data collection allows detection of seasonal environmental or ecological variables that may affect the shedding of ova within the host, and thus the rate of environmental contamination.

Methods

Study Site and Animals

Yaku macaques are a subspecies of Japanese macaque endemic to Yakushima, a mountainous island of *ca.* 500 km², lying 60 km from the south-western tip of Kyushu, southern Japan (31°N, 131°E). The entire island is designated as a National Park, with 10,747 ha also designated as a UNESCO World Heritage Site, primarily due to the sub-tropical to sub-alpine forest range and ancient Japanese cedar trees. *Yaku* macaques were recently re-assessed as Least Concern in the IUCN Red List (Watanabe 2008), although they are recognized to be at some risk due to their restricted range and ongoing habitat fragmentation due to conifer plantation and farming.

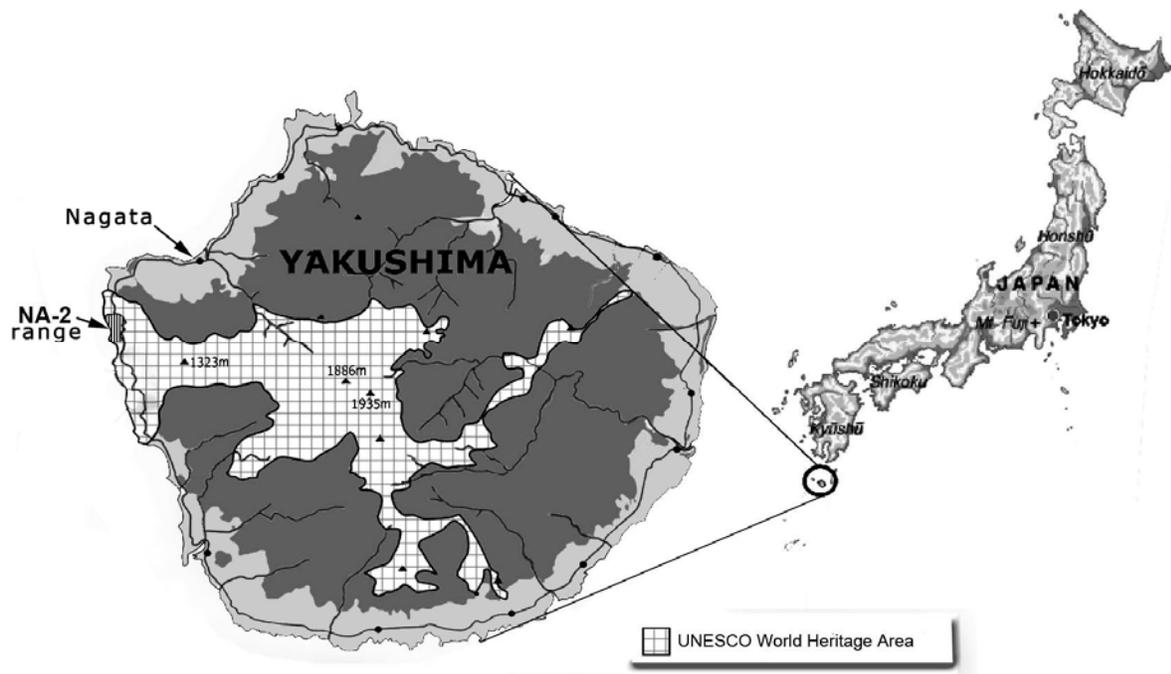


Figure 2.1 – Location of Yakushima showing UNESCO World Heritage Area and Nina A-2 Troop Home Range.

This study was carried out in the north-western coastal forest area of Yakushima (**Figure 2.1**). The Western Forest Road (*Seburindo*) area constitutes a Special Wildlife Protection Area, and the macaques have minimal contact with humans except for along the road. Macaques have been studied in

this area since 1975, when Maruhashi first habituated a group (Maruhashi 1980). Unlike nearly all other Japanese macaque research areas, the *Yaku* macaques have never been provisioned, but most groups in the research area are habituated to the presence of observers, allowing approach to within a few meters without overt response.

I focused on 10 adult female *Macaca fuscata yakui* of Nina-A2 group (NA-2), ranging in age from 5 yrs to approximately 23 yrs. Individual ages were calculated from long-term ranging and census data collected by the Kyoto University Field Research Station on the island. After the death of the female ‘*Shinju*’ in May 2007 at approximately 23 yrs of age, I incorporated ‘*Nagaramé*’ (21-23 yrs old) as a replacement for focal observation from June-August, to maintain 10 focal subjects. The group consisted of 12 adult females, 6 adult males, plus 24 sub-adults, juveniles and infants during the 2007 census (Dagg and Sugaira, unpub. data). The group inhabits approximately 73 ha of warm, temperate broad-leaved forest between sea level and 200m. Although the upper boundary of their range approximately follows the road, which was occasionally used for resting or transit, I observed no direct interaction with tourists, including feeding. Much of the central range is comprised of secondary forest due to the inhabitation of the area by charcoal burners until the 1950s, and includes the remains of terraced fields and introduced plant species. To the north and south of the range, the terrain becomes steeper with signs of human habitation restricted to charcoal-burning pits at the base of larger valleys.

Dr. Shin-Ichiro Aiba of Kagoshima University maintains a weather station at the research station in nearby Nagata village (**Figure 2.1**), and kindly shared his data. Although daily rainfall can be highly localized on Yakushima due to the high mountains, these data are representative of broader climatic patterns on the western coast of the island. Temperature was measured every 10 seconds and averaged to provide daily figures. Rainfall data for July 15th-31st were not reliable due to damage from a typhoon which struck the island at this time.

Study Period and Observational Data Collection

I collected the data presented here between 5th September 2006 and 29th August 2007, accommodating a full year's climatic variables, parasite life cycles and primate foraging seasonality (Stuart and Strier 1995). I collected behavioral data using full-day focal follows from first focal contact after dawn until 1600 or later, for two days per focal per month, when possible. Days with less than three hours of observations (e.g., due to heavy rain), were excluded from behavioral analyses. I recorded activity during day-long focals as point samples with a 10 min interval. Main activity categories were comprised of locomotion, grooming and foraging (including proximal searching, harvesting and ingestion, but excluding processing of cheek pouch contents). During foraging activity, I recorded the food plant species and part being ingested. Insectivorous foraging was classified by substrate due to the difficulty in visually identifying insect species. I determined rank relations between adult females by recording dyadic displacement and/or avoidance *ad libitum* during observation days. The degree of social linearity was calculated with Landau's Index of Linearity (Vries 1995).

Fecal Collection and Analysis

I collected fresh fecal samples from focal animals twice daily, morning and afternoon. In addition, I collected a further sample from each individual per month as opportunity allowed, increasing the replication to a maximum of five samples per focal per month (n=360 total samples). I selected only samples that were free from soil contamination, and collected them with a clean, sharpened stick into a screw-top plastic vial. I analyzed samples that evening or refrigerated them for up to 24 hrs at 5°C, removing the need for preservatives, and allowing the observation of motile larva.

I performed quantitative analysis on each sample using a modified, non-centrifugal McMasters floatation technique (Pereckiene *et al.* 2007). Approximately 4 g of fresh (or <24 hr refrigerated) feces were vigorously mixed in 28 ml of saturated salt/sugar solution (SG=1.28). The resulting suspension was strained, gently stirred again, and 0.3 ml examined in each of two 2-cell McMasters floatation slides. All helminth ova within the grids were counted, averaged between the two slides, and multiplied up (1:25) to

give a value of eggs per gram (epg) for each species present in that sample. This therefore allowed 2x replication of analyses within each sample and within each observation day, accommodating both diurnal and within-sample variation. To provide qualitative analyses and detection of non-floating ova and larvae, a sample of 0.2 g of fresh feces was homogenized with 0.5 ml isotonic saline. Using a small syringe, a sample of fluid suspension was drawn off, avoiding large particulates, and a single drop placed on a standard slide with a cover-slip. This wet-mount was examined at 100x - 400x. A count of ova and larvae was kept to allow a crude estimation of relative abundance. From one of the daily samples, approximately 2 g were separated and frozen at -30°C. Each month and in a batch, these samples were dried at 100°C for 24 hrs and then reweighed to determine percent water content. The presence, species and abundance of any adult parasites or undigested leaf matter in the feces was noted, as was the overall consistency of the feces (wet/med/dry).

From the floatation and wet-mount preparations, I identified ova and larvae by taxon based on characteristic morphology (Acha and Szyfres 2003, Desowitz 1980) and recorded the abundance of each taxon. Any unknown taxa were photographed, and subsequently identified through consultation with Dr. Hasegawa, a professor of parasitology in the Faculty of Medicine, Oita University. To confirm the species identity of the ova observed, I used a modified filter-paper coproculture (Intapan *et al.* 2006) to isolate filariform (L3) larva of *O. aculeatum* and *S. fuelleborni*, both of which would hatch and mature rapidly into infective larvae in the external environment. I spread 1 g of fresh feces thinly and evenly across half the surface of a 4" diameter filter paper. The paper was then folded and inserted into a test tube containing 2 ml water, such that the feces was held uppermost in the tube. I lightly sealed the tubes and incubated them at room temperature (27-30°C) for 10 days. Nematode ova hatched within the feces and migrated down the wet paper into the liquid reservoir at the base of the tube. After incubation, I removed the paper, agitated the reservoir and larvae, and drew a sample for examination at 100x.

Statistical Analyses

To determine whether the fecal sampling frequency was providing an accurate representation of the number of parasite taxa present, I compared the cumulative taxa prevalence using 1, 2, 3, 4, and 5 of the samples collected per individual per month via paired t-tests. The Kolmogorov-Smirnov Normalcy test indicated that there was significant departure from normality ($p < 0.01$) in the daily abundance (epg) of all parasite taxa, and so I used the non-parametric Friedman's analysis to determine the effect of the annual cycle (by month) on the abundance of ova in the feces (Martin and Bateson 1993). I used Pearson's correlations to compare intra-individual prevalence for each taxa, and to relate the mean monthly abundance of each taxa with mean monthly rainfall and temperature. I compared intra-individual prevalence and mean individual abundance with social dominance rank using Spearman's ranked correlations, and these variables to individual age using Pearson's correlations. To test a non-linear model of social rank versus parasite load, the 11 adult females were assigned a relative rank diverging from the median. Thus, the mid-ranking female was assigned a value of 1, while the alpha and lowest ranked females were both assigned 6. This value was similarly tested against parasite abundance and prevalence with Spearman's ranked correlations.

I define the *prevalence* of a particular taxon as the number of focal individuals infected divided by the total number of individuals tested (percentage) (Stuart and Strier 1995). *Intra-individual prevalence* for each focal animal is defined as the number of months with positive detected infection divided by the number of months sampled (for each taxon) (Setchell *et al.* 2007). *Abundance* is the recorded number of parasite ova per gram of sampled material (feces).

Limitations of Fecal Sampling

Researchers can only obtain limited gastrointestinal parasitological information through fecal sampling. Advantages of this method lie in ease of collection, non-invasive sampling and relatively simple analytical techniques. Although floatation analysis can give an estimate of the presence and ovulation rate of a taxon in a sample, it may not be directly related to the number of individuals infecting

the host animal; i.e., the intensity of infection. Some parasitic taxa, including *Oesophagostomum* sp., have been found to lower fecundity during winter months, thus displaying variable fecal abundance without altering the actual infection intensity (Fossing *et al.* 1995). False negative results may also occur due to lack of analytical sensitivity, and due to temporal variation in ova deposition or uneven distribution of ova in feces. These last two factors may be minimized through adequate replication within a short time period, and an analytical technique, such as the McMaster procedure used here, which relies on the homogenization of a larger amount of feces, from which a measurement sample is taken.

The abundance of heavy ova, such as *Streptopharagus pigmentatus*, was not measurable by floatation, and so a relative abundance was estimated through their observation frequency under wet-mount. While this figure may be used here to explore seasonal variation in ova abundance, the absolute value should not be compared directly with any other study.

Results

Total observation time with NA-2 was 1373 hours over 191 days. Two gaps of over 6 days interrupted my observations: Dec 30th – Jan 14th and May 12th - 29th. Temperature displayed distinct seasonality during the observation period, ranging from a minimum of 2.5°C in December to a peak of 36°C in August (lowest monthly mean = January:12.3°C, highest mean = August:28.0°C). Rainfall, however, is high year round, with a mean of 218 mm/month, and reaching 730 mm in late July, although this peak was associated with a typhoon which damaged the rain gauge, and may be inaccurate (Aiba 2007, unpublished data). These seasonal conditions are represented in **Figure 2.2**.

The data from 60 dyadic interactions suggested a linear hierarchy amongst NA2 females (Landau's Index of Linearity, $h = 0.68$) (Vries 1995), in accordance with previous analyses (Hernandez *et al.* 2009, Yamagiwa and Hill 1998). Twenty percent of paired inter-individual interactions were unrecorded, however, and one was reversed during the season. For the purposes of this study, linearity was assumed and unobserved relationships were inferred from transitive logic (Martin and Bateson 1993).

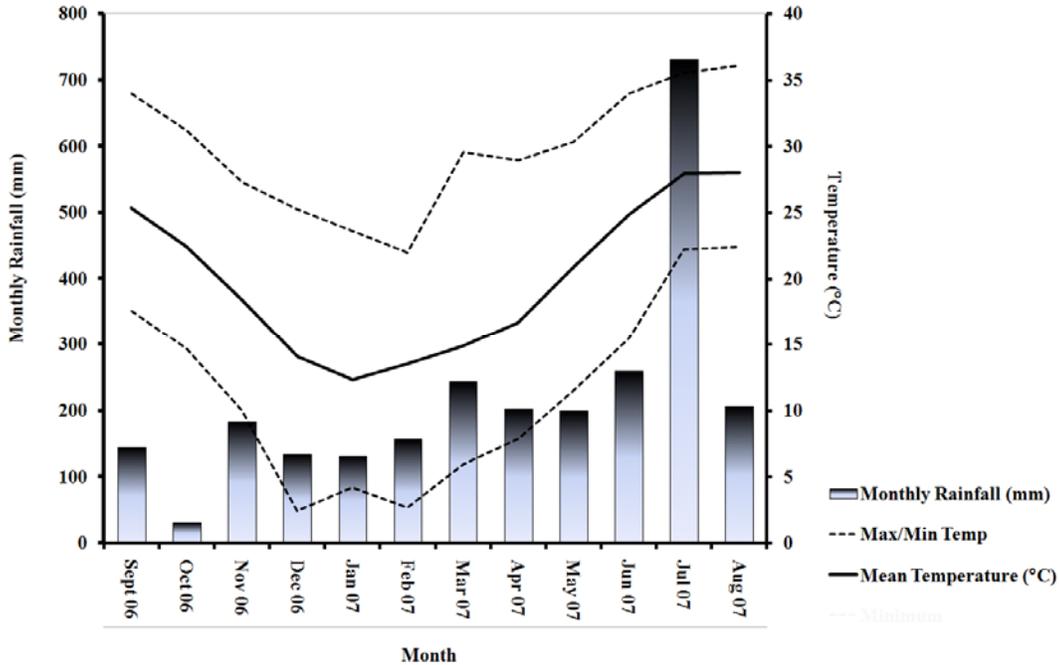


Figure 2.2 – Monthly rainfall and temperature recorded at Nagata Field Station (Aiba 2007, pers. comm.)

Parasite Taxa Identified

I recorded ova of five nematode taxa: *Oesophagostomum aculeatum* [OE], *Strongyloides fuelleborni* [STR], *Streptopharagus pigmentatus* [SP], *Trichuris* sp. [TR] and *Gongylonema* sp.[GO]. I confirmed the identities of OE and STR by successful larval culture and distinctive morphology. OE and STR ova were also distinguishable under floatation by size range. I also observed motile STR larvae under wet-mount, but rarely under floatation due to osmotic deformation caused by the saturated medium. I was able to identify SP ova by morphology in wet mount but the thick-shelled, embryonated ova rarely appeared under floatation. TR ova are highly distinctive due to their barrel shape and twin mucoid plugs, and I observed them primarily under floatation. GO ova were rarely observed (7 samples). The most prevalent taxa were OE and SP, which were detected in all individuals, whereas GO was detected in 6/11 individuals but only at very low abundance. I summarize the nematode species range in **Table 2.1**.

Table 2.1 - Nematodal taxa detected in NA2 Troop, September 06-August 07
(Acha and Szyfres 2003, Desowitz 1980, Gotoh 2000)

Taxon	Prevalence (n=10 macaques)	Frequency in samples (n=360)	Range of abundance per sample	Pathology	Transmission
<i>Oesophagostomum aculeatum</i>	100%	77%	0-650 epg	Diarrhea, malnutrition, intestinal ulceration	Direct: Infective larvae ingested
<i>Streptopharagus pigmentatus</i>	100%	70%	0-35 ova per wet mount slide (estimate 0-1400 epg)	Often asymptomatic but can cause malnutrition and obstruction under heavy loads	Indirect: ingested with intermediate hosts (coprophagous insects)
<i>Trichuris</i> sp.	45%	24%	0-212 epg	Mostly asymptomatic. Heavy load can cause diarrhea, lesions	Direct: fecal-oral ova.
<i>Strongyloides fuelleborni</i>	73%	21%	0-87 epg	Diarrhea, hemorrhage, Cutaneous larva migrans	Direct: Free-living larvae infect cutaneously. Also transmammmary, oral
<i>Gongylonema</i> sp.	55%	2%	<25 epg	Usually asymptomatic but can cause chronic mucosal inflammation	Indirect: ingested with intermediate hosts (coprophagous insects)

Cumulative Sampling Accuracy

The number of parasite taxa detected in an individual over a month increased significantly between the first and second sequential samples (n=105, t=5.75, p<0.001), the second and third (n=73, t=3.57, p=0.01), and third and fourth (n=47, t=3.07, p=0.04). A paired test showed that the addition of a fifth sample did not significantly add to the taxa detected (n=18, t=1.00, p=0.331)(**Figure 2.3**). Four sequential fecal samples were thus shown to fully ascertain the prevalence of parasitic nematode taxa, validating the methodology of two samples per focal day, twice monthly. Sampling difficulties arising from steep terrain and coprophagous deer, however, lowered the mean samples per individual to 3.01 per month. The prevalences recorded here, therefore, may be slight underestimates.

Prevalence

The prevalence of *STR*, *SP* and *GO* varied significantly across the annual cycle, with most widespread infection occurring in autumn (*STR* and *SP*) and early summer (*GO*) (**Figure 2.4**). No

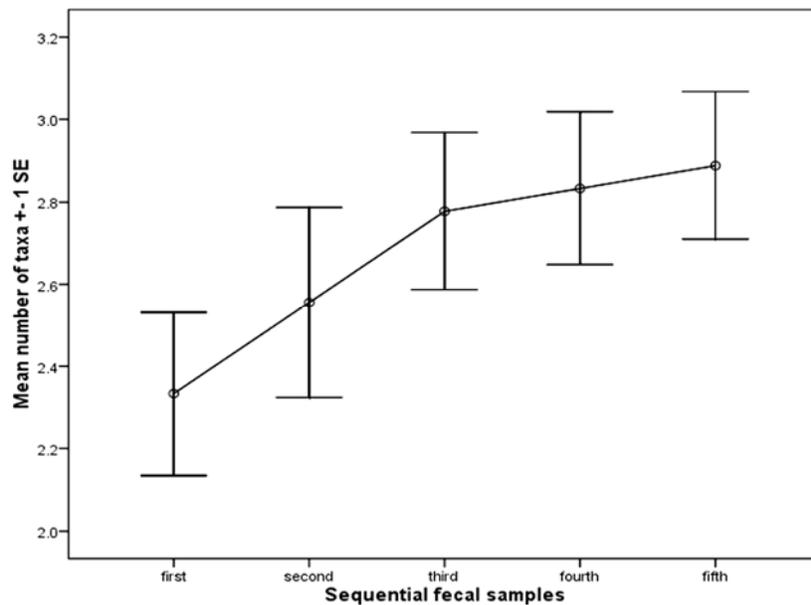


Figure 2.3 - Mean (\pm SEM) cumulative number of taxa found vs. number of sequential samples examined

significant relationship was detected between any parasite taxon's prevalence and monthly rainfall or temperature. However, Friedman's test revealed a significant temporal effect on the number of species detected each month (all focals: $df = 11$; $X^2=20.342$, $p=0.041$). Samples contained between 0 and 4 taxa, with the greatest richness recorded in October and May, and the least taxa detected during the winter and hottest summer months (**Figure 2.5**).

When correlated against age, only the intra-individual prevalence of *STR* showed a significant relationship, displaying lower prevalence of infection in older females ($r=-.724$, $p=.012$), and notably illustrated by the 0%, and 0.4% intra-individual prevalence in the two oldest individuals (approx. 21-23 yrs old). Intra-individual prevalence showed no significant linear relationship with social rank for any parasitic taxa. However, when relative rank divergence from the median (1-6) was applied, a Spearman's rank correlation demonstrated a significant relationship between relative social rank and *OE* intra-individual prevalence ($r=0.69$, $p=0.018$) (low and high rankers are more consistently infected). The data, therefore, fit a more non-linear model of social rank and parasite load.

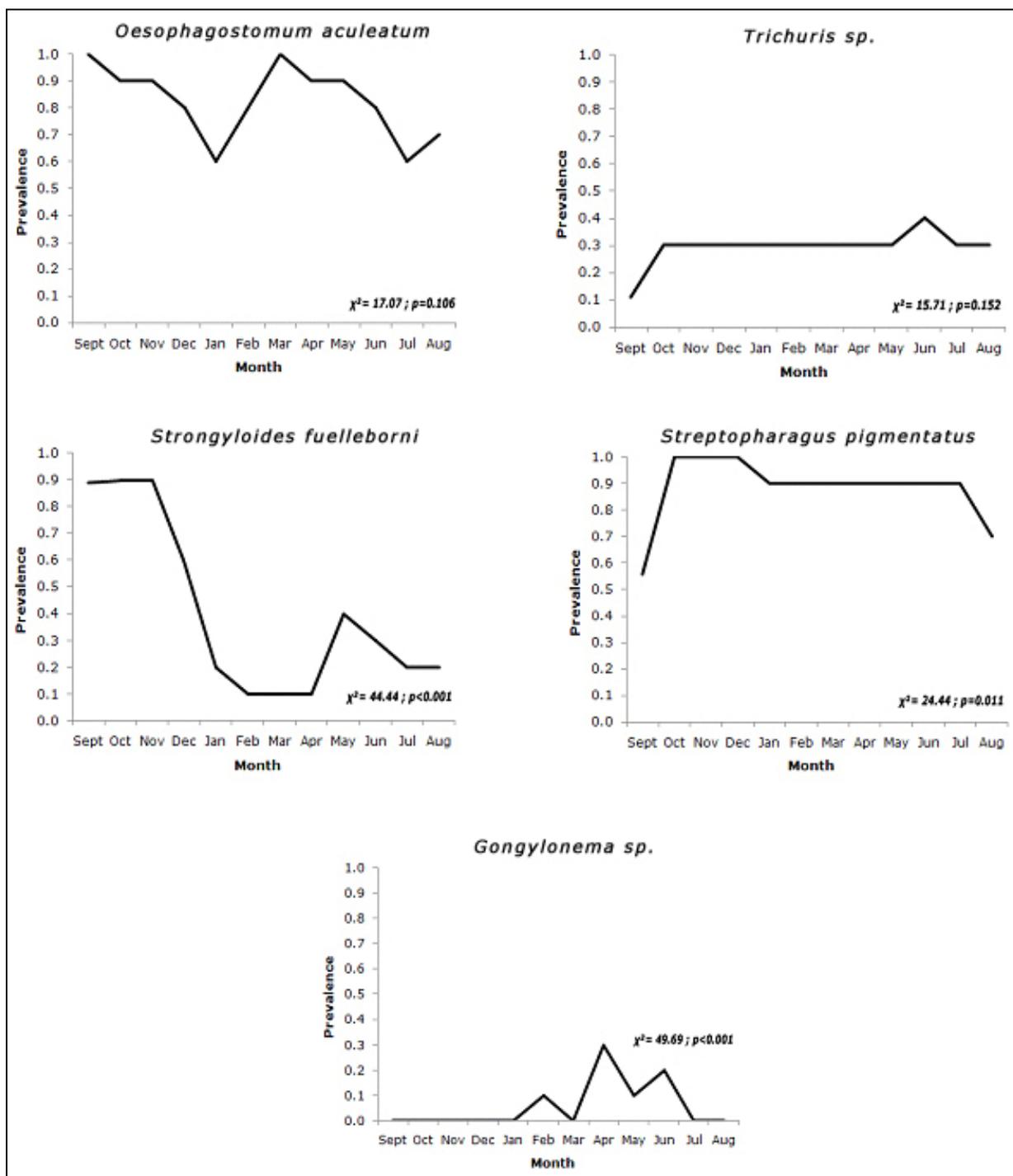


Figure 2.4 – Parasite prevalence across the annual cycle. (Monthly sample size ranges are 18-39, mean = 30.2). Statistical variability calculated by Friedman’s analysis

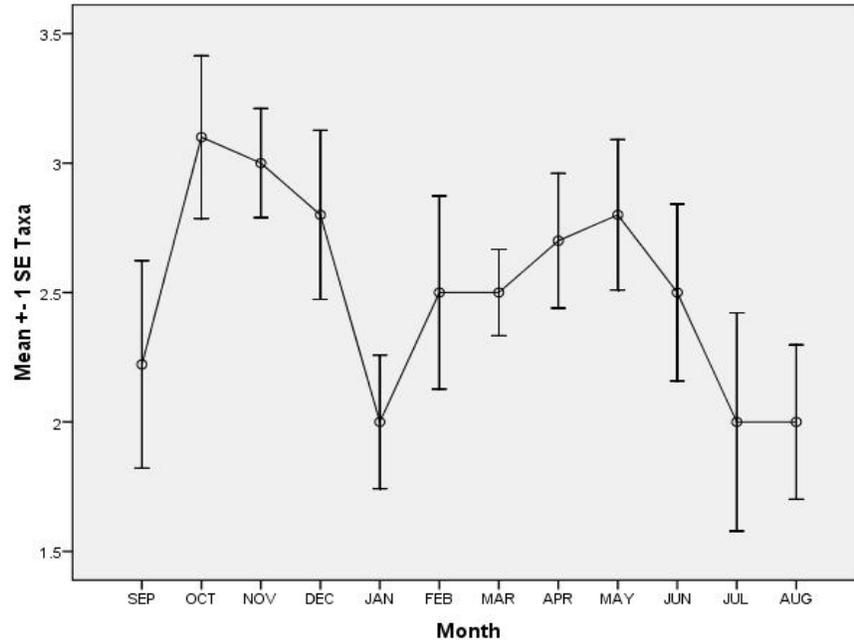


Figure 2.5 – Mean number of nematode taxa detected per individual, across the observational timespan.

Abundance

Figure 2.6 represents the annual variation in the mean abundance recorded for the five parasite taxa (average abundance across 10 focals, mean of 30.1 samples per month). Friedman’s analyses revealed significant temporal variation in the abundance of *OE*, *STR* and *SP*. *OE* displays high abundance in September and peaks again in May. *STR* abundance begins high in September and declines thereafter, but may be partly due to false positive identification early in the field period. *SP* displays two distinct peaks of abundance, in December and June. *TR* fluctuates throughout the year, but displays no clear pattern. *GO* abundance is very low throughout the year.

I detected no significant relationship between the fecal abundance of any parasite taxa and monthly rainfall or temperature, even when the seasonal variation was offset by the known pre-patent periods of *OE* (ca.30 days (Veglia 1924, cited in Anderson 2000)), *SP* (ca.60 days (Machida *et al.* 1978)) and *STR* (>17 days (Abadie 1963)). The detected monthly abundance of *STR*, however, correlated significantly with that of *OE* ($r=0.78$, $p=0.004$), suggesting a possible common ecological co-factor.

