

Biological and ecological foundations of primate behavioral tradition

10.1 Introduction

267

An interest in nonhuman primate behavioral traditions has existed since the beginning of primatology, with some of the earliest details coming from the Japanese macaque (*Macaca fuscata*). When Kyoto University researchers began their investigations in 1948, under the leadership of Denzaburo Miyadi and Kinji Imanishi (Asquith, 1991), animals were considered to act on instinct and such concepts as tradition or culture were considered to be a uniquely human trait (de Waal, 2001; Kroeber and Kluckhohn, 1952). Imanishi (1952) predicted the presence of “culture” in animals even before the results of these observations had begun to be published. He emphasized that, unlike instinct, culture in animals should be viewed as the expression of developmentally labile behaviors. He reasoned that, if one defines culture as behavior transmitted to offspring from parents, differences in the way of life of members of the same species, whether they are human, monkey, or wasp, belonging to different social groups could be attributed to culture. Imanishi’s general argument still holds today, albeit with greater refinements in our overall view of the phenomenon (e.g., Avital and Jablonka, 2000; de Waal, 2001; McGrew, 2001). Currently, healthy debate over whether culture or tradition in humans and animals is really the same is ongoing (e.g., Boesch and Tomasello, 1998; Galef, 1992; Tuttle, 2001; see also Ch. 6).

We use the term behavioral tradition in this chapter to denote those behaviors for which social context contributes to their acquisition by new practitioners and which are maintained within a population through social means (as defined by Fragaszy and Perry in Ch. 1; McGrew, 2001). Operationally, we define a behavioral innovation as any single (or set of)

species-typical voluntary action performed in a novel context that has not previously been observed to be performed by members of that group. Thus, new behavioral traditions arise from behavioral innovations (typically made up of existing behaviors) and can diffuse within a group through any of several possible processes, including local enhancement, social facilitation, observational learning, and imitation (Galef, 1976; Whiten, 2000). Visalberghi and Fragaszy (1990) have pointed out the importance of knowing the history of a behavioral innovation to assess the contribution of any one of these possible processes in its diffusion within a group. In nature, only under the best of long-term observational conditions is it possible to know with any degree of certainty whether or not one is documenting a behavioral innovation. Likewise, the diffusion of a new behavioral innovation into a group can be a long process. Rarely have such events been observed and documented in detail. Controlled experimentation sometimes allows us to avoid such difficulties and gain a better appreciation of the situation in nature (e.g., Hirata and Morimura, 2000; Inoue-Nakamura and Matsuzawa, 1997; Matsuzawa, 1994). Laboratory studies have also provided useful insights into the details of social diffusion, providing various parsimonious interpretations of the learning processes involved (e.g., Lefebvre, 1995; Visalberghi and Fragaszy, 1990; Chs. 6 and 7). Even under the best of experimental conditions, however, it is difficult to reconstruct faithfully the complex ecological and social conditions under which innovation and its subsequent diffusion may occur in natural populations.

Innovation and diffusion of new behaviors within a troop, and the establishment of group-specific behavioral traditions, have been topics of great interest from the beginning of research on Japanese macaques (e.g., Huffman, 1984, 1996; Huffman and Quiatt, 1986; Itani, 1958; Itani and Nishimura, 1973; Kawai, 1965; Kawamura, 1959; Watanabe, 1994). By the early 1950s, at well-known sites such as Koshima, Takasakiyama, Arashiyama, and Minoo, provisioning and individual recognition of all troop members was accomplished, starting off the practice of long-term comparative research of troops across the country (see Huffman, 1991; Takahata *et al.*, 1999; Yamagiwa and Hill, 1998). Provisioning provided the first outdoor laboratory situation for recording the process of behavioral innovation and diffusion of behaviors in a novel environment. Research at these sites has contributed much to our understanding of the patterns of diffusion of innovative behavior in monkeys (see Itani and Nishimura, 1973; Nishida, 1987; Thierry, 1994). A growing number of

long-term studies of chimpanzee populations have also revealed an array of behavioral variation between populations that has been attributed to social learning. Currently, a set of behavioral traditions within a group and differences in such sets of tradition between groups is given by some as evidence for chimpanzee culture (see Whiten *et al.*, 1999).

In chimpanzees, the uniqueness of a particular behavior to a given group is often highlighted (e.g., McGrew and Tutin, 1978; Nakamura *et al.*, 2000; Nishida *et al.*, 1983). As more sites have reached long-term study status, however, a number of these behaviors, shown to be practiced regularly within a group and socially transmitted to each new generation, are frequently found to occur in more than one population. In both Japanese macaques and chimpanzees, there are examples of behavioral traditions that occur in geographically isolated groups of the same species and or among different subspecies. They include foraging skills (ant dipping, leaf sponge, honey dipping), self-medication (leaf swallowing), social conventions, communicative signals (hand clasp grooming, leaf clipping) and a form of object play (stone handling) (see Boesch, 1996; Huffman, 1996, 1997).

While it is accepted that behavioral innovations can be passed to future generations via social learning, the foundations of behavioral innovations themselves, which form the basis of behavioral traditions in any species, including our own, have been little discussed. How can it be that behavioral innovations socially transmitted amongst members of one group can also occur in other groups for which social diffusion of behavior cannot possibly occur? A historical explanation would assume that such behavioral traditions are extremely old, implying that ecological, geographical, or even subspecies barriers now close previously open pathways of intergroup behavioral transmission. While plausible, in some cases this may often be difficult to demonstrate, and it does not explain cases where a particular behavioral innovation is observed to emerge simultaneously in more than one group under geographically isolated conditions. Alternatively, a biological explanation assumes that members of the same or closely related species possess common behavioral propensities, leading to a greater than random probability of a behavioral innovation based on them to arise independently in more than one group. This can happen simultaneously or at greatly different points in time. The biological explanation helps to explain why the same or similar behavioral traditions, which apparently arise from innovation, can occur in more than one group.

This strongly suggests that aspects of species-typical behavior can and do indeed shape the pattern of behavioral innovation among members of a taxonomic group. Here, both historical and biological explanations can be complimentary, and indeed important for a clear understanding of the phenomena. However, we have excluded the overly parsimonious assumption that behavioral innovations depend only upon species-wide, latent tendencies and require only the appropriate stimuli to bring them out independently in all individuals of a group or species.

This chapter addresses the various factors supporting behavioral innovation and discusses the interaction between the biological and environmental variables (both social and ecological) that influence the diffusion of such innovations in free-living populations. We synthesize research on Japanese macaque behavioral traditions to discuss the possible effects of group size and behavior type on the rate of diffusion and the pathways of transmission. New information is also presented from long-term multisite comparative studies of two behavioral traditions: stone handling in Japanese macaques (Huffman, 1984, 1996; Huffman and Quiatt, 1986) and leaf swallowing in the African great apes (Huffman, 1997; Huffman *et al.*, 1996; Huffman and Caton, 2001). This exploration of the biological and ecological foundations of animal traditions is intended to improve our understanding of fundamental aspects of social learning, and the role behavioral traditions may play in the survival of the organism.

10.2 Biological basis of behavioral innovation: behavioral predispositions

Given enough time to familiarize oneself with a particular social group of animals, it is apparent that each individual has its own unique personality. Further time spent comparing two or more groups will invariably lead one to the conclusion that different groups in different regions of the distribution of a species can differ strikingly from one another in some details of their social or feeding habits. These population differences in the overall behavioral repertoire of a species are what we most readily identify as behavioral traditions. At the same time, there are inescapable similarities between groups and the individuals within groups, which make them recognizable as members of the same species. That is, the better you get to know the behavior of individuals in one group, the easier it becomes to predict with a relatively high

level of accuracy just how any individual of that species will respond in a particular social situation elsewhere. An individual's unique qualities, based on personal experience and other attributes, coexists with a relatively high species-level predictability (behavioral predisposition) (see also Mendoza and Mason, 1989). We argue that this constitutes the biological foundations of behavioral traditions in any animal species, including humans.

Keeping these factors in mind, we make six basic assumptions about the role of species-level behavioral predispositions as important biological features of behavioral innovation and discuss their possible role in the emergence of behavioral traditions arising in geographically distinct populations of the same species.

1. The basic motor units of behavior evident in a species are shared by all members of that species. These basic behavioral units are the product of adaptation to social and ecological challenges in its evolutionary past and are shared by all members of a species in the present.
2. In order to survive and reproduce, animals have to be good at reading and appropriately responding to the behavior of conspecifics and to changing environmental conditions. These behavioral units are the basic building blocks of behavioral traditions.
3. The reliability with which a behavior occurs in a species is based on the predictability of the response to stimuli in the social and ecological environment and the reliability with which a particular set of environmental conditions occurs. (See Ch. 2, for a model of the rate of environmental change.)
4. Predictable behavior across individuals of a species reflects reliable production of a finite set of behavioral units. The capacity for innovation is limited to the possible number of permutations of such behaviors an individual can produce.
5. While the possible number of behavioral permutations is influenced by biological constraints (physiological and morphological), an individual may never fully exploit the full potential of its species in any given environment. This is considered to be the source of a behavioral innovation and intergroup variability upon which behavioral traditions are based.
6. A shared repertoire of behavioral units and a shared degree of predictability in the production of particular behaviors in particular situations makes it possible for common behavioral innovations to appear and common behavioral traditions to arise among socially and geographically isolated groups.

The juxtaposed predictability and unpredictability of behavior in a species becomes more apparent the more familiar we become with that species. Behavioral predispositions make members of a species interact with their environments in a relatively predictable way. At the same time, we fully recognize the dual importance of individual differences and the novelty of social and ecological contexts in which behavioral innovations arise. An innovation is likely to arise when an individual(s) or group is faced with new social or ecological challenges for which it currently has no workable solution in its existing behavioral repertoire.

Intuitively, the more generalist a species, the greater array of behavior it is likely to exhibit and, therefore, the more flexible to environmental change it should be. As a general principle then, those species found to exist in a wide range of social, climatic, and ecologically diverse environments should be expected to exhibit the greatest array of behavioral traditions. They may also be better social learners, but this is a different issue. Reader (Ch. 3) reports a significant positive correlation between the incidence of reports of social learning, innovation, and tool use and the absolute "executive" brain volume and the ratio of "executive" brain over brainstem in nonhuman primates. If these measures are a robust indicator of adaptability and intelligence, regardless of relative phylogenetic positions, behavioral traditions will be more frequent among generalist species than among specialists. This should hold true for any animal species and behavior in which social context contributes to behavioral acquisition.

10.3 Innovative behaviors in Japanese macaques

10.3.1 Phases of behavioral diffusion

In macaques, and presumably other social animals, the diffusion of a behavioral tradition can be divided into three distinct phases: transmission, tradition, and transformation (Huffman and Quiatt, 1986). The *transmission phase* is the period of early dissemination of a behavior and is typically similar from group to group and presumably species to species, at least among primates. The first individual(s) to display a behavior may do so repeatedly and perhaps for increasingly longer periods of time. The behavior is first acquired by a network of spatial-interactional associates of the innovator. The membership of this network is directly influenced by the nature of the behavior being performed and its context (e.g., feeding,

resting, traveling, mating, etc.). Laland's (1999) work on the transmission of digging behavior in rats, however, suggests that the innovator of a new behavior is not always easy to detect, and multiple individuals may exhibit the behavior almost simultaneously.

A behavioral tradition need not diffuse to all members of a social group. The more specialized the functions and context of the behavior is, the more limited will be the subgroup of individuals (age, sex, rank, etc.) that will acquire it. Diffusion rate and the distribution of the behavior across age-sex classes should, therefore, vary according to the behavior in question. For example, if the behavior were a form of sexual display, like leaf clipping in Mahale chimpanzee, then we would not expect it to be acquired by sexually immature or postreproductive individuals at any phase of the diffusion process. In such cases, the behavior would never spread to 100% of a group. If, however, the context of leaf clipping were to be altered at some point to a general solicitation of intent or to attract attention of others, for example, we could expect to see an increase in the proportion of the population exhibiting the behavior starting from that point in time.

The *tradition phase* is the period in which a behavior is passed down from mother to offspring or along other multigeneration lines. At this time, the rate of diffusion will depend upon the direction of diffusion and once again upon who the target of the behavior is in the transmission phase.

The *transformation phase* is a period in which prolonged practice and acquired familiarity with a behavioral pattern is gained. Increased behavioral variety brought about by more active manipulation occurs largely among younger age groups, which naturally tend to be more physically active and explorative. This can be a period of behavioral drift or easily changing fads. An example of this is the divergence in behavioral patterns that developed for wheat washing and potato washing in the 20 or more years following its initial spread at Koshima (Watanabe, 1994). Details on the direction of diffusion at this stage, however, are not clear. In this case too, diffusion is expected to be influenced by the innovator's network of spatial-interactional associates.

Among the reported cases of the diffusion of behavioral innovations in Japanese macaques, most are in one way or another related to food or foraging activity, including the acquisition of new foods and food-processing techniques. In general, information regarding food should be of importance to all members of a group and, therefore, foraging innovations are

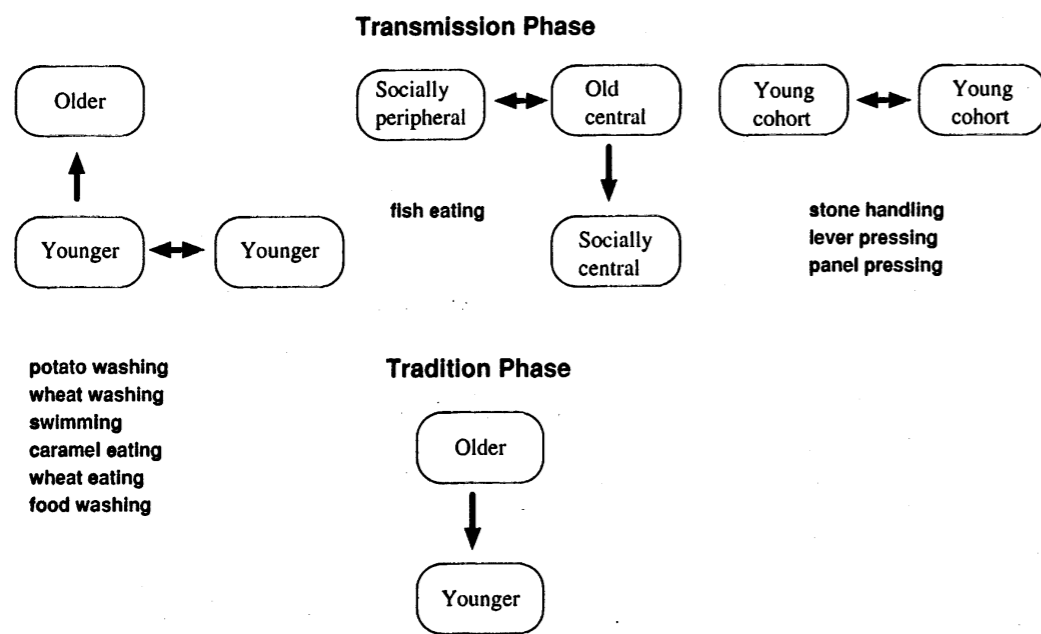


Fig. 10.1. Pathways of diffusion by behavioral type exhibited in Japanese macaques.

expected to diffuse widely. However, socio-ecological factors affecting access to novel foods or more preferred food can enhance or impede the rate and routes of diffusion (e.g., Giraldeau, 1997; Giraldeau, Caraco, and Valone, 1992; Tokida *et al.*, 1994; Watanabe, 1989).

10.3.2 Pathways of diffusion

Three basic pathways of behavioral diffusion in the transmission phase have been recorded: younger to older, peripheral to central group members, and between young of the same cohort. These pathways are shown in Fig. 10.1 along with the characteristic older to younger pathway inherent in the tradition phase. Some examples of the different behaviors associated with each pathway are also given. Other pathways are likely to exist, but to our knowledge they have yet to be reported. Pathways are dependent upon the nature of the behavior and the relationships of those individuals most likely to be in proximity with each other when the behavior is being practiced. In many, but not all, cases, this is characteristic of the transmission phase. As a general rule, behavioral type and the context of the behavioral innovation in question will strongly determine this pathway and thereby to some extent also the rate of diffusion.

Of the behaviors noted in Fig. 10.1, food-related behavioral traditions were most consistently associated with the initial lateral transmission among young, followed closely by the upwardly vertical transmission

to older kin members and then across kin boundaries to other adults.

The pathway of transmission of fish eating at Koshima is different in that it first appeared in an adult male living in the troop's social periphery. An old female of the troop eventually acquired the habit next. She was reported to be the link by which the behavior then diffused to some members within the troop's social central area (Watanabe, 1989). This food habit was acquired in response to a drastic reduction in provisioned foods. Those individuals most directly affected by the food shortage, who had less access to provisioned foods, and who had social contact with the peripheral adult male were reported to be the first to acquire the behavior. Fish eating was adopted by about a third of the population and is an interesting case of a putative behavioral tradition where diffusion within the group is limited by need (Watanabe, 1989).

Stone handling was first observed being performed by a young juvenile female, Glance 6774, in December of 1979. By June 1985, stone handling was found to have diffused throughout 60% ($n = 142$) of the 236 member B troop (Huffman, 1984). Of these 142 individuals, 80% were born between 1980 and 1983; that is after stone handling was first observed in the troop. Only limited diffusion to individuals older than the first female seen stone handling occurred, suggesting the recent emergence of this behavior within the troop: at least since provisioning was first begun at this site in 1954. It also shows that the behavior did not spread to adults within the troop. Those three individuals older than Glance-6774 that acquired stone handling were two of her female cousins, Glance-6775 and Glance-6774 (sisters), and one lower-ranking non-kin-related female, Blanche-596475.

By 1985, these four females and two others (Oppress-7078, Momo-5978), all then 10 years or older, had one or more offspring of their own. All 13 of these females' offspring also acquired stone-handling behavior. In 1986, B troop divided, becoming E and F troops (Huffman, 1991). By August 1991, 12 years after the first appearance of stone handling, every individual under the age of 10 years in E troop was verified to have acquired the behavior (F troop gradually stayed away from the provisioning site and observations on them were stopped). Stone handling had spread to the young of every kin group in the troop. Unlike potato washing or wheat washing, however, no individual 5 years of age or older in 1979 (when the behavior first appeared at Arashiyama) ever acquired stone handling later on.

Long-term observations made on stone handling have revealed that the social network of diffusion has varied over time as a function of the age of individuals exhibiting the behavior and the social context of the behavior itself. In the initial transmission phase of stone handling, this network included a very small group of cousins, sisters and non-kin playmates. Very shortly thereafter, however, the behavior began to spread more widely between play groups composed of kin and non-kin as it diffused downward to younger individuals from mother to offspring, older to younger siblings, etc. in the tradition phase (Fig. 10.1).

Consequently, in the first few years, infants of stone-handling mothers were exposed to the behavior earlier than other infants. However, according to the 1985 census, even those infants whose mothers had not acquired stone-handling behavior began to pick up or scatter stones on the ground as early as 10 weeks. In all these infants, the behavior was exhibited by older siblings indicating that stone handling can also be acquired via older siblings. However, since then and up to the time of writing this paper, all infants acquire the behavior within the first 6 months of life. Multiple modes are suspected to have played a role at different stages of the behavior's history, with some form of social facilitation no doubt playing a central role.

From this, we would predict that no one particular age, sex, or rank class had a monopoly on innovation skill. Rather, the type of innovation is likely to be influenced by the unique position of each individual within its social and ecological environment.

10.3.3 Behavior type, group size, and rates of diffusion

Earlier evolutionary and population-level models of cultural transmission assumed rapid and, as discussed by Laland and Kendal (Ch. 2), temporally accelerating rates of behavioral diffusion within a group, producing a sigmoid curve (Boyd and Richardson, 1985; Cavalli-Sforza and Feldman, 1981; Pulliam, 1983). Most of these models assume that, as each new individual acquires the behavior, the rate of diffusion will increase as a function of an increase in the number of demonstrators who can influence the remaining naïve individuals. Laland and Kendal (Ch. 2) disagree and suggest that the shape of the curve of diffusion is not always consistent with the pattern of learning (social versus asocial). They conclude that the shape of the diffusion curve may not allow us to identify the learning process. Lefebvre

(1995) found supporting evidence for an increase in the rate of diffusion with the increase in number of demonstrators in an analysis of the rates of acquisition of innovative behaviors reported in the primate literature, including potato washing, wheat washing and fish eating in the Japanese macaque. He found accelerating rates of diffusion as the number of practitioners of a behavior increased in some but not all behaviors. Conversely, Lefebvre and Giraldeau (1994) also found that large group size could have a negative effect; many naïve bystanders could slow down diffusion. However, we must not assume that a behavioral innovation will be of relevance to every individual in the group. In social learning models, we cannot assume *a priori* that all behaviors will reach 100% diffusion within a group. As seen from fish eating, not even food-related innovations are totally free from such considerations.

Behavior type and group size are not typically included in models of cultural transmission. The question we ask here is, "What effect do these variables have on the rate of diffusion?" From our discussions above, we know that the pathway of diffusion is affected by behavior type and that the function of the behavior determines the type of individual and, therefore, the total number of individuals within a group that will acquire it. Based on this evidence, we predict that group size alone does not have an over-riding effect on the rate of diffusion. To test this, we calculated the theoretical rate of increase in the number of individuals performing 12 novel behaviors reported in Japanese macaques to estimate the time it would have taken each behavior to spread to 50% of the group. Here we assume a constant rate of increase. The number of days necessary to diffuse to 50% of the population in these 12 behaviors was not found to be significantly related to group size alone (Spearman rank correlation coefficient (r_s) = 0.38; n = 12; not significant). This pattern remained constant even when we excluded experimentally induced behaviors (caramel eating and lever and panel pressing; r_s = 0.49; n = 7; not significant). No consistent pattern was found with regards to troop size (Fig. 10.2). However, we did find a significant difference in the number of days to diffuse to 50% of the population when these 12 new behaviors were grouped into four behavioral types (Kruskal-Wallis ANOVA: $H_{(3,12)} = 8.1$; $p < 0.05$) (Fig. 10.3). Food processing and play were much slower to diffuse (over 1400 days) than accepting a new food and experimental tasks (less than 200 days). As predicted, behavioral type does have an important effect on the rate of diffusion and the effect of group size is inconsistent, even when using a linear model (see also Ch. 2).

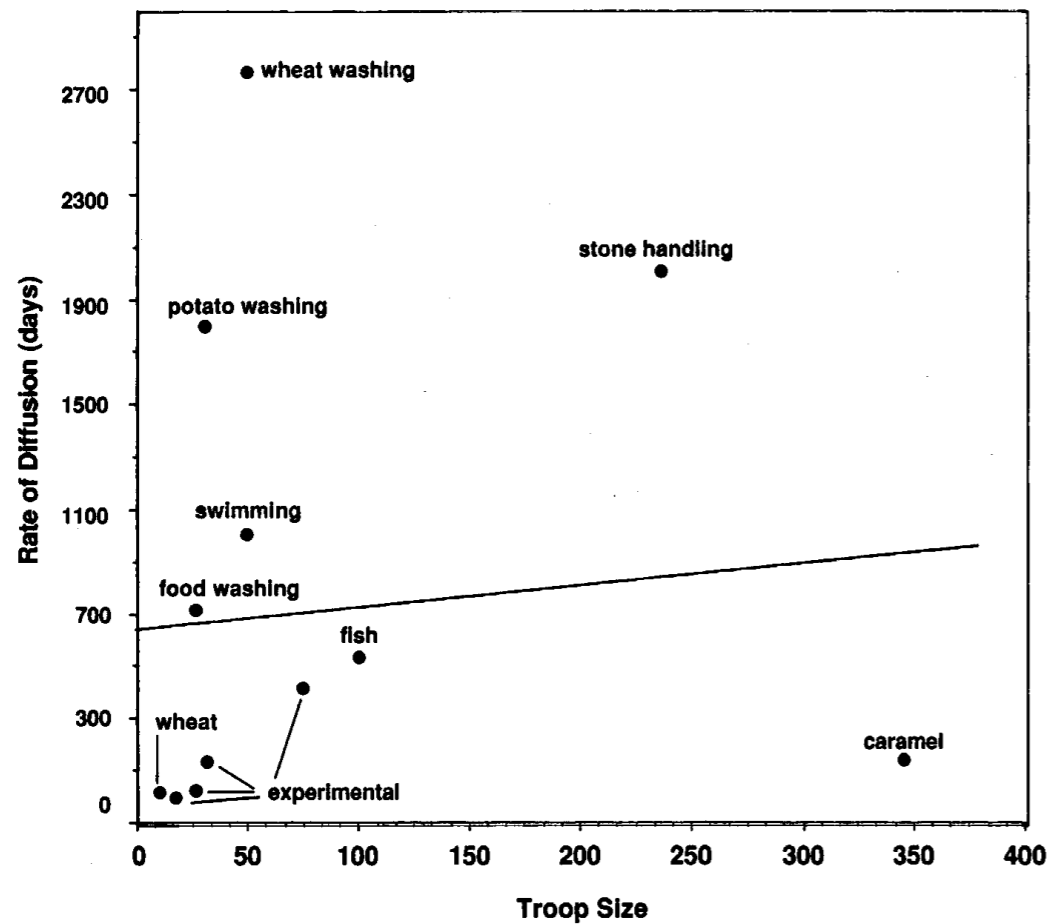


Fig. 10.2. Correlation between troop size and estimated rate of diffusion among 50% of the population for 12 behavioral traditions observed in Japanese macaques.

This difference in rates of diffusion can be explained by the fact that accepting a new food item or manipulating an experimental device for immediate food reward does not compete with an existing way of handling a problem (e.g., already available food). In the case of play, the new behavior is likely to be acquired only by a specific subset of the population, constraining the rate and defining the level of diffusion into the group.

Previously, a slow rate of diffusion has been considered an argument in favor of the more parsimonious mechanism of individual learning supporting the acquisition of some behaviors in Japanese macaques (Galef, 1991, 1992). However, in light of our empirical analysis discussed above and supported by the theoretical discussion of Laland and Kendal (Ch. 2), we conclude that variations in the rate of diffusion do not necessarily reflect more or less reliance on social context in learning.

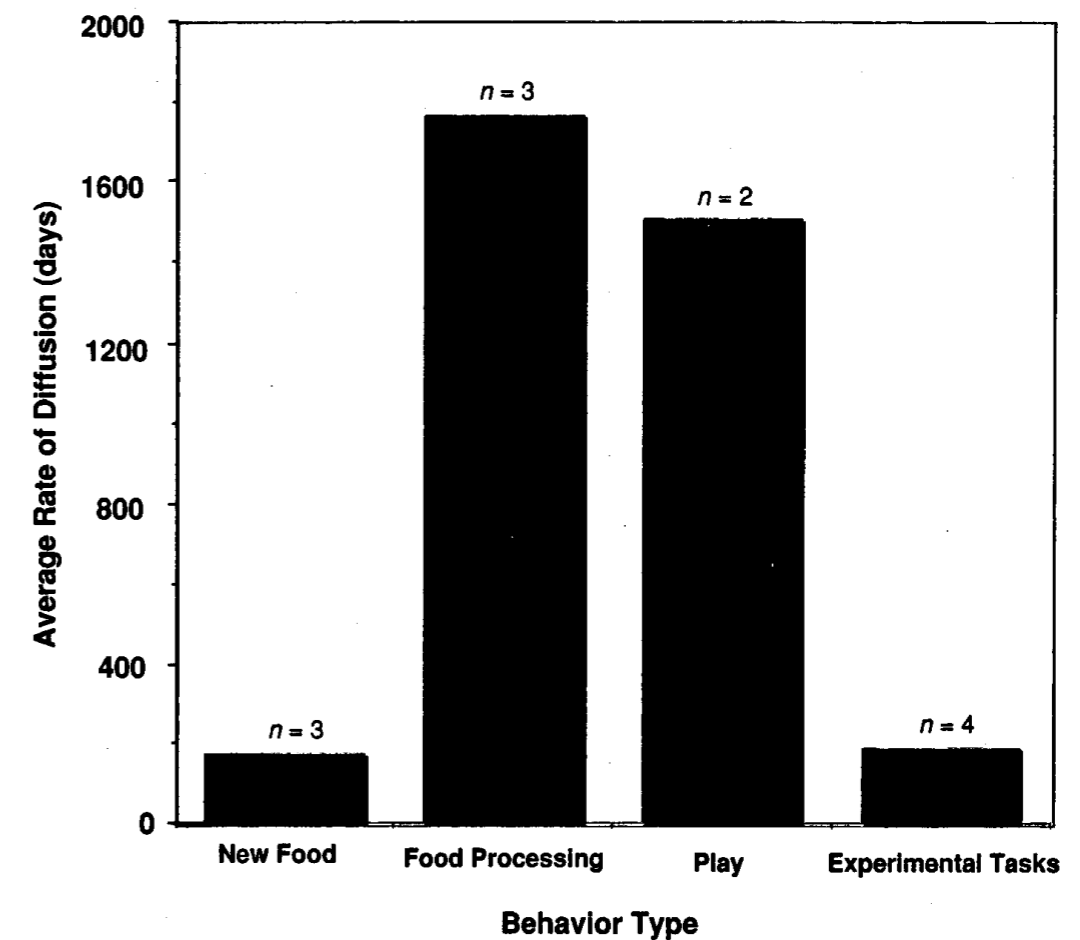


Fig. 10.3. Average rate of diffusion to 50% of the population by behavior type. New Food, acquisition and consumption of food; food processing, new way to process food; play, play-related behavior, such as stone handling and swimming; experimental tasks, new behavior induced by the introduction of a novel experimental setting.

10.4 Factors influencing the innovation, diffusion, and maintenance of primate behavioral traditions

10.4.1 Appearance and disappearance of behavioral traditions

Behavioral traditions can appear, disappear, and even reappear sometimes in slight variation within the same group over time (see Ch. 14). Very few studies have been able to document such change given the long time investment required. Some behaviors, while practiced by a few individuals in any population, may not endure very long. For example, stone handling was observed at Arashiyama and Takasakyama for the first time in late 1979, after over 30 years of close observation at both sites by a number of researchers and park employees (Huffman, 1984, 1996). Interestingly, before provisioning was started at Arashiyama in 1954, young macaques were sometimes seen to play with inedible hard-covered citrus fruits in

a fashion resembling some of the current stone-handling patterns (see Huffman, 1984). The initial circumstances bringing about the practice of stone handling and other new behaviors at these sites are unknown. Site differences in the frequency of provisioning and the size of the feeding area may have some influence on the relative daily frequency of occurrence as well as the overall opportunities for others to observe and take up the practice of the behavior habitually. Although not completely understood, some of the possible factors are discussed below.

10.4.2 Provisioning

Early research on Japanese macaques used provisioning as a way of speeding up the process of habituation and to lure them out into the open for better observation. This brought about a change in the life habits of the monkeys, providing them with access to new foods and environments previously not encountered. These types of change preceded such innovations as potato washing, wheat washing, and altered swimming behavior at Koshima (Kawai, 1965).

With provisioning comes a tendency for a more sedentary lifestyle. More time is spent around the feeding area and that too can have profound effects on behavioral and dietary innovation (see Fa and Lindburg, 1996). At Arashiyama, Huffman (1984) found, on the one hand, that the natural diet of the troop decreased from its early provisioning period (1954–1958) level of approximately 192 species of plants (Murata and Hazama, 1968) to as few as 67 species between 1979 and 1980. On the other hand, while the diversity of their natural diet decreased and dependence on provisioned foods increased, at least 17 new natural and introduced plant foods were acquired in the process of adjusting to life in the newly exploited 1 km radius of the feeding site.

Provisioning can also bring about other changes in behavior. With less time spent actively searching for food, more time is left for other activities such as play and socializing. Provisioning improves reproductive potential in females and causes a shortening of the interbirth interval. This, in turn, can have an effect on a number of behaviors, ranging from infant care practices to modification of matrilineal dominance-rank systems (Hill, 1999; Itani, 1959; Kutsukake, 2000). Little attention has yet been paid to the possible relationship of behavioral innovations and changes in population structure. This should be a fruitful area of future investigation.