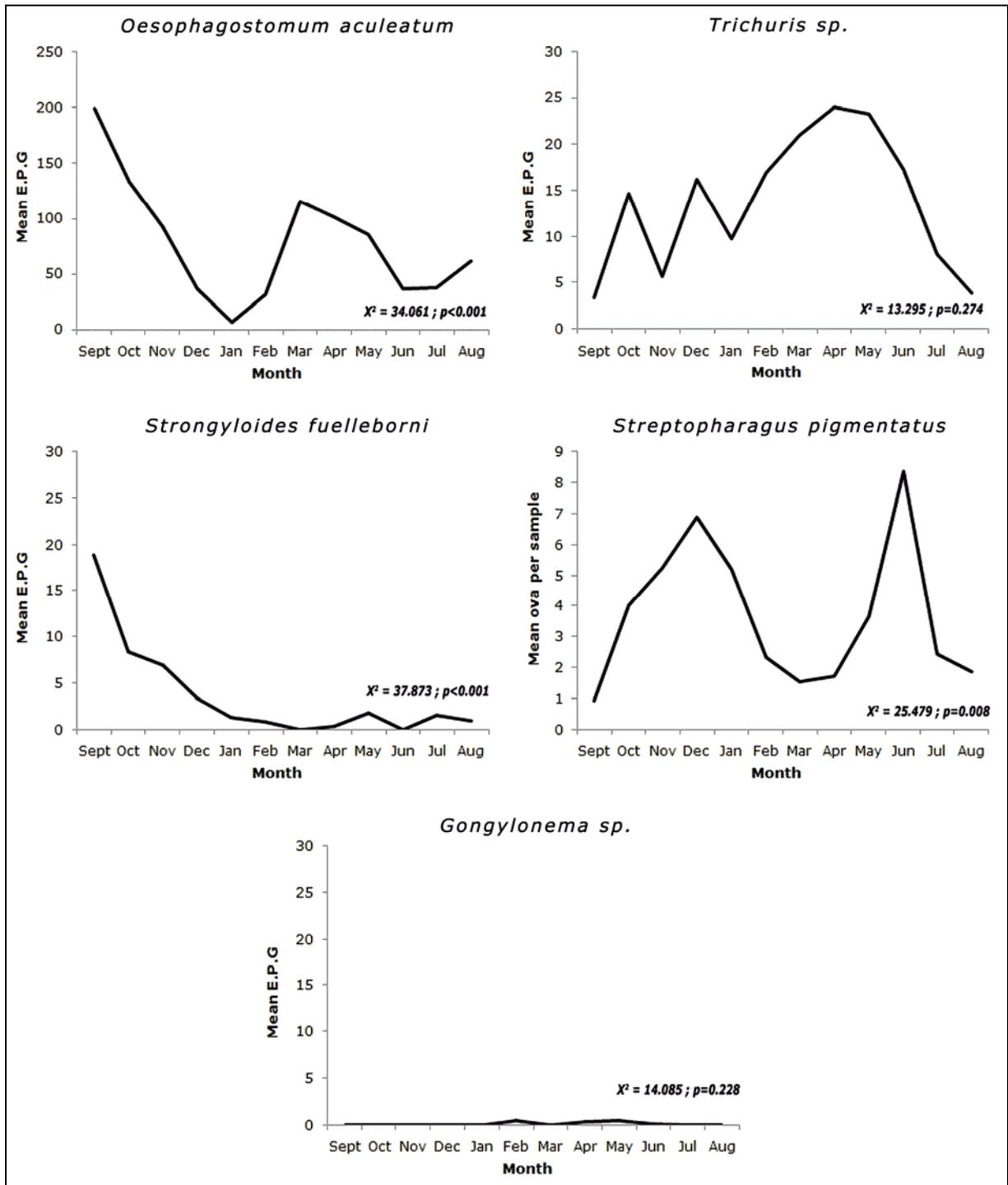


Figure 2.6- Parasite fecal abundance of all detected taxa, across the annual cycle. (Monthly sample size ranges are 18:39, mean = 30.2). Statistical variability calculated by Friedman's analysis

The monthly abundance of *SP* demonstrated a strong, significant correlation to monthly frequency of ground insect foraging by the macaques (Pearson's; $r=0.81$, $p=0.01$) (**Figure 2.7**), while none of the other taxa showed a relationship to diet. This relationship also held when individual daily figures were analyzed, with ingestion of ground invertebrates a significant predictor of *SP* fecal abundance (linear regression: $t=3.281$, $p=0.001$). This relationship did not hold between individuals' mean annual *SP* abundance and overall frequencies of ground invertebrate foraging.

Pearson's correlation revealed a significant relationship between individual age and *SP* infection intensity ($r=0.81$, $p=0.002$). Older individuals presented higher fecal abundance of ova of this parasite, notably the focal female 'Shinju' (approx. 23 yrs old), who died during the observation period.

I detected no linear relationship between individual rank and abundance of any parasite taxa. The abundance of *OE*, however, suggested a more complex relationship with individual social rank, with both high and low ranking individuals demonstrating the highest intensity of infection (**Figure 2.8**). Using the assignment of social rank relative to the median, a Spearman's rank correlation revealed a significant relationship between relative social status and mean annual *OE* abundance ($r=0.699$, $p=0.017$). The lowest severity of infection appeared in the middle ranks, and the highest occurred in the low and high ranking females.

Symptoms of Parasitosis

The mean water content of desiccated fecal samples was 69.85% (range=53-92%, $n=170$). A visual appraisal of fecal consistency (1-3: firm/med/soft) correlated positively with water content (Pearson's correlation: $t=0.323$, $p<0.001$). Neither measure, however, related to the fecal abundance of any nematodal ova. Only 3% of samples were categorized as soft or diarrheal and these were not associated with high parasite abundance. On only two occasions did focal macaques display visible symptoms of colic or malaise, and these were not accounted for by high parasite virulence.

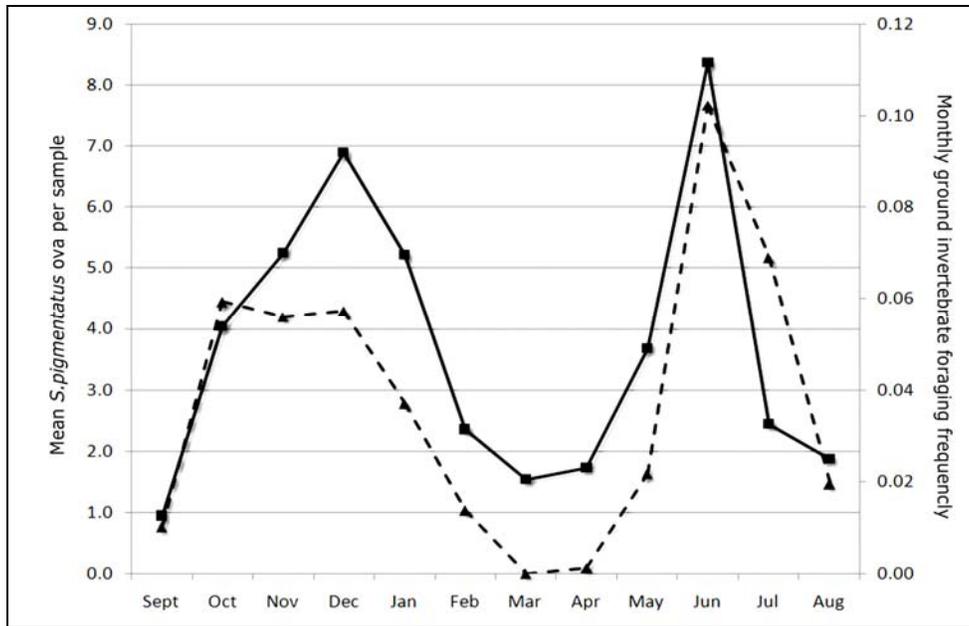


Figure 2.7 – Monthly fecal abundance of *Streptopharagus pigmentatus* ova (solid line), and mean monthly frequency of terrestrial invertebrate foraging (dashed line).

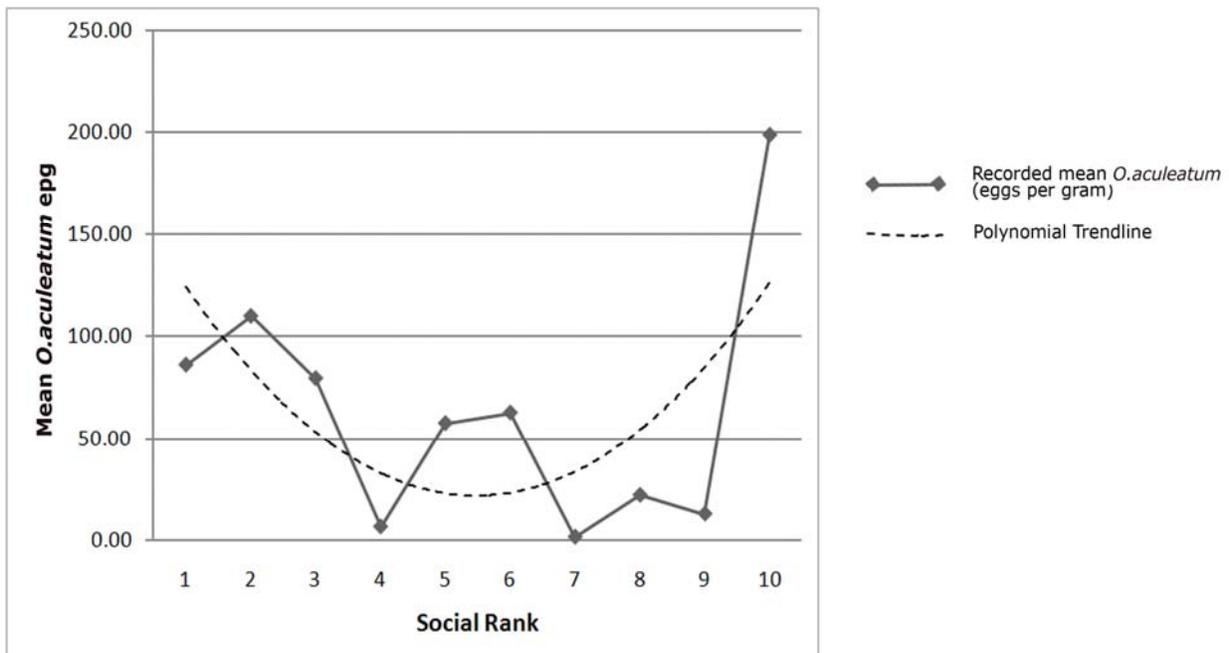


Figure 2.8 - Individual mean *Oesophagostomum aculeatum* fecal abundance by social rank and non-linear trend.

Discussion

Parasite Taxa Identified

All of the nematode taxa detected are known to infect other non-human primate species. *Oesophagostomum* spp. have been detected in mona, patas and green monkeys, olive baboons (de Gruijter *et al.* 2006), colobus monkeys (Gillespie and Chapman 2008) and chimpanzees (Huffman *et al.* 1996, Muehlenbein 2005), although *O. aculeatum* has only been reported in Asian macaques (Dewit *et al.* 1991, Kuntz and Myers 1969). *Trichuris* spp. are widespread among African (Gillespie *et al.* 2005, Muriuki *et al.* 1998) and Asian (Gotoh 2000, Kuntz and Myers 1969) monkeys. *Streptopharagus pigmentatus* has been found in Formosan (Kuntz and Myers 1969) and Japanese macaques (Machida *et al.* 1978), and the genus is widespread across Old World monkeys (Dewit *et al.* 1991, Gillespie *et al.* 2004, Hahn *et al.* 2003, Karere and Muneneb 2002). *Strongyloides fuelleborni* has been recorded in several taxa of African (Gillespie and Chapman 2008, Hahn *et al.* 2003, Legesse and Erko 2004) and Asian (Knezevich 1998, Kuntz and Myers 1969) monkeys. *Gongylonema* sp. is rarely found in humans and primates (Acha and Szyfres 2003, Molavi *et al.* 2006), being more prevalent among birds, rodents and ruminants. Among Japanese macaques, it has only been detected in the southern-most populations of Yakushima and Kyushu (Gotoh 2000)

The taxa vary in their means of transmission. *TR* can be acquired through direct ingestion of ova, while *OE* and *STR* develop into infective larvae in soil and may be ingested directly, or in the case of *STR*, also can infect a host transcutaneously. *Yaku* macaques spend considerable time on the ground, foraging, resting or in locomotion, providing ample opportunities for infection via foodstuffs or contaminated hands. *SP* and *GO* require invertebrate intermediate hosts, primarily coprophagous insects that acquire infective ova from mammalian feces. The *Yaku* macaques ingest a range of beetles, cockroaches and other invertebrates from leaf litter, providing a route of reinfection. *Yakushika* deer (*Cervus nippon yakushimae*) also frequently ingest macaque feces, and when tested, deer feces contained *OE* ova. Deer feces are ubiquitous across the forest floor, especially in flatter areas, and thus it is likely that deer serve as a reservoir for *OE* infection and contribute significantly to widespread environmental contamination.

Factors Affecting Parasitism

a. Ecological variables

I observed variation in the prevalence and fecal abundance of parasite taxa, although these did not correlate directly to any common climatic variable. Yakushima does not have a clearly defined 'dry' season, with rainfall being relatively high year-round. During this field season there was a brief respite from rain in October, but in all other months the precipitation and soil moisture were adequate for successful environmental contamination and re-infection from macaque feces. Both recorded seasonal prevalence and fecal abundance of OE, however, indicate a decrease in ova deposition during January and July, almost the coolest and hottest months of the year, with minimum temperatures of 4°C and a maximum of 36°C respectively. Survival and infection risk of many nematodes, including *Oesophagostomum* sp. (Fossing *et al.* 1995) and *Strongyloides* sp. (Premvati 1958) have been previously shown to decrease at environmental temperatures below 15°C and above 30°C. The measure of fecal abundance, however, is not directly indicative of infection load, or environmental risk, but instead indicates parasite fecundity within the host, resulting in environmental contamination (Gillespie *et al.* 2005). This suggests that, in the absence of a clear wet/dry season, the seasonal fecundity of this species may be locally adapted to a tolerable range of temperature for external survival (spring and autumn). This hypothesis may be supported by the observation that fecal abundance of OE and STR correlate strongly. Infections of both taxa are propagated by environmental contamination with ova, which must then hatch into infectious-stage larvae before they can re-infect a host. This may suggest a similar seasonally timed shedding of eggs into the environment, based on suitable conditions for larval hatching, maturation and reinfection, or an inhibition of the same during adverse conditions.

This phenomenon has support in existing studies, especially of domestic ruminants. Across many helminth species, arrested development (hypobiosis) has been recorded during local seasonal conditions most adverse for the free-living stages of the parasite concerned, be that either cold winter or dry summer, with normal development resuming once suitable transmission conditions return (Eysker 1993). The variation of parasite fecal abundance in Czech sheep was most closely explained by seasonal day length,

temperature, or rainfall depending on the parasitic taxa (Langrová *et al.* 2008). In Norwegian reindeer, Trichostrongyle nematode fecundity was found to decrease measurably in winter months when successful transmission is very unlikely, and is independent of infection intensity (Stien *et al.* 2002). This effect is particularly prevalent at high and low latitudes, where seasonal extremes of cold or dryness most significantly reduce the survival of free-living larvae (Davies and McKerrow 2003). In *Yaku* macaques, therefore, the maturation or fecundity of *OE* would appear to be similarly adapted to conserve energy during local conditions of inhospitable heat or cold, and resume environmental contamination during more temperate months.

In an analogous pattern, the fecal abundance of *SP* ova seems to follow the frequency of ground invertebrate foraging, undoubtedly including coprophagous intermediate host species. As the pre-patent period for this taxon is up to 60 days, it is unlikely that this pattern could be caused by fresh infection from intermediate hosts. Nor are these measurements likely to be of unhatched ova, recently reingested, as they hatch rapidly in the insect intermediate host. Equally, this pattern cannot be a cumulative multi-season effect of individual foraging behavior, since the annual means of foraging habits and *SP* infection are not related. In a phenomenon analogous to environmental tracking by *OE*, this seasonal profile may illustrate an adaptive synchronization of ovulation with the period of greatest abundance of intermediate hosts, or a reduction in fecundity during periods of scarce transmission opportunities. A survey of *Leishmania infantum* infections in Spanish dogs (Fernandez-Bellon *et al.* 2008), largely concerned with prevalence, did not detect any significant variation despite the strong seasonality of the sandfly vector and might seem to refute the tracking of vector abundance. The authors noted, however, that clinical signs of infection increased during the sandfly season, as did humoral immunological factors. The immune response may be a reaction to increased reproduction in and lysis of macrophages, significant both to transmission opportunities and the development of life-threatening visceral symptoms. That the prevalence remained steady throughout the year might suggest that the variation in virulence is an adaptive strategy coordinated with the abundance of vectors, rather than a consequence of recent re-

infection. The quantitative correlation found in *Yaku* macaques between *SP* fecundity and invertebrate foraging may indicate a similar phenomenon. The methods employed here, however, did not allow the measurement of invertebrate abundance, nor the identification of the beetle and cockroach species that are suitable intermediate hosts for *SP*. The systematic trapping and identification of foraged invertebrate species, such as has been carried out recently on Yakushima by Hernandez (unpublished data), would be required for conclusive testing of this hypothesis.

b. Social Rank

I found no linear relationship between NA2 social rank and parasitism, but propose a modified analytical model to potentially account for the apparent relationship between both high and low rank and *OE* virulence. The putative connection between these two factors is the 'stress' response - a suite of neurological responses evoked by a physical or psychosocial (perceived) threat to physical homeostasis. The response typically involves the release of catecholamines from the adrenal medulla and the sympathetic nervous system, and glucocorticoids such as cortisol from the adrenal cortex (Sapolsky 2005). Although adaptive in dealing with short term threats, chronic stress responses have been demonstrated to produce deleterious effects, including cardiovascular dysfunction, infertility and immunosuppression (Abbott *et al.* 2003, Khansari *et al.* 1990). Cortisol is easily measured in urine or feces and has been linked with increased incidence of infectious disease in humans (Flinn and England 1997) and non human primates (Sapolsky 2005). The relationship between catecholamines, stress and health is less known due to the difficulty in measuring these more rapidly metabolized hormones.

The interaction of social rank and psychophysical stress is complex. Both high and low ranking individuals may be subject to stressful stimuli, and the relative significance of these will depend on the particular social structure of that species and the ecological context of the population (Sapolsky 2005). For instance, egalitarian or inherited status (such as is found in Japanese macaques) would require less antagonistic maintenance than 'despotically' established rank. Abbott *et al.* (2003) developed a decision tree model to take account of these variables in anticipating stress dynamics within primate groups.

Despite the recognition of such complexities, such existing models of social rank and stress assume a linear relationship, such that analyses of social organization can have one of only three outcomes: 1) dominant individuals have higher stress; 2) subordinates have higher stress; or 3) there is no relationship between stress and social status.

If the greatest likelihood of stress lies in the extremes of the social order, it could follow that the mid-range individuals are relatively buffered from such effects. Indeed, my field observations suggest that there are several sources of elevated stress in high ranking females, including inter-matriline competition, social defense of lower ranking adult allies, especially within the same matriline, and defense of offspring. Simultaneously, sources of stress in low ranking females include frequent aggressive displacement from resources, displaced aggression from higher rank disputes, lack of social support, and chronic cutaneous conditions due to reduced access to grooming partners.

In this scenario, one can visualize a U-shaped stress-rank model, with high stress effects at each end, and lowest effects around the median. Linear models such as that of Abbott *et al.* (2003) could determine the relative amplitude of either extreme, but miss the mid-range dynamics (**Figure 2.9**). Transformation of the numerical rank into divergence from the median makes this distribution amenable to Spearman's rank analysis, which in this case supported this model (**Figure 2.8**). Observed *OE* fecal abundance increased with divergence from the median rank, consistent with elevated immunosuppressive stress at the extremes of social status.

The interaction between social rank, physiological stress and immunology is complex, however, and is unlikely to fit a simple causal model. Stavinsky *et al.* (2001) found no relationship between rank and baseline cortisol in stable groups of *Macaca fascicularis*, although the usual antagonistic behaviors were displayed by both dominant and subordinate individuals. The authors suggest that such a pattern is only found in socially unstable groups. In contrast, Gust *et al.* (1993) found social rank in captive *M. mullata* to be predictive of baseline cortisol only in well established groups, due to the higher rate of grooming in newly formed unstable groups.

To focus solely on the effect of stress on immunology, however, ignores several other rank-related factors that may influence parasite infection risk. While high ranking individuals may enjoy priority access to food resources, improving overall physical condition and immunological robusticity, some parasites are acquired from ingestion of those same dietary items, increasing infection risk (Halvorsen 1986). This effect may be particularly pertinent in parasite cycles involving intermediate hosts, which may be ingested as a dietary item, or which may inhabit commonly foraged plant parts. Similarly, high ranking individuals may occupy a smaller geographical range than peripheral subordinates, increasing the risk of fecal reinfection (Ezenwa 2004). By potentially reducing the competitive fitness of high-ranking individuals, parasites may in fact play a significant role in maintaining the genetic heterogeneity of a population, or act as mechanisms of stabilizing selection (Halvorsen 1986).

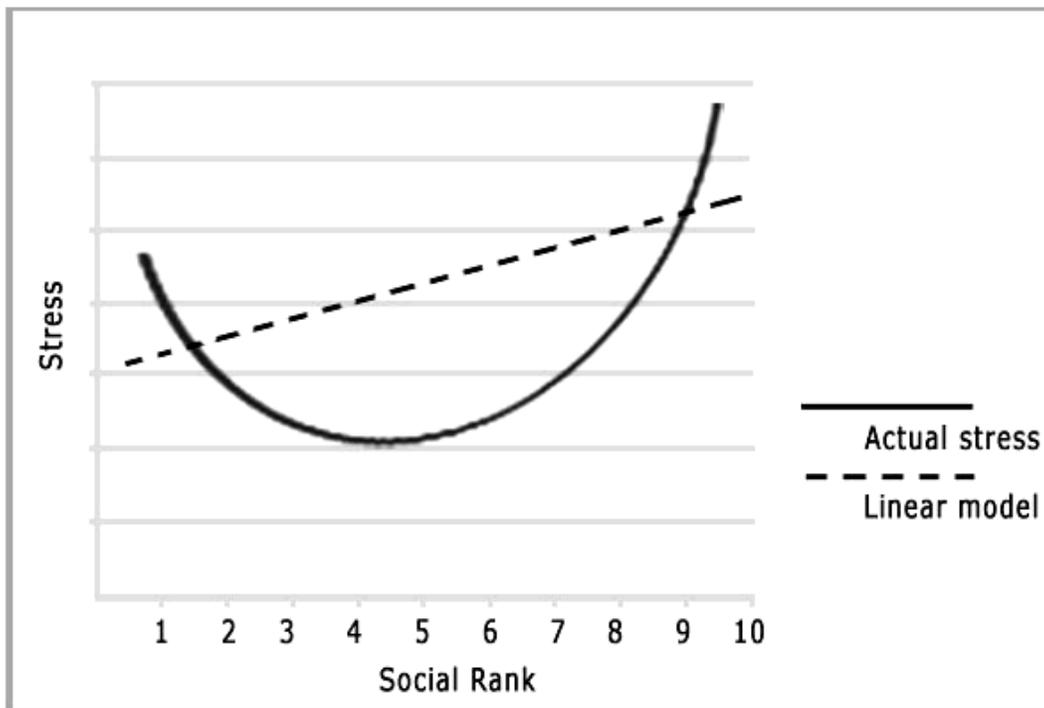


Figure 2.9 - Simplified model of U-shaped Stress distribution by Social Rank. Dotted line shows linear model assumption with the result: "subordinates have higher stress."

c. Age

The only significant relationship between age and intra-individual prevalence occurred in *STR*, the larvae and ova of which were more consistently detected in fecal samples from younger females. This result is in line with previous findings that younger macaque individuals tend to have a heavier and more species rich parasite burden than older individuals (Knezevich 1998), possibly due to immunological naïvety. In one of the few long-term experimental studies on the topic, Cattadori (2005) recorded a similar peak of *Trichostrongyle* infection in juvenile rabbits, which decreased thereafter with age and acquired immunity. The few studies directly relating age to *Strongyloides* infection in humans found a significantly greater prevalence of infection in older subjects (Arakaki *et al.* 1992, Lindo *et al.* 1995), but this is likely to be confounded by generational changes in societal standards of hygiene and exposure, immigration and emigration (Stolk *et al.* 2004). As these factors are not applicable to *Yaku* macaques, a model of acquired immunity may be confidently accepted in this case.

In contrast to the above taxon, *SP* displayed a significantly greater abundance of ova in older individuals, although the prevalence of infection was not significantly affected. Neither abundance nor prevalence, however, was related significantly with an individual's overall frequency of ground invertebrate foraging, discounting the potential increased infection risk of what could be a more terrestrial foraging pattern in older monkeys. Other contributing factors may include a depressed immune response in elderly individuals, and cumulative infection over several seasons. The large size of adult *SP* (and the relatively slow developmental period, Machida *et al.* 1978) may suggest that the species is long lived, allowing for a gradual multi-year accumulation of adult worms in the intestine, even with a constant infection rate.

Conclusions

The NA2 troop of *Yaku* Japanese macaques are host to five nematodal parasite taxa, of which, *Oesophagostomum aculeatum* and *Streptopharagus pigmentatus* are the most prevalent. These two are most likely candidates as health risks within this population, and may have contributed to the 1998 mass mortality event (Hanya *et al.* 2004). Other taxa present are *Strongyloides fuelleborni*, *Trichuris* sp. and *Gongylonema* sp. Although fecal floatation analysis is unable to directly measure parasite infection intensity, certain population dynamics are observable in the variable abundance of fecal ova deposition over time and between host individuals. The lack of distinct wet and dry seasonality on Yakushima limits the predictable effect of humidity on parasite infection risk, but there is some suggestive evidence of parasite ovulation rates tracking ecological conditions relevant to interhost transmission: a tolerable temperature range in *Oesophagostomum aculeatum* and *Strongyloides fuelleborni*; and intermediate host abundance in *Streptopharagus pigmentatus*. On an individual level, infection risk and severity may have a complex relationship with social rank, *Oesophagostomum aculeatum* seeming to most severely affect those at the high and low extremes of the hierarchy. *Strongyloides fuelleborni* infection appears more frequently in younger females, while *Streptopharagus pigmentatus* infections appeared more severe in older females. Lack of symptoms of acute parasitosis, however, suggest that under normal conditions these infections are well tolerated by the monkeys.

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CHAPTER 3

THE INGESTION OF *MISCANTHUS SINENSIS* AS AN ANTIPARASITIC ADAPTATION IN YAKUSHIMA JAPANESE MACAQUES (*MACACA FUSCATA YAKUI*)²

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Abstract

A growing body of literature in behavioral, ecological and pharmacological sciences suggests that animals, including primates, use plant materials in the control of parasitic and other diseases. This study investigates the use of medicinal plants by Yakushima Japanese macaques (*Macaca fuscata yakui*) in relation to nematodal parasitic infection. Behavioral observations and parasitological analyses of ten adult females of the non-provisioned Nina-A2 group were carried out over one year and focused around a set of candidate medicinal plants. Infections with five parasitic taxa were detected: *Oesophagostomum aculeatum*, *Strongyloides fuelleborni*, *Streptopharagus pigmentatus*, *Trichuris* sp. and *Gongylonema* sp. The abundance of *S.pigmentatus* was associated with the ingestion and defecation of whole leaves of *Miscanthus sinensis* (Gramineae) in focal animals. A fecal sample was discovered in E-Troop consisting of a grassy bolus and entangled, adult *S.pigmentatus* nematodes, further reinforcing evidence of this relationship. These observations suggest that *M.sinensis* ingestion is a behavior which functions to control *S.pigmentatus* infection, with a mechanism analogous to 'leaf swallowing' in chimpanzees. Significant quantities of pharmacologically active materials were ingested from other plant sources without evidence of parasitological stimuli, but which may contribute to the low incidence of symptomatic parasitosis in the group. These results may be valuable in planning of conservation areas and captive breeding programs.

Introduction

Conservation biologists are increasingly recognizing infectious disease as a challenge to the conservation of wildlife (Deem *et al.* 2001). Intestinal parasites, such as nematodes, are common throughout the tropics, and produce a range of debilitating symptoms including chronic diarrhea, hemorrhage, dehydration and malnutrition. Novel parasites and pathogens are also being introduced to many threatened populations due to the proximity of hunters, domestic animals or introduced species, often with devastating consequences. This consideration is central to the application of conservation medicine, an emerging multidisciplinary field investigating the effect of disease in threatened populations, both as short-term challenges and long-term coevolution (Aguirre *et al.* 2002). Central to this field is the recognition that health is a state involving not just human populations, but domestic animals, wild animals and the functioning of the ecosystem as a whole. This field is of increasing importance in the face of ecological disruption, novel interspecies contact, and anthropogenic pathogen pollution (Cunningham *et al.* 2003). Although direct veterinary intervention has been suggested as a counter-measure to the risks these pathogens pose to threatened populations, the costs and logistics of such action are often prohibitive. Alternatively, a preventative strategy involves the identification of disease risks, and the use of habitat conservation strategies to minimize the threat posed by disease agents.

Many primate species are particularly susceptible to parasitic disease because they live in large, close-knit social groups, providing a high risk of infection during social grooming and from contamination of foodstuffs (Hart 1990; Stoner 1996). Many primate populations are also in close proximity to humans, including tourists, hunters and farmers, producing the risk of inter-species infection (Cunningham *et al.* 2003). Wild primates employ a range of behavioral counter-strategies to minimize the risk and severity of parasitic infections, however, including the use of specific defecation sites (Gilbert 1997), the rotation of sleeping sites or foraging areas (Hart 1990), grooming, and fur-rubbing (Lozano 1998). In addition, ecological and pharmacological studies have indicated that primate diets include plant parts that are ingested without apparent nutritional benefit, but which may instead be ingested for the secondary chemicals they contain (Huffman 2001; Jansen 1978). The field of zoopharmacognosy

investigates the ingestion of such materials and their potential medicinal properties, and has revealed self-medicative behaviors in many taxa, including several primate species, bears, parrots and sheep (Engel 2002). Often these pharmacognitive strategies are targeted against parasite infections. The most detailed studies to date have involved chimpanzees in Tanzania and Uganda, which have been observed ingesting the bitter pith of *Vernonia amygdalina* (containing the anthelmintic steroid glucosides *Vernonioside A1-3*) (Huffman *et al.* 1993; Huffman and Seifu 1989), and swallowing whole leaves (e.g., *Aspilia mossambicensis*) (Wrangham and Nishida 1983) whose rough surfaces have been convincingly shown to expel intestinal parasites (Huffman and Caton 2001), and may alleviate associated abdominal pain (Wrangham 1995).