In a restricted sense, provisioning can be considered to be synonymous with dramatic changes in a more natural habitat. That is to say, changes

in the distribution, defensibility, and abundance of food or other sought-after resources in nature are also expected to trigger changes in social organization, group behaviors, and diet.

10.4.3 Competition

As shown in this chapter, the rate of diffusion of innovative behaviors and their longevity is a complex issue. The relative abundance of resources associated with the innovation also affects which individuals will acquire a new behavior that another practices. The type of behavior under consideration is very important. This will directly influence who is most likely to acquire the behavior, and in the end how widely a behavior will spread among members of a group. If, for example, a behavior allows an individual to obtain a resource previously denied because of sex, age, or rank, the behavior is not likely to spread widely, passing only very slowly to others in the same social situation who are tolerated by the innovator. An example of this is tool manufacture by a chimpanzee to rouse a squirrel out of its hiding place in the hole of a tree (Huffman and Kalunde, 1993). Meat is a highly prized food resource by chimpanzees, with access controlled by a few adult males of the group based upon social and sexual status of the potential recipient (Nishida *et al.*, 1992; Stanford, 1999). The manufacture and use of tools to drive a squirrel out of hiding is an extremely rare behavior at Mahale. The orphan adolescent female observed performing this behavior would normally have no chance to obtain meat from others or to hunt larger prey on her own. Hunting in the presence of others increases the likelihood of the catch being taken away from a subordinate, and, therefore, such activities tend to be done in secret (Huffman and Kalunde, 1993). Here, the lack of social tolerance (see Ch. 11) indirectly encourages efforts to obtain a meat source not highly open to competition. At the same time, this suite of characteristics of the individual and the behavior inhibits the diffusion of the behavior to more powerful individuals in the group. Consequently, although the behavior is potentially important to all, it is not likely to diffuse widely or be observed frequently, because of both limited opportunities for observation by others and the intolerance of subordinate individuals to competitors for a limited resource. This is in contrast to ant fishing, where resources are more widely distributed and abundant, resulting in less competition. By comparison, behaviors with a clear benefit to all members are more likely to spread throughout an entire group and be maintained indefinitely if the resources required for its performance are widely available (e.g., potato washing).

10.4.4 Maintenance of “neutral” behaviors

In some cases, traditions emerge that seem to have no (or even mildly negative) immediate adaptive consequences (e.g., capuchin hand sniffing, Ch. 14). Presumably these behaviors are maintained because of some internal consequences that we cannot as yet measure. Stone handling is a case in point (Huffman, 1996). Unlike the leaf-swallowing behavior exhibited by chimpanzees in the wild, the significance of stone-handling behavior to a Japanese macaque is difficult to interpret. Individually, motivation to perform the behavior simply may be the social value placed on these items by others in the group (Huffman, 1984). The immediate motivation to act as others do and the long-term motivation to continue performing a behavior may be different at both the individual and group levels. This is an especially important area for theorists to consider, because assumptions about motivation and performance should be approached from both short- and long-term perspectives. For example, during the tradition phase (Fig. 10.1) of behavioral transmission, when behaviors are acquired by the very young from their mothers or older siblings, the motivation to perform a behavior is likely to be quite different from that which induced the innovator(s), and perhaps subsequent early initiators in the propagation phase, to acquire the behavior. Furthermore, once a behavioral habit is acquired, individuals may continue to perform it even after the original conditions for promoting its adoption are no longer present if there is no cost to performing it. They are continued merely out of habit.

10.5 A behavioral tradition in multiple troops of the same species: stone handling among Japanese macaques

10.5.1 Behavioral description

Classified as a form of object manipulation or play (Candland, French, and Johnson, 1978), stone handling has so far never been observed in a nonprovisioned troop (Huffman, 1984, 1996). However, there are also several provisioned troops where stone handling has never been recorded (Fig. 10.4). In provisioned and nonprovisioned troops where stone handling has never been seen, prolonged physical contact with stones is absent. At sites where it does occur, stone handling is habitual and occurs most predictably just after feeding time. In this situation, individuals have gathered all the food (often grains of wheat or soy beans) they can

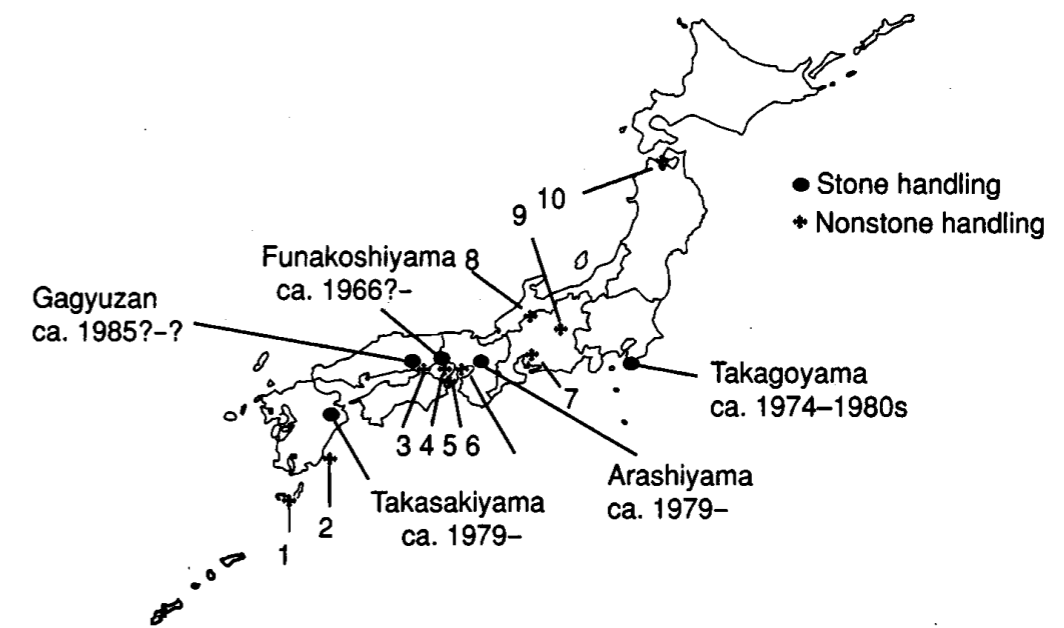


Fig. 10.4. Distribution of study sites where stone handling has been observed in free-ranging, provisioned populations of Japanese macaques. Sites denoted by numbers are provisioned sites where stone handling has not been observed; 1, Yakushima; 2, Koshima; 3, Katsuyama; 4, Shodoshima; 5, Awajishima; 6, Minoo; 7, Hanyama; 8, Hakusan; 9, Jigokudani; 10, Shimokita.

at one time and are slowly pushing the items from the cheek pouch back into the mouth to be chewed before swallowing. Stone handling can be interspersed with bouts of feeding but begins to taper off in most individuals when the food has been completely consumed. The general mood of individuals is relaxed with an intense concentration directed toward the activity, sometimes ignoring or refusing the solicitations for play or mating. There is nothing to stop them from feeding on provisioned food or from moving into the forest to feed on natural vegetation, yet they choose to manipulate stones first.

Some individuals will continue to carry stones around for several minutes after feeding time has finished, depositing the stones in piles at the feeding site, at the base of trees on slopes in the forest, or even sometimes in the fork of a tree. Based on the analyses of video surveys conducted in the winter of 1989 at Takasakiyama and the summer of 1991 at Arashiyama, a highly significant negative correlation was found between age and total handling time per stone-handling session at both sites (Arashiyama: $n = 167$; $r_s = -0.435$; $p = 0.0001$; Takasakiyama: $n = 53$, $r_s = -0.488$; $p = 0.0004$; Huffman, 1996). For both males and females, the decline in stone-handling activity with age appears to be closely correlated with social and biological life-history variables (Huffman, 1996).

Stone handling replaces other activities that normally follow or are interspersed with foraging. In a nonprovisioned troop, and indeed among individuals that do not handle stones in provisioned troops, this is a time for social grooming and play. Only the most dominant individuals are likely to continue feeding until the last bit of provisioned food has disappeared. Mothers can be seen grooming the backs of their offspring who are handling stones, while they themselves are processing the food they have stored in their own cheek pouches.

Macaques in general may have an intrinsic propensity towards manipulatory activity concurrent with foraging. If so, stone-handling activity fits nicely into that time slot. In nonprovisioned groups, foraging activity is likely to take longer because food resources are less abundant in any one location and are more spread out, making it necessary to feed for a longer time to obtain the same amount of food. Interspersed with traveling between food patches, this would leave little time for such leisure activities as stone handling.

10.5.2 Intergroup and interspecies behavioral comparison of stone handling

Stone handling has been observed to occur independently in at least five free-living provisioned populations across Japan (Fig. 10.4) and in two captive groups kept at the Kyoto University Primate Research Institute (Huffman, 1984, 1996; Huffman and Quiatt, 1986).

The spatial and temporal distribution of known stone-handling sites demonstrates the behavioral tradition's independent origin in each group. There is no geographical or temporal pattern of emergence to suggest that the behavior spread between provisioned troops within a region (Fig. 10.4). The behavior does not reliably occur in neighboring populations within the same regions where it does occur. An interesting example from this perspective is that stone handling appeared in both the Arashiyama and Takasakiyama populations at around the same time in 1979, while it is thought to have started much earlier at the intermediate location of Funakoshiyama, around 1966. Separated by an ocean barrier and several hundred kilometers of land, it is implausible that the behavior was transmitted between these different populations on Kyushu and Honshu islands.

Only sketchy details are known about most of the other sites where stone handling has been observed. Hiraiwa (1975) made the first brief report of stone handling in Japanese macaques from her observations of

the Takagoyama troop. The frequency of occurrence was low and only subadults, younger than 3 years of age, exhibited the behavior. Later on in 1984 when provisioning was stopped, the practice of stone handling gradually ceased (T. Fujita, personal communication). On the island of Koshima in the early 1980s, a 15–16-year-old male named Ira was frequently observed carrying and clacking stones together along the rocky shoreline, but the behavior never spread to other group members (K. Watanabe, M. Kawai, and S. Mito, personal communications). Written records do not exist for the first occurrence of stone handling at the Funakoshiyama Monkey Park, but the caretaker in charge of provisioning this free-ranging troop remembers seeing the behavior as early as 1966 (I. Narahara, personal communication). Stone handling has not spread widely within this troop with approximately 300 members. Details of stone handling at Gagyuzan are even scarcer. The behavior apparently underwent a couple of periods in which its visibility rose and subsequently dropped again in frequency (F. Fukuda, personal communication).

At Arashiyama, where the most detailed studies of stone handling have been conducted, 17 basic behavioral types have been classified (see Table 10.1 for these and the abbreviations). The first five behaviors (GA, PU, SC, RIH, and RT) are commonly exhibited by macaques in general when manipulating objects in their environment such as twigs and acorns or novel human-introduced objects with which they come into contact. The last three behaviors (TW, MP, and GW), connected with moving, are considered to reflect a growing familiarity with stones and are a product of human habitats where hard-packed ground, roofing, or concrete is available.

With the exception of one behavior (PUD), all of the Arashiyama behavioral types have been observed at Takasakiyama (Huffman, 1996), and at relatively similar frequencies (Fig. 10.5). The most common behavioral patterns observed at both Arashiyama and Takasakiyama were SC, RWH, GW, and GH. At Takagoyama, the relative frequency of occurrence was low and the behavioral patterns limited to GA, CD, PU, RT, and RIH (Hiraiwa, 1975), five of the same eight basic behaviors first observed at Arashiyama (Huffman, 1984). The general visibility of stone handling at Funakoshiyama, as observed in the mid-1990s, was much lower than at either Arashiyama or Takasakiyama, despite the troop's large size. The behavioral patterns observed (GA, RWH, RT, RIH, GH, etc.) were identical to those recorded in the other groups (K. Kaneko,

Table 10.1. The 17 basic behavioral patterns of stone handling observed at Arashiyama

Behavioral pattern	Characteristics
Gathering (GA)	Gathering stones into a pile in front of oneself
Pick up (PU)	Picking up and placing stones into one hand
Scatter about (SC)	Scattering stones about on the ground in front of oneself
Roll in hands (RIH)	Rolling stones in the hands
Rubbing stones together (RT)	Rubbing stones together
Clacking (CL)	Clacking two stones together
Carrying (CA)	Carrying stones from one place to another
Cuddling (CD)	Holding or cradling stones
Pick up and drop (PUD)	Pick up repeated over and over
Rub on surface (ROS)	Rubbing stones on tin roofing, cement surfaces, etc.
Flinting (FL)	Striking one stone against another held stationary
Pick up small stones (PUs)	Resembling the picking up of wheat grains or soy beans
Rub with hands (RWH)	Similar to potato-washing behavior
Grasp with hands (GH)	Clutching a pile of stones gathered and placed in front of oneself
Toss walk (TW)	Repeated tossing ahead and picking up of a stone(s) while walking
Move and push (MP)	Pushing a stone with both hands while walking forward
Grasp walk (GW)	Walking with one or more stones in the palm of one or both hands

unpublished report; J. Itani, M. A. Huffman, unpublished observations). These comparisons demonstrate that the behaviors that make up stone handling are based on the wide behavioral repertoire of the species.

The behaviors exhibited in stone handling appear to be a predisposition shared by macaques in general. Two sites where stone handling is seen in semiprovisioned troops of long-tailed macaques (*Macaca fascicularis*) in Indonesia and Thailand have recently been brought to the authors' attention. Stone handling occurs in a free-ranging, potato- and fruit-provisioned troop of long-tailed macaques inhabiting the sacred monkey forest of Padangtegal, Ubud Bali. Here, CL, SC, PUD, RWH, ROS, and RIH are the behaviors observed most often (A. Fuentes, personal communication). These macaques have also been observed to exhibit food-washing behavior similar to that on Koshima (Wheatly, 1988) and other forms of object-rubbing behavior (Fuentes, 1992). Another troop living along the coast in Prachuap Province, Thailand are opportunistically

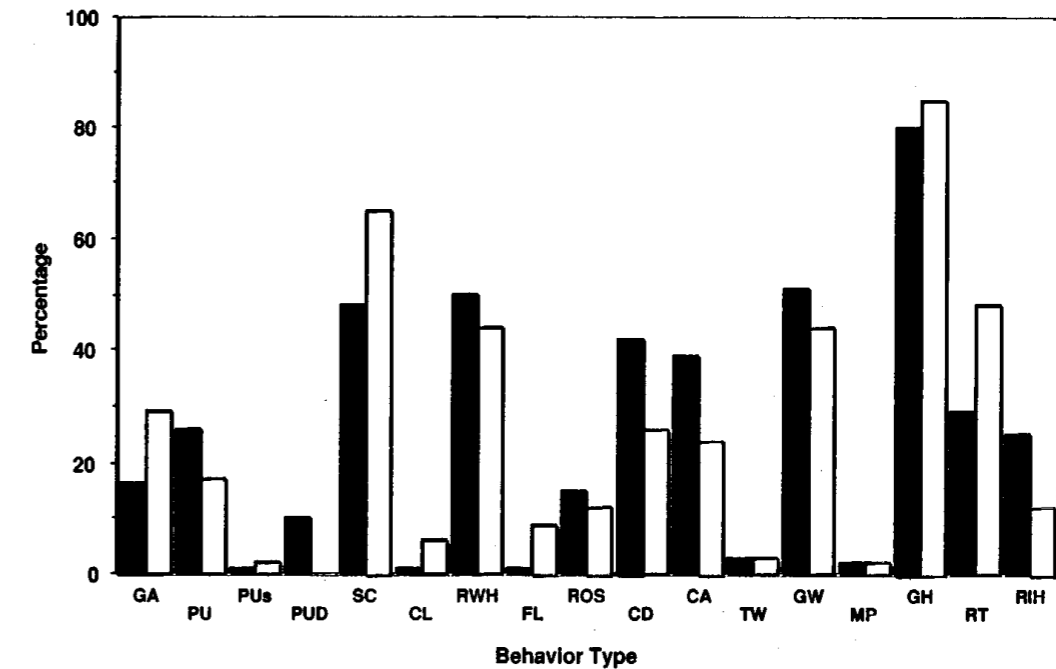


Fig. 10.5. Comparison of the frequency of behavioral patterns of stone-handling displayed by Arashiyama (■) and Takasakiyama (□) Japanese macaques. See Table 10.1 for the behavior types for these abbreviations.

fed bananas and peanuts, which are sold to tourists. At this site, the stone-handling patterns exhibited are ROS, CA, POD, TW, and MP (K. Bauers, personal communication).