This last example, notably, does not rely on secondary chemicals, but instead the physical properties of the leaves which stimulate gastrointestinal motility and dislodge adult nematodes. Leaf-swallowing has provided a strong case for self-medication as a cultural tradition in chimpanzees, and illustrates how such a behavior may be acquired by an individual through a combination of environmental affordance (presence of suitable material), social learning, and positive psychosocial associations (Huffman and Hirata 2003). When experimentally introduced to a naïve captive chimpanzee population, unusually hispid leaves were immediately folded and swallowed whole by two individuals, while others rejected them. Within one week, however, this behavior spread through observational learning to 6 of 11 animals in the group, even in the absence of ill-health (Huffman and Hirata 2004).

This paper reports the use of whole blades of the grass *Miscanthus sinensis* to ameliorate parasite stress by wild Japanese macaques. Previous related research in this species has been restricted to geophagy in Arashiyama monkey park, which was convincingly shown to be a response to indigestion caused by provisioning (Wakibara *et al.* 2001). Although there are several plant species of significance in East Asian medicine among the recorded dietary range of Japanese macaques, there has been no direct investigation of their role other than nutrition. This research conducted the first systematic investigation of phytochemical self-medication in this species.

Methods

Site and subjects

Yaku Japanese macaques, *Macaca fuscata yakui*, are a subspecies of Japanese macaque known locally as *Yakuzaru* (Yaku-monkey). They are endemic to Yakushima, a mountainous island of approximately 500 km², lying 60 km from the southwestern tip of Kyushu, Japan (31°N, 131°E). The island is part of the Kirishima-Yaku National Park, with 10,747 ha also designated as a UNESCO World Heritage Site, primarily on the basis of the sub-tropical to sub-alpine forest range and ancient Japanese cedar trees (*Yakusugi*). *Yaku* macaques were recently re-assessed as Least Concern in the IUCN Red List (Watanabe 2008), although they are recognized to be at some risk due to their restricted range and ongoing habitat fragmentation due to conifer plantation and farming.

This study was carried out in the north-western coastal forest area of Yakushima (**Figure 2.1**). The Western Forest Road (*Seburindo*) area constitutes a Special Wildlife Protection Area, and the macaques have minimal contact with humans except for along the road. Macaques have been studied in this area since 1975, when Tamaki Maruhashi first habituated a group (Maruhashi 1980). Unlike most Japanese macaque research areas, the *Yaku* macaques have never been provisioned, but many groups in the research area are habituated to the presence of observers, allowing approach to within a few meters without overt response.

The study focused on 10 adult female *Macaca fuscata yakui* of Nina-A2 group (NA-2), ranging in age from 5-23 years. The group consisted of 12 adult females, 6 adult males, plus 24 sub-adults, juveniles and infants during the 2007 census (Sugaira *et al.*, unpub. data), with one adult female dying and three infants being born during the preceding year of observation. After the death of the female ‘*Shinju*’ in May 07 at approximately 23 yrs of age, ‘*Nagaramé*’ (*ca.*21-23 yrs old) was incorporated as a replacement for focal observation from June-August, maintaining 10 focal subjects. The group inhabits approximately 71 ha of warm, temperate broad-leaved forest between sea level and 200 m. Although the upper boundary of their range approximately follows the road, and this area was occasionally used by the macaques for

resting or transit, no direct interaction was observed with tourists, including feeding. Much of the central range is comprised of secondary forest due to inhabitation by charcoal burners until the 1950s, and includes the remains of some terraced fields and introduced species. To the north and south of the range, the terrain becomes steeper, and understory vegetation denser, with signs of human habitation restricted to charcoal pits at the base of larger valleys.

Behavioral Observations

The data presented here were collected between 5th September 2006 and 29th August 2007. Total observation time with NA-2 was 1373 hours over 191 days. Two gaps of over 6 days interrupted observations: Dec 30th – Jan 14th and May 12th - 29th. I collected behavioral data utilizing day-long focal follows, from first contact with the day's focal subject after dawn until 1600 or later, for two days per focal animal per month, when conditions allowed (mean observation duration = 10.53 hours per individual per month). Whenever possible, the same focal animal was followed for two successive days, providing paired data separated by 24 hrs and a record of short-term changes in health state. This was achieved on 50 occasions, including all focal animals, for a mean of 4.54 paired observations per individual across the year. Days with less than three hours of observations (e.g., due to heavy rain), were not included in behavioral analyses. Activity was recorded as point samples with a 10 minute interval. Main activity categories were comprised of moving, grooming, processing cheek pouch contents and foraging (including proximal searching, harvesting and ingestion, but excluding processing of cheek pouch contents). During foraging activity, the plant species and part being ingested were recorded. Invertebrates and fungi were not identified by taxa but were, instead, subdivided by substrate location (arboreal/terrestrial/on or in fallen wood). To increase the likelihood of recording rare self-medication, the identity of the focal animal was allowed to change immediately on the observation of patently sick behavior in another individual, such as diarrhea, lethargy or signs of abdominal pain. This *symptom-initiated follow* was only employed once, however, due to the general lack of overt illness in the group.

From the entire range of the *Yaku* macaque diet, a set of candidate plants and materials were selected that were most likely to be involved in self-medicative behavior. Four plant species were selected for focused observational measurement from a combined list of 135 plant species (Hanya 2003, 2004; Hanya *et al.* 2003; Hill 1997; Otani and Shibata 2000): *Melia azedarach*, *Rhus succedanea*, *Trema orientalis*, and *Zanthoxylum ailanthoides*. These species were selected based on their appearance in traditional east-Asian pharmacopoeias (Dash 1991; Duke and Ayensu 1985; Li 2006), published phytochemical and pharmacological analyses (Cheng *et al.* 2005; Cheng *et al.* 2003; Duke 1996; Jassim and Naji 2003; Lee *et al.* 2004; Morita and Shimizu 1966; Tokita *et al.* 2007; Wu *et al.* 2002), and zoopharmacognosy literature (Huffman and Wrangham 1994) (properties and sources summarized in **Table 5.1**).

In addition, distinctive ethological signifiers were recorded during the field observations, as suggested by the C.H.I.M.P.P. group (Chemo-ethology of Hominoid Interactions with Medicinal Plants and Parasites), to identify potential medicative behavior and allow the selection of further plant species for candidate status (Huffman, 1996). These behaviors include: ingestion of plant part of minimal nutritional value (e.g., bark); non-nutritional ingestive behavior (e.g., chewing-discarding or whole leaf swallowing); evidence of indigestibility of plant or whole-leaf swallowing by appearance in feces; bitter or pungent taste, and focal distance from the group during ingestion. These observations led to the incorporation of two additional species for candidate observation: *Lagerstroemia fauriei* (bark) and *Miscanthus sinensis* (leaf).

When a focal animal approached a candidate plant, the interaction was recorded *ad libitum* until the focal animal moved out of arm's reach of the material. Recorded variables included: location; proximity time (focal animal within reach of plant, seconds); the duration of focused manipulation, mastication and ingestion of the material (in seconds); part ingested; number of mouthfuls (when clearly seen); and the occurrence of 'intense observation' by infants indicating opportunities for social learning of these behaviors (Tarnaud and Yamagiwa 2008).

Parasitology

Fresh fecal samples were collected from focal animals twice daily, typically in both morning and afternoon. In addition, a further sample was collected from each individual per month as opportunity allowed, increasing the replication to a maximum of 5 samples per focal per month (N = 360 total samples). Since these additional samples were not collected during focal follows, they were used only for characterization of general parasitological patterns, and not in the analysis of potential self-medicative stimuli. Samples were selected that were free from soil contamination, and were collected with a clean, sharpened stick into a screw-top plastic vial. Samples were analyzed on the evening they were collected, or refrigerated for up to 24 hrs at 5°C, removing the need for preservatives, and allowing the observation of motile larva.

Fecal samples were analyzed as follows:

The presence, species and abundance of any adult parasites or undigested leaf matter in the feces was noted, and each sample was graded for gross consistency (1- firm, 2-medium, 3-loose).

Each sample was subjected to quantitative analysis using a modified, non-centrifugal McMaster floatation technique (Gordon and Whitlock 1939; MAFF 1986; Pereckiene *et al.* 2007). Approximately 4 g of fresh (or <24 hr refrigerated) feces were vigorously mixed in 28 ml of saturated salt/sugar solution (SG=1.28). The resulting suspension was strained, gently stirred again, and 0.3 ml examined in each of two 2-cell McMasters floatation slides (0.15 ml per cell). All helminth ova within the grids were counted, averaged between the two slides, and multiplied up (x25) to give a value of eggs per gram (epg) for each species present in that sample.

To provide qualitative analyses and detection of non-floating ova and larvae, a sample of 0.2 g of fresh feces was homogenized with 0.5 ml isotonic saline. Using a small syringe, a sample of fluid suspension was drawn off, avoiding large particulates, and a single drop placed on a standard slide with a cover-slip. This wet-mount was examined at 100x -400x. A count of ova and larvae was kept to allow a crude estimation of relative abundance.

Diarrhea is the most common and easily detectable symptom of parasitosis and a hypothesized physiological stimulus for self-medication. Loose watery stool is often associated with virulent parasite infections, and is linked to the major health risks of these conditions – dehydration, intestinal or colonic ulceration, and hemorrhage (Acha and Szyfres 2003). From one of the daily samples, approximately 2 g were separated and frozen at -30°C. Each month and in a batch, these samples were dried at 100°C for 24 hrs and then reweighed to determine percent water content.

From the floatation and wet-mount preparations, ova and larvae were identified by taxon based on characteristic morphology (Acha and Szyfres 2003; Desowitz 1980) and the abundance of each taxon was recorded. Any unknown taxa were photographed, and subsequently identified through consultation with Dr. Hasegawa, a professor of parasitology in the Faculty of Medicine, Oita University.

Limitations

The study of health and disease in wild populations has inherent methodological challenges that limit the accuracy of any scientific picture generated. Sick animals may modify their behavior in such a way that makes them more or less difficult to locate and observe (Hart 1990), thus causing the observer to under or overestimate the prevalence of disease in the population. Long term observation of a single group may alleviate this error through close familiarity with the subjects, but physiological data are vital in gaining accurate insight into the health of the group.

Unfortunately, researchers can only obtain limited gastrointestinal parasitological information through fecal sampling. The advantages of this method lie in ease of collection, non-invasive sampling, and relatively simple analytical techniques. Although floatation analysis can give an estimate of the presence and relative abundance of a taxon in a sample, this may not be directly related to the number of parasite individuals infecting the host animal – i.e., the intensity of infection. For example, some parasitic taxa, including *Oesophagostomum* sp., have been found to lower their fecundity during winter months (Fossing *et al.* 1995). False negative results may also occur due to lack of analytical sensitivity, temporal variation in ova deposition, or uneven distribution of ova in feces (Pereckiene *et al.* 2007; Stuart and

Strier 1995). These last two factors may be minimized through adequate replication within a short time period, and an analytical technique, such as the McMaster procedure used here, which relies on the homogenation of a larger amount of feces, from which a smaller analytical sample is taken. Still, the figures presented here are not absolute, and while they may be used to accurately compare records within this study, they should not be compared directly to those collected utilizing other methods in other sites.

Statistical analyses

Due to the small sample size of observed individuals, the non-parametric Friedman's analysis was used to determine the effect of the annual cycle on 1) the abundance of each nematodal taxon recorded in the macaque feces; and 2) the daily duration of candidate plant use (Martin and Bateson 1993). To determine the role of parasitosis as a stimulus for ingestion of plants, daily records of parasitic abundance were regressed against daily ingestion of candidate materials (seconds), controlling for the length of observations on each day (weighted least squares regressions).

To test for efficacy of potential self-medication, the overnight (24 hour) difference in parasite abundance was calculated, where paired observations existed, and this was then regressed against the previous day's ingestion of candidate material. Positive medicinal efficacy would be evident in a consistent reduction of parasitic fecal abundance after consumption of a candidate plant.

To validate the association between measured fecal water content and diarrheal symptoms, percentile water content was correlated with the visual grading of each fecal sample (Pearson's correlation). To assess the pathological relationship between parasite abundance and diarrheal symptoms, the water content in fecal samples was regressed against the corresponding counts of ova and larvae for each parasite taxon. If a consistent relationship was found between diarrheal symptoms and parasite abundance, then any outlying deviations from the trend would be examined and compared with putative self-medicative behavior.

I used SPSS 16.0 for all statistical analyses. Significance was set at $p < 0.05$.

Results

General dietary patterns

Monkeys of NA-2 troop ingested a large variety of food items, and are broadly omnivorous, their diet including large numbers of invertebrates, a wide variety of plant parts, fungi and soil (**Figure 3.1**).

Appendix A.1 lists the full 71 food plant species that I recorded during daylight hours over this study period.

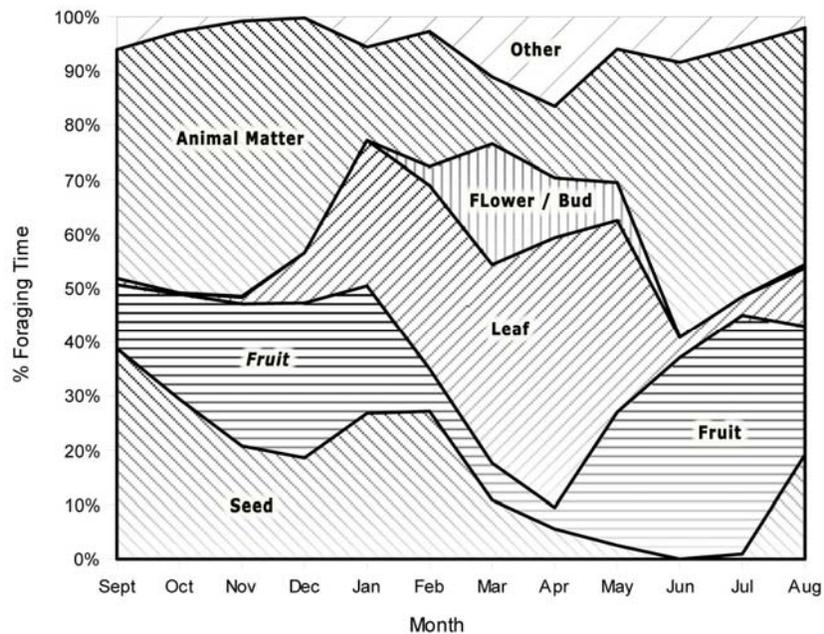


Figure 3.1 - Seasonal variation in diet of NA-2 macaques: Frequencies are based on 10 minute point samples collected September 06 – August 07

Parasitology

Fecal analyses identified active infections of five nematodal taxa: *Oesophagostomum aculeatum*, *Strongyloides fuelliborni*, *Streptopharagus pigmentatus*, *Trichuris* sp. and *Gongylonema* sp. Of these, *O.aculeatum* and *S.pigmentatus* were the most prevalent, occurring in all focal animals. *Gongylonema* ova were detected only in very low abundances in 6 out of the 11 monkeys and were excluded from further analyses. **Table 2.1** summarizes the prevalence and disease ecology of these taxa. Individual samples contained 0-4 taxa, with significant peaks in diversity occurring in October and May (Friedman's: $X^2=20.342$, $p=0.041$).

Figure 2.6 presents the annual variation in the mean fecal abundance recorded for the five parasite taxa (average abundance across 10 focals, mean of 30.1 samples per month). Friedman's analyses revealed a significant temporal variation in the abundance of *Strongyloides fuelleborni*, *Streptopharagus pigmentatus* and *Oesophagostomum aculeatum*. *O.aculeatum* displayed high abundance in September and peaked again in May ($X^2=34.061$, $p<0.001$). *S.fuelleborni* abundance was high at the inception of the study in September, and declined thereafter ($X^2=37.873$, $p<0.001$), which may indicate some false positive identifications early in the observation period. *S.pigmentatus* displayed two distinct peaks of abundance, in December and June ($X^2=25.479$, $p=0.008$). *Trichuris* sp. abundance was generally low, with a weak peak in April/May ($X^2=13.295$, $p=0.274$). *Gongylonema* abundance was extremely low throughout the year ($X^2=14.085$, $p=0.228$).

Fecal water content (%) was consistent with the observed sample consistency (firm/med/soft) (Pearson's $r=0.323$, $p<0.001$), confirming this measure as a reasonable indicator of diarrheal symptoms. The measured abundance of all analyzed parasite taxa, however, demonstrated no significant relationship to these symptoms. Only 3% of samples were categorized as soft or diarrheal and these were not associated with high parasite abundance. On only two occasions did focal macaques display visible symptoms of colic or malaise and these also were not accounted for by parasite fecal abundance.

Use of candidate plants

No relationship was found between parasite abundance and the frequency of ingestion of the candidates *Melia azedarach*, *Rhus succedanea*, *Trema orientalis*, *Zanthoxylum ailanthoides* or *Lagerstroemia fauriei*. Focal macaques did, however, consume notable quantities of *T.orientalis* leaves (33 events, 5 hrs 35 mins total) and *Z.ailanthoides* bark and exuded gum (88 events, 42 mins total), and active dosages of antibacterial and anthelmintic compounds may have consequently been ingested. *L.fauriei* was also associated with highly anomalous foraging behavior, as the bark was chewed and discarded without being swallowed.

The grass, *Miscanthus sinensis*, is found in rocky, exposed areas across NA-2 range, including road cuttings, between riverbed rocks and on small crags. On 9-16-06, the 7 yr old adult female "Shipi" was observed to ingest the leaf blades of this coarse grass for 30 sec. This behavior subsequently was repeated on 18 other occasions by 6 of the 11 focal adults, mainly during September and October 2006, and with a further distinct peak in June 2007 (**Figure 3.2a**). Variation in foraging time across the season was significant when analyzed through Friedman's test ($X^2 = 21.73$; $p=0.027$). These observations were accompanied by 26 fecal samples containing up to 80 cm whole undigested *M.sinensis* leaf during a similar date range in fall-winter and early summer (**Figure 3.2b**). Leaf-containing fecal samples were obtained from all focal animals, indicating that some ingestion was not observed, probably occurring before first contact in the morning. The leaf blades of this grass are tough and fibrous, and are taken whole into the mouth and chewed only briefly, perhaps insufficiently for break-down of the fibers, accounting for their appearance in feces. The leaf margins are lined with sharp, directional trichomes, clearly discernible by touch and confirmed by microscopic examination (**Figure 3.3**). In 6 cases (32%), infants were seen closely observing (Tarnaud and Yamagiwa 2008) the ingestion of *M.sinensis* by their mother, but never subsequently ingested it themselves. On a single occasion, a 1 year old male juvenile joined his mother in eating the grass without first investigating her behavior.

Focal records of daily *M.sinensis* leaf swallowing (duration in seconds) demonstrated a positive predictive correlation with fecal appearance of undigested leaves on that same day ($t=3.062$; $p<0.001$). This relationship, however, did not extend to the feces collected on the subsequent day of a paired sample ($t=0.250$; $p=0.804$). This plant demonstrated the only statistically significant relationship with parasite ova abundance by a candidate plant in this study. Fecal discovery of undigested *M.sinensis* leaves was significantly predicted by the fecal abundance of *S.pigmentatus* ova ($t=2.351$; $p=0.02$), while observed ingestion of *M.sinensis* leaves (sec/day) was correlated with daily *Trichuris* sp. ova abundance ($t=2.388$; $p=0.018$).

In addition to these observational data, a single fecal sample was collected by Hayakawa from the neighboring E-Troop (11/17/06, 11:04 am), whose range follows the road forming the eastern border of NA-2. The sample came from “Nancy,” an 8 year old female in estrus who appeared constipated but otherwise in good health. The feces constituted half of a sample collected for hormonal analysis (Hayakawa, in prep.) and appeared as a solid bolus of *Miscanthus sinensis* leaves and large nematodes (Figure 3.4).

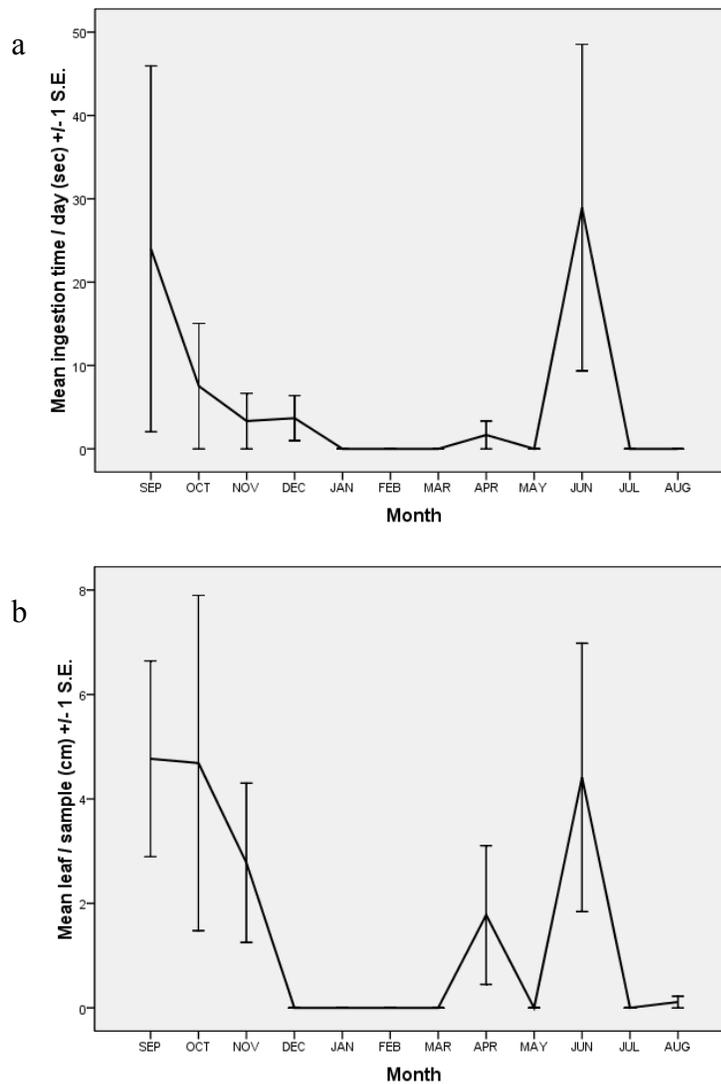


Figure 3.2 – Occurrence of *Miscanthus sinensis* ingestion (a) and leaf-containing fecal samples (b).

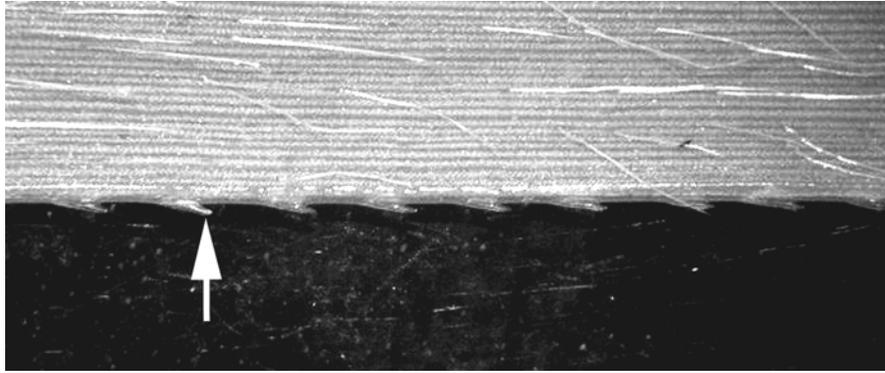


Figure 3.3 - Margin of *Miscanthus sinensis* leaf blade (10x). (Photo by Dagg).

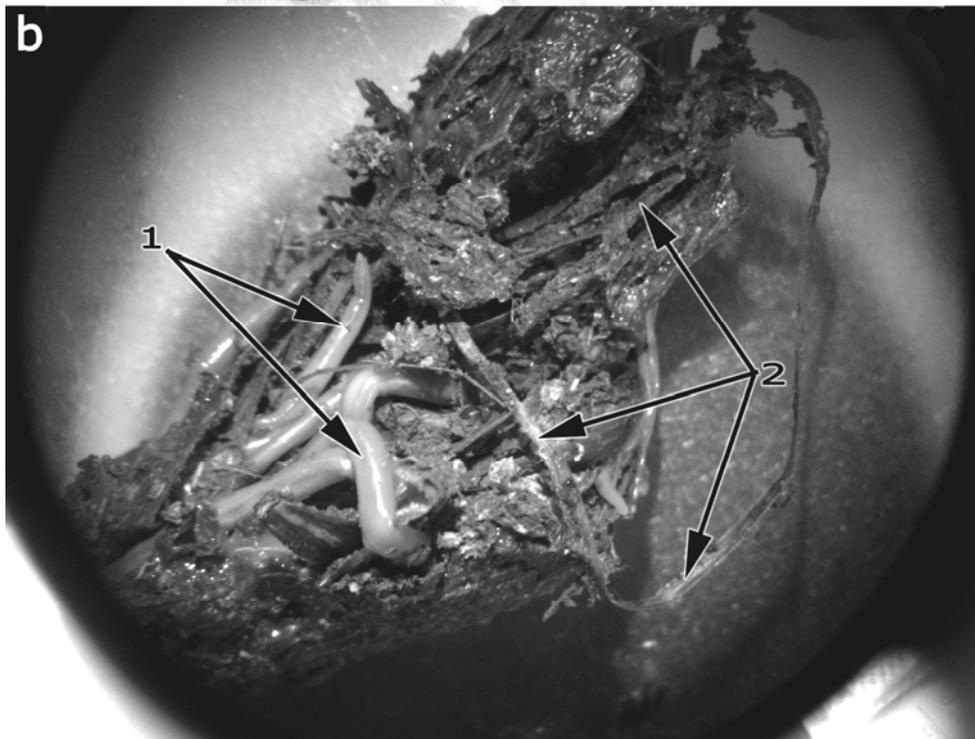
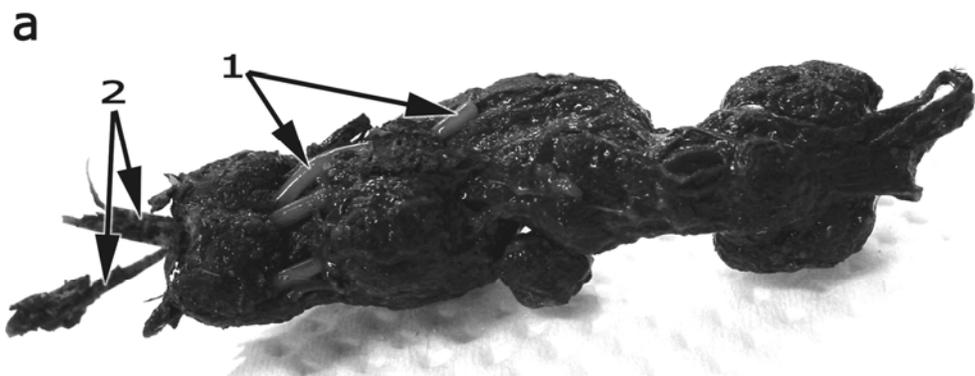


Figure 3.4 – E-Group fecal sample whole (a) and after partial dissection (4x) (b), comprised of adult *Streptopharagus pigmentatus* (1) within a grassy bolus of *Miscanthus sinensis* leaves (2). (Photos by Dagg)

When dissected, the bolus comprised by weight: 52% homogenous dry fecal matter, 27% *Miscanthus sinensis* leaves (20 pieces of mean length 8 cm, totaling approximately 160 cm) and 21% adult nematodes (four of 7 cm length, one of 4 cm). The worms were immotile at the time of collection. With assistance from Professor Hasegawa of Oita University, the nematodes were identified as adult *Streptopharagus pigmentatus*. Analysis of the fecal matter recorded *Streptopharagus pigmentatus* ova (20), *Strongyloides fuelleborni* larva (1) and *Oesophagostimum aculeatum* ova (1) under wet-mount, and 75 eggs per gram *O.aculeatum* through McMaster's floatation. The number of *S.pigmentatus* ova recorded was greater than any NA-2 sample in this study, and when calibrated by a comparative quantitative analyses carried out by Hernandez (unpublished data), was equivalent to approximately 5800 ova per gram, a heavy load. No other, similarly grassy feces were seen during observations of E-Troop.

Discussion

Of the set of candidate plant species investigated, only *Miscanthus sinensis* demonstrated evidence of use and function relating to parasite infection in *Yaku* macaques. Similar to the hispid leaves selected by chimpanzees, the margins of this grass have sharp, directional trichomes (**Figure 3.3**) that may both adhere to parasitic worms, 'scour' the gut mucosa, and stimulate purgative motility. In this study, the rapid appearance of grass blades in fecal samples following ingestion may be indicative of such an effect on gut motility. Similar leaf-swallowing behavior in chimpanzees decreased gut transit time to a mean of 6.2 hours, 26% of that recorded on a fruit-only diet, contributing to the expulsion of adult *Oesophagostimum aculeatum* (Huffman and Caton 2001). Japanese macaque mean gut transit time has been recorded as approximately 48 hours (Chalmers, per. comm.), and if accelerated by a similar degree, could result in gut transit within 12 hours, and account for the apparent expulsion of ingested leaves within the same day.

The statistical relationship between *Streptopharagus pigmentatus* fecal abundance and leaf-blade ingestion in NA-2 macaques is supported by the direct association of adult nematodes and *Miscanthus sinensis* leaves in the E-Troop fecal sample. These parallel observations are highly suggestive of parasite

load being a significant stimulus for the behavior. Whether the mechanism is specific to *S.pigmentatus* is less certain. The adults of this species are the largest of the nematodal taxa recorded in *Yaku* macaques (Machida *et al.* 1978), and in high numbers may cause gastro-intestinal obstruction or malnutrition (Gotoh 2000). The stimulus for the ingestion of *M.sinensis* may therefore be generalized gastric discomfort caused by such obstruction. This may serve to expel adult *S.pigmentatus* in E and NA-2 troops, but it is equally possible that the same behavior may be demonstrated by other groups in response to other locally specific stressors such as parasitosis caused by other taxa.

In interviews with traditional Japanese herbalists conducted in Kyoto and Nara (Dagg 2009), *M.sinensis* was recognized amongst the interviewees (11/20), but is rarely used in current medical practice, with most stated uses dating from historical tradition. The leaf was previously used to treat bloat and acid indigestion, and secondarily was used for vaginal discharge and amenorrhea. The root was stated by one to be used as a febrifuge and external anti-inflammatory. The pith was a known foodstuff, with two pharmacists remembering eating it themselves as children. Interestingly, three respondents mentioned that cats and dogs preferred this species for the well-known grass-emetic behavior, and one claimed that village dogs were known to seek this plant specifically to treat nematode infections.

The stems of *Miscanthus sinensis* (inseparable from the leaf blades) are used in Taiwan to treat hypertension (as a diuretic), coughs and vaginal discharge, while the related *M.floridulus* is used as an antidiarrheal (Li 2006). The presence in feces of whole undigested leaves of this species is perhaps the clearest indication of a non-dietary ingestive behavior in *Yaku* macaques. Strongly analogous behavior has been recorded in the ingestion of sedge grass (*Carex* spp) by Alaskan brown bears to effectively rid themselves of tapeworms in the weeks leading up to hibernation, and by Canadian snow geese prior to migration (Huffman 1997). Similarly, grass blades are ingested year-round by both wolves and Indian tigers, which have both been observed to defecate grass-boli droppings, often associated with whole roundworms and tapeworms (Engel 2002). In chimpanzees (*Pan troglodytes schweinfurthii*), the grass *Hyparrhenia cymbaria* is reportedly swallowed at Kibale, Uganda, and a sedge-grass (Cyperaceae) swallowed at Tai, Ivory Coast (Huffman 1997). More recently, almost identical grassy boli have been

found in the feces of chimpanzees (*Pan troglodytes vellerosus*) in Nigeria, along with unchewed leaves of *Desmodium gangeticum*, and adult *Oesophagostomum* sp. nematodes (Fowler *et al.* 2007). Undigested leaves in fecal samples were also the earliest indications of the well-known leaf-swallowing medication in chimpanzees (Wrangham and Nishida 1983).

The evidence from chimpanzees, bears, and geese suggests that the repeated passing of whole hispid leaves may be an effective strategy to reduce worm burdens during times of greatest infection or health risk (Huffman and Caton 2001). The observations of this study suggest that this behavior may be a seasonal strategy by *Yaku* macaques to manage *S.pigmentatus* worm burden at the times of greatest virulence, and may be significant in the maintenance of this population's health.

Prophylaxis and Dietary Phytochemicals

Although by its nature, prophylaxis is extremely difficult to distinguish from field observations of dietary activity (Carrai *et al.* 2003), the inclusion of pharmacologically active chemicals in ingested materials may indicate at least a potential health benefit gained from that food, without inferring any awareness or intentionality on the part of the animals. Such plants may have significance in conservation medicine, beyond that of simple nutritional items, and should be considered as higher priority resources in reserve design. In this study, the ingestion of significant amounts of pharmacologically active material from *Trema orientalis* and *Zanthoxylum ailanthoides*, for example, may still affect the transmission and symptomology of parasite infections. As such, their exclusion from a protected area may negatively impact the health of a threatened population, just as would the exclusion of a key nutritional resource.

Conservation Health

Parasite infection is highly prevalent in *Yaku* macaques, including potentially pathogenic taxa. The adult females of NA-2 group, however, seem to be in generally good health, the low occurrence of diarrhea suggesting that the infections are well tolerated. Such infections may, however, cause a more significant threat to population health under certain abnormal conditions. Nutritional stress due to a dry

fruiting season may depress immunological defenses and cause catastrophic mortality as occurred on Yakushima between August 1998 and August 1999 (Hanya *et al.* 2004). During this period, 56% of the population of five studied troops died, with two troops suffering extinction. Patterns of variable mortality in this instance suggested the mutual effects of starvation and epidemic disease. Habitat fragmentation or group relocation might also isolate populations from resources such as *Miscanthus sinensis* that may play a role in the management of parasite load and diarrheal symptoms, thereby increasing their susceptibility to severe disease risks.

In the calculation of risk assessment for threatened primate populations, studies of anthropogenic habitat modification focus largely on major food species (e.g., Kinnaird 1992). While nutritional resources are clearly vital, the widespread harvesting of wild medicinal plants may also prove to be a significant area of ecological conflict between human and primate populations. This consideration is even more significant in highly fragmented habitats, where the removal of a single tree may have a serious effect on the resident primates (Cowlshaw and Dunbar 2000), and contribute to their vulnerability to local extinction events.

The importance of grass ingestion for Yakushima macaques could be investigated through a natural experiment conducting an epidemiological and ecological comparison of two or more groups from differing forest zones. *Yaku* macaques live in all altitudes, from sea-level to the dwarf bamboo zone at *ca.* 1500 m, including several distinct ecological zones of both primary and secondary forest. This diversity of habitat across their range would suggest that groups have varying access to the rocky outcrops or road cuttings where *M.sinensis* is found. Alternatively, the introduction of this plant species to existing captive populations of Japanese macaques may allow a controlled pre/post experimental design such as that employed by Van Asseldonk and Hass (1997, 2006) to assess the health benefits of carminative plants in zoo enclosures. Such zoo enclosure enrichment with medicinal plants may also benefit captive breeding efforts through a reduced necessity for veterinary intervention and consequent animal distress, the provision of sensory enrichment for the animals (Vermeer 1995) and enhanced public education opportunities (Davey *et al.* 2005).

Conclusions

Parasite infection is highly prevalent in Yakushima Japanese macaques but the infections seem to be well tolerated insofar as could be determined non-invasively. These observations add Japanese macaques to the range of animal species that appear to use coarse grass blades to scour intestinal tracts of large parasitic taxa. In this case, the leaves of *Miscanthus sinensis* appear to be ingested as a strategy to control *Streptopharagus pigmentatus* in NA-2 and E troops of *Yaku* macaques on Yakushima. The data are consistent with previous research that suggests an effective combined mechanism of physical entanglement and increased gut motility.

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CHAPTER 4

GEOPHAGY IN YAKUSHIMA JAPANESE MACAQUES (*MACACA FUSCATA YAKUI*): AN INVESTIGATION OF PARASITOLOGICAL AND DIETARY FUNCTIONS³.

³ Dagg, C. To be submitted to *Primates*

Abstract

Geophagy, the deliberate ingestion of soil or clay, has been recorded in many primate species, and several hypotheses of adaptive functions suggested for this behavior. I observed the occurrence of geophagy over one year (September 06 – August 07) in a wild, free-ranging group of ‘Yaku’ Japanese macaques (*Macaca fuscata yakui*) on the Japanese island of Yakushima. All focal animals (adult females) ingested small amounts of clay throughout the observation period. In the majority of events, the monkeys ate orange or tan subsurface clay, but on rare occasions were seen to ingest topsoil. The frequency and duration of geophagy was tested against concurrent parasitological and foraging data. Neither symptoms nor severity of parasite infection could be demonstrated as a stimulus for geophagy, although clay ingestion did affect the abundance of *Oesophagostomum aculeatum* and *Trichuris* sp. ova after 24 hours, and may serve a prophylactic role against diarrhea. I found significant relationships between the daily frequency of geophagy and foraging for invertebrates, *Lithocarpus edulis* acorns and *Myrica rubra* fruit. Possible functions of geophagy in this population, therefore, may include detoxification of invertebrate chemical defenses and acorn tannins, or relief from generalized gastrointestinal distress.

Introduction

The practice of geophagy (the deliberate ingestion of soil) is widespread across the mammalian and avian orders, including human (Johns and Duquette 1991a) and non-human primates (see review in Krishnamani and Mahaney 2000). For many years, however, the behavior was regarded as a form of pica, an abnormal psychological condition causing an appetite for inedible items such as hair, stones or feces (Ziegler 1997). Several primatologists and anthropologists have proposed hypothetical adaptive functions for the occurrence of geophagy in human and non-human primates. These hypotheses are recognized to be non-exclusive and the relative role of each function may vary between species and populations due to their differing adaptive value. Indeed, the various published evidence of the phenomenon in non-human primates has supported each of the possible hypotheses (Krishnamani and Mahaney 2000; Wilson 2003). Proposed stimuli include: 1) relief from diarrhea caused by gastrointestinal parasites (Bicca-Marques and Calegare-Marques 1994; Knezevich 1998; Vermeer and Ferrell 1985); 2) relief from digestive discomfort/acidosis (Davies and Baillie 1988; Mahaney *et al.* 1997; Oates 1978; Wakibara *et al.* 2001); 3) dietary supplementation of mineral micronutrients (Abrahams 1997; Danford 1982; Wiley and Katz 1998); and 4) detoxification of dietary plant toxins (de Souza *et al.* 2002; Dominy *et al.* 2004; Gilardi *et al.* 1999; Johns 1986; Johns and Duquette 1991b; Mahaney *et al.* 1995).

Parasitic infection is one of the most ubiquitous health stressors in tropical and sub-tropical latitudes, with symptoms including malnutrition, diarrhea and chronic dehydration - thus offering a substantial selective advantage to its effective management (Lozano 1998). In the majority of analyzed geophagic material, the ingested soil contains a high amount of 1:1 Al:Si kaolinitic/metahalloysitic clays, and to a lesser extent, 2:1 clays such as smectite and illite (e.g., Aufreiter *et al.* 2001; Davies and Baillie 1988; de Souza *et al.* 2002; Mahaney *et al.* 1997). Each of these groups has high cation-exchange properties, proffering a very high adsorptive ability for dietary and entero-toxins, as well as for water (Dominy *et al.* 2004). This property has proven therapeutic in cases of dietary or infectious diarrhea in humans and monkeys (Beck *et al.* 1977; Leber 1988). In one study of rhesus macaques (*Macaca mulatta*)

Knezevich (1998) found widespread helminthic infection, but a surprisingly low incidence of diarrhea, which the author attributed to the widespread practice of kaolinitic geophagy in that population.

Existing studies of geophagy in Japanese macaques are limited to Arashiyama, where the macaques are provisioned to facilitate observation. Such practice is common amongst Japanese macaque research areas, and associated indigestion was convincingly shown to be the primary cause of geophagy in that population (Wakibara *et al.* 2001). In contrast, the macaques on the island of Yakushima have never been provisioned and as such their digestive health, social and foraging behaviors are not affected by the anthropogenic schedule and composition of supplementary food (Hill 1999).

This study investigates the occurrence of geophagy in Yakushima Japanese macaques (*Macaca fuscata yakui*) as part of a wider study of self-medication and parasite ecology under natural foraging conditions (Dagg in prep. a; Dagg and Hayakawa in prep. b). In addition to testing a primary antiparasitic/antidiarrheal hypothesis for clay ingestion in this population, the methodology allowed evaluation of the digestive and detoxification hypotheses listed above, through temporal association with dietary items. Ethnomedicinal data collected by the author in 2003 on the use of clay by traditional Japanese pharmacists are reviewed to help evaluate therapeutic conclusions drawn from the macaque observations.

Methods

Site and subjects

Macaca fuscata yakui are a subspecies of Japanese macaque endemic to Yakushima, a mountainous island of approximately 500 km², lying 60 km from the south-western tip of Kyushu, Japan (31°N, 131°E). The island is part of the Kirishima-Yaku National Park, with 10,747 ha also designated as a UNESCO World Heritage Site. Due to population stability, *Yaku* macaques have recently been re-assessed from Endangered to Least Concern in the IUCN Red List (Watanabe 2008), although they are still recognized to be at risk due to their restricted range and ongoing habitat fragmentation by plantations and agriculture.

This study was carried out in the northwestern coastal forest area of Yakushima (**Figure 2.1**). The Western Forest Road (Seburindo) area constitutes a Special Wildlife Protection Area, and the macaques have minimal contact with humans except for along the road. Macaques have been studied in this area since 1975, when Maruhashi first habituated a group (Maruhashi 1980). Although the *Yaku* macaques are not provisioned, most groups in the research area are habituated to the presence of observers, allowing approach to within a few meters without overt response.

This study focused on 10 adult female *Macaca fuscata yakui* of Nina-A2 group (NA-2), ranging in age from 5- 23 years. The age of individuals was determined from long-term census data collected at the University of Kyoto Yakushima Field Research Station (Sugaira *et al.*, unpub. data). The group consisted of 12 adult females, 6 adult males, plus 24 sub-adults, juveniles and infants during the 2007 census. After the death of the female ‘*Shinju*’ in May 07 at approximately 23 yrs of age, ‘*Nagaramé*’ (21-23 yrs old) was incorporated as a replacement for focal observation between June and August, maintaining 10 focal subjects. NA-2 inhabits approximately 73 ha of warm, temperate broad-leaved forest between sea level and 200m. Although the upper boundary of their range approximately follows the road, which was occasionally used by the macaques for resting or transit, no direct interaction with humans was observed. Much of the group’s central range is comprised of secondary forest due to inhabitation by charcoal burners until the 1950s, while the terrain becomes steeper to the north and south of the range, with denser understory vegetation, and signs of human habitation restricted to charcoal pits at the base of larger valleys.

Behavioral Data collection

Total observation time with NA-2 was 1373 hours over 191 days, between 5th September 2006 and 29th August 2007. Two gaps of over 6 days interrupt the observations: Dec 30th – Jan 14th and May 12th - 29th. Behavioral data was collected using day-long focal follows, from first contact with the focal individual after dawn until 1600 or later, for two days per focal animal per month, when conditions allowed (mean observation duration = 10.53 hours per individual per month). Whenever possible, the

same focal animal was followed for two successive days, providing paired data separated by 24 hrs and a record of short-term changes in health state. This was achieved on 50 occasions (100/191 observation days), including all focal animals, for a mean of 4.54 paired observations per individual across the year. Days with less than 3 hours of observations (e.g., due to heavy rain), were not included in behavioral analyses. Activity was recorded as point samples with a 10 min interval. Main activity categories were comprised of moving, grooming, processing cheek pouch contents and foraging (including proximal searching, harvesting and ingestion, but excluding processing of cheek pouch contents). During foraging activity, the plant species and part being ingested were recorded. Invertebrates and fungi were not identified by taxa, but were, instead, subdivided by location (arboreal, terrestrial, on/in dead wood).

When a focal animal approached a clay mining site, the interaction was recorded *ad libitum* until the focal moved out of arms-reach of the site. Recorded variables included: location; duration of proximity to the site (in seconds); the duration of focused manipulation, mastication and ingestion of the material (in seconds); visual appearance and strata of ingested clay; and occurrence of 'intense observation' by infants that might indicate opportunities for social learning of such behaviors (Tarnaud and Yamagiwa 2008).

Parasitological Analyses

Fresh fecal samples were collected from focal animals twice daily, typically in both morning and afternoon. In addition, a further sample was collected from each individual per month as opportunity allowed (N = 360 total samples). Since these additional samples were not collected during focal follows, however, these were used only for general parasitological patterns, and a total of 312 samples were used in the analysis of geophagy. Samples were selected that were free from soil contamination, and collected with a clean sharpened stick into a screw-top plastic vial. Samples were analyzed that evening or refrigerated for up to 24 hrs at 5°C, removing the need for preservatives, and allowing the observation of motile larva. The presence, species and abundance of any adult parasites or undigested leaf matter in the feces was noted, and each sample was graded for gross consistency (1- firm, 2-medium, 3-loose).

Quantitative analysis was performed on each sample using a modified, non-centrifugal McMaster's floatation technique (Gordon and Whitlock 1939; MAFF 1986; Pereckiene *et al.* 2007) using approximately 4 g of fresh (or <24 hr refrigerated) feces in 28 ml of saturated salt/sugar solution (SG=1.28), and two 0.3 ml McMasters 2-cell floatation slides. Nematode ova counts were multiplied up (x25) to give a value of eggs per gram (epg) for each species present in the sample. Wet-mount analysis was performed on a single drop of fecal suspension (0.2 g in 0.5 ml isotonic saline), observed at 100-400x under a standard slide coverslip. This provided a qualitative diagnosis of parasite species prevalence, and detected larvae and non-floating ova that were not suitable for floatation analysis. A count of ova and larvae was kept to allow a crude estimation of relative abundance. From one of the daily samples, approximately 2 grams were frozen at -30C. Each month in a batch, these samples were dried at 100°C for 24 hrs and reweighed to reveal percent water content as a measure of diarrheal symptoms.

Ethnographic Interviews

During the summer of 2003, I interviewed 14 practitioners of traditional herbal medicine in Kyoto and Nara, and one expert of ethno-mineralogy at a private geological museum in Kyoto (Dagg, unpub. data). Both cities have historical districts that have housed *Kanpō* (Chinese style) pharmacies for centuries (Lock 1980). Informants were identified through recommendations from the University of Kyoto Pharmacy Department, directory searches and chain-referrals (Bernard 2000). With the assistance of student translators from the School of Pharmacy, each consenting establishment was engaged in a semi-structured interview to ascertain what materials were used for the treatment of parasitic infection, whether any of these were based on clay minerals, and if not, whether they used clay at all in their pharmacy. These interviews were designed to collect free-list data on antiparasitic treatments in *Kanpō* medicine, with the potential to rank individual medicines in terms of cultural importance. Those interviewees that did use clay in their practice were asked to complete a questionnaire covering specifics of clay pharmacy, parasitic diagnosis and treatment. Samples of materials discussed were collected whenever possible.

Statistical Analyses

Due to the small number of focal individuals in the macaque group, the non-parametric Friedman's analysis was used to assess the variance across the year in the monthly occurrence of geophagy, and Kruskal-Wallis to compare individuals (Martin, P. and Bateson 1993). The effect of age on the frequency of geophagy was determined using a Spearman's ranked correlation. To determine the role of parasitosis symptoms as stimuli for geophagy, daily records of parasitic fecal abundance were regressed against the total daily duration of clay ingestion (in seconds), controlling for the length of observations on each day (weighted least squares regressions). To investigate alternative dietary and digestive functions, the daily frequency of foraging for dietary staples (items contributing >10% of total foraging time in any given month) were regressed against the daily duration of geophagy, again controlling for variation in the daily observation duration.

Data obtained from the interviews with *Kanpō* medicine practitioners concerning antiparasitic medications and clay use were collated as raw free-lists (Martin, G. J. 1995) and analyzed in the *Anthropac* software package (Borgatti 1996) for list frequency and salience rankings of each recorded substance. The output data were subjected to consensus analysis using the same software package, which compares the degree of agreement and disagreement within respondents to determine whether their knowledge is consistent within a single cultural/cognitive domain.

Results

Patterns of Geophagy

All focal individuals ingested soil or clay at least once (mean = 7.55 events, range 1-18). The mean duration per hour was greatest during the month of October, but showed no significant annual variation (Friedman's: $X^2=16.874$; $p=0.112$), (**Figure 4.1**). The mean duration of observed geophagy varied significantly between individual focal animals, ranging from 0.1 sec/hr to 2.15 sec/hr (Kruskal Wallis: $X^2=26.867$, $p=0.003$), and when ranked by age, was significantly less evident in older individuals (Spearman's ranked correlation: $r=-0.651$; $p=0.03$: **Figure 4.2**). Mining bouts were generally brief,

ranging from 1 to 64 seconds (mean = 11.61 sec, n=83, SD = 9.90). Mining sites ranged from eroded banks, to small, hand-sized holes and loose topsoil. During the field season I recorded 34 discrete sites within the NA2 range (**Appendix B2**), of which 15 were visited on more than one occasion. Most frequently, mining sites were on steep ground, where natural erosion had revealed an expanse of subsoil. The most common substrate ingested was orange clay subsoil (69%), followed by brown or ochre subsoil (27%). On four occasions (5%), three focal individuals ingested dark organic topsoil. The duration of mining did not, however, differ significantly between clay types (Kruskal Wallis: $X^2=3.218$, $p=0.200$).

Parasite Ecology

Through fecal analyses I identified active infections by five nematodal taxa - *Oesophagostomum aculeatum*, *Strongyloides fuelleborni*, *Streptopharagus pigmentatus*, *Trichuris* sp. and *Gongylonema* sp. Of these, *O.aculeatum* and *S.pigmentatus* were the most prevalent, occurring in all focal animals (Dagg in prep. a). *Gongylonema* sp. ova were detected only in very low abundances in 6 out of the 11 monkeys and are excluded from further analyses due to the very low sampling frequency. **Table 2.1** summarizes the prevalence and transmission strategies of these taxa. Individual samples contained 0-4 taxa, a diversity which varied significantly across the season, peaking in October and May (all focals: $X^2=20.342$, $p=0.041$). I found no significant statistical relationship between daily geophagy duration and daily mean fecal abundance of any parasite taxa, nor with fecal water content (diarrheal symptoms). Geophagy did, however, show significant effect over two successive days of sampling and observation. Data from the 50 cases where two consecutive days of observation recorded the same individual provided a value of change in parasite abundance over 24 hours. A linear regression analysis, weighted by observational day length, showed a significant relationship between geophagy duration and an overnight reduction in individual *O.aculeatum* fecal abundance ($t=-2.449$, $p=0.025$), and between geophagy duration and an increase in *Trichuris* sp. ova fecal abundance ($t=7.602$, $p<0.001$).

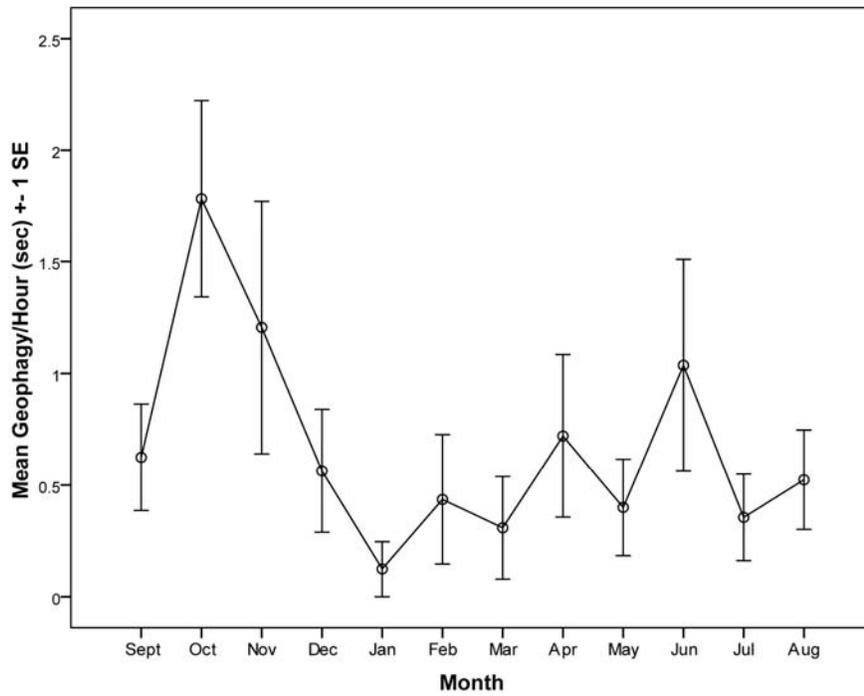


Figure 4.1 - Occurrence of geophagy in NA2 group across the observation period (September 06 – August 07)

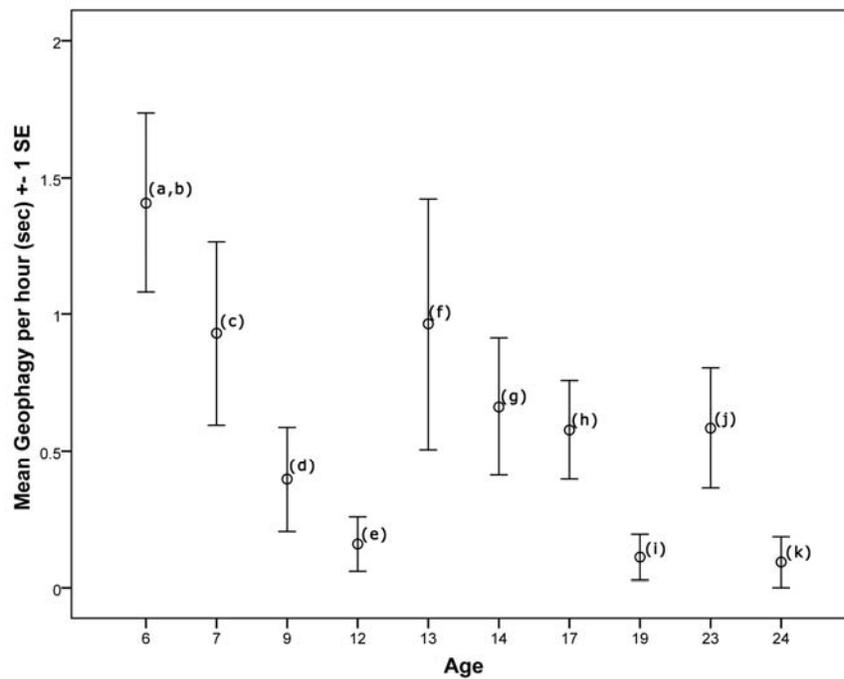


Figure 4.2 - Observations of geophagy (mean sec / hr +/- 1 SE) in NA-2 adult females, ranked by individual age : (a) Namaste; (b) Masala; (c) Shipi; (d) Noriko; (e) San; (f) Nasube; (g) Man; (h) Sangogi; (i) Namida; (j) Nagaram (June-Aug); (k) Shinju (died May 07)

Geophagy and Diet

Monkeys of NA2 troop ingested a large variety of food sources, and are broadly omnivorous, their diet including large numbers of invertebrates, a wide variety of plant parts, fungi and soil (**Figure 3.1, Appendix A**). Overall, the most time was spent collecting and ingesting animal matter, largely invertebrates, which accounted for approximately half of all foraging time in both November and June (monthly mean = 34.7%, range 12.3%-50.1%). Among plant species, significant contributions were made by *Lithocarpus edulis* seeds between September and November (and again in February, recovered from leaf litter), *Rhus succedanea* fruit from December-February, *Ardisia sieboldi* fruit in December, *Cinnamomum camphora* buds in March, *Eurya emarginata* leaves in April, *Myrica rubra* fruit in May and June, and *Ficus superba* fruit in July and August (**Table 4.1**). The daily focal duration of geophagy was significantly predicted by the concurrent daily foraging frequencies of three staple food items listed above: invertebrates ($t= 2.86, p=0.01$), and in particular, those found in the forest canopy ($t=3.36, p=0.001$), *Lithocarpus edulis* acorns ($t=2.02, p=0.045$), and *Myrica rubra* fruit ($t=2.10, p=0.037$) (multiple least squares regression).

Twenty-seven percent of geophagy events ($n=22$) were closely observed by infants or young juveniles. Ten of these (12%) were followed by ingestion of clay by the youngster, while in 5 cases (6%) juveniles initiated geophagy behavior before the adult. On one occasion an infant initiated clay mining behavior, rapidly followed by the removal of the material from its grasp by the adult. No relationship was found between age of immatures and observational interest or subsequent ingestion.

Clays in traditional Japanese medicine

Ten of the fifteen *Kanpō* practitioners interviewed said that they used some form of medicinal mineral in their practice (between one and five types per practice). The most commonly cited was *Sekko* (a white, fibrous gypsum), followed by *Ka-seki* (pale halloysite), *Bukuriukan* (loess) and *Shakutsekishi* (red, ferric halloysite).