In the late 1980s, at the Primate Research Institute, Inuyama Japan, the habit of clacking hard food pellets together was seen to spread from one to other individually caged rhesus macaques (A. Mikami, personal communications). Stone handling or its proximate behavior with other objects appears to be a genus level behavioral propensity associated with provisioning and a sedentary lifestyle.

10.5.3 Factors influencing the rate of diffusion of stone handling

The rate of diffusion of stone handling in Arashiyama was estimated at two time points from surveys conducted in 1983 (B troop) and 1991 (E troop) (Fig. 10.6). The natural logarithm of the yearly total number of individuals for which stone handling was observed was plotted against time to compare the slopes of the linear regression equations (Sokal and Rohlf, 1994). The rate of diffusion was significantly higher in B troop ($y = -0.9 + 0.6x$) than in E troop ($y = 2.8 + 0.2x$) ($p < 0.001$).

In 1986, B troop divided, producing E and F troops (Huffman, 1991). Regardless of the smaller size of E troop, the age-sex class structure of stone

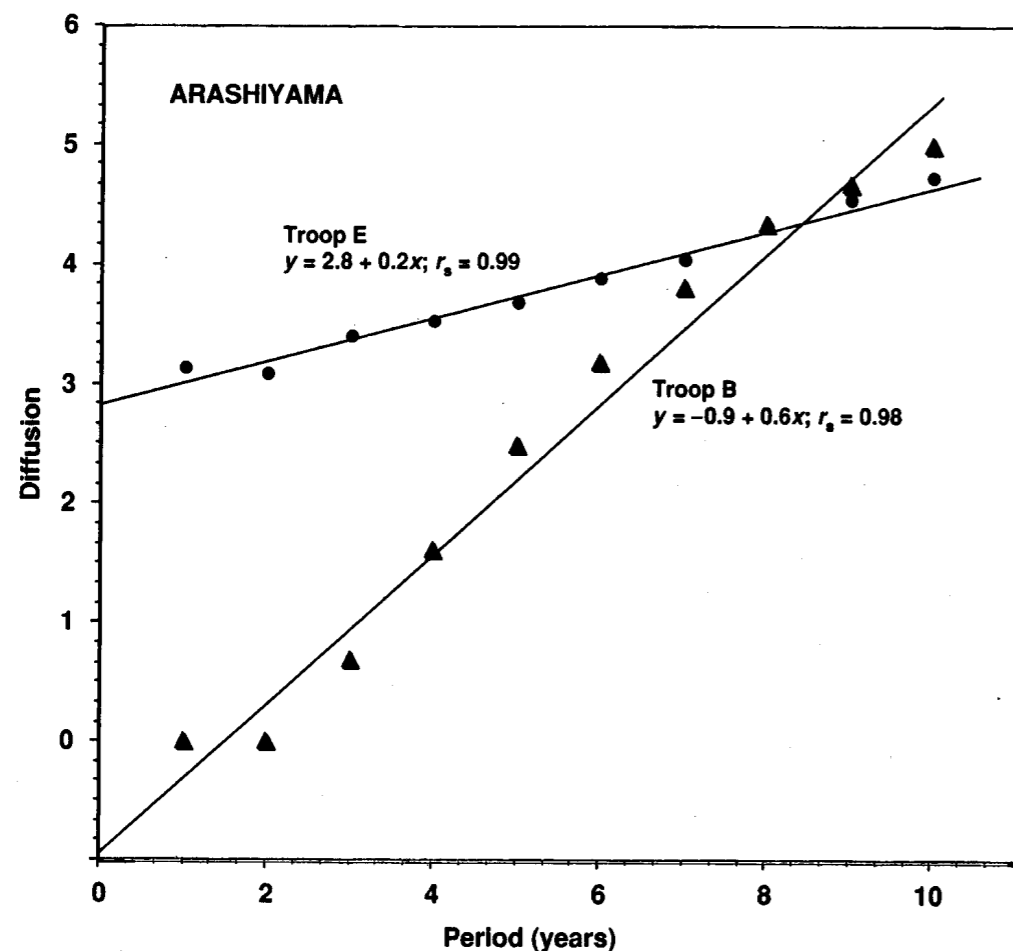


Fig. 10.6. Diffusion of stone handling in two Arashiyama troops of different sizes (troop E with 139 and troop B with 236 members). Each time period on the x axis represents one year. The number of individuals performing stone handling for each period was estimated on the basis of two surveys conducted in 1983 (B troop) and 1991 (E troop). The natural logarithm of the yearly number of individuals performing stone handling (diffusion) was calculated; the significance of the difference in slope between the two linear regression equations was $p < 0.001$.

handlers remained basically the same. Rather than the rate of diffusion being a function of group size, these estimated differences in the rate of diffusion are the result of the current phase of transmission. At this phase, acquisition of stone handling was only occurring among infants, because all individuals acquired the behavior within their first 6 months of life. Therefore, the increase in new stone handlers after this point in time is purely a function of new births.

If the period of innovation of stone-handling behavior had not been observed in the detail achieved at Arashiyama, an investigator seeing the behavior for the first time today would find it difficult to conclude that stone handling is a behavioral innovation. Although phylogenetic and group-history factors are difficult to establish in field research, it is important to

keep in mind the importance of both these factors when interpreting the origin of a behavior.

10.6 Behavioral tradition in multiple groups and among subspecies: leaf swallowing, a self-medicative behavior in African great apes

10.6.1 Behavioral description and its context of performance in the wild

Attention was first brought to leaf-swallowing behavior by Wrangham and Nishida (1983). They pointed out that leaf swallowing was unlikely to provide any nutritional value as they noticed a pattern of folded, undigested leaves of *Aspilia* spp. in the dung of chimpanzees at both Gombe and Mahale.

Leaves are most commonly swallowed early in the morning or shortly after climbing out of the night nest, often by visibly ill individuals, as one of the first items ingested after waking (Huffman and Caton, 2001; Huffman *et al.*, 1996; Wrangham and Goodall, 1989; Wrangham and Nishida, 1983). Leaf swallowing is a form of animal self-medication (Huffman, 1997) and has been documented in the greatest detail in chimpanzees at four study sites in East Africa (Mahale, Gombe, Kibale and Budongo see Fig. 10.7). At these sites, the behavior is strongly associated with the expulsion of adult intestinal nematodes and or cestode proglottids (Huffman and Caton, 2001; Huffman *et al.*, 1996; Wrangham, 1995). The gastrointestinal tract responds to the swallowed leaves by rapidly expelling the undigested leaves approximately 6 hours after swallowing. Repeated periodically throughout peak periods of infection, leaf swallowing was projected to have a significant impact on the level of *Oesophagostomum* sp. infection (Huffman and Caton, 2001).

10.6.2 Species comparison and geographical distribution of leaf swallowing

The evidence that great apes practice leaf-swallowing behavior as a form of self-medication has stimulated researchers to look for this anomalous feeding habit among apes across Africa. At the writing of this paper, leaf-swallowing behavior involving the use of more than 34 different plant species has been noted in at least 22 social groups at 13 great ape study sites in Africa (Fig. 10.7). Represented by these observations are

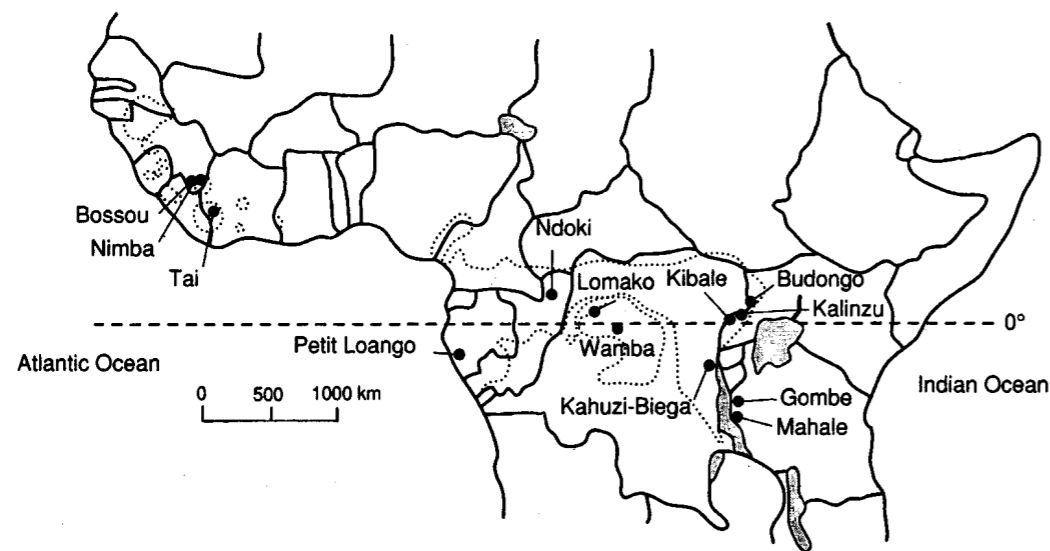


Fig. 10.7. African great ape study sites where whole leaf swallowing has been observed (Based on Huffman, 1997 and M. A. Huffman unpublished data). Species represented by each study site are as follows; bonobo (Lomako, Wamba), chimpanzee (Bossou, Nimba, Tai, Petit Loango, Ndoki, Mahale, Gombe, Kahuzi-Biega, Kalinzu Forest, Kibale, Budongo Forest), eastern and western lowland gorillas (Kahuzi-Biega, Lope).

three subspecies of chimpanzee, the bonobo, and both western and eastern lowland gorilla species. The behavioral details described above are basically the same at other sites, where leaf swallowing has been directly observed (Tai: Boesch, 1995; Bossou and Mt. Nimba: Matsuzawa and Yamakoshi, 1996; Sugiyama and Kohman, 1992; Mahale and Gombe: Huffman, 1997; Takasaki and Hunt, 1987; Wrangham and Nishida, 1983; Lomako: Dupain *et al.*, 2002).

Self-medication is most likely a very old behavior and, therefore, widespread throughout the distribution of the species that practice it. One source of behavioral variation in leaf swallowing among sites is the species of plant selected for leaf swallowing (Huffman, 1997; Huffman and Wrangham, 1994). Local variation may not be manifest in the behavioral pattern itself, but in the materials used; the species selected for use in the wild appear to be transmitted among individuals within a group. The local, regional and pan-African patterns of plant species selected for leaf swallowing suggest that transmission of information about which particular plant species are used also occurs between neighboring groups. Local and regional level similarities, not explainable by plant distribution alone, suggest that social learning and intergroup diffusion of the behavioral tradition exists for leaf swallowing (Huffman, 2001).

10.7 Future prospects and directions

To-date, the majority of behavioral traditions described in the literature have been related to food or foraging activity. While a good case for adaptive value can be made for such foraging-related behavioral traditions, it need not be an absolute criterion for the emergence or the continued existence of a behavioral tradition, as long as the behavior is not maladaptive. Pleasure seeking, stress release, even addictions are the motivation behind widespread human behavioral traditions, such as using worry beads, smoking, alcohol consumption, bungee jumping, or automobile racing. Versions of these traditions exist in practically every human culture. These behaviors are, in part, based on common propensities rooted in the evolutionary past of our species. Other species also have a great range of possibilities for traditions. New examples from emerging long-term studies on capuchin species are presented for the first time by Perry *et al.* (Ch. 14). Boinski *et al.* (Ch. 13) provide interesting new examples of social interactions, foraging techniques, and object manipulation that are suggestive of behavioral traditions.

One of our tasks for the future is to evaluate the potential impact of behavioral traditions on the survival of the individual and the group. At the same time, it will be productive to identify the ecological variability and biological foundations upon which these behaviors may be based and to look for similarities and differences among taxonomically related species. The knowledge gained from such research, when integrated into the current theoretical models used to explain the dynamics of behavioral transmission, should provide a broader understanding of the role of animal traditions in the survival of the species.

10.8 Acknowledgements

We are indebted to the many people and institutions that helped the research on which this chapter is based, in particular N. Asaba, J. Itani, T. Matsuzawa, T. Nishida, and Y. Sugiyama. We wish to give our sincere appreciation to Massimo Bardi for his help in the statistical analyses and in preparing some of the figures, for commenting on the manuscript at various stages, and for his overall technical and intellectual input. We are deeply indebted to the staff of the Arashiyama facilities, who generously provided immeasurable logistical support and friendship over the years. This study benefited in many ways from our colleagues and the

facilities of the Primate Research Institute, Kyoto University. Our sincere thanks go to the people and Government of Tanzania for their long-term cooperation and commitment to chimpanzee research. In particular the Tanzanian National Scientific Research Council, Tanzanian National Parks, Serengeti Wildlife Research Institute, Mahale Mountains Wildlife Research Centre, Gombe Stream Research Centre, and the University of Dar es Salaam. To all these people and institutions, we extend our sincerest thanks.