Table 4.1: Staple food items consumed by *Yaku* macaques, contributing >2% of annual foraging time and >10% maximum monthly contribution. Multiple regression analysis between daily foraging frequency (%) with daily duration of geophagy (in seconds). (* = Significant at p<0.05)

Food Item	Annual Contribution to foraging duration	Maximum monthly contribution	Regression Coefficient	
			t	p
Invertebrates	34.7%	50.1% (June)	2.86*	0.01*
<i>Canopy invertebrates</i>	21.79%	37.37% (Sept)	3.36*	0.001*
<i>Terrestrial invertebrates</i>	8.44%	20.87% (June)	-0.03	0.973
<i>Deadwood invertebrates</i>	4.46%	10.69% (Dec)	0.54	0.588
<i>Lithocarpus edulis</i> seed	7.61%	27.5% (Sept)	2.02*	0.045*
<i>Rhus succedanea</i> fruit	4.59%	10.61% (Aug)	0.21	0.832
<i>Ficus erecta</i> fruit	4.46%	30.38% (Jul)	0.49	0.622
<i>Myrica rubra</i> fruit	3.75%	28.99% (June)	2.10*	0.037*
<i>Eurya emarginata</i> leaf	3.58%	20.99% (Apr)	1.42	0.159
<i>Ardisia seiboldii</i> fruit	2.42%	14.01% (Dec)	0.89	0.375
<i>Ficus superba</i> fruit	2.26%	12.97% (Jul)	-0.95	0.346
TOTAL	63.38%			

The sedative and antifebrile properties ascribed to *Sekko* exemplify the food-medicine dualism in traditional Chinese and Japanese medicine. Its qualities are said to be derived from its high calcium content, and while this is a nutritive property, its action is stated as directly therapeutic. It is prescribed for colds and fevers, as well as mental anxiety and atopic dermatitis, which was claimed to be relieved by a lowering of skin temperature. Although translated by some informants as kaolinum, *Ka-seki* is more accurately classified as halloysite, a clay of equivalent molecular formula $[Al_2Si_2O_5(OH)_4]$ but different crystalline structure. It is soft, white, odorless clay and is most commonly used as a diuretic and antifebrile for urinary conditions such as cystitis, haemaurea, urinary stones and bladder infections. The stated qualities of *Bukuriukan* (also known as *Uo-do* and ‘yellow earth’) much more resemble the conventionally accepted properties of kaolinitic clay in the west. It is, however, a form of Loess, fine sediment gathered from river-beds. Its specific use during pregnancy stems from both its absorptive

qualities (preventative against pregnancy toxicity), buffering ability (anti-emetic) and nutritional iron supplementation ('increases blood'). It is used both as a simple medicine and as a component of the compound medicine known as *Koyokuin*, prescribed for the relief of pregnancy-related nausea. Further reported actions include anti-diarrheal, including that caused by parasitosis, and the stemming of internal bleeding (anti-hemorrhagic). A close relative of *Ka-seki* is *Shakutsekishi* (also known as *Uyoryo*), a ferrous halloysite, and found in the form a very soft red powder. It is primarily used in the treatment of chronic diarrhea, including that caused by parasitic infection, and intestinal hemorrhage. In Nara there exists a local variant of this material, *Manjuishi*, which although mineralogically identical, is found only within hollow igneous 'eggs' from the dormant volcano there. The clay interior is streaked with iron deposits and is highly regarded in Nara as a powerful anti-diarrheal, although its rarity is causing its use to decline.

Cultural consensus analysis determines the degree of agreement between respondents to identical questions in order to identify cognitive domains of knowledge and the relative expertise of respondents (Weller 2007). The interview data collected did not conform to a model of cultural consensus, largely due to the number of informants, within this small sample, who said they used no minerals in their practice. This analysis of free-listing data did not provide a sufficient Eigen ratio (3.0) for the pseudo-reliability value to be significant. (Eigen Value 1:2 ratio = 2.576, pseudoreliability = 0.811).

Discussion

Although geophagy demonstrated a statistically significant effect over two successive days of fecal analyses (a reduction in *Oesophagostomum aculeatum* abundance and an increase in *Trichuris* sp. abundance), no proximal stimuli were evident, as the behavior was not related to parasitic activity (fecal abundance) or symptoms (diarrhea) on individual days. It is not possible, therefore, to suggest that geophagy in *Yaku* macaques is a direct behavioral response to parasitic stress, although it is plausible that the fecal abundance of the more ubiquitous *O. aculeatum* is reduced as a incidental consequence of clay

ingestion. The increase in *Trichuris* sp. evident over two days may be caused by physical expulsion of ova by the clay (Engels 2002). Equally plausible, however, is that the increase is due to ingestion of soil contaminated with *Trichuris* sp. ova, and the direct passage of unhatched ova. Most soil is mined from banks or holes, and is thus unlikely to be directly infected by fecal matter, but in some cases lumps of dry soil were selected from the surface, where it may have been in direct contact with infected feces. The 24 hour paired data, however, were unable to demonstrate such a pattern in relation to soil location, and so the possibility that this is merely a sampling artifact cannot be discounted.

A long-term prophylactic effect of habitual geophagy may be evident in the general lack of symptomatic parasitosis (diarrhea, etc.) in the group. Despite near ubiquitous infection by known pathological taxa such as *Oesophagostomum aculeatum* and *Strongyloides fulleborni*, only 4% (n=13) of fecal samples from NA-2 group were classified as loose and diarrheal. Such a phenomenon was noted previously in rhesus macaques on Cayo Santiago (Knezevich 1998). In that case, habitual geophagy was also implicated in the pattern of reduced parasitic abundance in older individuals, through progressive thickening of gut mucosa. This would have an inhibitory effect on burrowing nematodal taxa such as *Oesophagostomum* sp. and *Strongyloides* sp. This age effect may also be evident in this population, as intra-individual prevalence of *Strongyloides fulleborni* was found to decrease with age (Dagg in prep. a). *Streptopharagus pigmentatus*, in contrast, was more abundant in older individuals, but inhabits only the gut lumen and therefore would be unaffected by mucosal thickening.

In the absence of a parasitic stimulus for geophagic behavior, there are several other existing functional models that could be applied, perhaps the most common being micro-nutritional and the detoxification of food. A superficial consideration of mineral supplementation is afforded by the apparent 2:1 preference for orange-colored clays over brown or tan. Orange and red pigmentation is often indicative of high mineral iron content, and this has been proposed as a contributory function of geophagy in several primate species (Davies and Baillie 1988; Heymann and Hartmann 1991; Mahaney *et al.* 2005). On closer inspection, geophagy in NA2 troop does not seem to be related to seasonal dietary fluctuations that might cause deficiencies. No significant relationship was found between mean monthly geophagy and

variation in major food sources. The data collected here, however, are insufficient to allow speculation on either the nutritional mineral content of the clays, or mineral inadequacies in the rest of the macaque diet.

The daily focal duration of geophagy was most significantly predicted by the daily frequency of invertebrate ingestion, but since this study was not designed to accurately record the ingestion of animal material at the species level, this relationship must be regarded as putative. It does, however, suggest a hypothesis for future enquiry. Invertebrates can employ a range of anti-predator strategies, including chemical defenses. Grasshoppers (Orthoptera) and caterpillars (Lepidopteran larvae) are particularly noteworthy for the range of material they employ, including the regurgitation of plant materials (Sword 2001), excretion of irritant/toxic chemicals from abdominal glands (Krall *et al.* 1999), and the accumulation of pungent or toxic feces (Weiss 2006). The macaques in NA2 troop were observed to ingest many invertebrate species, including caterpillars, grasshoppers, centipedes, cockroaches, cicadas and moths. The single greatest food item contribution to annual foraging time in NA2 was the moth, *Geisha distinctissima*, found abundantly in the canopy from summer to late autumn. Little seems to be known about the biochemistry of this species, but their pale color does not suggest a cryptic defense strategy. The concurrent ingestion of absorptive clays may ameliorate the toxic defenses in one or more of these food species, allowing them to be safely exploited. Geophagy has not yet been unequivocally documented as a response to insect chemical defenses, which is somewhat surprising since more than half of all insect orders contain species with toxic defenses (Glendinning 2007).

A weaker, but still significant, relationship was demonstrated between daily geophagy and ingestion of *Lithocarpus edulis* acorns and *Myrica rubra* fruit. Although processed to remove tannins before human consumption (Kunkel 1984), *Lithocarpus edulis* seeds are relatively low in antifeedants, compared to others in the genus. Tarnaud (pers. comm.) found 2.3% condensed tannins in the cotyledon of this species, sampled in a nearby area on Yakushima. This can be compared with up to 14% in *Quercus glauca*, which are an autumn staple food item in Arashiyama macaques (Wakibara *et al.* 2001). *L. edulis* seeds are a major food staple in Yakushima during the autumn, contributing of 25-28% of total foraging time in NA2 group during September and October, and 7.6% of total annual foraging time. This species

thus represents the greatest time allocation to a single plant-based foodstuff. Hill (1997) also previously reported that over half of total seed eating in a nearby group involved this species. When ingested in such quantities, the moderate tannin levels, along with other phenolic antifeedants, may still pose a digestive challenge to macaques, which could be alleviated through a detoxification strategy such as geophagy.

Such detoxification has precedent in some traditional human food processing techniques. Meyers *et al.* (2007) found there to be just 0.5% condensed tannins restricted to the pericarp of *Lithocarpus densiflora* (Tanoak), with 7.1-8.6% hydrolysable tannins in the cotyledon. Despite the relatively lower toxicity of the hydrolysable tannins, the Pomo natives of California leach the ground cotyledon to remove tannins before cooking this staple foodstuff (Meyers *et al.* 2007). Johns and Duquette (1991b) report a similar use of clay by the Pomo in the partial adsorption of tannic acid from *Quercus* spp. acorns used for bread flour. Cooking further facilitates the detoxification through adsorption and catalysis, resulting in up to a 77% reduction in protein inhibition. The detoxification of chemical defenses of seeds has also been proposed as the primary function of geophagy by neotropical parrots (Gilardi *et al.* 1999). In all these cases, the use of detoxification techniques allows exploitation of valuable but otherwise chemically inaccessible dietary items, and can therefore be seen as analogous to the biocultural 'keys' in Katz's (1987) model of human diet.

Wakibara *et al.* (2001) reported that clays ingested by Arashiyama macaques bound moderately to tannins (32-58% adsorption), which is in accord with in-vitro models such as that of Dominy *et al.* (2004), who found $\leq 25\%$ adsorption of *quebracho* (a condensed tannin from the bark of *Schinopsis balansae*) and $\leq 30\%$ adsorption of hydrolysable tannic acid with pure kaolin. The aforementioned study by Wakibara *et al.* (2001) may also elucidate the seemingly anomalous ingestion of topsoil, on four occasions, by *Yaku* macaques. The authors reported that organic topsoil, ignored by the Arashiyama macaques, was more effective in binding tannic acid than was the kaolinitic subsoil, due to the tannin's high affinity to the protein contained therein. On Yakushima, three of the four topsoil ingesting events observed here were during the autumn period of intensive *Lithocarpus edulis* ingestion and coincided with mid-upper quartile daily ingestion frequencies. Although these limited data cannot be assessed

statistically, such observations suggest a potential function of geophagy that does not involve the subsurface or excavated clays more typically ingested by primates and other mammals.

Alternatively, the ingestion of large numbers of acorns may result in more generic acid indigestion or gastrointestinal discomfort, aspects of which may also account for the relationship between geophagy and *Myrica rubra* fruit ingestion. *Lithocarpus edulis* acorns are rich in soluble carbohydrate (Asada and Ochiai 1996), and are the largest seed ingested by *Yaku* macaques, at an average 1.99g per acorn (Otani and Shibata 2000). The nearest comparable published nutritional analysis was for *Quercus* sp. acorns, which are comprised of 40.75% carbohydrate and 6.15% protein (U.S. Department of Agriculture 2007). Wakibara *et al.* (2001) concluded that indigestion caused by rich provisioning foods at Arashiyama was the most likely stimulus for the high levels of geophagy observed in that group. The commonly provisioned foods contained highly available carbohydrate and protein, with low fiber, known to produce clinical indigestion through accelerated fermentation, increased volatile fatty acids and changes in microbial populations. Knezevich (1998) also noted a strong temporal association between ingestion of monkey chow and geophagy on Cayo Santiago. Although *Lithocarpus edulis* is a naturally foraged foodstuff, its acorns share some of the same nutritional characteristics as the Arashiyama provisions, and as such it is feasible that intensive ingestion of this foodstuff would cause acid indigestion, which may be effectively alleviated by geophagy.

Myrica rubra fruit consist of a thin, juicy pericarp firmly attached to a large (~1 cm) indigestible seed, which is passed undamaged in the feces. During mast fruiting years, the fruit of this species is sufficiently abundant to substantially alter *Yaku* macaque ranging behavior (Agetsuma and Noma 1995). Up to 48 seeds per fecal sample were recorded during the peak of foraging in 2005 (Terakawa *et al.* 2008), constituting the majority of the sample. Such heavy passage of seeds is unusual in the *Yaku* macaque diet and could produce severe gastrointestinal discomfort. Furthermore, the intensive ingestion of some fruits can have a laxative effect (Murray, *et al.* 1994). In such cases, geophagy may be a generalized response to gastrointestinal discomfort, or a functionally significant anti-diarrheal strategy.

The data presented here demonstrate the potential transmission of geophagic behavior required for the phenomenon to be considered a cultural tradition: the close observation of parental geophagy by infants, potentially facilitating emulative learning through local enhancement. My observations failed, however, to show a progressive adoption of the behavior by older juveniles, which has been cited as evidence for ‘cultural’ behavior in primates (Huffman and Hirata 2003; Nishida 1987). Tarnaud and Yamagiwa (2008), however, recorded a high positive ‘Solid Food Interest Index’ (Perry and Ordonez-Rimenez 2006) for geophagy in Dongori and Umi group juveniles (1-3 yrs old), indicating a high observational interest in soil consumption by an adult model, compared to its actual contribution to the diet. This contrasted with observation patterns in the infants aged 1 yr or less. For these younger animals, soil ingestion produced a negative score on the Food Interest Index.

Red ferrous kaolinite and halloysite are both used in traditional Japanese medicine (*Ka-seki* and *Shakusekishi*, respectively), the latter for the treatment of diarrhea, especially chronic or hemorrhagic. The raw clay is dried, ground and a 10-20g dose mixed in water (Wu 2005). In traditional Chinese medicine it is reported to protect the intestines and absorb “abnormal fermented foods” (Hsu 1986). Long term use is said to increase bodyweight, suppress hunger and lengthen lifespan (Li and Luo 2003). This traditional use is in accordance with other cultures. The West African medicine *eko*, and the western pharmaceutical preparation Kaopectate™ are both based on the highly similar kaolinite, and used to treat stomach complaints such as indigestion and diarrhea (Voros *et al.* 2001). Such clays are composed of flat layered aluminum-silicate matrices, affording a large surface area of negatively charged cation-exchange sites; and when ingested, they absorb both water and (positively charged) toxins (Glendinning 2007) and promote their safe excretion (Wilson 2003).

Halloysitic and kaolinitic clays are largely found as weathered volcanic sub-soils, and this classification is consistent with the orange and tan sub-soils ingested by the *Yaku* macaques. *Shakusekishi* provided by interviewees ranged from deep red to pale tan (in the case of *manjuishi*). The color variation may be accounted for by variable iron content.

To determine more conclusively the primary stimulus for geophagy in *Yaku* macaques, samples of both ingested and eschewed (control) soils need to be tested for geochemical content (particle size, clay mineralogy, cat-ion content), for their ability to adsorb alkaloids and tannins under *in vitro* gastric conditions, and their acid buffering properties.

Conclusions

Yakushima Japanese macaques engage in low levels of geophagy throughout the year, and are infected with several taxa of nematodal endoparasites. Several geochemically distinct clays also occur within the traditional Japanese *Kanpo* pharmacopoeia, with applications ranging from the treatment of fever to diarrhea. The hypothesis that geophagy in *Yaku* macaques functions to alleviate symptoms of parasitosis was, however, not supported. There is suggestive evidence that associates the occurrence of geophagy with invertebrate foraging, and with the ingestion of *Lithocarpus edulis* acorns and *Myrica rubra* fruit. Two potentially adaptive functions, one protecting the animals from invertebrate defensive toxins or tannin toxicity, and the other buffering gastrointestinal discomfort, are promising lines of enquiry but require further research for clarification.

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CHAPTER 5

CANDIDATE MEDICINAL PLANTS IN MACAQUE ECOLOGY AND HUMAN TRADITIONAL MEDICINE

Introduction

This chapter covers material included in the research design, but which was unsuitable for inclusion in the primary research articles (Chapters 2-4). This includes negative results, intriguing but inconclusive observations, and the confluence between macaque behavioral observations and ethnographic interviews conducted on Yakushima, and in Kyoto and Nara. Comparative analyses between macaque and human pharmacopoeias provides valuable qualitative information on the materials involved, but also has the ability to assess the degree of confidence assignable to the putative function of that behavior. This methodology has been applied in previous multidisciplinary studies of primate self-medication. Data collected on local ethnomedicinal practices were used to strengthen support for the hypothesized efficacy and adaptive function of leaf swallowing and bitter pith ingestion in chimpanzees (Huffman *et al.* 1996; Huffman and Wrangham 1994).

I selected six candidate plant species from the *Yaku* macaque dietary range, based on literature and behavioral indicators as described in Chapter 3. **Table 5.1** summarizes the published ethnobotanical and ecological information for each candidate. Of these, only one, *Miscanthus sinensis*, demonstrated a clear association with parasitosis during field observations, and is discussed fully in Chapter 3. Others remained intriguing, due either to the ingestion of known pharmacologically active parts, or through ingestion behavior suggesting a non-nutritional function, but provided insufficient concrete evidence to be

included in the former chapters. Here I review the behavioral findings for each species, the patterns of foraging, the responses accrued from the ethnomedicinal interviews, and the existing literature. I thus assess the potential significance of each for macaque health, and make suggestions for future studies on Yakushima.

Methods

Ethnobotanical Methodology

In order to assess the degree of overlap between human and *Yaku* macaque pharmacopeias, ethnomedicinal data gathered in semi-structured interviews and questionnaires were compared quantitatively with the characteristics of macaque plant use, as observed in the field.

Pharmacists, practitioners or experts of traditional Japanese *Amatsu* and *Kanpō* medicine were identified on Yakushima and in Kyoto and Nara cities through recommendations from the University of Kyoto Pharmacy Department, directory searches and chain-referrals. I conducted two periods of interviews, the first in 2003, focusing on geophagy (see Chapter 4), and the second in September 2007, concentrating on the candidate plants discussed in this chapter. During the 2007 interviews, participants were shown unlabeled samples of each candidate plant and asked to identify them. After the names of any unidentified species were revealed, they were asked to elaborate on the pharmacy of those plants within their practice, including parts used, medicinal properties, indications and uses, or their appearance in folklore and folk knowledge, including their use by animals. In this way, I was not only able to build a broad perspective on the use of these plants in Japanese traditional medicine, but also analyze the results quantitatively based on the degree of recognition and cultural salience that each plant enjoys within medicinal practice.

Phillips and Gentry (1993) proposed a method of quantitative scoring of ethnobotanical plant species across a range of indexed properties, in order that their cultural significance could be statistically compared between a broad range of biological, ecological and human categories, such as between informants, ethnic groups or plant communities. This procedure assigns a numerical value to each plant

based on a cumulative list of indices which illustrate its significance within a culture or a specific area of interest. These indexes may be defined by the informants themselves or, as in this case, by the researcher. Following these principles, the use of each candidate plant by each interviewee was ranked according to a salience score, illustrated below, which had deliberate bias for medicinal, and specifically antiparasitic uses. This procedure was repeated with the macaque observational data, using the most comparable indices applicable to behavioral observations and treating the entire year of behavioral observations as a single ‘respondent.’ The data were insufficient to calculate medicinal salience for each focal animal, since the use of foraging frequencies would not distinguish between dietary and medicinal significance.

To normalize the data to allow direct cross-taxa comparison, the scores resulting from each system were converted to percentiles of the range of salience recorded in each species. This transformed, combined matrix of salience scores for each plant species was subjected to consensus analysis using ANTHROPAC software (Borgatti 1996), both within humans only, and then including the macaques. Cultural consensus analysis determines the degree of agreement between respondents to identical questions in order to identify cognitive domains of knowledge and the relative expertise of respondents (Weller 2007). In this software package, an Eigen Value of greater than 3.0 indicates that the respondents conform to a model of cultural consensus.

From each ethnomedicinal interview, salience scores were calculated for each plant species as follows. The maximum ‘recognition’ score for a plant is 2, while the other values are open-ended:

- Visual recognition of unlabelled sample (2pts) *or* Recognition of name (1pt)
- Medicinal plant parts used (2pts each) *and* non-medicinal part used (1pt each)
- Antiparasitic/anti-diarrheal application (2pts each) *and* other medicinal (1pt each)
- Folkloric citation (1pt)
- Animal self-medicative anecdote (1pt)

An equivalent set of macaque salience scores were derived from focal behavioral data as follows:

- Known ethno-medicinal part ingested (2pts each) *plus* other parts ingested (1pt each)
- Associated non-nutritional behavior observed (e.g., whole leaf swallowing) (1pt)
- Statistical association with parasite fecal abundance (2pts)
- Field evidence of efficacy against parasite infection (2pt)

Ecological Measurements

Arguably the most common determinant of plant ingestion is its availability. To account for this variable, the distribution of each candidate plant species within the NA-2 range was mapped and the seasonal productivity of each was assessed bimonthly. During preliminary surveys, a 50 m x 50 m resolution grid was plotted over the initially identified home range of NA-2 troop (total area = 41.75 ha). East-west transects were walked along grid rows, recording all visible candidate plant specimens from the center of each grid square and allowing the plotting of density distributions of the candidate species (**Appendices B3-B8**). This survey did not extend across the entire NA-2 home range (*ca.* 71 ha) as the northern and southern extremes were not used by the group until later in the season, but encompassed the core area (**Appendix B1**). Every two weeks, I recorded the relative abundance of leaves, flowers and fruit for each candidate species that had been identified on the basis of existing literature. Five grid locations containing candidate plants were randomly selected, an individual plant selected on first sight within those squares, and the following abundance measures carried out on that sample: leaves were recorded as percent occlusion of the sky as viewed through a 10x10cm square held at arm's length from the base of the trunk; the presence of fruit and flowers were recorded on a scale of abundance from 0-5⁴; and fruit

⁴ Relative abundance measurements subjective to observer, defined as :

- 0 – Not present
- 1 – Single incidence
- 2 – Sparse, occasional single or pairs
- 3 – Very small clusters or sparse clusters
- 4 – Many clusters but not at every node, or clusters incomplete
- 5 – Plant seems at maximum production, every node loaded.

ripeness was recorded as the percentage of fruits that were visibly fully ripe. Since phytochemical analyses were beyond the scope of this study, non-seasonal resources such as bark, roots, etc., were assumed to be constant with regards to their biochemical resources. During behavioral data analysis, the monthly mean ingestion of specific plant parts was correlated against recorded abundance (Pearson's correlations) to determine the role of annual variability on their appearance in the foraging record.

Results

The following species were recorded as candidate species in the macaque behavioral observations, and included in ethnobotanical interviews. For each species I report the ecological and behavioral data from Yakushima and the interview responses, review the existing literature, and offer preliminary interpretations or hypotheses concerning the plant's significance for *Yaku* macaque health and ecology.

Lagerstroemia fauriei or *L.subcostata* var. *fauriei* (Lythraceae) is a deciduous tree known in Japanese as "*Yakushima-sarusuberi*" (Monkey Slip Tree) due to its characteristic orange or olive green bark which sloughs annually in long strips to leave a smooth surface. While this species is not listed by the IUCN, it is classed as Near Vulnerable in the Japanese Red List of threatened species (M.O.E. 1997). It is found at low densities in the central NA-2 range (n=21, mean = 0.5/ha), most commonly on flat ground by rivers or in seasonal stream-beds (**Appendix B4**). I recorded several isolated occurrences when monkeys ingested or chewed on the bark of this tree. On 8-23-06, before the onset of full focal observations, the 13yr old female "*Nasube*" chewed on and discarded a 2x5cm piece of bark from the lower trunk of *L.fauriei*. On 8-25-06 the 14yr old female "*Man*" moved 50m away from the grooming group, manually peeled a 15cm strip of bark from the same species, chewed the end for a few seconds and, again, discarded it. On each of these occasions, only one individual ingested the bark, while nearby group members ignored the activity. On 5-8-07, a group of 1-2 year old juveniles licked and bit at the bark of *L.fauriei* near a small damaged area for around a minute. Under some loose sections of bark there

was a thin smear of resin of bitter taste. I was unable to collect fecal samples on those occasions. On only one occasion did a focal individual chew the bark during their observation period – the 6yr old female "Masala," daughter of "Man," for 7 seconds on 9-8-06. On that day her fecal sample contained 645 epg of *Oesophagostomum aculeatum*, the second highest daily count for the year, but the significance of this figure is lessened as it was based on a single incident. When I investigated the trees, no significant invertebrates could be found behind strips of bark. The wound resulting from the ingestion on 8-23-06 produced a pungent resin, and similarly, peeled strips of bark had a strong bitter taste. This taste would normally be expected to deter feeding. Along with the unusual chew-spit behavior and lack of apparent nutritional reward, this suggests a non-nutritional, perhaps medicinal function.

Interviewees demonstrated basic recognition of the species name (10/20) but few knew of any medicinal use. One archaic use of the powdered bark of this species was mentioned as part of an external anti-inflammatory ointment. One respondent with pan-Asian expertise cited the use of this bark as an anti-diarrheal and antibacterial.

Bark and wood are characteristically highly fibrous, relatively indigestible, sometimes toxic, and usually nutrient poor (Huffman 2003). Chimpanzees and gorillas have been reported to ingest bark, including that of several species known to be plasmodicidal, purgative, antiparasitic and antidiarrheal, although little is known about their dietary or medicinal role (Huffman 2003). The ingestion of *L.fauriei* bark by *Yaku* macaques has not been recorded in previous dietary surveys (e.g., Hanya 2004; Hill 1997), nor has any information been published on its medicinal properties or phytochemical constituents. In Taiwan, the root and flowers of the closely related *L.subcostata* are used for the treatment of abdominal pain and poisoning (Li, T. S. C. 2006). The bark of *L.speciosa* (native to India) is used to treat diarrhea and abdominal pain, while that of *L.indica* (native to China) is used as a stimulant and febrifuge (Khare 2007). Due to the great rarity of this ingestive behavior in this study, statistical methods were unsuitable for investigating its efficacy or identifying stimuli which might have induced its ingestion. What remains is a highly unusual behavior greatly deserving of closer examination. On all occasions of bark chewing, the target was unequivocal, with no invertebrate or other apparent nutritional reward. The bitter taste of

the bark, and the pungent, bitter resin would all normally be expected to deter feeding by stereotypical taste aversion reflexes, unless some positive physiological or psychological reward is forthcoming (Mattes and Beauchamp 2000). That the bark seemed to be largely discarded after a short time is further indicative of a non-nutritional function, and is reminiscent of the behavior recorded in orangutans by Gladikas (1988). In that case several species of bark were similarly chewed or sucked, and the fibers spat out, eliciting interest from several zoopharmacological experts (Huffman *et al.* 1998). Although the reported ethnobotanical uses of *L.fauriei* have not yet been substantiated in pharmacological analyses, it is feasible that a positive neuro-cognitive association be established, by the macaques, between its distinctive bitter taste and some therapeutic benefit (Hart 2005). A careful assay of bark extracts against common parasitic infections, both nematodal and protozoan, would be revealing.

Melia azedarach (Meliaceae; ‘China Berry’) is a tall canopy / emergent tree, and abundant throughout the central NA-2 range (N=254 from transect survey, mean = 6.1/ha), especially in wet valleys or around watercourses (**Appendix B5**). Neither the anthelmintic leaves nor the toxic fruit were observed to be ingested by any NA-2 individual. On six occasions, mostly in August, I observed focal animals ingesting small amounts of gum from deep wounds in the trunk of this species (mean = 57 sec). No statistical relationship was found between this behavior and parasite abundance of any taxon, and indeed parasite ova abundance was uniformly low in all involved individuals on those days. The canopy of this species, however, was amongst the tallest in the area, and individual focal activity was extremely difficult to observe. Ingestion of plant parts could have taken place here, but completely out of sight. As a result, I regarded the observational data from this species to be unreliable for behavioral analysis.

A dose of 5g of leaves has been recorded as an effective anthelmintic agent in humans (Duke 2002), extracts of leaves and seeds have demonstrated activity against ova and larvae of *Haemonchus contortus* in sheep (Maciel *et al.* 2006), and drupe extracts have been reported to outperform commercial anthelmintics against a range of nematodes *in vitro* (Szewczuk *et al.* 2006). In traditional Chinese medicine, the bark and fruit are used as a strong anthelmintic and external parasiticide (Li, S. *et al.* 2003),

due to the presence of the bitter triterpenoids nimbin and sendanin (Hsu 1986). The flowers have been used to treat prickly-heat, and the leaves to treat hernia (Li, S. and Luo 2003). Only gum was observed to be ingested by *Yaku* macaques, however, and this has no reported medicinal activity aside from the potential immunostimulant effect of gum polysaccharides discussed with regards to *Z.ailanthoides*, below. Indeed, Hedrick (1972) records that a cooling drink is made from the gum of this species in India. Based on current observations, the most plausible interpretation for ingestion of this gum by *Yaku* macaques would be that it is primarily nutritional, and related to the carbohydrate content of the exudate.