References

- Asquith, P. J. 1991. Primate research groups in Japan: orientations and East–West differences. In *The Monkeys of Arashiyama. Thirty-five Years of Research in Japan and the West*, ed. L. M. Fedigan and P. J. Asquith, pp. 81–98. Albany, NY: SUNY Press.
- Avital, E. and Jablonka, E. 2000. *Animal Traditions: Behavioral Inheritance in Evolution*. Cambridge: Cambridge University Press.
- Boesch, C. 1995. Innovation in wild chimpanzees (*Pan troglodytes*). *International Journal of Primatology*, **16**, 1–16.
- Boesch, C. 1996. Three approaches for assessing chimpanzee culture. In *Reaching into Thought: The Minds of the Great Apes*, ed. A. E. Russon, K. Bard, and S. Taylor Parker, pp. 404–429. Cambridge: Cambridge University Press.
- Boesch, C. and Tomasello, M. 1998. Chimpanzee and human cultures. *Current Anthropology*, **39**, 591–613.
- Boyd, R. and Richardson, P. J. 1985. *Culture and the Evolutionary Process*. Chicago, IL: University of Chicago Press.
- Candland, D. G., French, D. K., and Johnson, C. N. 1978. Object-play: test of a categorized model by the genesis of object-play in *Macaca fuscata*. In *Social Play in Primates*, ed. E. O. Smith, pp. 259–296. New York: Academic Press.
- Cavalli-Sforza, L. L. and M. W. Feldman 1981. *Cultural Transmission and Evolution: A Quantitative Approach*. Princeton, NJ: Princeton University Press.
- de Waal, F. B. M. 2001. *The Ape and the Sushi Master: Cultural Reflections of a Primatologist*. New York: Basic Books.
- Dupain, J., van Elsaker, L., Nell, C., Garcia, P., Ponce, F., and Huffman, M. A. 2002. *Oesophagostomum* infections and evidence for leaf swallowing in bonobos (*Pan paniscus*): indication for self-meditative behavior? *International Journal of Primatology*, **23**, 1053–1062.
- Fa, J. E. and Lindburg, D. G. 1996. *Evolution and Ecology of Macaque Societies*. New York: Cambridge University Press.
- Fuentis, A. 1992. Object rubbing in Balinese macaques (*Macaca fascicularis*). *Laboratory Primate Newsletter*, **31**, 14–15.
- Galef, B. G., Jr. 1976. Social transmission of acquired behavior: a discussion of tradition and social learning in vertebrates. In *Advances in the Study of Behavior*, Vol. 6, ed. J. R. Rosenblatt, R. A. Hinde, E. Shaw and C. Beer, pp. 77–99. New York: Academic Press.
- Galef, B. G., Jr. 1991. Tradition in animals: field observations and laboratory analyses. In *Interpretation and Explanation in the Study of Animal Behavior*, ed. M. Bekoff and D. Jamieson, pp. 74–95. Boulder, CO: Westview Press.
- Galef, B. G., Jr. 1992. The question of animal culture. *Human Nature*, **3**, 157–178.
- Giraldeau, L.-A. 1997. The ecology of information use. In *Behavioral Ecology: An Evolutionary Approach*, 4th edn, ed. J. R. Krebs and N. B. Davies, pp. 42–68. Oxford: Blackwell Scientific.
- Giraldeau, L.-A., Caraco, T., and Valone, T. J. 1992. Social foraging: individual learning and cultural transmission of innovations. *Behavioral Ecology*, **5**, 35–43.
- Hill, D. A. 1999. Effects of provisioning on the social behavior of Japanese and rhesus macaques: implications for socioecology. *Primates*, **40**, 187–198.
- Hiraiwa, M. 1975. Pebble-collecting behavior by juvenile Japanese monkeys. [In Japanese] *Monkey*, **19**, 24–25.
- Hirata, S. and Morimura, N. 2000. Naive chimpanzees' (*Pan troglodytes*) observation of experienced conspecifics in a tool-using task. *Journal of Comparative Psychology*, **114**, 291–296.
- Huffman, M. A. 1984. Stone-play of *Macaca fuscata* in Arashiyama B troop: transmission of a non-adaptive behavior. *Journal of Human Evolution*, **13**, 725–735.
- Huffman, M. A. 1991. History of Arashiyama Japanese Macaques in Kyoto, Japan. In *The Monkeys of Arashiyama. Thirty-five Years of Research in Japan and the West*, ed. L. M. Fedigan and P. J. Asquith, pp. 21–53. Albany, NY: SUNY Press.
- Huffman, M. A. 1996. Acquisition of innovative cultural behaviors in non-human primates: a case study of stone handling, a socially transmitted behavior in Japanese macaques. In *Social Learning in Animals: The Roots of Culture*, ed. B. G. Galef, Jr. and C. Heyes, pp. 267–289. Orlando, FL: Academic Press.
- Huffman, M. A. 1997. Current evidence for self-medication in primates: a multidisciplinary perspective. *Yearbook of Physical Anthropology*, **40**, 171–200.
- Huffman, M. A. 2001. Self-meditative behavior in the African great apes: an evolutionary perspective into the origins of human traditional medicine. *BioScience*, **51**, 651–661.
- Huffman, M. A. and Caton J. M. 2001. Self-induced increase of gut motility and the control of parasitic infections in wild chimpanzees. *International Journal of Primatology*, **22**, 329–346.
- Huffman, M. A. and Kalunde, M. S. 1993. Tool-assisted predation by a female chimpanzee in the Mahale Mountains, Tanzania. *Primates*, **34**, 93–98.
- Huffman, M. A. and Quiatt, D. 1986. Stone handling by Japanese macaques (*Macaca fuscata*): implications for tool use of stone. *Primates*, **27**, 427–437.
- Huffman, M. A. and Wrangham, R. W. 1994. Diversity of medicinal plant use by chimpanzees in the wild. In *Chimpanzee Cultures*, ed. R. W. Wrangham, W. C. McGrew, F. B. deWall, P. G. Heltne, pp. 129–148. Cambridge, MA: Harvard University Press.
- Huffman, M. A. Page, J. E., Sukhdeo, M. V. K., Gotoh, S., Kalunde, M. S., Chandrasiri T., and Towers, G. H. N. 1996. Leaf-swallowing by chimpanzees: a behavioral adaptation for the control of strongyle nematode infections. *International Journal of Primatology*, **72**, 475–503.
- Imanishi, K. 1952. Evolution of humanity. [In Japanese] In *Man*, ed. K. Imanishi. Tokyo, Mainichi-Shinbunsha.
- Inoue-Nakamura, N. and Matsuzawa, T. 1997. Development of stone tool use by wild chimpanzees (*Pan troglodytes*). *Journal of Comparative Psychology*, **111**, 159–173.
- Itani, J. 1958. On the acquisition and propagation of a new food habit in the troop of Japanese monkeys at Takasakiyama. In *Japanese Monkeys: A Collection of*

- Translations*, ed. K. Imanishi and S. Altmann, pp. 52–65. Edmonton: University of Alberta Press.
- Itani, J. 1959. Paternal care in wild Japanese monkeys, *Macaca fuscata fuscata*. *Primates*, **2**, 61–93.
- Itani, J. and Nishimura, A. 1973. The study of infrahuman culture in Japan. In *Symposia of the Fourth International Congress of Primatology*, Vol. 1, ed. E. W. Menzel Jr., pp. 26–60. Basel: Karger.
- Kawai, M. 1965. Newly acquired pre-cultural behavior of a natural troop of Japanese monkeys on Koshima Island. *Primates*, **6**, 1–30.
- Kawamura, S. 1959. The process of sub-human culture propagation among Japanese macaques. *Primates*, **2**, 43–60.
- Kroeber, A. L. and Kluckhohn, C. 1952. Culture: a critical review of concepts and definitions. *Papers of the Peabody Museum of American Archeology and Ethnology*, **47**, 41–72.
- Kutsukake, N. 2000. Matrilineal rank inheritance varies with absolute rank in Japanese macaques. *Primates*, **41**, 321–336.
- Laland, K. N. 1999. Exploring the dynamics of social transmission with rats. In *Mammalian Social Learning: Comparative and Ecological Perspectives*, ed. H. O. Box and K. R. Gibson, pp. 174–187. Cambridge: Cambridge University Press.
- Lefebvre, L. 1995. Culturally transmitted feeding behavior in primates: evidence for accelerating learning rates. *Primates*, **36**, 227–239.
- Lefebvre, L. and Giraldeau, L.-A. 1994. Cultural transmission in pigeons is affected by the number of tutors and bystanders present. *Animal Behaviour*, **47**, 331–337.
- Matsuzawa, T. 1994. Field experiments on use of stone tools by chimpanzees in the wild. In *Chimpanzee Cultures*, ed. R. W. Wrangham, W. C. McGrew, F. B. de Waal, and P. G. Hiltne, pp. 351–370. Cambridge, MA: MIT Press.
- Matsuzawa, T. and Yamakoshi, G. 1996. Comparison of chimpanzee material culture between Bossou and Nimba, West Africa. In *Reaching into Thought: The Minds of the Great Apes*, ed. A. Russon, K. A. Bard and S. Taylor, pp. 211–232. Cambridge: Cambridge University Press.
- McGrew, W. C. 2001. The nature of culture: prospects and pitfalls of cultural primatology. In *Tree of Origin: What Primate Behavior Can Tell Us About Human Social Evolution*, ed. F. B. M. de Waal, pp. 229–254. Cambridge, MA: Harvard University Press.
- McGrew, W. C. and Tutin, C.E.G. 1978. Evidence for a social custom in wild chimpanzees? *Man*, **13**, 234–251.
- Mendoza, S. and Mason, W. 1989. Primate relationships: social dispositions and physiological responses. In *Perspectives in Primate Biology*, Vol. 2, ed. P. K. Seth and S. Seth, pp. 129–143. New Delhi: Today and Tomorrow's Printers and Publishers.
- Murata, G. and Hazama, N. 1968. Flora of Arashiyama, Kyoto, and plant foods of Japanese monkeys. [In Japanese] *Iwatayama Shizen Kenkyujo Chosa Kenkyu Hokoku*, **2**, 1–59.
- Nakamura, M., McGrew, W. C., Marchant, L. F., and Nishida, T. 2000. Social scratching: another custom in wild chimpanzees? *Primates*, **41**, 237–248.
- Nishida, T. 1987. Local traditions and cultural transmission. In *Primate Society*, ed. B. B. Smuts, D. L. Cheney, R. M. Seyfarth, R. W. Wrangham, and T. T. Struhsaker, pp. 462–474. Chicago, IL: University of Chicago Press.

- Nishida, T., Wrangham, R. W., Goodall, J., and Uehara, S. 1983. Local differences in plant-feeding habits of chimpanzees between the Mahale Mountains and Gombe National Park. *Journal of Human Evolution*, **12**, 467–480.
- Nishida, T., Hasegawa, T., Hayaki, H., Takahata, Y., and Uehara, S. 1992. Meat-sharing as a coalition strategy by an alpha male chimpanzee? In *Topics in Primatology*, Vol. 1, *Human Origins*, ed. T. Nishida, W. C. McGrew, P. Marler, M. Pickford, and F. D. M. de Waal, pp. 159–174. Tokyo: University of Tokyo Press.
- Pulliam, H. R. 1983. On the theory of gene-culture co-evolution in a variable environment. In *Animal Cognition and Behavior*, ed. R. Melgren, pp. 427–443. Amsterdam: North Holland.
- Sokal, R. R. and Rohlf, F. J. 1994. *Biometry*. New York: Freeman.
- Stanford, C. B. 1999. *The Hunting Apes: Meat Eating and the Origins of Human Behavior*. Princeton, NJ: Princeton University Press.
- Sugiyama, Y. and Kohman J. 1992. The flora of Bossou: its utilization by chimpanzees and humans. *African Studies Monographs*, **13**, 127–169.
- Takahata, Y., Huffman, M. A., Suzuki, S., Koyama, N., and Yamagiwa, J. 1999. Male-female reproductive biology and mating strategies in Japanese macaques. *Primates*, **40**, 143–158.
- Takasaki, H. and Hunt, K. 1987. Further medicinal plant consumption in wild chimpanzee? *African Studies Monographs*, **8**, 125–128.
- Thierry, B. 1994. Social transmission, tradition and culture in primates: from the epiphenomenon to the phenomenon. *Techniques and Culture*, **23–24**, 91–119.
- Tokida, E., Tanaka, I., Takefushi, H., and Hagiwara, T. 1994. Tool-using in Japanese macaques: use of stones to obtain fruit from a pipe. *Animal Behaviour*, **47**, 1023–1030.
- Tuttle, R. H. 2001. On culture and traditional chimpanzees. *Current Anthropology*, **42**, 407–408.
- Visalberghi, E. and Fragaszy, D. M. 1990. Food-washing behaviour in tufted capuchin monkeys, *Cebus apella*, and crab eating macaques, *Macaca fascicularis*. *Animal Behaviour*, **40**, 829–836.
- Watanabe, K. 1989. Fish: a new addition to the diet of Japanese macaques on Koshima Island. *Folia Primatologica*, **52**, 124–131.
- Watanabe, K. 1994. Precultural behavior of Japanese macaques: longitudinal studies of the Koshima troops. In *The Ethnological Roots of Culture*, ed. R. A. Gardner, A. B. Chiarelli, B. T. Gardner, and F. X. Plooji, pp. 81–94. Dordrecht, the Netherlands: Kluwer Academic.
- Wheatly, B. P. 1988. Cultural behavior and extractive foraging in *Macaca fascicularis*. *Current Anthropology*, **29**, 516–519.
- Whiten, A. 2000. Primate culture and social learning. *Cognitive Science*, **24**, 477–508.
- Whiten, A., Goodall, J., McGrew, W. C., Nishida, T., Reynolds, V., Sugiyama, Y., Tutin, C. E. G., Wrangham, R. W., and Boesch, C. 1999. Cultures in chimpanzees. *Nature*, **399**, 682–685.
- Wrangham, R. W. 1995. Relationship of chimpanzee leaf-swallowing to a tapeworm infection. *American Journal of Primatology*, **37**, 297–303.
- Wrangham, R. W. and Goodall, J. 1989. Chimpanzee use of medicinal leaves. In *Understanding Chimpanzees*, ed. P. G. Heltne and L. A. Marquardt, pp. 22–37. Cambridge, MA: Harvard University Press.

Wrangham, R. W. and Nishida, T. 1983. *Aspilota* spp. leaves: a puzzle in the feeding behavior of wild chimpanzees. *Primates*, **24**, 276–282.

Yamagiwa, J. and Hill, D. 1998. Intraspecific variation in the social organization of Japanese macaques: past and present scope of field studies in natural habitats. *Primates*, **39**, 257–273.

Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants

Michael A. Huffman

Section of Ecology, Primate Research Institute, Kyoto University, 41–2 Kanrin, Inuyama Aichi 484–8506, Japan

Early in the co-evolution of plant–animal relationships, some arthropod species began to utilize the chemical defences of plants to protect themselves from their own predators and parasites. It is likely, therefore, that the origins of herbal medicine have their roots deep within the animal kingdom. From prehistoric times man has looked to wild and domestic animals for sources of herbal remedies. Both folklore and living examples provide accounts of how medicinal plants were obtained by observing the behaviour of animals. Animals too learn about the details of self-medication by watching each other. To date, perhaps the most striking scientific studies of animal self-medication have been made on the African great apes. The great ape diet is often rich in plants containing secondary compounds of non-nutritional, sometimes toxic, value that suggest medicinal benefit from their ingestion. Chimpanzees (*Pan troglodytes*), bonobos (*Pan paniscus*) and gorillas (*Gorilla gorilla*) are known to swallow whole and defecate intact leaves. The habit has been shown to be a physical means of purging intestinal parasites. Chimpanzees and man co-existing in sub-Saharan Africa are also known to ingest the bitter pith of *Vernonia amygdalina* for the control of intestinal nematode infections. Phytochemical studies have demonstrated a wide array of biologically-active properties in this medicinal plant species. In light of the growing resistance of parasites and pathogens to synthetic drugs, the study of animal self-medication and ethno-medicine offers a novel line of investigation to provide ecologically-sound methods for the treatment of parasites using plant-based medicines in populations and their livestock living in the tropics.

African great apes: Self-medication: Diet: Ethno-medicine: Parasite control

In recent years a growing body of evidence has given momentum to the study of self-medication in animals, often referred to as ‘zoopharmacognosy’ (for example, see Rodriguez & Wrangham, 1993; Huffman, 1997, 2001; Engel, 2002). The amount of detailed information on self-medication in animals gathered thus far is greatest in primates, in particular the African great apes (see Huffman, 1997, 2001). The basic premise of zoopharmacognosy is that animals utilize plant secondary compounds or other non-nutritional substances to combat or control disease. The hypothesis being developed from investigations of self-medication in the great apes is that such behaviour aids in the control of intestinal parasites and provides relief from related gastrointestinal upset (for example, see Huffman *et al.* 1993, 1996b; Wrangham, 1995; Huffman & Caton, 2001). However, given the obvious adaptive importance that self-medication implies, it is expected to occur in response to a variety of illnesses throughout the animal kingdom.

Parasite infection and other diseases can have a strong effect on the behaviour and reproductive fitness of an individual, making the need to counteract such pressure of extreme importance to survival. Anti-parasitic behaviour is one such adaptive response, with examples ranging from arthropods to primates, and is undoubtedly the product of a long evolutionary process (for example, see Harborne, 1978; Swain, 1978; Blum, 1981; Boppré, 1984; Huffman, 2001). Ethnographic literature and recent ethno-medicinal research suggests that man has long been aware of the use of medicinal plants by animals and has looked to them for clues about the medicinal properties of plants.

The growing problem of antibiotic and anthelmintic resistance is an increasingly serious problem in human health care and livestock husbandry in Africa and around the world. The study of self-medication in nature and tradition-based diet and herbal medicines in man can provide alternative and important insights into dealing with these

Corresponding author: Dr Michael Huffman, fax +81 568 63 538, email huffman@pri.kyoto-u.ac.jp

problems. Detailed behavioural, ethno-medicinal, pharmacological and parasitological investigations are currently underway to elucidate the full potential of self-medication in animals for the prevention and control of illness. Multi-disciplinary investigations of chimpanzee behavioural strategies in the wild and ethno-veterinary and ethno-medicinal surveys of traditional African medicine are being conducted by a multi-regional multi-disciplinary research consortium, The CHIMPP Group (Huffman, 1994). The present paper reviews some of the literature and recent findings in this area and suggests future directions of research.

Animal self-medication and ethno-medicine

Throughout the history of man animals have been looked to for sources of herbal medicines and narcotic stimulation (for example, see Brander, 1931; Riesenber, 1948). Anecdotal reports of the possible use of plants as medicine by wild animals such as the elephant, civet, jackal and rhinoceros are abundant (Table 1). The Navajo living in the southwestern USA acknowledge the bear for their knowledge of the anti-fungal, antiviral and antibacterial properties of the Umbelliferae, *Ligusticum porteri* (Moore, 1979; Grisanzio, 1992).