Rhus succedanea (Anacardiaceae; ‘Wax Tree’) is highly abundant in NA-2’s home range (n=342 from transect survey, mean = 8.2/ha), particularly in open areas and ridges (**Appendix B6**). Eight of the eleven focal individuals ingested leaves of *R.succedanea*, and nine ingested the latex-rich shoots and petioles of new leaf growth. Foraging of both parts peaked significantly in April and early May (leaf: $\chi^2=47.914$, $p<0.001$; shoots: $\chi^2=64.039$, $p<0.001$), when they seemed to become a staple dietary item, with up to 51 minutes dedicated to leaf foraging per day, and 43 minutes to petioles/shoots. From daily data, no significant relationship was apparent between ingestion items from this tree and the abundance of any tested parasitic ova. Pearson’s correlations found no significant relationship between *R.succedanea* leaf ingestion and overall leaf availability, but a clear spike in ingestion time occurred just as new leaves were emerging in spring, suggesting that this may be the primary explanation for the temporal peak in ingestion (**Figure 5.1**).

Those interviewees who were familiar with the species (10/20) largely stressed the manufacture of wax, or ointment base, from the fruit. The ointment base was used to apply other active materials, including *Zanthoxylum sp.*, but was not reported to have medicinal properties of its own. Only one mentioned the use of leaves, in older practice, for burns, fever and eye-infections. The bark was recognized by two as being high in tannins and an effective antidiarrheal.

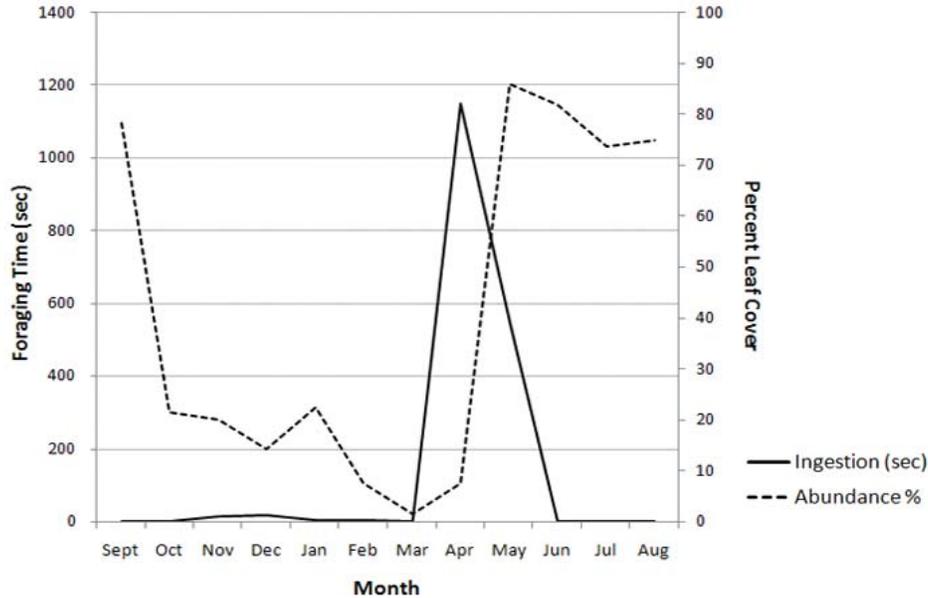


Figure 5.1 – Foraging of *Rhus succedanea* leaves by NA-2 macaques, by seasonal availability September 06 – August 07

The leaves of this tree have exhibited antiviral (Jassim and Naji 2003) and anticancer properties, but are also listed as a caustic and poison (Duke 2002): the leaves and latex are known to cause blistering on contact (Khare 2007) like the plant's more familiar relative, poison oak (*Toxicodendron diversilobum*, syn. *Rhus diversiloba*). It is not known, however, whether the allergenic reaction for which these species are known in humans is reproduced in the Cercopithecoidea. Leaves of the mainland relative, *R. chinensis* (*Wu Bei Zi*, Chinese sumac), are used to treat chronic intestinal infections (Li, T. S. C. 2002), and the white bark is used as a vermifuge and antidiysenteric (Li, S. and Luo 2003). Both are also regarded as poisonous. Galls are also collected from the leaves and used as an astringent antidiarrheal (Hsu 1986). Despite their toxicity, the young leaves, petioles and buds of *R. succedanea* were used extensively by *Yaku* macaques for food, and there were no indications of medicinal use. It may be that the young materials largely chosen as foraging items are less caustic than the stem latex or mature leaves, and that this influenced their foraging pattern.

Trema orientalis (Ulmaceae) is an evergreen tree up to 18 m in height and is rare in the range of NA-2 (n=17 across the central range, mean = 0.41/ha), found most frequently by the road, close to rivers or in disturbed patches (**Appendix B7**). The individual trees found in the macaques' range are large and mature. Six of the focal macaques ingested the fruit of this species, while all ingested *T.orientalis* leaves; leaf consumption showed significant monthly variation, with peaks in time allocation in January and May ($\chi^2=19.74, p=0.05$). Statistical analyses revealed no significant relationship with fecal parasite abundance, and no overnight effect on parasite abundance was detectable. Although Pearson's correlations found no relationship between *T.orientalis* leaf abundance and foraging, the maximum ingestion time corresponded with the winter predominance of leaves in the diet, and with the emergence of new young leaves in spring (**Figure 5.2**). Leaves were often ingested in large volumes, and chewed well, all supporting a supposition that nutrition was the primary role of foraging for these leaves. While four interviewees were familiar with the species' name, none knew it by sight (the lowest recognition score: 4/20) and none used it in medicinal practice.

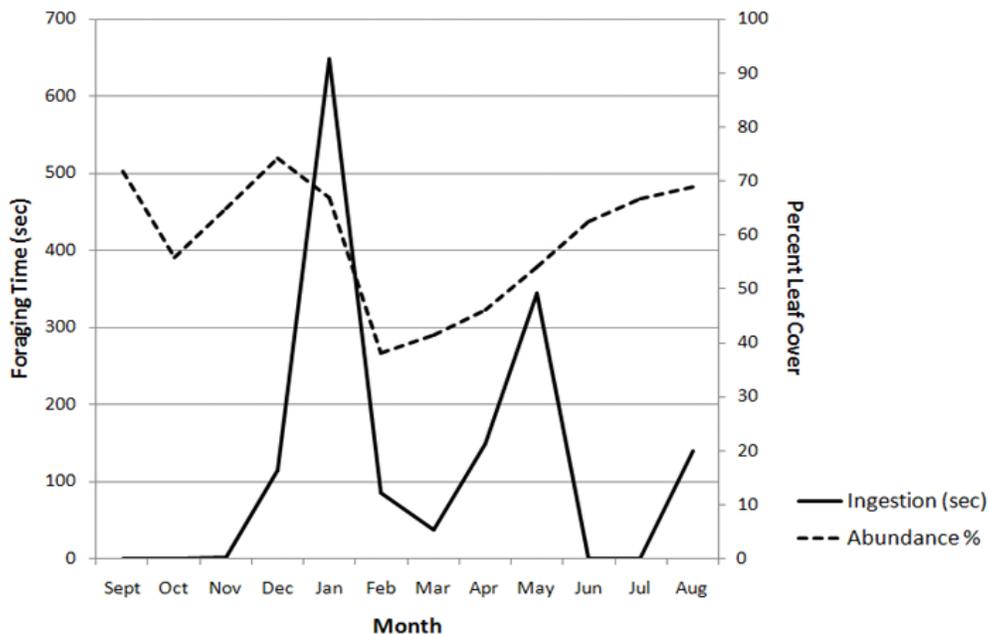


Figure 5.2 - Foraging of *Trema orientalis* leaves by NA-2 macaques, by seasonal availability September 06 – August 07

Several parts of this tree are used medicinally across South-east Asia and tropical Africa. Both leaf and bark decoctions are used to treat parasitic and diarrheal conditions in humans, as well as for the de-worming of dogs (Perry, L. M. and Metzger 1980). The root is used in Taiwan to treat both internal and external bleeding (Kuo *et al.* 2007), while the bark is used as an analgesic (Khare 2007) and is attracting pharmaceutical attention as a possible source of anti-diabetic compounds (Dimo *et al.* 2006). More unusually, the leaves of this species are among those observed to be swallowed whole by chimpanzees as a well documented anti-parasitic behavior (Huffman 2001).

Despite its established reputation as a medicinal plant, it was not known as such by any interviewee. Its absence from the Japanese pharmacopoeia may be due to its restricted range in only the southernmost parts of the archipelago. Japanese *Kanpō*, although based on Chinese medicine, was altered during the years of Japanese isolationism to use largely plants that did not require importation. This species is not present around the major centers of Japanese medicine, such as Kyoto, and so may have fallen out of use. Similarly, there was no significant evidence of antiparasitic, or indeed any non-nutritional use by the *Yaku* macaques. Leaf foraging was notably absent during the periods of highest *O. aculeatum* abundance, and no "leaf-swallowing" behavior was observed. The ingestion of large quantities of leaves suggests a nutritional function, particularly during the winter season when leaves played a large part in the total diet.

This case does, however, provide a good example of how potentially therapeutic plant products may be concealed within the diet, and any therapeutic benefit accrued prophylactically, without requiring awareness by the animals. Based on the above ethnomedicinal literature, anti-parasitic benefit may be gained from intensive folivory on this species, and if so, loss of access to this resource could have detrimental health consequences for *Yaku* macaques. Furthermore, the mature leaves of this tree are ingested largely as a fall-back food item in periods of scarcity (winter), and this is one of the routes to medicinal innovation suggested by Huffman (2001). During poor fruiting years, sick animals may resort to eating familiar leaves during times of high parasitosis risk, and potentially associate recovery with that action. Although this study revealed no medicinal use of *Trema orientalis* by *Yaku* macaques, it would be

worth maintaining as a candidate in other similar studies and observations of other macaque groups on Yakushima.

Zanthoxylum ailanthoides (Rutaceae) is a small tree, abundant across the NA-2 range (n=205 from transect survey, mean = 4.9/ha), especially on sheltered slopes and in gullies (**Appendix B8**). All NA-2 individuals ingested bark and/or gum of *Zanthoxylum ailanthoides* September-December 06 and June-August 07, the duration of which varied significantly across the annual cycle ($\chi^2=31.902, p=0.001$). The precise target of ingestion was unclear at times, due to the proximity of the two parts, but gum is extruded distinct from the bark during summer months, and the animals spent up to several minutes harvesting it, while proportionally more bark was ingested in early winter. The precise seasonal variation in gum exudation was not recorded, however. The daily ingestion of *Z. ailanthoides* was not related to the daily fecal parasite abundance of any taxon, nor with the 24 hour change in parasite abundance. There is no evidence, therefore, of *Z.ailanthoides* ingestion being either stimulated by, or functioning to alleviate, nematodal parasitosis.

Interview data revealed this species to be by far the most salient of the candidate species in Japanese and *Kanpō* medicine, largely due to the widespread use of its cultivated relative, *Sanshou* (山椒; *Z.piperitum*). Few, however, were able to recognize the tree by sight (recognition 10/20). The Japanese name for the species, *Karasusanshou*, roughly translates as "useless *Sanshou*" - only good for "dogs and crows," although it is recognized to have qualities similar to those of the commercial species. Rather than the gum, the most frequently mentioned products were the fruit and fruit-casing, used as digestive and circulatory stimulants, as well as a spice to counter a range of gastric ailments, including food poisoning, parasitosis and diarrhea. The bark and gum were only mentioned by one expert, who stressed their anti-bacterial, anti-diarrheal and insecticidal properties.

Little published information is available on the medicinal use of this particular species in Japan, due to it being firmly in the shadow of its commercial cousin. The seed pods of *Sanshou* are used across

South-East Asia as a culinary spice, and in medicinal mixtures for use as a gastro-intestinal stimulant (Tokita *et al.* 2007), to treat “cold” colic and dysentery (Li, S. and Luo 2003), and as an anthelmintic (Hsu 1986). The leaves, bark and root-bark of *Z.ailanthoides* are recorded in traditional medicine as a circulatory and digestive stimulant, and used for colds, bruises and stasis (Cheng *et al.* 2003; Li, T. S. C. 2006), as well as an antirheumatic (Hsu 1986). Specifically, the bark-resin is reported to be antitussive, carminative and stimulant (Li, S. *et al.* 2003). The bark and root-bark of *Z.ailanthoides* have been found to contain a wide array of potentially active chemicals, including coumarins, alkaloids, quinoline derivatives, flavinoids and tetrahydroberberine (Cheng *et al.* 2003), which have been reported to show activity against infectious diarrheal diseases such as cholera, giardia (Kaneda *et al.* 1991) and leishmaniasis (Ghosh *et al.* 1985). Both the bark and resin of the plant have a yellow appearance, which is congruent with the published berberine content of its western relatives (Duke 1992), although Li (2006) does not list it as a constituent. Recent research has revealed anti-oxidant (Chung *et al.* 2006), and anti-HIV activity (Cheng *et al.* 2005) in the leaves and root-bark, respectively, while other members of the genus are known in Africa as anti-malarials (e.g., Tabuti 2008).

The use of *Zanthoxylum ailanthoides* by *Yaku* macaques showed no pattern suggesting behavioral stimulus by nematodal parasitosis. However, due to a typhoon, there were no fruit of *Z.ailanthoides* available in NA-2’s range during this field season. As the fruits are the most well-known medicinal part of the tree, and previously recorded as a dietary item, future similar studies of *Yaku* macaques should continue observation of this candidate species. It would be interesting to compare the ingestion of gum from this season with that during a year of plentiful *Z.ailanthoides* fruit. Considerable effort was employed by the macaques to harvest the gum and bark, and this has been previously assumed to be motivated by the sugar content of the gum. However, the periods of greatest ingestion, September-December and June-August, were during the seasons of plentiful fruit sugars (**Figure 3.1**), and one interviewee, a specialist in resins and aromatics, asserted that the available sugar in exuded gums was relatively low. In concurrence with this opinion, Nash (1986) makes the important distinction between a *sap*, which contains easily digestible photosynthetic sugars but requires the ability to gouge into the wood

to harvest it, and a *gum*, which is exuded on the surface of trees in response to breakage or insect damage, and contains highly polymerized complex polysaccharides. These complex carbohydrates are relatively indigestible for most vertebrates, requiring either gastro-intestinal fermentation or specialist enzymes. Bonnet macaques (*M.radiata*) produce amylases and isozymes in their cheek pouches which allow polysaccharide digestion (Rahaman *et al.* 1975), but a similar adaptation has not been identified in Japanese macaques. Many of the commonly found polysaccharides are known to be immunostimulants, however, suggesting a potential medicinal benefit (Berry *et al.* 1995). Certainly it would seem that a significant amount of a several medicinal and potentially antiparasitic components are ingested by these macaques, but what benefit they may proffer and any stimuli involved remain to be determined.

Traditional Antiparasitic Pharmacy

The antiparasitic remedy most commonly cited by the interviewed pharmacists was *Kayaninso* (*Digenea simplex*), or *Macuri*, which is a combination of the former with dried plum. Despite the reportedly unfortunate side effect of yellow-tinted vision, the use of this seaweed was widespread until the middle of the 20th Century, when synthetic antiparasitics and improved post-war living hygiene caused a significant reduction in endoparasitic infections in Japan. Amongst stated anti-diarrheal remedies, the most salient were *Genshuko* (*Geranium thunbergii*), an astringent folk-medicine reputed to be effective against amoebic dysentery, and *Obaku* bark (*Phellodendron amurense*), a strongly bitter antibiotic containing berberine (Duke 2002). Although one interviewee stated that *Genshuko* was frequently used in Yakushima, none of these species have been recorded in the *Seburindo* area of Yakushima.

Comparison of Human and Macaque Medicine

The ethnomedicinal data discussed above were gathered from interviews with 10 individuals, one on Yakushima itself, seven in Kyoto and two in Nara. Their occupations included pharmacist, wholesaler, factory manager, holistic therapist and one researcher. Their area of expertise or practice was largely (8 of

the 10) Chinese-style *Kanpō* medicine, while three professed knowledge of traditional Japanese *Amatsu* medicine, which is often integrated with acupuncture therapy.

Kanpō is the predominant traditional medicine practiced in modern Japan and was brought to Japan by Buddhist priests from China and Korea in the 6th Century CE. During the years of Imperial isolationism, the practice adopted the use of a more Japanese pharmacopeia, but still relies on many cultivated plant species originating in China. The indigenous folk-medicine of Japan (*Minkanyoku*), while still practiced in more rural areas, is in severe decline due to lack of apprenticeship, while *Kanpō* enjoys institutional support and integration with western-style medical care. Practitioners of herbal medicine in Japan are required to obtain a license in western medicine, and then train under a practicing *Kanpō* doctor (Lock 1980).

When each of the 10 interviewees' responses were evaluated, they afforded a salience score, for each of the five candidate plants, ranging from 0 (no recognition or knowledge) to 14 (several parts known for medicinal use, including some antiparasitic). **Table 5.2** lists all the uses described for the candidate plants, including *Miscanthus sinensis* (see **Chapter 3**). Although responses varied, when all salience scores were placed into a respondent-subject matrix, the set conformed to a cultural consensus model (Eigen ratio = 3.257; Reliability = 0.917), indicating agreement between respondents and reliability of their knowledge within a common cultural tradition (Romney *et al.* 1986). Overall, the highest combined salience was attributed to *Zanthoxylum ailanthoides* (59), largely due to its similar but more effective and well-known cousin, *Z.piperitum*, followed in descending rank by *Miscanthus sinensis* (32), *Rhus succedanea* (31), *Lagerstroemia fauriei* (24) and *Trema orientalis* (4). Only *Z.ailanthoides* was specifically mentioned as an antiparasitic, while *R.succedanea* and *L.fauriei* were said to be anti-diarrheal. *M.sinensis* was not reported as an antiparasitic in humans, but its use as an antiparasitic and emetic by domestic animals was noted.

The equivalent salience of each plant in NA-2 *Yaku* macaques differed from the human medicinal use mainly in the reversal of the two most important species. The highest score (9) was afforded to *Miscanthus sinensis*, due to its presence, undigested, in feces; a statistical relationship with

Streptopharagus pigmentatus abundance; and an unequivocal association with the fecal expulsion of adults of that same taxon in the E-Troop sample. Second-greatest salience was afforded to *Z.ailanthoides*, due to the ingestion of bark, known to be rich in pharmacologically active chemicals, although this species may be under-rated here due to the lack of fruit parts during the observation period. These were followed by *R.succedanea/L.fauriei* (tied) and *T.orientalis*. When both human and macaque scores were converted to percentiles and combined, they no longer conformed to a cultural consensus model (Eigen ratio = 2.956; Reliability = 0.912). This suggests that the use of these plants occupies a different cognitive domain in humans and macaques, and as such, that it is unlikely that ethnomedicinal uses of these species were derived from historical observations of such animals, as has been suggested for several items of traditional medicine in North America, Sub-Saharan Africa, and central Asia (Huffman 2003).

The main area of convergence between macaque and human data was the use of *Zanthoxylum ailanthoides*, by virtue of its better known cousin, although the unusual lack of *Karasusanshou* fruit within the NA-2 range during the study period limited the possible agreement over its use. The low level of interviewee knowledge covering other plants ingested by *Yaku* macaques may stem in part from the geographical and ecological distance between the field site and the centers of traditional medicine in Kyoto and Nara. Although folk medicine may retain more localized botanical knowledge than the institutionalized *Kanpō*, it was impossible to interview a suitable local informant during the study, and the medicinal tradition on Yakushima has long been specialized into the commercial production of *Ukon*, a medicinal variety of turmeric, for which the island is renowned.

The one expert found on Yakushima demonstrated the greatest recognition of candidate plant species, and cited species found on the island as antiparasitic: *Sendan* bark (*Melia azaderach*), and antidiarrheal: *Fu-uro*, and *Kaki* (*Diospyros* spp). Of the above, *Diospyros morrisiana* was observed as part of the NA-2 diet (fruit and seed), and is worthy of candidate classification in future studies. The fruit, seed and leaf of the cultivated persimmon *Diospyros kaki* have been found to contain betulinic acid and astringent tannins. Fruit that are left peeled in the sun develop a white encrustation, used as an anthelmintic and febrifuge (Duke and Ayensu 1985) and the fruit and seed are used as an antidiarrheal

(Li, T. S. C. 2002, 2006). Despite abundant ripe fruit, NA-2 macaques were frequently observed to eat only a few mouthfuls before ceasing consumption, unlike their more typical frugivorous behavior where available ripe fruit were ingested to satiation.

Informational Transfer and Learning

The degree of close infant observation of ingestive behavior varied greatly between the candidate materials, and qualitatively seems to correspond with the medicative significance of each behavior. While the entire study was not designed to accurately measure the frequency of such events, it was possible to apply a modified “Solid Food Interest Index” to the observations of candidate species, as described by Perry and Ordonez-Rimenez (2006) and used on Yakushima by Tarnaud and Yamagiwa (2008). This method compares the ‘expected’ frequency of close infant observation of adult ingestibles (if all items were observed randomly) with the observed frequency in the field. In this case, the index was calculated only within recorded incidences of candidate plant ingestion. For each plant, an ‘expected’ value was calculated by multiplying the proportion of all candidate events which involved that species, by the total number of infant observation events. This figure was then subtracted from the observed number of infant observation events, to provide a Food Interest Index for that plant. A positive index value would demonstrate a greater than ‘expected’ frequency of interest, while a negative index value would indicate the converse. The significance of the results were evaluated using a Chi-squared proportions test. **Table 5.3** summarizes the values calculated for the range of candidate plant species, and for geophagy.

In this case, significantly positive indexes were shown for both geophagy (+12.34) and *Miscanthus sinensis* (+3.79) ingestion, suggesting some cultural importance of these behaviors in the Yaku macaque population, and supporting the conclusions of Chapters 3 and 4 concerning their therapeutic significance. While functionally beneficial behaviors, the ingestion of these two materials does not present a neurophysiological ‘flavor’ associated with nutritious food, each being non-organic or tough and fibrous. Repeated cultural reinforcement through local enhancement and emulative observation may be required for their adoption into the dietary range of a young macaque. Conversely, the strongly

negative index calculated for *Rhus succedanea* leaves (-10.73) indicated an unusual paucity of infant and juvenile observation of this plant part, and may relate to its reported toxicity. The non-significant, near zero values calculated for the other plant species support the position that they are utilized primarily for basic nutritional need, a behavior which is quickly or semi-innately acquired by offspring through common taste cues, and requires no additional observational experience. For statistical confirmation of the role of these plants in the entirety of NA2 group culture, however, a specifically designed survey of close infant observation and foraging activity akin to that by Tarnaud and Yamagiwa (2008) would be required.

Conclusions

Self-medication is an inherently rare behavior, and is difficult to observe reliably and confirm statistically. This challenge to researchers has often made them vulnerable to accusations of relying on anecdotal evidence (e.g., Sapolsky 1994). While early observations of unusual feeding behaviors are in themselves not concrete proof that an animal is self-medicating, they are, however, of value in the process of inquiry, and allow more focused experimental designs to be directed at the most promising phenomena. The ingestion of pharmacologically active substances from *Lagerstroemia faurei* and *Zanthoxylum ailanthoides*, while demonstrating no causal relationship with the parasites recorded in this study, may yet be demonstrated to play a significant role in the chemical ecology of *Yaku* macaques and their parasitic symbionts, and certainly warrant further investigation.

While comparison between animal plant use and ethnomedicine may serve to strengthen self-medicative hypotheses, the Chinese cultural origins of most Japanese traditional medicine illustrates the caution with which researchers must approach apparent areas of confluence. Just as analogous biological traits may emerge through convergent evolution and exaptation, so may aspects of material culture such as medicines develop under profoundly differing selective pressures. In such situations, assumptions of similar function may be dangerously misleading. In this study, the pharmacopeia of Japanese pharmacists was ecologically distinct from the plant diversity present on Yakushima. While providing valuable information on the potential activities of some plant products, the ethnographic material presented here should not be used to imply that *Yaku* macaques have a functionally similar use for the plants concerned.

Table 5.1: Ethnobotany and ecology of candidate plant species.

† Key to plant parts: ft – fruit; fl – flower; lf – leaf; sh – shoot; bk – bark; ph – pith; gm – gum

Bold entries indicate observed candidate plant foraging, *italic* entries indicate part ingestion not observed during current study.

* In addition to cited references, information was gathered from Duke's Ethnobotanical Database (sun.ars-grin.gov/duke)

Species name	Common names	Growth Form	Parts eaten by Yaku macaques †	Ethnobotanical and phytochemical activity	Published phytochemical components	References *
<i>Lagerstroemia fauriei</i> (Lythraceae)	<i>Yakushima-sarusuberi</i> (Jp) Monkey-slip tree	Deciduous tree	Bk	Purgative, stypic, stimulant, antiseptic, anti-dysentery [data from <i>L.indica</i>]	Lythrine, cryogenine, lythridine (lf)	(Kaoru <i>et al.</i> 1978)
<i>Melia azaderach</i> (Meliaceae)	<i>Sendan</i> (Jp) China Berry	Deciduous tree	Gm, sd	Anthelmintic, stomachic, astringent (bk,lf), vermifuge (ft, rt), antirheumatic (sd), poisonous (ft)	Vanillic Acid, Cinnamic Acid (bk, ft, rt), Carvacrol (oil)	(Duke and Ayensu 1985; Hill 1997)
<i>Miscanthus sinensis</i> (Graminaceae)	<i>Susuki</i> (Jp) Japanese Silver Grass	Grass	Lf, Ph, Sd	Diuretic, anti-hypertensive (lf/sh)	Flavonoids, misrathoside, prunia, tricin, Diphenhydramine	(Li 2006)
<i>Rhus succedanea</i> (Anacardiaceae)	<i>Hazenoki</i> (Jp) Wax Tree	Deciduous tree	Sd, Lf, Ph	Astringent, Diarrhea Dysentery, Expectorant Fever, Stimulant, Tonic. Antiviral (lf), Anti-cancer (lf), Antioxidant (gm), cytotoxic (gm), Antibacterial (lf)	Heptadecenyhydroquinone (gm), tannins (lf)	(Hanya <i>et al.</i> 2003; Hill 1997; Jassim and Naji 2003; Lee <i>et al.</i> 2004; Wu <i>et al.</i> 2002)
<i>Trema orientalis</i> (Ulmaceae)	<i>Urajiroenoki</i> (Jp)	Evergreen tree	Ft, Lf, Sh	Anti-Diarrheal, Anti-Dysenteric, Vermifuge, Coughs and sore throats, Astringent (lf)	Xanthones, saponins, condensed tannins.	(Hanya 2003; Hill 1997; Huffman and Wrangham 1994)
<i>Zanthoxylum ailanthoides</i> (Rutaceae)	<i>Karasuzanshou</i> (Jp)	Deciduous tree	Ft, Sd, Bk, Gm	Antidote, Carminative (bk), Diarrhea, Dysentery, GI Stimulant (bk,ft), Stomachic (ft), anti-oxidant,	Berberine (pl), sesquiterpines, xanthyletin, hesperidin (rt bk),	(Cheng <i>et al.</i> 2003; Cheng <i>et al.</i> 2005; Morita and Shimizu 1966; Tokita <i>et al.</i> 2007)

Table 5.2 – Ethnographic interview summary of candidate plant species.

* Recognition score (out of a possible 20) / Total salience score.

Key to plant parts: ft – fruit; lf – leaf; bk – bark; gm – gum; rt – root

Species name	Japanese names	Salience*	Stated Medicinal Uses	Preparation	Non-Medicinal	Other
<i>Lagerstroemia fauriei</i>	Yakushima-sarusuberi	10 / 22	anti-inflammatory, antidiarrheal, antifungal, antibacterial (bk), tonic (lf)	Ointment, infusion	Ornamental plant	
<i>Miscanthus sinensis</i>	Susuki	11 / 31	Bloat (lf), vaginal discharge (lf), ammenorrhea (lf), carminative (lf), reduce heat (lf), anti-acid (lf), ext anti-inflammatory (rt), febrifuge (rt)	Liquid extract	Childrens food (pith), ornamental, used in <i>Tsukimi</i> moon-festival.	Dogs and cats eat as antiparasitic and emetic
<i>Rhus succedanea</i>	Haze-noki	10 / 29	Antifebrile (lf), burns, eye infections, astringent (bk), antidiarrheal, antibacterial (gm),		Wax and ointment base (ft)	Ointment base often used with <i>Sanshou</i> . Old literature classed the lfs as non-toxic
<i>Trema orientalis</i>	Urajiroenoki	0 / 4	None	None	None	
<i>Zanthoxylum ailanthoides</i>	Karasuzanshou	10 / 65	Antiparasitic (ft) , digestive stimulant (ft) , anti-toxin (ft) , antibacterial (ft, gm, lf), analgesic (ft), stomachic (ft), stomachache (ft), constipation (ft), balance colonic flora (ft), anti-diarrheal (ft, rt, bk, lf), haemorrhoid (rt), post partum heamorrhage / prolapse (rt), circulatory stimulant (ft)	Spice (ft) infusion (ft), decoction (rt, bk),often with ginseng and ginger	Ritual new year drink "Odosoo"	Insecticidal (lf, gm), mould-resistant tools (wood).

Table 5.3 – Solid Food Interest Index applied to close infant observation of candidate medicinal materials.