Tabernanthe iboga (Apocynaceae) contains several indole alkaloids, and is used as a powerful stimulant and aphrodisiac in many secret religious societies in Gabon (Harrison, 1968). Harrison (1968) speculated that because of the widespread reports from local inhabitants of gorilla (*Gorilla gorilla gorilla*), bush pig and porcupine going into wild

frenzies after digging up and ingesting the roots, they probably learned about these peculiar properties of the plant from watching the animals' behaviour. The most active principle, found in the root, is ibogaine, which has been shown to affect the central nervous system and the cardiovascular system. Two other known similarly-active compounds in the plant are tabernanthine and iboluteine. The stimulating effects are similar to those of caffeine (Dubois, 1955). The sloth bear and local population of central India are noted to become intoxicated after eating the fermented madhuca flowers (Brander, 1931) and reindeer and the indigenous Lapps consume fly agaric mushrooms (*Amanita muscaria*) known for their intoxicating effects (Phillips, 1981).

One version of the discovery of coffee is that the chance observation by a shepherd that goats became stimulated after grazing on the berries of wild coffee plants in the highlands of Ethiopia provided the clue for man to exploit the plant as a stimulant. Dr Jaquinto, the trusted physician to Queen Ann, wife of James I in 17th century England, is said to have made systematic observations of domestic sheep foraging in the marshes of Essex, which led to his discovery of a successful cure for consumption (Wilson, 1962). In the foothills of the Himalayas near Mt. Everest the use of the roots of 'chota-chand' as a potent antidote for a snake bite is said to have been learned by observing mongooses feeding on the plant before fighting with cobra (Balick & Cox, 1996). All these examples suggest the occurrence of self-medication in a variety of animal species and ways that man may learn about the medicinal value of plants from them.

Table 1. Some anecdotal evidence for self-medication in animals

Species	Plant specie (Family)	Comments	References
Malay elephant	<i>Entada schefferi</i> (Leguminosae):	Foramina before long walk, possible pain killer	Hubback (1941), Janzen (1978)
African elephant	Boraginaceae sp.	Induce labour; used by Kenyan ethnic group to induce labour and abortion. Similar story related to Huffman about observations made in Tanzania	Cowen (1990), MS Kalunde (personal communication)
Indian bison	<i>Holarrhena antidysenterica</i> (Apocynaceae)	Bark regularly consumed. Species name suggests anti-dysenteric action	Ogilvie (1929)
Wild Indian boar	<i>Boerhavia diffusa</i> (Nyctaginaceae) called pig weed	Roots are selectively eaten by boar and is a traditional Indian anthelmintic	Janzen (1978), Dharmkumarsinhji (1960)
Pigs	<i>Punicum granatum</i> (Punicaceae) pomegranate	Root sought after by pigs in Mexico	Janzen (1978), McCann (1932)
Indian tigers, wild dogs, bears, civets, jackals	<i>Careya arborea</i> (Barringtonaceae), <i>Dalbergia latifolia</i> (Leguminosae) etc.	Fruits of various species eaten by large carnivores. Possibly helps in elimination of parasites ingested along with contents of intestines of herbivore prey	McCann (1932), Burton (1952), Janzen (1978)
South American wolf	<i>Solanum lycocarpum</i> (Solanaceae)	Rotting fruit said to be eaten to cure stomach or intestinal upset	DAO Courtney and Kirby (personal communication)
Asiatic two-horned rhinoceros	<i>Ceriops candoleana</i> (Rhizophoraceae)	Tannin-rich bark eaten in large amounts enough to turn urine bright orange. Possible use in control of bladder and urinary tract parasites	Hubback (1939)
Black howler monkey, spider monkey		Indigenous peoples living in primate habitats of the Neotropics claim that some monkey species are parasite-free because of the plants they eat	Vitazkova (personal communication), M Pavelka (personal communication)

Table 2. Some common plant secondary compounds and their effects on animals (after Wink *et al.* 1993; Howe & Westly, 1998)

Class of compounds	Effects and comments
Terpenoid alkaloids	Modulation of ion channels (highly toxic)
Isoquinoline alkaloids	DNA intercalation, interaction with receptors, causes spasms (toxic and bitter)
Quinolizidine alkaloids	Binding to ACH receptor (toxic and bitter)
Tropane alkaloids	Inhibition of ACH receptor (highly toxic)
Pyrrrolizidine alkaloids	Mutagenic and carcinogenic (liver toxic)
Cyanogenic glycosides	Inhibition of respiration
Cardiac glycosides	Inhibition of Na ⁺ /K ⁺ -ATPase (highly toxic)
Terpenes	Diuretic (bitter taste)
Volatile terpenes	Antibiotic, irritant
Volatile monoterpenes	Antibiotic (aromatic smell)
Saponines, amines	Detergent for biomembranes (bitter)
Triterpene saponines	Detergent for biomembranes (toxic, emetic)
Sesquiterpenes, pyrrolizidines	PA are mutagenic and carcinogenic, irritant (cytotoxic, liver toxic)
Convallatoxin	Inhibition of Na ⁺ /K ⁺ -ATPase (highly toxic and bitter)
Antraquinones	Purgative (toxic)
Phenolics	Astringency, reduces digestibility
Cellulose, hemicellulose, lignins, silica	Undigestible

ACH, acetylcholine; PA, pyrrolizidine alkaloids.

Why should any of this information really be a surprise? After all, from an evolutionary standpoint, preservation of health is a basic principle of survival, and all species living today can be expected to have evolved a variety of ways of protecting themselves from predators and parasites, large and small, in their environment.

Where did this process all begin? In the plant world a common line of defence is to produce a variety of toxic secondary compounds such as sesquiterpenes, alkaloids and saponins (Table 2) that prevent predation by animals (for example, see Swain, 1978; Howe & Westley, 1988). At some point in their co-evolutionary history, probably starting with the arthropods, animals began to take advantage of the plant kingdom's protective chemical arsenal to protect themselves from predators and parasites and to enhance their own reproductive fitness (see Blum, 1981). For example, adult danaine butterflies of each gender utilize pyrrolizidine alkaloids for defence against predators, and males have also been shown to depend on it as a precursor for the biosynthesis of a pheromone component needed for courtship (Boppré, 1978, 1984). The monarch butterfly is reported to feed on *Asclepias* species containing cardiac glucosides that make birds sick, conditioning them not to feed on the species (Brower, 1969). Such tri-trophic level interactions are likely to have provided the foundation for the evolution of a more sophisticated level of self-medication seen in the higher vertebrates.

The impact of parasites on the evolution of self-medicative behaviour

Parasitism has played an important role in the evolution of host behaviour throughout the animal kingdom (for example, see Anderson & May, 1982; Futuyama & Slatkin, 1983; Clayton & Moore, 1997). Co-evolution between host and parasite has resulted in the development of mechanisms by which the host limits parasitic infection and the parasite increases its chance of infecting the host (see Hart, 1990; Toft *et al.* 1991; Ewald, 1994). At the primary level, the

host's immune response (innate and acquired) normally controls infections. However, some parasites invariably establish themselves by undergoing antigenic variation, thus disguising themselves with the host's antigens, or by interfering directly with the immune response (see Cox, 1993; Wakelin, 1996).

Where physiological adaptation is not enough, hosts have developed behavioural responses to avoid or limit contact with parasites and other pathogens. These behaviours are widespread among vertebrates and include such strategies as regular changing of sleeping or feeding sites and differential use of drinking sites by baboons (*Papio cynocephalus*) and mangaby monkeys (*Cercocebus albigena*), use of antiparasitic leaf material to line nests or dens occupied over long periods by sparrows and wood rats, and the direct application of aromatic substances to repel fur- and feather-infesting parasites in coatis, capuchin monkeys (*Cebus capucinus*), brown bears, sparrows and starlings (for example, see Kummer cited by Nelson, 1960; Freeland, 1980; Sengputa, 1981; Hausfater & Meade, 1982; Seigstadt cited by Cowen, 1990; Clark, 1991; Grisanzio, 1992; Gompper & Holyman, 1993; Baker, 1996). Learned aversion of foods or tastes associated with illness, parasite infection and compensatory changes in host dietary preferences induced by parasites has been demonstrated in the laboratory and field for a wide range of vertebrates (for example, see Gustavson, 1977; Keymer *et al.* 1983; Kyriazakis *et al.* 1994).

These learned aversions are another level at which the host avoids prolonged exposure to pathogens. Diet modification has also been proposed as a means of altering or controlling internal parasite load. A causal relationship between a sudden change in diet and reduced tapeworm load has been suggested for black bears (Rausch, 1954, 1961). Another example of this type of behaviour involves the ingestion by chimpanzees (*Pan troglodytes*) of specific plant parts (with little or no nutritional value) for their antiparasitic qualities, which may be either pharmacological (Huffman *et al.* 1993, 1998; Ohigashi *et al.* 1994) or

physical (Huffman *et al.* 1996b; Huffman & Caton, 2001). A discussion of these two behaviours will be presented later (see p. 375).

Food as medicine in animals and man

Many traditional human societies around the world are still very much dependent on plants for both food and medicine. Food and medicine greatly overlap in the diet, sometimes making the difference difficult to perceive. It has been found that 30 % of the plant species identified as foods among the Hausa of Nigeria in western Africa are also used as medicine. Similarly, 89 % of the species they use to treat symptoms of malaria are also used in a dietary context (Etkin & Ross, 1983; Etkin, 1996).

From an evolutionary perspective, it seems likely that many proposed medicinal plants used by animals are derived from the ingestion of rarely eaten or fall-back foods (eaten in periods of main food shortage) with supportive medicinal properties. One of the challenges and difficulties of interpreting self-medication in animals is distinguishing between possible indirect medicinal benefits derived from plants rich in secondary compounds that are assumed to be ingested for their nutritional value *v.* limited and situation-specific ingestion of items that are processed only for their medicinal properties. When consumed on a fairly regular basis throughout the year or in seasons of wide availability, food and medicine may be one in the same. Indeed, in man, many traditional spices, condiments and vegetables of Asian cuisine used today, such as ginger (*Zingiber officinale*) root, marine algae and various herbs contain important sources of anti-tumour agents (Ohigashi *et al.* 1992; Murakami *et al.* 1994, 1996) that may also have an active role in the suppression of parasitic and viral infections.

Many of these 'food-medicines' are beneficial beyond their nutritional value because of the variety of plant secondary compounds they contain. As discussed earlier, these compounds are considered as being produced by the plant to deter herbivores from ingesting them (for example, see Ehrlich & Raven, 1964; Feeny, 1976; Howe & Westley, 1988; Wink *et al.* 1993), but in small amounts such compounds will likely be of some benefit to the consumer. A number of plant foods found in the diet of the great apes appear to share these properties, as can be seen from the diet of Mahale chimpanzees (Fig. 1).

Several species from the genus *Aframomum* are major food plants for gorillas and chimpanzees throughout the lowland rainforests and in many mountain areas (Nishida & Uehara, 1983; Sugiyama & Koman, 1992; Wrangham *et al.* 1993; Idani *et al.* 1994; Moutsamboté *et al.* 1994; Tutin *et al.* 1994; Yumoto *et al.* 1994). An extensive literature survey of the pharmacological properties of the *Aframomum* by Cousins & Huffman (2002) revealed a wide variety of considerable biological activity, including potent bactericidal activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Bacillus subtilis*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Serratia marcescens*. Fungicidal activities inhibited *Candida albicans*, *Trichophyton mentagrophytes*, *Aspergillus niger*, *Botryodiplodis theobromae* and species of *Cladosporium*

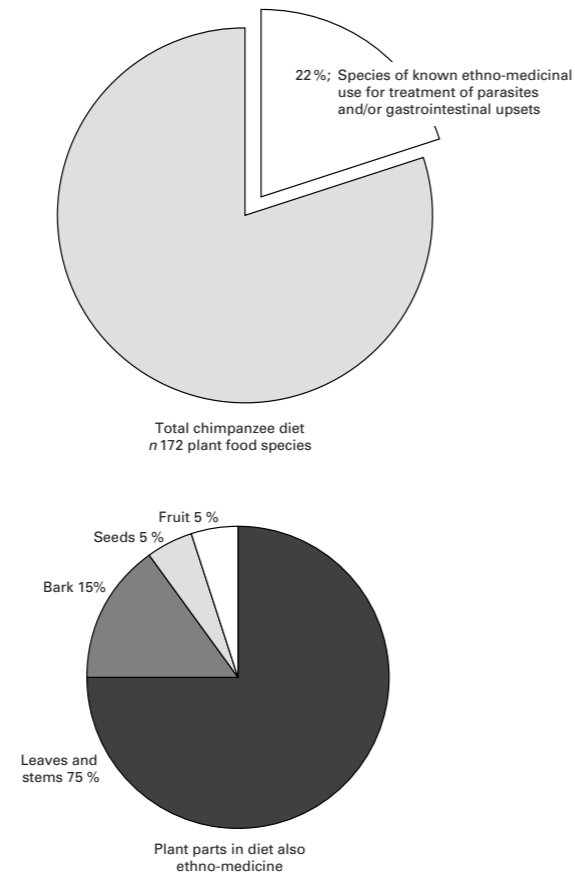


Fig. 1. Percentage contributions and plant parts in the Mahale chimpanzee diet with anthelmintic and other gastrointestinal-relieving properties recognized in the ethno-medicinal and pharmacological literature.

cladosporioides (Oloke *et al.* 1988). Bioassays of the extract of *Aframomum danielli* have been made by Adegoke & Skura (1994), revealing active growth inhibitors of *Salmonella enteritidis*, *Pseudomonas fragi*, *Pseudomonas fluorescens*, *Proteus vulgaris*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus ochraceus* and *A. niger*.

The berries of *Phytolacca dodecandra* L. Herit (Phytolaccaceae) are an abundant and frequently-ingested food item of the Kanyawara group of chimpanzees in Kibale, western Uganda (Wrangham & Isabirye-Basuta cited by Huffman & Wrangham, 1994). These bitter-tasting berries are a concentrated source of at least four toxic triterpenoid saponins (lemmatoxin, lemmatocin-C, oleanoglycotocin-A, phytolacca-dodecandra glycoside). Consumption of about 2 g of the material is fatal in mice and rats. Other known properties of these triterpenoid saponins include antiviral, antibacterial, anti-fertility, spermicidal and embryotoxic activities (Kloos & McCullough, 1987).

The tips of the young leaves of *Thomandersia laurifolia* (*T. Anders. ex Benth.*) Baill. (Acanthaceae) are on rare occasions chewed by western lowland gorillas in the Ndoki

forest of northern Congo (S Kuroda, personal communications). According to Kuroda and colleagues, the local human inhabitants use these leaves as a treatment for parasites and fever. Weak anti-schistosomal activity has been found from crude leaf extracts (Ohigashi, 1995).

Bark and wood are characteristically highly fibrous, heavily lignified, sometimes toxic, relatively indigestible and nutrient poor (Waterman, 1984). Chimpanzees and gorillas are known to infrequently ingest the bark and wood of several plant species (for example, see Huffman & Wrangham, 1994).

While the list of plant species whose bark is ingested is extensive, little is actually known about the contribution of bark to the diet and general health. The literature on African ethno-medicine suggests some medicinal benefits from its consumption. The bark of *Pycnanthus angolensis* (Welw.) Warb. (Myristicaceae) avidly ingested by chimpanzees at Mahale in western Tanzania is used by West Africans as a purgative, laxative, digestive tonic, emetic and reliever of toothaches (Abbiw, 1990). In Tanzania the chimpanzees of Gombe National Park occasionally eat the bark of *Entada abyssinica* Steud. ex A. Rich. (Mimosaceae). In Ghana the bark is used by the human population to treat diarrhoea and as an emetic (Abbiw, 1990). The bark of *Erythrina abyssinica* DC (Papilionacea) is occasionally eaten by Mahale chimpanzees. Marked plasmodicidal and anti-schistosomal activities have been demonstrated for the bark of this species collected at Mahale (Ohigashi, 1995; C Wright, C Phillipson, DV Kirby, MA Huffman and H Ohigashi, unpublished results). The bark of *Gongronema latifolium* Benth. (Asclepiadaceae) occasionally eaten by the chimpanzees of Bossou Guinea is extremely bitter, and the stems are used by the human population in West Africa as a purge for colic, stomach pains and symptoms connected with intestinal parasite infection (Burkill, 1985).

The present brief overview of some of the fruit, leaf, bark and wood items ingested by the great apes serves to demonstrate the diversity of secondary compounds or inferred pharmacological activity present in their diet. The total effect of ingesting these items is still unclear, but it is unrealistic to assume nutritional gain alone as the utility of ingestion when little nutritional value is likely to be obtained.

Use of plants as medicine by chimpanzees in the wild

Recent evidence from the African great apes suggests that certain plants are ingested, not incidentally but directly, for their considerable medicinal value. The hypothesis currently being developed is that the behaviours aid in the control of intestinal parasites (*Oesophagostomum stephanostomum*, *Bertiella sturdi*) and/or provide relief from related gastrointestinal upset (Wrangham, 1995; Huffman *et al.* 1996b; Huffman, 1997; Dupain *et al.* 2002). These observations provide the clearest systematic evidence collected thus far for self-medication in animals. Perhaps due to their phylogenetic closeness, man and chimpanzees select some of the same plants when displaying similar symptoms of illness (Huffman *et al.* 1996a). Unquestionably, the implications of self-meditative behaviour are of extreme interest when considering the

nutritional and medicinal habits during the evolution of great apes, early hominids and *Homo sapiens*.

Whole-leaf swallowing and the physical expulsion of parasites

The first documentation of a putative self-meditative behaviour in the great apes is known as leaf-swallowing behaviour. It was the lack of any apparent nutritional value in swallowing leaves whole that brought this behaviour to the attention of primatologists (Wrangham & Nishida, 1983). Leaves are frequently swallowed early in the morning, often as the first, or one of the first, items ingested (Wrangham & Nishida, 1983; Wrangham & Goodall, 1989; Huffman *et al.* 1997; Huffman & Caton, 2001). Leaf swallowing is the slow and deliberate swallowing, one at a time, of whole leaves that are folded between tongue and palate, and pass through the gastrointestinal tract visibly unchanged.

Initially, the self-meditative value of leaf swallowing and the possible link between the behaviour and parasite expulsion was not recognized. A chemical action was first proposed *ad hoc* on the basis of the ethno-medicinal uses of species in the genus *Aspilia*, the first observations being documented by Wrangham & Nishida (1983). Subsequently, preliminary chemical analysis of plant material led researchers to propose an antibiotic and/or nematocidal component to the behaviour (Rodriguez *et al.* 1985; Rodriguez & Wrangham, 1993). This hypothesis has since been rejected in light of more extensive and detailed studies in the laboratory that have failed to replicate the earlier results (Page *et al.* 1992, 1997; Huffman *et al.* 1996b; Messner & Wrangham, 1996). Field observations have positively linked leaf swallowing with the expulsion of parasites (Wrangham, 1995; Huffman *et al.* 1996b), but its mode of action is considered to be largely a physical one, in which parasites are purged from the host by means of self-induced reduction in gut transit time and diarrhoea (Huffman & Caton, 2001). Independent analysis of this behaviour in two populations of eastern long-haired chimpanzees (*Pan troglodytes schweinfurthii*) has reported a correlation between the swallowing of whole leaves and the expulsion of the strongyle nematode *O. stephanostomum* at Mahale, Tanzania, and a species of the tapeworm (*Bertiella sturdi*) at Kibale, Uganda (Wrangham, 1995; Huffman *et al.* 1996b).

Leaf-swallowing behaviour is now known to occur widely in the African great apes. Chimpanzees, bonobos (*Pan paniscus*) and lowland gorillas use a wide range of plant genera, represented by more than thirty-four species (Huffman, 1997, 2001). Evidence suggests that similar behaviour may also have evolved convergently in at least two other different vertebrate taxa; the snow goose (*Anser caerulescens*) and brown bear (see Huffman, 1997).

Vernonia amygdalina and bitter-pith chewing behaviour

The hypothesis that bitter-pith chewing has medicinal value for chimpanzees was first proposed after detailed behavioural observations and parasitological and phytochemical analyses of patently-ill chimpanzees ingesting *Vernonia*

amygdalina Del. (Compositae) at Mahale (Huffman & Seifu, 1989; Huffman *et al.* 1993). These observations are the first reported systematic observations to verify illness and subsequent improvement in health of an animal ingesting medicinal plants.

V. amygdalina occurs throughout tropical sub-Saharan Africa (Watt & Breyer-Brandwijk, 1962). Bitter-pith chewing of other *Vernonia* species has been observed at Gombe, Tanzania (*V. colorata* (Willde.) Drake; Huffman & Wrangham, 1994; Hilali, unpublished results cited by J Wallis, personal communication) and Kahuzi-Biega, Congo-Kinshasa (*V. hochstetteri* Schi-Bip., *V. kirungae* Rob. E. Fries; Yumoto *et al.* 1994; AK Basabose, personal communication). At Tai, Ivory Coast, the bitter piths of *Paliosota hirsuta* (Thunb.) K. Schum. (Commelinaceae) and *Eremospath macrocarpa* (Mann & Wendl.) Wendl. (Palmae) are chewed (C Boesch, personal communication cited by Huffman, 1997).

When ingesting the pith from young shoots of *V. amygdalina*, chimpanzees meticulously remove the outer bark and leaves to chew on the exposed pith, from which they extract the extremely bitter juice and residual amounts of fibre. The amount of pith ingested in a single bout is relatively small, ranging from portions of 50–1200 mm × 10 mm. The entire process, depending on the amount ingested, can take from <1 to 8 min. Mature conspecifics in proximity to an individual chewing *Vernonia* bitter pith or leaf swallowing show little or no interest in ingesting the pith (Huffman & Seifu, 1989; Huffman *et al.* 1997), but will on occasion approach the individual and watch it. Infants have been observed to taste the pith discarded by their ill mothers. Interestingly, one instance of a healthy mother refusing her infant access to the discarded pith of an ill chimpanzee has also been observed at Mahale (MA Huffman, unpublished results). In this way, group individuals are exposed to both the behaviour and the extremely bitter taste of the pith from a very young age.

At Mahale, use of *V. amygdalina* has been recorded in all months except June and October (late dry season), demonstrating its year-round availability (Nishida & Uehara, 1983). However, despite this factor, its use by chimpanzees is highly seasonal. It is most often used during the rainy season months of December and January, the time when parasite re-infection by *O. stephanostomum* and other nematodes is at their peak (Huffman *et al.* 1997).

The ethno-medicine and phytochemistry of bitter-pith chewing

V. amygdalina is used by numerous African ethnic groups across the continent as medicine (Table 3). A concoction made from this species is prescribed treatment for malarial fever, schistosomiasis, amoebic dysentery, several other intestinal parasites and stomach-aches, and a variety of other ailments (Dalziel, 1937; Watt & Breyer-Brandwijk, 1962; Burkill, 1985; Huffman *et al.* 1996a). The Tongwe of Mahale use this plant as a treatment for intestinal parasites, diarrhoea and stomach upset.

Phytochemical analysis of *V. amygdalina* samples collected at Mahale from plants that are known to be used by chimpanzees revealed the presence of two major classes of

bioactive compounds. A total of four known sesquiterpene lactones, seven new stigmastane-type steroid glucosides and two freely-occurring aglycones of these glucosides have been isolated by our group (Ohigashi *et al.* 1991; Jisaka *et al.* 1992a,b, 1993a,b). The sesquiterpene lactones present in *V. amygdalina*, also found in *V. colorata* and a number of other *Vernonia* spp., are well known for their anthelmintic, anti-amoebic, anti-tumour and antibiotic properties (Toubiana & Gaudemer, 1967; Kupchan *et al.* 1969; Asaka *et al.* 1977; Gasquet *et al.* 1985; Jisaka *et al.* 1992a, 1993b). From crude methanol extracts of the leaves Koshimizu *et al.* (1993) also found inhibition of tumour promotion and immuno-suppressive activities.

In vitro tests on the anti-schistosomal activity of the pith's most abundant steroid glucoside (vernonioside B₁), and sesquiterpene lactone (vernodaline), showed marked inhibition of movement of the adult parasites and adult females' egg-laying capacity (Jisaka *et al.* 1992b). These findings are consistent with the observed decline (from 130 to fifteen eggs per g faeces; 88 % decrease) in the *O. stephanostomum* faecal egg count level measured 20 h after an adult female chimpanzee at Mahale ingested *V. amygdalina* pith. The normal egg count fluctuation recorded during the same period for seven other individuals was an increase in egg count of 69.9 (SD 84, range 5–236; Huffman *et al.* 1993). The sesquiterpene lactones showed marked *in vivo* plasmodicidal activity, while that of the steroid glucosides was weaker (Ohigashi *et al.* 1994). *In vivo* test trials of *V. amygdalina* by Nfi *et al.* (1999) validated the ethno-veterinary use of this plant by Fulani pastoralists as an anthelmintic, observing >52 % reduction in faecal egg count for nematodes, including *Oesophagostomum* spp. following treatment with a decoction.

Some of the species with bitter piths ingested by chimpanzees at Gombe, Kahuzi-Biega and Tai also have a number of ethno-medicinal and pharmacological properties. *V. colorata* and *V. amygdalina* are not distinguished from each other ethno-medicinally in relation to their medicinal properties and folk classification (Burkill, 1985). Alkaloids occur in the pith, as well as flower and leaf of *V. hochstetteri* (Smolenski *et al.* 1974). *P. hirsuta* and *E. macrocarpa* are used in west African ethno-medicine for the treatment of upset stomachs, colic, as an antiseptic and analgesic, and for venereal disease (Abbiw, 1990; Neuwinger, 1996). Moluscicidal activity has also been reported for *P. hirsuta* (Okunji & Iwu, 1988).

A link between animal self-medication and ethno-medicine

The ethno-medicinal uses of *V. amygdalina* and the conditions under which ill chimpanzees have been observed to ingest this species are similar in many respects (Huffman *et al.* 1993, 1996a). In the two most detailed documented cases of use by chimpanzees described earlier, the rate of recovery (20–24 h) was comparable with that of indigenous human inhabitants of Mahale and neighbouring regions (within 24 h), who use *V. amygdalina* for the treatment of parasitosis and gastrointestinal upset.

This group, the Tongwe, typically make their medicine from a cold-water decoction of two to three crushed fresh

leaves (approximately 10–15 g fresh weight) in 300–400 ml water. Due to the plant's toxic effect on the patient, this treatment typically comprises one dose, but smaller amounts spread over the day(s) are sometimes also prescribed (MS Kalunde, personal communication). An analysis replicating this traditional method (three trials) yielded 3.3–5.0 mg versonioside B₁ (Huffman *et al.* 1993). This finding was compared directly with chimpanzee intake by quantitative analysis of pith collected from the plant used by the adult female chimpanzee that had experienced a drop in parasite load 20 h after its ingestion. The amount of pith she ingested (600 mm, approximately 50–100 g fresh weight) was found to contain approximately 3.8–7.6 mg versonioside B₁; an amount approximately equal to that normally obtained by a Tongwe patient in a single full dose (Huffman *et al.* 1993).

Both man and chimpanzees appear to recognize the important physiological activity of this plant, and evidence suggests that chimpanzees ingest *V. amygdalina* when they experience some of the same symptoms. From an ethno-botanical viewpoint, the greater number of different cultures that recognize a single plant species as having some kind of medicinal property, the more likely that species is to contain marked physiological activity. The example of *V. amygdalina*, with its widely-recognized medicinal value in Africa, takes this process one step further by bridging the gap between apes and man (Table 3).

Tongwe ethno-zoology and health care

A key collaborator in my long-term research at Mahale is Tanzanian National Park game officer Mohamedi Seifu

Table 3. Ethno-medicinal uses of *Vernonia amygdalina* in Africa*

Application	Plant part used	Region used	Comments
General intestinal upsets:			
Enteritis	Root, seeds	Nigeria	
Constipation	Leaves, sap	Nigeria, Tanzania, Ethiopia	As a laxative
Diarrhoea	Stem, root bark, leaves	West Africa, Zaire	
Stomach upset	Stem, root bark, leaves	Angola, Ethiopia	
Parasitosis:			
Schistosomiasis	Root, bark, fruit	Zimbabwe, Mozambique, Nigeria	Sometimes mixed with <i>Vigna sinensis</i>
Malaria	Root, stem bark, leaves	East Africa, Angola, Guinea, Nigeria, Ethiopia	A quinine substitute
Trematode infection	Root, leaves	East Africa	Treatment for children used as a suppository
Amoebic dysentery	Root bark	South Africa	
Ringworm	Leaves	Nigeria	Ringworm and other unidentified epidermal infections
Unspecified	Leaves	Nigeria	Prophylactic treatment for nursing infants, passed through mother's milk
	Root, seeds	Nigeria	Worms
	Leaves	West Africa	Crushed in water and given to horses as a vermifuge, livestock fodder supplement for treating worms
Tonic food	Leaves	Ghana	Purgative
	Leaves	Cameroon, Nigeria	Boiled or soaked in cold water, prepared as soup or as a vegetable fried with meat, 'n'dole', 'fatefate', 'mayemaye', leaves sold in markets and cultivated in home gardens
Other ailments:			
Amenorrhoea	Root	Zimbabwe	
Coughing	Leaf	Ghana, Nigeria, Tanzania	
Diabetes	All bitter parts	Nigeria	
Fever	Leaves	Tanzania, Kenya, Uganda, Congo-Kinshasa	Leaves squeezed and juice taken
Gonorrhoea	Roots	Ivory Coast	Taken with <i>Rauwolfia vomitoria</i>
'Heart weakness'	Root	West Africa	Vernonine is a cardiotonic glycoside comparable with digitalin
Lack of appetite	Leaf	West Africa	Leaves soaked in cold water to remove bitterness and then boiled in soup
Pneumonia	Leaf	Ivory Coast	Taken with <i>Argemone maxicana</i> or used in a bath
Rheumatism	Stem, root bark	Nigeria	
Scurvy	Leaves	Sierra Leone, Nigeria, West Cameroon	Leaves sold in markets and cultivated in home gardens
General hygiene:			
Dentifrice	Twig, stick	Nigeria	Chew stick for cleansing and dental caries
Disinfectant	Not given	Ethiopia	
Soap	Stems	Uganda	

*Data from Dalziel (1937), Irvine (1961), Watt & Breyer-Brandwijk (1962), Kokwaro, (1976), Palgrave (1983), Burkill (1985), Abebe (1987), Nyazema (1987), Akah & Okafor (1990), Muanza, *et al.* (1993), Huffman, unpublished results from interviews in Uganda and Tanzania.

Kalunde, who comes from a long family line of traditional healers. During the course of collaborative work starting in 1987, he has taught me much about the medicinal plants used by his people, the Tongwe, some of which are also found in the diet of chimpanzees in the Mahale M group. His narratives have provided me with important insights into the origins of some of the Tongwe's most important medicinal treatments, notably acquired through the observation of sick wild animals such as the elephant, porcupine and bush pig.

An example is 'munyonga nTembo', which is used by the Tongwe as a treatment for general stomach upsets. The leaves are crushed and placed in water. The resulting solution is drunk or the crushed leaves are used as a suppository. The origin of the use of this plant for medicinal purposes has been preserved in its name. The Tongwe verb 'kunyonga' means to twist and pull off. Elephants (tembo in both Swahili and Tongwe) twist and pull off the leaves of this plant before ingesting them. It is said that one day an elephant visibly suffering from stomach upset was observed obtaining a bunch of leaves from this particular species. The elephant put a few bunches of leaves in its mouth, and after chewing on them a bit, drew up some water and blew it into its mouth. After holding the water in its mouth together with the leaves for sometime, it blew the leaves out of its nose and swallowed the water. The observer interpreted the elephant's behaviour as using a cold-water concoction from the leaves. The leaves of this plant are thus prepared as a Tongwe treatment for stomach upset.

Another important medicinal plant of the Tongwe, 'mulengele' (*Aeschynomene* sp., Leguminosae), was first discovered by Mohamedi's grandfather, babu Kalunde, as a treatment for diarrhoea with blood, from watching a sick young porcupine ingest the roots of this plant and recover from these symptoms. The story goes as follows. On one occasion babu Kalunde was out checking his snares for captured prey. He came upon a female porcupine in one of them. He killed the porcupine, but found out too late that she had dependent young hidden in the bush nearby. Babu Kalunde took the young home to care for them. He put them in a small enclosure so that they would not stray off. The young porcupines grew ill with passing time, displaying, among other symptoms, bloody diarrhoea. These symptoms were the same as those showing up in members of the village, and babu Kalunde was looking for something with which to cure them. During this time one of the ill porcupines escaped and wandered off into the forest. Following it to see what it would do, babu Kalunde observed it dig up and chew on the roots of mulengele. The species had never been used as medicine before, but was known and avoided because of its highly toxic qualities. He observed that the porcupine became better over time, convincing babu Kalunde that he should try it on the sick people of his village. He told the villagers what he had seen and asked to be allowed to test it on the sick. People were at first reluctant because of the plant's reputation, but it was agreed that if babu Kalunde himself would first take some they too would submit to treatment. He took a small decoction and they were satisfied. The medicine was put to work to cure many people in the village afflicted with bloody diarrhoea. From that time the plant has become an

important medicine of the Tongwe in regions of western Tanzania where the plant is found.

Mohamedi has experimented with the plant on various illnesses and has also found it effective against syphilis and gonorrhoea. It has also recently been learned that a traditional healer in Mpanda, western Tanzania, has used the root to treat AIDS-related symptoms. All this evidence suggests that the plant may possess marked antibiotic activity. Laboratory investigations are currently underway to determine the types of compounds present in the roots and to ascertain their pharmacological properties.

Mohamedi's mother, Joha Kasante, also a traditional healer, experimented with an antidote for snake bite by asking Mohamedi to follow the snake that bit his younger brother and bring back leaves of the plant that the snake subsequently chewed on. The leaves are said to prevent the snake's venom from circulating throughout the body. After chewing on the leaves his brother immediately vomited, but suffered no further ill consequences from the snake bite. Separated by great distances and cultures, this story closely resembles that of 'chota-chand' from the Himalayas of Nepal, noted earlier. Is this similarity a coincidence, or have populations around the world obtained similar knowledge from watching the behaviour of similar animal species? Given the fact that animals of the same or similar species and genera distributed around the globe share behavioural traits in common with one another, this explanation does seem to be possible. For example, the leaf-swallowing habit of bears is reported to occur in Eurasia and North America (see Huffman, 1997).

Future studies and directions of research

As the present paper has shown, the disciplines of animal behaviour, parasitology, pharmacognosy and ethno-medicine have the unique potential to provide important leads to future sources of nutraceuticals and medicine for the control and/or treatment of parasitosis, together with a number of other interesting possibilities. In addition, a closer look into the manner in which animals use natural plant products in combination with antiparasitic behaviour may provide new insights into alternative strategies for suppressing or slowing down the rate of acquisition of chemo-resistance by these parasites in livestock and human populations living in the tropics.

The next step in this research is to conduct *in vivo* tests to determine direct efficacy in a wide range of parasites, using a number of different host species. Differences in metabolism, drug pharmacokinetics etc. between single-stomached animals and ruminant livestock necessitates screening activities in a broad range of animal species. Protocols for testing herbal preparations against the nematode parasites of economically-important farm animals (both ruminant and single-stomached animals) as a model system would be of great benefit to agricultural sectors around the world.

This multi-disciplinary approach to research, in which biological activity of novel plant-derived compounds acquired from the study of animal behaviour and ethno-medicine are assessed against a whole range of parasite species found across a wide range of hosts, maximizes the

chance of success. Contributions from multiple disciplines and cultural traditions to the study of animal self-medication and ethno-pharmacology can bring about a rich understanding and appreciation of the value of Africa's cultural and biological diversity for the future of its population, as well as science in general. An ultimate objective of this research is to integrate our results into local health care and livestock management systems so that locally-available plants can be properly used to the benefit of all.

Acknowledgements

The author wishes to thank the many collaborators and their institutions around the world for untiring support, intellectual input and friendship. There are too many to name them all here, but I wish to thank in particular Guy Balansard, Mohamedi S. Kalunde, the late Junichiro Itani, Hosey Kayumbo, Mayunga H.H. Nkunya, Koichi Koshimizu, Charles Nshimo, Hajime Ohigashi, Ton Polderman, Shunji Gotoh and the following institutions: Kyoto University; Tanzanian National Parks; Tanzanian Commission for Science and Technology; Tanzania Wildlife Research Institute; Japan Ministry of Education, Science, Culture and Sports; the Wellcome Trust, UK; Louis Pasteur Institute, Kyoto, Japan; the Leakey Foundation. Without them, this paper could not have been written.

References

- Abbiw DK (1990) *Useful Plants of Ghana*. Kew: Intermediate Technology Publications and Royal Botanic Gardens.
- Abebe D (1987) Plants in the health care delivery system of Africa. In *Medicinal and Poisonous Plants of the Tropics*, pp. 79–87 [AJM Leeuwenburg, compiler]. Wageningen: Pudoc Wageningen.
- Adegoke GO & Skura BJ (1994) Nutritional profile and antimicrobial spectrum of the spice *Aframomum daniellii*. *Plant Foods for Human Nutrition* **45**, 175–182.
- Akah PA & Okafor CL (1990) Blood sugar lowering effects of *Vernonia amygdalina* in experimental rabbit model. In *The State of Medicinal Plant Research in Nigeria. Integrative Seminar on Natural Products Research and Development of Plant Based Drugs*, p. 27 [A Sofowora, editor]. Kumasi, Ghana: University of Science and Technology.
- Anderson RM & May RM (1982) *Population Biology of Infectious Diseases*. Berlin: Springer-Verlag.
- Asaka Y, Kubota T & Kulkarni AB (1977) Studies on a bitter principle from *Vernonia anthelmintica*. *Phytochemistry* **16**, 1838–1839.
- Baker M (1996) Fur rubbing: Use of medicinal plants by capuchin monkeys (*Cebus capucinus*). *American Journal of Primatology* **38**, 263–270.
- Balick MJ & Cox PA (1996) *Plants, People, and Culture*. New York: WH Freeman and Co.
- Blum MS (1981) *Chemical Defenses of Arthropods*. New York: Academic Press.
- Boppré M (1978) Chemical communication, plant relationships, and mimicry in the evolution of danaid butterflies. *Applied Experimental Entomology* **24**, 264–277.
- Boppré M (1984) Redefining 'Pharmacophagy'. *Journal of Chemical Ecology* **10**, 1151–1154.
- Brander AAD (1931) *Wild Animals in Central India*. London: Edward Arnold.
- Brower LP (1969) Ecological chemistry. *Scientific American* **22**, 22–29.
- Burkill HM (1985) *The Useful Plants of West Tropical Africa*, vol. 1, 2nd ed. Kew: Royal Botanical Gardens.
- Burton RW (1952) The tiger as fruit eater. *Journal of the Bombay Natural History Society* **50**, 649.
- Caius JF (1940) The pomegranite. *Journal of the Bombay Natural History Society* **42**, 13–37.
- Clark CC (1991) The nest protection hypothesis: the adaptive use of plant secondary compounds by European starlings. In *Bird-parasite Interactions: Ecology, Evolution, and Behaviour*, pp. 205–221 [JE Loye and M Zuk, editors]. Oxford: Oxford University Press.
- Clayton DH & Moore J (1997) *Host-parasite Evolution. General Principles & Avian Models*. Oxford: Oxford University Press.
- Cousins D & Huffman MA (2002) Medicinal properties in the diet of gorillas – an ethnopharmacological evaluation. *African Study Monographs* **23**, 65–89.
- Cowen R (1990) Medicine on the wild side. *Science News* **138**, 280–282.
- Cox FEG (1993) *Modern Parasitology*, 2nd ed. Oxford: Blackwell Scientific Press.
- Dalziel JM (1937) *The Useful Plants of West Tropical Africa. Appendix to Flora of West Tropical Africa* [J Hutchinson and JM Dalziel, editors]. London: Whitefriars Press.
- Dharmakumarsinhji RS (1960) Indian wild boar (*Sus scrofa cristatus* Wagner) feeding on *Boerhavia diffusa* Linn. *Journal of the Bombay Natural History Society* **57**, 654–655.
- Dubois L (1955) Tabernanthe Iboga Baillon. *Bulletin Agricole du Congo Belgique* **46**, 805–829.
- Dupain J, Van Elsacker L, Nell C, Garcia P, Ponce F & Huffman MA (2002) New evidence for leaf swallowing and *Oesophagostomum* infection in bonobos (*Pan paniscus*). *International Journal of Primatology* **23**, 1053–1062.
- Ehrlich P & Raven PH (1964) Butterflies and plants: A study in coevolution. *Evolution* **18**, 586–608.
- Engel C (2002) *Wild Health*. Boston, MA: Houghton Mifflin.
- Etkin NL (1996) Medicinal cuisines: diet and ethnopharmacology. *International Journal of Pharmacology* **34**, 313–326.
- Etkin NL & Ross PJ (1983) Malaria, medicine, and meals: Plant use among the Hausa and its impact on disease. In *The Anthropology of Medicine: From Culture to Method*, pp. 231–259 [L Romanucci-Ross, DE Moerman and LR Tancredi, editors]. New York: Praeger.
- Ewald PW (1994) *Evolution of Infectious Disease*. Oxford: Oxford University Press.
- Feeny P (1976) Plant apparency and chemical defense. *Recent Advances in Phytochemistry* **10**, 1–40.
- Freeland WF (1980) Mangaby (*Cercocebus albigena*) movement patterns in relation to food availability and fecal contamination. *Ecology* **61**, 1297–1303.
- Futuyma DJ & Slatkin M (1983) *Coevolution*. Sunderland, MA: Sinauer Associates Inc.
- Gasquet M, Bamba D, Babadjamian A, Balansard G, Timon-David P & Metzger J (1985) Action amoebicide et anthelmintique du vernolide et de l'hydroxyvernolide isolés des feuilles de *Vernonia colorata* (Willd.) Drake (Amoebicidal and anthelmintic action of vernolide and hydroxyvernolide isolates of the leaves of *Vernonia colorata* (Willd.) Drake). *European Journal of Medical Chemistry* **2**, 111–115.
- Gompper ME & Holyman AM (1993) Grooming with *Trattinnickia* resin: possible pharmaceutical plant use by coatis in Panama. *Journal of Tropical Ecology* **9**, 533–540.
- Grisanzio JA (1992) Fur-bearing pharmacists. *Animals* September–October issue, 26–30.
- Gustavson CR (1977) Comparative and field aspects of learned food aversions. In *Learning Mechanisms in Food Selection*,

- pp. 23–43 [LM Barker, MR Best and M Domjan, editors]. Baylor, TX: Baylor University Press.
- Harborne JB (editor) (1978) *Biochemical Aspects of Plant and Animal Coevolution. Phytochemical Society of Europe Symposia Series* no. 15. London: Academic Press.
- Harrison GP (1968) *Tabernaemontana iboga*: an African narcotic plant of social importance. *Economic Botany* **23**, 174–184.
- Hart BL (1990) Behavioral adaptations to pathogens and parasites: Five strategies. *Neuroscience and Biobehavioral Review* **14**, 273–294.
- Hausfater G & Meade BJ (1982) Alternation of sleeping groves by yellow baboons (*Papio cynocephalus*) as a strategy for parasite avoidance. *Primates* **23**, 287–297.
- Howe HF & Westley LC (1988) *Ecological Relationships of Plants and Animals*. Oxford: Oxford University Press.
- Hubback TB (1939) The two-horned Asiatic rhinoceros (*Dicerorhinus sumatrensis*). *Journal of the Bombay Natural History Society* **40**, 594–617.
- Hubback TB (1941) The Malay Elephant. *Journal of the Bombay Natural History Society* **42**, 483–509.
- Huffman MA (1994) The C.H.I.M.P.P. Group: A multi-disciplinary investigation into the use of medicinal plants by chimpanzees. *Pan Africa News* **1**, 3–5.
- Huffman MA (1997) Self-medication in primates. *Yearbook of Physical Anthropology* **40**, 171–200.
- Huffman MA (2001) Self-medicative behavior in the African Great Apes – an evolutionary perspective into the origins of human traditional medicine. *BioScience* **51**, 651–661.
- Huffman MA & Caton JM (2001) Self-induced increase of gut motility and the control of parasitic infections in wild chimpanzees. *International Journal of Primatology* **22**, 329–346.
- Huffman MA, Gotoh S, Izutsu D, Koshimizu K & Kalunde MS (1993) Further observations on the use of *Vernonia amygdalina* by a wild chimpanzee, its possible effect on parasite load, and its phytochemistry. *African Study Monographs* **14**, 227–240.
- Huffman MA, Gotoh S, Turner LA, Hamai M & Yoshida K (1997) Seasonal trends in intestinal nematode infection and medicinal plant use among chimpanzees in the Mahale Mountains, Tanzania. *Primates* **38**, 111–125.
- Huffman MA, Koshimizu K & Ohigashi H (1996a) Ethnobotany and zoopharmacognosy of *Vernonia amygdalina*, a medicinal plant used by humans and chimpanzees. In *Compositae: Biology & Utilization*, vol. 2, pp. 351–360 [PDS Caligari and DJN Hind, editors]. Kew: The Royal Botanical Gardens.
- Huffman MA, Page JE, Sukhdeo MVK, Gotoh S, Kalunde MS, Chandrasiri T & Towers GHN (1996b). Leaf-swallowing by chimpanzees, a behavioral adaptation for the control of strongyle nematode infections. *International Journal of Primatology* **17**, 475–503.
- Huffman MA & Seifu M (1989) Observations on the illness and consumption of a possibly medicinal plant *Vernonia amygdalina* (Del.), by a wild chimpanzee in the Mahale Mountains National Park, Tanzania. *Primates* **30**, 51–63.
- Huffman MA & Wrangham RW (1994) The diversity of medicinal plant use by chimpanzees in the wild. In *Chimpanzee Cultures*, pp. 129–148 [RW Wrangham, WC McGrew, FB DeWaal and PG Heltne, editors]. Cambridge, MA: Harvard University Press.
- Idani G, Kuroda S, Kano T & Asato R (1994) Flora and vegetation of Wamba Forest, Central Zaire with reference to Bonobo (*Pan paniscus*) foods. *TROPICS* **3**, 309–332.
- Irvine FR (1961) *Woody Plants of Ghana*. London: Oxford University Press.
- Janzen DH (1978) Complications in interpreting the chemical defenses of tree against arboreal plant-eating vertebrates. In *The Ecology of Arboreal Folivores*, pp. 73–84 [GG Montgomery, editor]. Washington, DC: Smithsonian Institution Press.
- Jisaka M, Kawanaka M, Sugiyama H, Takegawa K, Huffman MA, Ohigashi H & Koshimizu K (1992a) Antischistosomal activities of sesquiterpene lactones and steroid glucosides from *Vernonia amygdalina*, possibly used by wild chimpanzees against parasite-related diseases. *Bioscience, Biotechnology and Biochemistry* **56**, 845–846.
- Jisaka M, Ohigashi H, Takagaki T, Nozaki H, Tada T, Hirota M, Irie R, Huffman MA, Nishida T, Kaji M & Koshimizu K (1992b) Bitter steroid glucosides, vernoniosides A1, A2, and A3 and related B1 from a possible medicinal plant *Vernonia amygdalina*, used by wild chimpanzees. *Tetrahedron* **48**, 625–632.
- Jisaka M, Ohigashi H, Takegawa K, Hirota M, Irie R, Huffman MA & Koshimizu K (1993a) Steroid glucosides from *Vernonia amygdalina*, a possible chimpanzee medicinal plant. *Phytochemistry* **34**, 409–413.
- Jisaka M, Ohigashi H, Takegawa K, Huffman MA & Koshimizu K (1993b) Antitumor and antimicrobial activities of bitter sesquiterpene lactones of *Vernonia amygdalina*, a possible medicinal plant used by wild chimpanzees. *Bioscience, Biotechnology and Biochemistry* **57**, 833–834.
- Keymer A, Crompton DWT & Sahakian BJ (1983) Parasite induced learned aversion involving *Nippostrongylus* in rats. *Parasitology* **86**, 455–460.
- Kloos H & McCullough FS (1987) Plants with recognized molluscicidal activity. In *Plant Molluscicides*, pp. 45–108 [KE