Candidate Material	Ingestion events	Close infant observations	'Expected' observations	Food Interest Index	Proportions Test
<i>Geophagy (clay)</i>	83	22	9.66	12.34*	$\chi^2=20.47, p<0.001$
<i>Lagerstroemia fauriei</i>	1	0	0.12	-0.12	Insufficient cases
<i>Miscanthus sinensis</i>	19	6	2.21	3.79*	$\chi^2=7.62, p=0.005$
<i>Rhus succedanea</i>	118	3	13.73	-10.73*	$\chi^2=12.70, p<0.001$
<i>Trema orientalis</i>	44	1	5.12	-4.12	$\chi^2=3.68, p=0.06$
<i>Zanthoxylum ailanthoides</i>	88	9	10.24	-1.24	$\chi^2=0.15, p=0.70$

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CHAPTER 6

CONCLUSIONS AND FUTURE DIRECTIONS

Introduction

This dissertation has reported the quantitative assessment of parasitic health stressors in *Yaku* Japanese macaques, and the role of several ecological medicinal resources in their management. This final chapter will address the implications of these findings for the emergence of medicine in humans and animals, and for conservation medicine strategies. Finally, I suggest future directions for similar research that builds upon these findings and addresses some of the methodological limitations encountered during this study.

To briefly summarize the field research: I found female *Yaku* macaques of NA-2 group to be infected by five nematodal parasite taxa which are generally well tolerated by the monkeys, allowing them to maintain good overall health under observed conditions. In the absence of a clear rainy season, some parasites may synchronize ova deposition to other meteorological and ecological conditions optimal for their transmission. Some infections also seem to be affected by age and social rank.

This study illustrates the weakness in the assumption that ethnomedicinal plants ingested by animals necessarily have a medicinal function or stimulus. Of the plants pre-selected based on medicinal literature, three were behaviorally and statistically indistinguishable from food items, while the medicinal parts of the fourth were not observed to be ingested. More promising results arose from field observation of anomalous ingestive behavior and by careful fecal analyses.

There is convincing evidence that Yaku macaques swallow whole leaves of the grass *Miscanthus sinensis* as a strategy to manage infections by the large nematode, *Streptopharagus pigmentatus*. Other results from tested plants are more ambiguous. Significant dosages of physiologically active compounds may be ingested from *Trema orientalis* and *Rhus succedanea* leaves, but simply as a result of normal dietary foraging. The ingestion of *Zanthoxylum ailanthoides* gum also affords a range of pharmacologically active compounds, but both its effect and/or stimulus (if any) remain unclear. The rare ingestion of *Lagerstroemia fauriei* bark compounds, while certainly intriguing, requires more attention before any functional hypothesis can be tested.

The monkeys also ingest clay throughout the year, which may be an efficacious functional response to gastrointestinal distress caused by invertebrate toxins, plant antifeedants and large indigestible seeds. An incidental function may serve to ameliorate the diarrheal symptoms otherwise associated with the recorded levels of parasite infection.

These findings can be assessed in terms of the guiding hypotheses stated in Chapter 1:

H1. Ingestion of candidate plant species is stimulated measurably by helminthic endoparasite load.

Supported. The only firm association of this type found, however, was that of *Miscanthus sinensis* leaf swallowing and *Streptopharagus pigmentatus* load.

H2. The frequency of geophagy in Yaku Japanese macaques is stimulated measurably by helminthic endoparasite load.

Not supported. There was no evidence of symptoms of parasitosis being a physiological stimulus for geophagy, although it may serve to ameliorate some of the common symptoms.

H3. Observed ingestion of candidate plants constitutes an effective self-medicative response to parasitosis.

Not supported. There is insufficient evidence to conclude that the observed behaviors are effective. The paired 24 hour records did not show any consistent effect of the ingestion of any plant material. The association of *M. sinensis* and *S. pigmentatus*, however, and evidence

supporting a feasible mechanism of action are highly suggestive of an effect that could mediate parasite load.

H4. Observed ingestion of clays constitutes an effective self-medicative response to parasitosis.

Not supported. Although clay ingestion may indeed play a role in the amelioration of parasitic symptoms, the behavior appears to be a response primarily to dietary toxins.

H5. The self-medicative pharmacopoeia of Yaku Japanese macaques will be similar to that used in traditional medicine in Japan.

Not supported. Due to geographical and cultural distance between the field site and the centers of traditional medicine in Japan, very little overlap was recorded between macaque and human medicinal use of plants.

Despite failing to support several of the above hypotheses, the results of this study have broader theoretical implication in the emergence of medicine in humans and non-human primates, and the theory and application of conservation medicine.

Implications

The Emergence of Medicine in Humans and Non-Human Primates

Despite the presence of several plants of significant medicinal potential, the evidence thus far does not suggest that *Yaku* Japanese macaques possess a pharmacopoeia as complex or specific as has been found in some other primate species, such as chimpanzees. The two areas where the data convincingly suggested self-medication were those found in several other taxa of mammals and birds. The ingestion of grass to scour parasites has been recorded in bears, geese (Huffman 1997), tigers and wolves (Engel 2002), and it was reported in my ethnomedicinal interviews that Japanese dogs are thought to seek just this type of grass to perform the same function. The use of clays to detoxify dietary elements is also common to several other primate species, as well as parrots and African elephants (Engel 2002). This

finding is, perhaps, in keeping with the lack of tool use in Japanese macaques, outside the unusual ‘environmental tools’ demonstrated in one population by sweet potato washing and placer-mining. Certainly the biochemical ‘tools’ for the treatment of infections are available, but not consciously used. It may be that, given the general good health of the macaques, and their tolerance of the parasitic infections that exist throughout the population, the selective conditions have not arisen for the adoption of more complex behavior.

This apparent situation supports Johns’ (1990) model of the evolution of human medicine. A fundamental characteristic of medicinal culture is the use of exogenous materials to alter health, cognitively separate from those ingested as part of normal dietary activity. Johns posits, however, that medicine is most likely to have originated in broad spectrum plant foraging, and indeed may have emerged as an independent cultural phenomenon only after the adoption of agricultural staples caused a sharp decline in the range of secondary compounds in the diet. A residual effect of such a transition may be evident in the strong overlap between traditional dietary and medicinal plant species in many cultures worldwide (Etkin 1996) and the inclusion of wild taxa as spices and flavorings in regional cuisine.

The legendary folk-hero *Shen-nung* (Divine Peasant) is credited as the father of Chinese medicine and agriculture, which arose around 2700 BCE (Chan 1939). He is said to have personally tasted hundreds of herbs and categorized their medicinal or poisonous properties based on their taste and effect on his own body (before dying from poisoning at 160 years of age). It is intriguing that he is also credited with the introduction of agriculture, as this would have simultaneously removed a plethora of wild herbs from the pre-existing broad-spectrum diet, replacing them with starchy staple crops.

Primate foraging strategies vary in their degree of dietary neophobia (Johnson 2000; Visalberghi *et al.* 2002), depending on environmental and social factors (Drea 2006; Hikami *et al.* 1990; Visalberghi and Fragaszy 1995), and generally being more conservative in wild populations (Visalberghi *et al.* 2002). This can be seen as an adaptive protection against accidental poisoning, but dietary selection always involves a trade-off between satisfying nutritional requirements and risking phytochemical challenges (Glendinning 1994). Particularly in herbivores, many plant secondary chemicals are ingested and

tolerated/detoxified in order that adequate nutrition is obtained from low-quality forage. Thus, in contemporary primate and early hominid diets, there would exist a range of pharmacologically active substances with the ability to ameliorate infectious conditions without resorting to cultural medicine.

Although fur-rubbing and other external applications of medicine do occur in several species, many forms of human and animal medication involve the digestive tract, either through the control of pathogens, or detoxification of dietary items. Gastrointestinal malaise is a powerful stimulus for conditioned taste aversion (Bernstein 1991), and the relief from aversive symptoms has been similarly shown to condition taste preferences (Villalba *et al.* 2006). In order for a substance to be active, however, and for a situation to exist such that its effects may be learned, the material containing the substance must pass initial taste and olfactory discrimination in order to be ingested. By far the most likely candidate for this situation would be a plant already accepted as a food item, although Huffman (2001) points out that the dietary range of a population is not static, and many otherwise ignored ‘fall-back’ foods may be sampled during times of nutritional stress, such as droughts or fruit failure, when the selective benefit of recognizing their effects would also be amplified. By this model, therefore, the most likely candidates for medicinal innovation would be found on the margins of dietary acceptability, rarely ingested under normal conditions, but increasingly exploited during times of environmental stress.

In the case of *Yaku* macaques, the conditions are set for the emergence of conditioned or cognitive associations between health benefits and plant chemicals. The monkeys are widely infected with several taxa of potentially pathogenic parasite and their diet contains several plant species known as anthelmintics in traditional medicine. In addition, the population must endure rare seasons of fruit shortage, and associated mortality due to pathogenic infections, such as was recorded in 1998-1999 (Hanya *et al.* 2004). During such events, the ability to manage parasitic infection levels would become selectively advantageous. Under normal epidemiological and nutritional conditions, however, these substances and behaviors may remain concealed within the dietary repertoire, and although perhaps providing prophylactic benefit, may persist without biological or cognitive association of their activity.

A key element in the biological adaptation of both diet and medicinal behavior is sensory ecology. Most discussion concerning sensory discrimination of dietary items centers on the chemoreceptors of taste and smell. That these mechanisms do not encompass the significant range of sensory signals is amply illustrated by the fact that there exist no mammalian chemoreceptors for tannin astringency (Swain 1979), yet it is clearly a major factor in palatability and food discrimination. The role of textural cues has been explored by Huffman and Hirata (2004) with relation to hispid leaf swallowing in chimpanzees. They found that when analogous leaves were introduced to naïve captive animals, some immediately adopted the behavior seen in the wild, while others rapidly acquired the trait through observational learning. While there is insufficient evidence here to determine the ontogeny of grass blade swallowing in *Yaku* macaques, the chimpanzee example suggests that, like taste preferences, this may exist as a complex interplay of innate and learned responses. Like leaf-swallowing, it is unlikely that the behavior is first adopted in association with a self-medicative function, but rather acquired as part of social learning of the mother's broader foraging repertoire. With subsequent personal or observational experience, it is then possible for the established trait to become cognitively associated with a physiological condition, or the relief from it.

That no infant ingestion of *Miscanthus sinensis* was recorded during focal observations, despite repeated close observation of the behavior by youngsters, suggests that the grass blades are not within the initial sensory range of palatable foods adopted by exploratory young macaques. This is in accordance with the observation during Huffman and Hirata's (2003, 2004) controlled study of leaf swallowing. All but two of the experimental animals initially reacted aversely to the introduced leaves, rejecting them outright, and only later adjusted this assessment after some process of social learning. It may be the case that *Yaku* macaques instinctively swallow sharp edged or toothed leaves without the normal degree of mastication. The initial buccal acceptance, however, may still require learning to overcome its more typically unpalatable characteristics.

In their role as a novel, potentially therapeutic material in the diet of a macaque, physically active medicinal materials such as clay or grass blades may be less risky than a phytochemical of unknown

toxicity, sensitive pharmacodynamics and potential side effects. The sensory identifier may be less ambiguous also, compared to the vast range of activities found within ‘bitter’ tasting alkaloids. This ‘safe technology’ may account for the apparently more taxonomically widespread adoption of physical scours and geophagy than biochemical phytotherapy. The finer points of this mechanism are very difficult to illuminate through field studies. Like the chimpanzees involved in the above experiments, however, there exist several well established captive colonies of Japanese macaques, naïve to wild plant foraging. The controlled introduction of a grass species analogous to *Miscanthus sinensis* to such populations, even in the absence of parasitic infection, may reveal at least the relative significance of innate and ontogenic behaviors. If grass swallowing does occur spontaneously, subsequent observations might reveal the speed and dynamics of informational transfer as the trait spreads through the group.

Unlike the ethnomedicinal folklore recorded in many parts of the world (review in Huffman 2003), the interviews carried out in Japan did not reveal evidence of the acquisition of medicinal lore from animals. There was some recognition that animals are able to medicate themselves, as illustrated by the reports that Japanese dogs are familiar with the antiparasitic actions of *Miscanthus sinensis* (Chapter 3), but no admission that human medicine may have arisen from such a ‘lowly’ teacher. This may stem in part from the importation of much current Japanese herbalism from Chinese tradition (Lock 1980), and the subsequent geographical dissociation from the source of the pharmacopeia. In addition to this, there lies the apparent habit of attributing Chinese texts to an “ancient worthy” (Henricks 1998), whereupon the accumulated medicinal knowledge of generations may be credited to the inspiration of one author, such as *Shen-Nung*, the Devine Peasant discussed above (Chan 1939).

Conservation Medicine

Although parasite infections appear to be well tolerated in the observed *Yaku* macaques, they may cause a more significant threat to population health under certain abnormal conditions. Nutritional stress due to a poor fruiting season may depress immunological defenses, exacerbating the risk of infectious

disease agents. Reporting the mass mortality event amongst Yakushima macaques, Hanya *et al.* (2004) concluded that the extinction of two groups and widespread mortality in others was due both to nutritional stress in a drought season, and to heightened susceptibility to an unknown pathogen. In the face of current predictions of climate change and species loss, such catastrophic events may become a more frequent threat to endangered primates, especially those endemic to island habitats, such as Yaku macaques, Coiba Island howler monkeys (*Alouatta palliata ssp. coibensis*) (Cuarón *et al.* 2008), Pagai macaques (*Macaca mentaveensis*) (Whittaker and Mittermeier 2008), and the Peleng tarsiers (*Tarsius pelengensis*) (Shekelle *et al.* 2008).

Despite the advances in chemical ecology over the past three decades, the majority of studies of inter-order interactions are still focused on diet composition and nutritional ecology. In order to assess risk to threatened species, and focus amelioration strategies, the role of disease and existing behavioral counter-measures to pathogens must be elucidated in such populations. This assessment is, by necessity, multi-disciplinary and multi-dimensional. Such work should encompass longitudinal parasitological surveys, allowing identification of ecological and environmental correlates of infection risk or disease virulence. In parallel, key medicinal resources should be identified and their distribution mapped, and these should be assessed for their own vulnerability to habitat and climatic alteration.

Habitat fragmentation and degradation, or group relocation, may deprive a population of resources such as *Miscanthus sinensis* and kaolinitic clays that appear to play a role in the management of diarrheal symptoms and parasite load. Isolation from other potentially prophylactic medicinal resources, possibly in this case including *Trema orientalis*, *Zanthoxylum ailanthoides* or *Lagerstroemia fauriei*, may also cause a greater severity of infection and parasitosis. In protected areas it is vital, therefore, to identify medicinal resources that are part of the chemo-behavioral repertoire of the population and ensure that they are not isolated by further fragmentation, left outside the protected area, or subject to over-harvesting by human populations.

The result of ecological isolation from key medicinal resources may be illustrated by the increased susceptibility to disease often reported in captured animals (e.g., Dutton *et al.* 2008). Several

surveys of wild animal health have found either lower prevalence of infectious disease agents (Deem *et al.* 2005; Fujita and Kageyama 2007; Verschoor *et al.* 2008), or the tolerance of pathogenic agents that would be expected to cause significant mortality in captive populations (Gilardi *et al.* 1995). Direct, systematic comparisons of health and disease between captive and wild populations are scarce, however, and are complicated by differing pathogen pressure, immunological life-history trade-offs (Buehler *et al.* 2008), and routine veterinary intervention. Also, studies often report infection or antibody prevalence without determining the actual health state of the subjects, neglecting an important half of the picture (e.g., Munene *et al.* 1998). The example of gorilla fibrosing cardiomyopathy discussed in Chapter 1 is one case where the isolation from a single biochemical resource (*Aframomum* spp.) is thought to have had a measurably detrimental effect on captive populations (Dybas 2007). Controlled pre-post experiments introducing captive populations to *ecologically relevant* self-medicinal plants and clays may be able to illuminate the role that these resources play in health maintenance, and by inference, their importance for survival of endangered wild populations. Similarly, to reduce the burden of disease and cost of veterinary care in captive breeding or reintroduction programs, it would be valuable to identify the medicinal resources used in the corresponding wild populations. The provision of these plants in enclosures not only allows their therapeutic or preventative benefits to be enjoyed, but maintains the behavioral traditions of their use, which may have significant impact on the success of reintroduction programs.

Future Directions

The conclusions drawn from these analyses are based upon purely observational data of self-medication, as is often the case in the early evidence of such phenomena. As such, they are limited to the identification of associations and potential stimuli. For a zoopharmacognitive hypothesis to be more conclusively supported, the mechanisms of action must be determined, and the efficacy of the treatment demonstrated. In this case, budgetary constraints did not allow several procedures that would help elucidate this element, and which would be recommended in future research design in this area.

Chemical Ecology

Despite lacking detectable parasitic stimuli, components of several candidate plants may have a prophylactic effect on parasite loads in *Yaku* macaques. For a medicative model of plant use to be supported, however, it must be shown that bioavailable extracts of the ingested parts are indeed active against the parasite species present. Current data are unable to discount the possibility that the supposed active principles may be unreliably present (as was the case for thiarubrine A in *Aspilia* spp. (Page *et al.* 1997)), or deactivated either enzymatically or by another dietary component, such as geophagic clays. To this end, experimental assays of extracts may be conducted against *in vitro* populations of nematode adults, ova and larvae. Plants should be harvested during the season of use by the monkeys, to account for annual variation in plant chemistry. After drying and grinding, a crude extract should be made with ethyl alcohol, and dried for storage. Parasites may be tested for inhibition of motility and inhibition of ova hatching by incubation with varying concentrations of the extract in ethanol, dimethyl sulphoxide (DMSO), water or simulated gastrointestinal conditions (Dominy *et al.* 2004). By these means, the role of these plants in parasite dynamics and their significance in the overall health of the population may be more accurately determined.

The logical succession to a positive antiparasitic assay would be to perform comprehensive phytochemical analyses on the active plant materials, and this would also be recommended as a progression in the inquiry into *Lagerstroemia fauriei* bark substances and *Zanthoxylum ailanthoides* gum. In the absence of antiparasitic functions or stimuli, these intriguing and anomalous ingestive behaviors may be illuminated by their chemical constituents. A high concentration of other pharmacologically active elements, be they digestive, antibiotic, hormone analogues, or other such elements, would allow the design of future observational protocols to determine whether these hypothesized actions are responsible for their ingestion, and if they are indeed effective.

Similarly, the conclusions drawn here about the occurrence of geophagy in Yakushima macaques are solely based on observational data. In subsequent enquiry into the function and mechanisms of this

behavior, samples of eaten and eschewed clays would need to be mineralogically analyzed – for particle size, elemental content, proportional clay content, kaolin type mineral identification, and adsorptive capacity under physiological conditions (Dominy *et al.* 2004). Mineralogical analyses in previous studies have been inconsistent in their methodology, leading to some concern regarding the reliability of tests for significant components, such as halloysite (Wilson 2003). It has also been suggested that both textural consistency and soil color may serve as dual identifying traits for animals choosing mining sites. While taste and texture may be approximated by measures of clay content, color analysis may be performed by photographic color profile under controlled conditions.

In addition to the corroborative analyses described above, further field observations similar to those described in this dissertation should modify their techniques to compensate for encountered limitations and disadvantages of the original methodology. For instance, the technique employed here for the pre-selection of candidate plants relied on the overlap between macaque diet and published human medicine in surrounding geographical areas. While this information is widely available and quickly allows the entire range of dietary materials to be reduced to a shortlist of likelihood, more significant candidates were found during preliminary observations of foraging behavior, such as *Miscanthus sinensis*. While ethnobotanical comparisons may be useful in corroborating self-medicative hypotheses after observational testing, their importance should not be over-estimated. Observational methodology in future studies should immediately incorporate any observations of potential non-nutritive ingestion as new candidates. Indicators should include bark or wood ingestion, whole leaf swallowing, the investigation and ingestion of a plant by only a few individuals while others ignore it, or solitary ingestion distant from the group. Items ingested from one species that satisfied the latter conditions during this field season were the fruit and leaves of *Crataeva religiosa* (Capparidaceae). While the leaf was recorded as part of the *Yaku* macaque diet (Hill 1997), and is known as an antibacterial treatment for dysentery (Duke and Ayensu 1985), the pungent fruit is previously unrecorded and was unknown to station researchers of both monkey and deer. Between December and April, it was characteristically eaten only in very small amounts (1-2 bites before discarding the remainder and moving out of the tree after less than 10 minutes).

The first observation of such foraging involved one female (*Man*) leaving the group alone and travelling at speed for 10 minutes, directly to the stand of *C.religiosa* trees. The subsequent four records all involved a single female (*San*). No published chemical analyses are available for this fruit.

Disease ecology

The one parasite taxon associated in this study with anthelmintic behavior, *Streptopharagus pigmentatus*, was not susceptible to quantitative analyses by the floatation methods applied here, and quantitative assessment could only be estimated from crude wet-mount counts. Future studies of this phenomenon should employ methods of analysis equally sensitive to dense, non-floating ova, such as sedimentary concentration. This would probably entail the preservation of samples in formalin and their transport to more adequate laboratory facilities, including centrifuges. Careful quantification of sample sizes and fluid volumes allows techniques such as formalin-ether sedimentation to be calibrated and used to measure fecal abundance of ova (Elkins *et al.* 1986), despite the majority of applications of this method being simply to detect presence or prevalence of taxa.

A more complete epidemiological profile would be drawn through sampling and analytical methods that allow the detection of pathogens and health status beyond solely nematodal parasites. As *Yaku* macaques are in a natural habitat, and carefully habituated for behavioral analyses, only non-invasive techniques are suitable. Urine analysis is a relatively simple procedure due to the low cost availability of dipstrips, allowing close-to-patient diagnosis of urinary tract infections, kidney disease, liver conditions, symptoms of diabetes or dietary disruption in clinical (Bauer *et al.* 2008) and in field studies (Krief *et al.* 2005). More specific immunochromatography strips can be used to test for diseases such as leishmaniasis (Reithinger *et al.* 2002). Although dipstrips are largely qualitative and have been criticized for inaccuracies and the frequency of false-positive results (Tighe 1999), the tests are easily replicatable and simple enough to be used in the field without other specialized equipment. For more reliably calibrated diagnoses, electronic readers are available for commonly used dipstrip brands, rather than relying on eyesight in variable lighting conditions (Bauer *et al.* 2008). Urine may be collected with a

plastic bag on a forked stick, or a pipette from droplets on leaves and rocks (Krief *et al.* 2005). Collection of urine from wild Japanese macaques was successfully carried out by Hayakawa (2008), but considerable challenges must still be expected during rainy conditions.

Protozoan infections are highly prevalent in many mammalian species worldwide, ranging from harmless commensals to the dysenteric *Entamoeba histolytica*. The closely related and similarly pathogenic *E. dispar* was detected in Japanese macaques from Takasakiyama, Oita prefecture (Rivera and Kanbara 1999). *Balantidium coli* is also a common diarrheal agent in non-human primates (Hubbard 1995) and has been detected in Japanese macaques (Nakauchi 1999). Although protozoan cysts and trophozoites were observed in the fecal samples described in this dissertation, the author did not have the expertise to make specific diagnoses. Fecal floatation analyses reveal protozoan infections, but morphological identification is difficult, requiring polymerase chain reaction (PCR) genetic analyses for accurate detection (Rivera and Kanbara 1999).

Although many infections may be present, they may be tolerated asymptotically or their effects may vary with the overall stamina and fitness of the animal (Alados and Huffman 2000). Measures of parasite abundance, therefore, are insufficient to determine the degree of overall health or discomfort experienced by a focal subject. There is an emerging technique using fractal mathematical analyses of behavioral patterns which has the potential to non-invasively measure the subjective health state of individual animals (Alados *et al.* 1996). Video analyses of repeated behavioral events reveal variations that, while seemingly random, may correlate to health variables. For instance, Ibx predator vigilance behavior benefits from rhythmic unpredictability to optimize scanning efficiency during foraging. A predicted decline in fractal complexity in the behavior of parasitized animals is observed in the lessened unpredictability of head-lifting (Alados *et al.* 1996). Elderly humans or those suffering from Huntingdon's disease have been demonstrated to express a greater fractal dimension (complexity of fluctuation) in stride length than do healthy or young individuals (Hausdorff *et al.* 1997). Similarly, healthy female chimpanzees displayed a greater fractal dimension in multiple behavioral states than did sick individuals (Alados and Huffman 2000). This technique, once calibrated using available

measurements of physiological health, may be used to non-invasively monitor the subjective health status of animals in studies of self-medication. Since overall discomfort, rather than disease-specific diagnosis, is likely to be the motivational stimulus for self-medication, such measures may play a valuable role in identifying such phenomena.

Conclusions

There is increasing evidence that the practice of medicine has close precedents in non-human primates. The *Yaku* macaques studied for this dissertation were infected with five nematodal taxa, which were generally well tolerated without symptoms of parasitosis. They ingest a range of pharmacologically active plants and clays in their diet, but care must be taken not to draw self-medicative conclusions without evidence of specific stimuli for ingestion, or measurable efficacy against the target disease agent. While the ingestion of *Miscanthus sinensis* leaves and halloysitic clays appear to be practiced as an functional response to heavy parasite loads and dietary toxicity, respectively, some other materials may contribute to the management of nematodal parasites without requiring cognitive awareness of their properties. These results and the future related research that they may generate, increasingly demonstrate the importance of non-nutritional resources in the survival of primate populations, especially in the conservation of threatened species in the face of habitat alteration and climate change.

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APPENDIX A – TOTAL DIETARY RANGE RECORDED IN NA-2 GROUP SEPT 06-AUG07

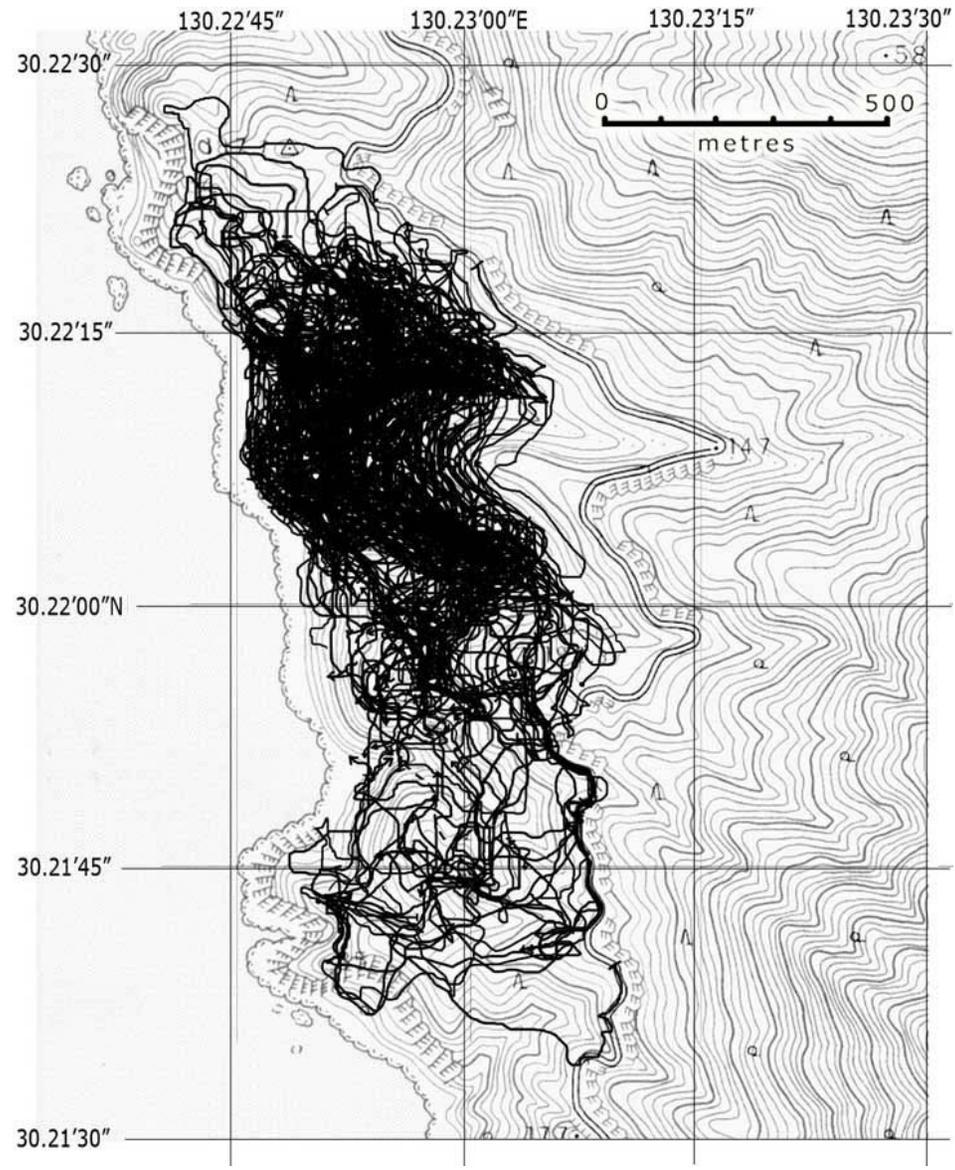
Species	Family	Form	Parts Ingested			
<i>Actinidia sp</i>	Actinidiaceae	Liana	Fruit	Leaf (young)		
<i>Actinodapne longifolia</i>	Lauraceae	Evergreen Tree	Seed			
<i>Ardisia seiboldii</i>	Myrsinaceae	Evergreen Tree	Fruit	Leaf		
<i>Callicarpa shikokiana</i>	Verbenaceae	Deciduous Bush	Fruit	Leaf	Flower	
<i>Camellia japonica</i>	Theaceae	Tree	Shoot			
<i>Castanopsis cuspidata</i>	Fagaceae	Evergreen Tree	Leaf	Seed		
<i>Cinnamomum camphora</i>	Lauraceae	Evergreen Tree	Seed	Leaf	Flower	
<i>Cinnamomum daphnoidea</i>	Lauraceae	Evergreen Tree	Fruit			
<i>Cinnamomum japonica</i>	Lauraceae	Evergreen Tree	Fruit			
<i>Crataeva religiosa</i>	Capparidaceae	Deciduous Tree	Fruit	Leaf		
<i>Daphniphyllum teijsmanni</i>	Daphniphyllaceae	Evergreen Tree	Fruit	Leaf		
<i>Dianella ensifolia</i>	Liliaceae	Herb	Leaf			
<i>Dicranopteris dichotoma</i>	Gleicheniaceae	Fern	Leaf			
<i>Diospyros morrisiana</i>	Ebenaceae	Evergreen Tree	Fruit	Seed		
<i>Distylium racemosum</i>	Hamamelidaceae	Evergreen Tree	Leaf			
<i>Elaeagnus glabra</i>	Elaeagnaceae	Evergreen Vine	Leaf	Fruit		
<i>Elaeocarpus japonicus</i>	Elaeocarpaceae	Evergreen Tree	Fruit	Leaf		
<i>Eurya emarginata</i>	Theaceae	Evergreen Tree	Fruit	Leaf		
<i>Eurya japonica</i>	Theaceae	Evergreen Tree	Fruit	Leaf		
<i>Euscaphis japonica</i>	Staphyleaceae	Deciduous Tree	Seed			
<i>Farfugium japonicum</i>	Compositae	Herb	Leaf			
<i>Ficus erecta</i>	Moraceae	Deciduous Tree	Fruit	Leaf	Flower	
<i>Ficus microcarpa</i>	Moraceae	Evergreen Tree	Fruit	Leaf		
<i>Ficus oxyphylla</i>	Moraceae	Evergreen Tree	Fruit	Leaf		
<i>Ficus spp</i>	Moraceae	Evergreen Tree	Fruit			
<i>Ficus superba</i>	Moraceae	Evergreen Tree	Fruit	Leaf	Flower	Shoot
<i>Firmiana simplex</i>	Sterculiaceae	Deciduous Tree	Leaf	Shoot		
<i>Glochidion obovatum</i>	Euphorbiaceae	Evergreen Tree	pith			

<i>Ilex pedunculosa</i>	Aquifoliaceae	Evergreen Tree	Fruit	Leaf		
<i>Ilex rotunda</i>	Aquifoliaceae	Evergreen Tree	Leaf	Flower		
<i>Lagerstroemia fauriei</i>	Lythraceae	Deciduous Tree	Bark			
<i>Lithocarpus edulis</i>	Fagaceae	Evergreen Tree	Seed	Leaf	Flower	Pith
<i>Litsea acuminata</i>	Lauraceae	Evergreen Tree	Seed			
<i>Lonicera hypoglauca</i>	Caprifoliaceae	Evergreen Vine	Leaf	stem		
<i>Machilus thunbergii</i>	Lauraceae	Evergreen Tree	Fruit	Leaf	Flower	
<i>Mallotus japonica</i>	Euphorbiaceae	Deciduous Tree	Seed			
<i>Marsdenia tomentosa</i>	Asclepiadaceae	Deciduous Vine	Leaf			
<i>Melia aseaderach</i>	Meliaceae	Deciduous Tree	gum			
<i>Michellia Compressa</i>	Rubiaceae	Herb	Fruit			
<i>Miscanthus sinensis</i>	Gramineae	Herb	Leaf			
<i>Morinda umbellata</i>	Rubiaceae	Evergreen Vine	Fruit	Leaf		
<i>Myrica rubra</i>	Myricaceae	Evergreen Tree	Fruit			
<i>Neolitsea sericea</i>	Lauraceae	Evergreen Tree	Fruit			
<i>Oreocnide pedunculata</i>	Urticaceae	Evergreen Tree	Fruit	Leaf	Flower	
<i>Parthenocissus tricuspidata</i>	Vitaceae	Deciduous Vine	Fruit			
<i>Pinus thunbergii</i>	Pinaceae	Evergreen Tree	Leaf			
<i>Piper Kadzura</i>	Piperaceae	Evergreen Vine	Leaf			
<i>Premna microphylla</i>	Verbenaceae	Deciduous shrub	Fruit	Leaf	Flower	
<i>Psychotria serpens</i>	Rubiaceae	Evergreen Vine	Fruit	Leaf		
<i>Quercus philairoides</i>	Fagaceae	Evergreen Tree	Seed			
<i>Rapanea nerifolia</i>	Myrsinaceae	Evergreen Tree	Fruit			
<i>Rhaphiolepis indica</i>	Rosaceae	Evergreen Tree	Fruit			
<i>Rhus succedanea</i>	Anacardiaceae	Deciduous Tree	Leaf	Pith	Seed	Petiole
<i>Rubus seiboldii</i>	Rosaceae	Herb	Fruit			
<i>Scurrula yadoriki</i>	Loranthaceae	Evergreen Vine	Fruit			
<i>Shefflera octophylla</i>	Araliaceae	Evergreen Tree	Flower	Leaf		
<i>Symplocos glauca</i>	Symplocaceae	Evergreen Tree	Leaf			
<i>Symplocos kanoki</i>	Symplocaceae	Evergreen Tree	Leaf			
<i>Symplocos myrtacea</i>	Symplocaceae	Evergreen Tree	Leaf			

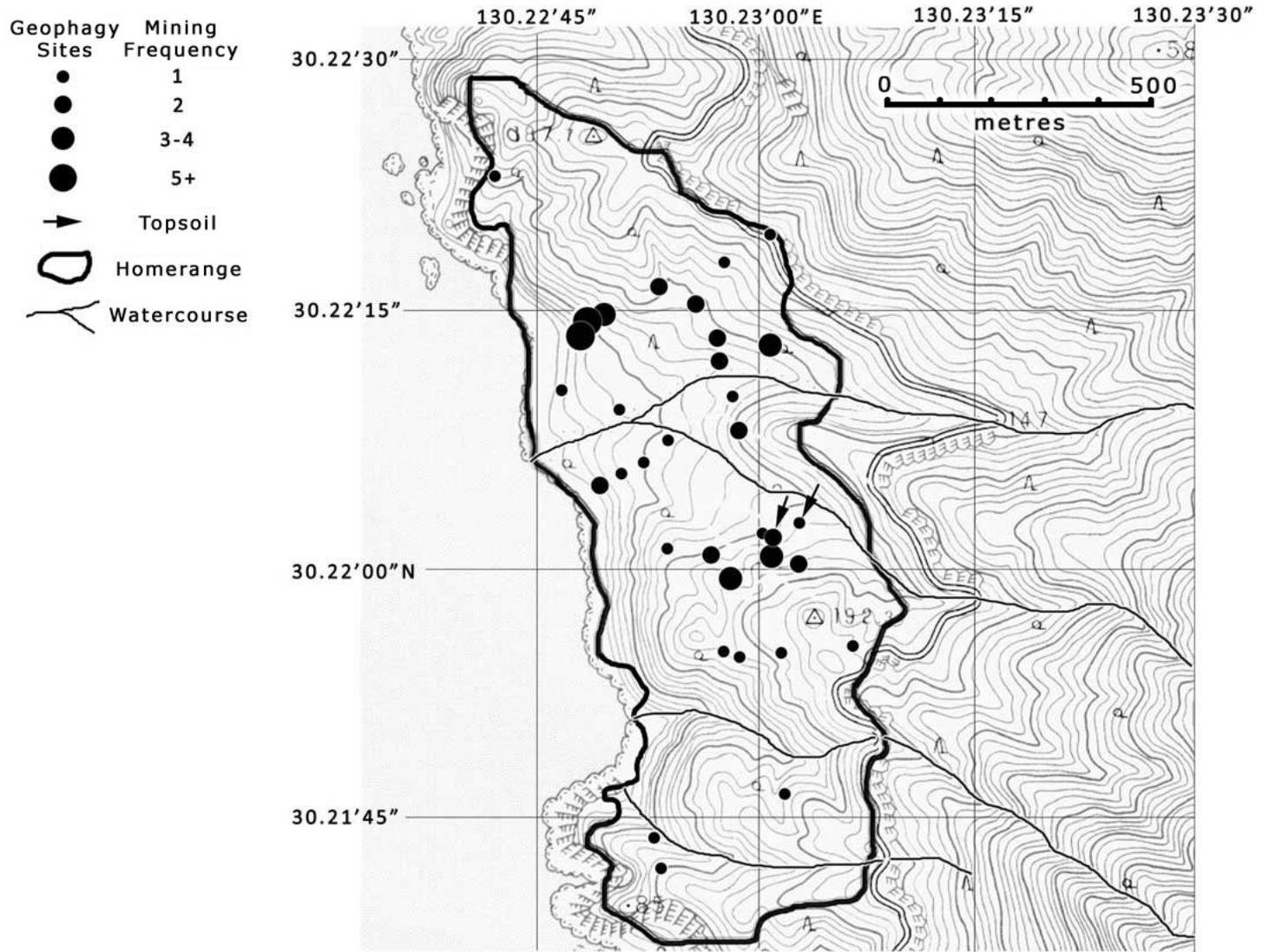
<i>Taxillus yadoriki</i>	Loranthaceae	Evergreen Tree	Seed			
<i>Ternstroemia gymnathera</i>	Theaceae	Evergreen Tree	Fruit			
<i>Trema orientalis</i>	Ulmaceae	Evergreen Tree	Fruit	Leaf		
<i>Viburnum seiboldii</i>	Caprifoliaceae	Evergreen Tree	Fruit			
<i>Vitis ficifolia</i>	Vitaceae	Liana	Fruit			
<i>Zanthoxylum ailanthoides</i>	Rutaceae	Deciduous Tree	Gum	Bark		

APPENDIX B - MAPS

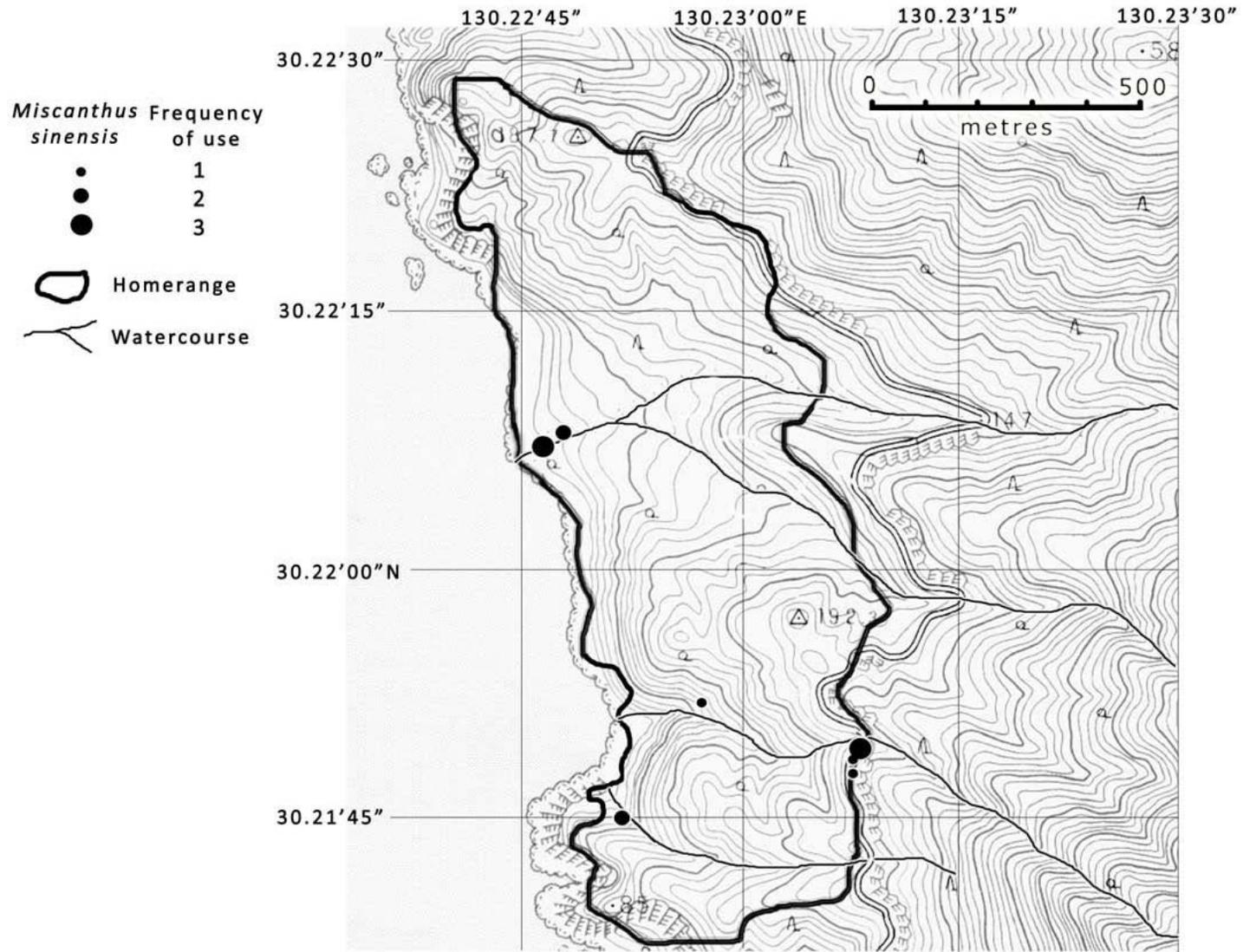
Appendix B.1 – Cumulative NA2 ranging data Sept 06 – Aug 07



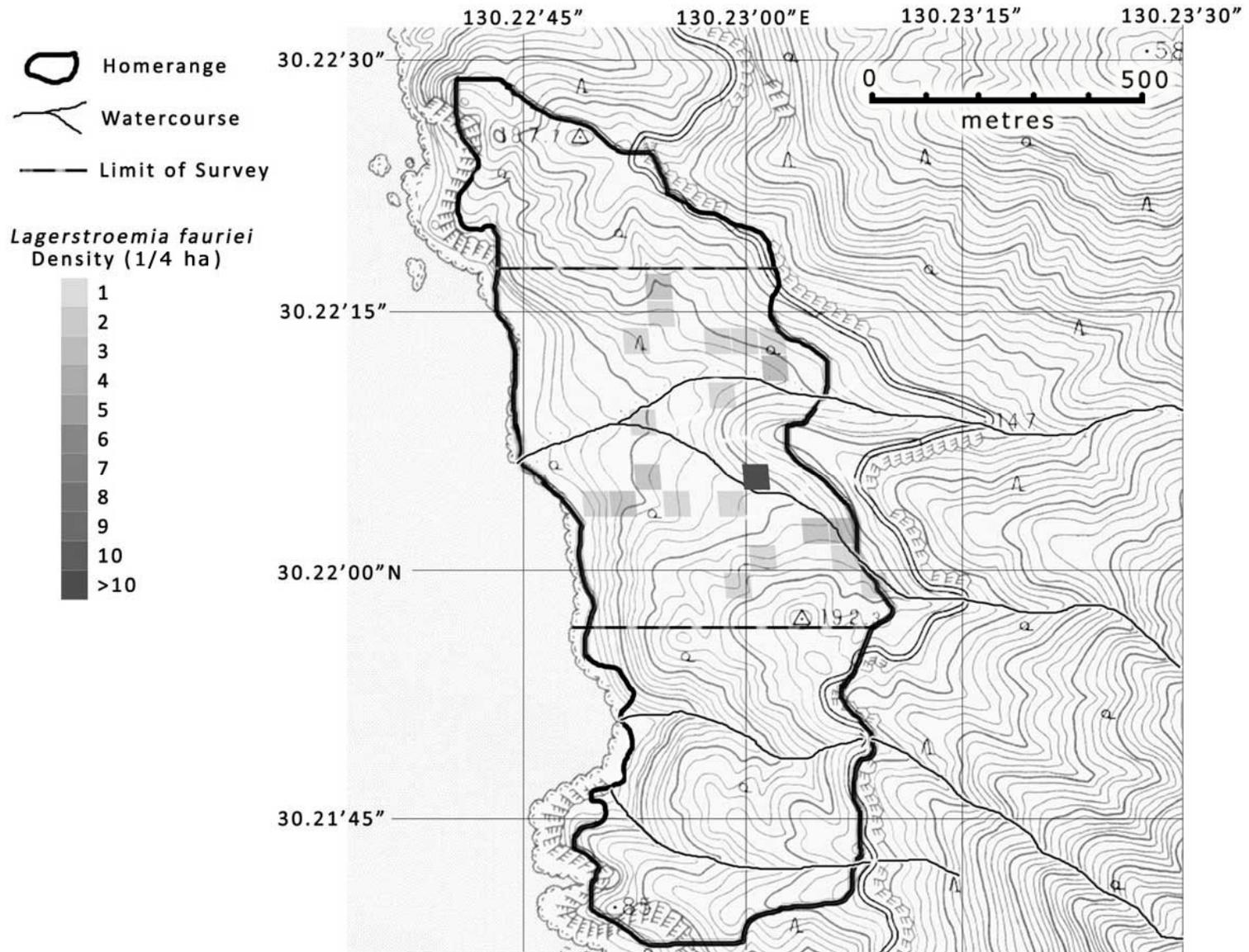
Appendix B.2 – Distribution of Geophagy Mining Sites



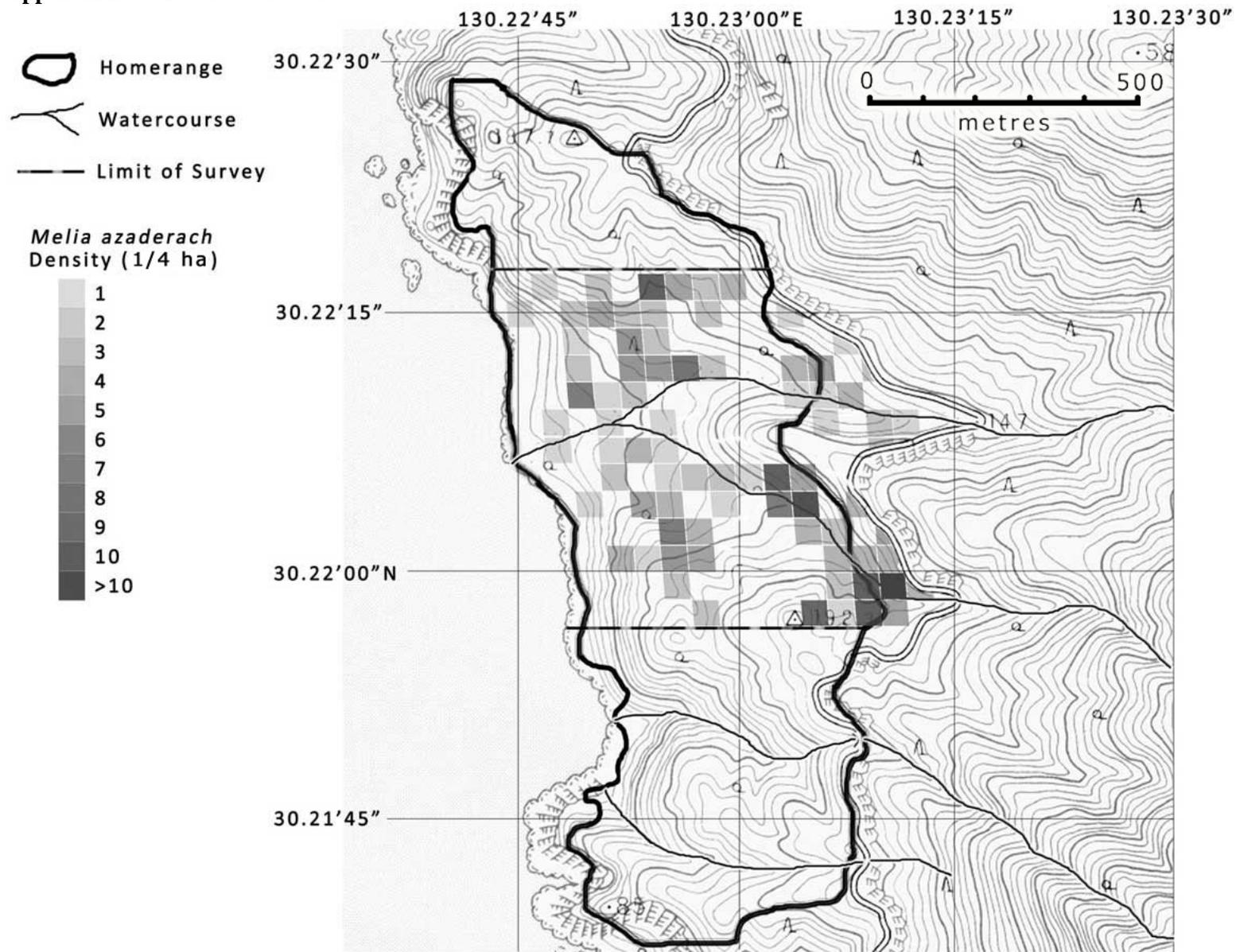
Appendix B.3 – Distribution of *Miscanthus sinensis*



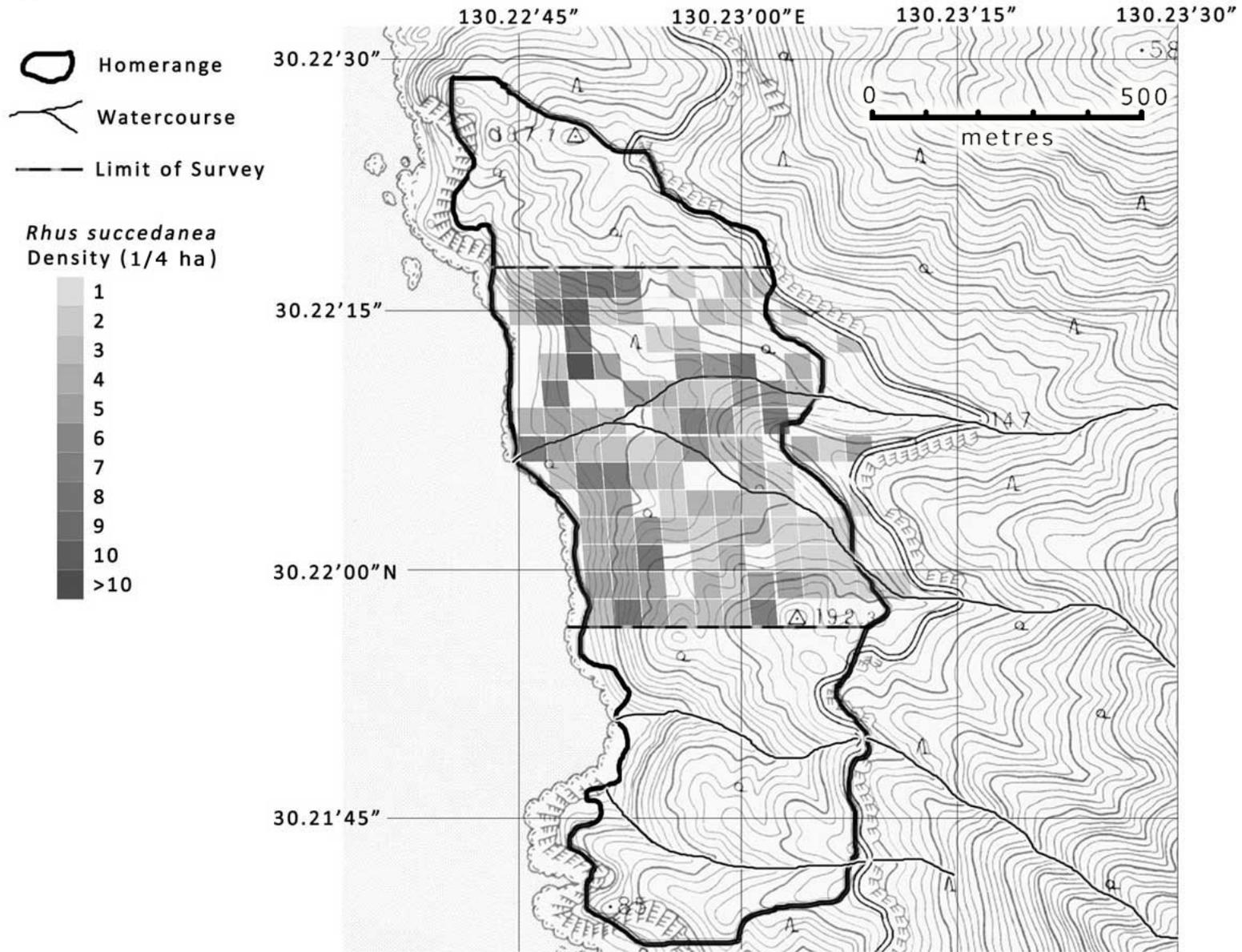
Appendix B.4 – Distribution of *Lagerstroemia fauriei*



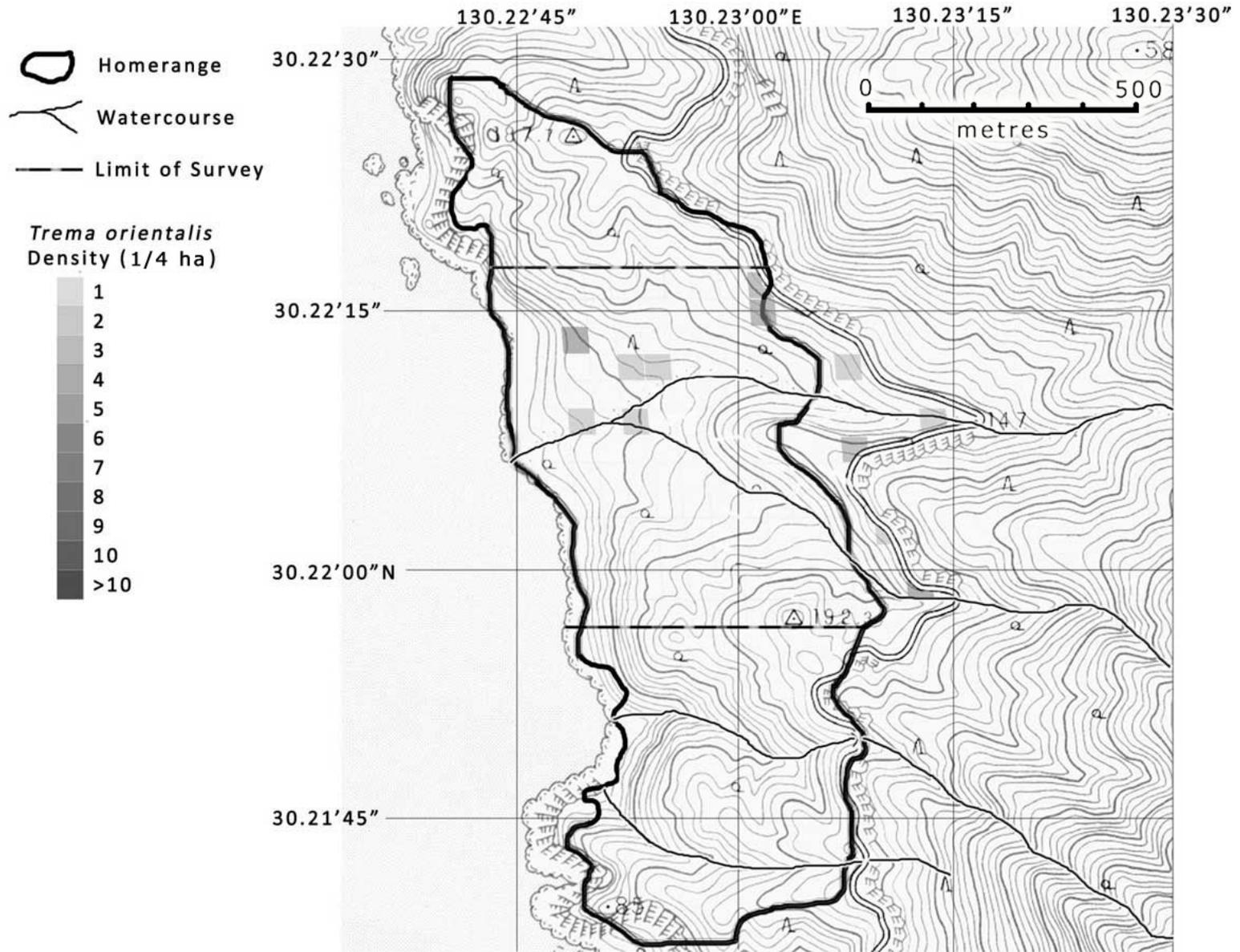
Appendix B.5 – Distribution of *Melia azaderach*



Appendix B.6 – Distribution of *Rhus succedanea*



Appendix B.7 – Distribution of *Trema orientalis*



Appendix B.8 – Distribution of *Zanthoxylum ailanthoides*

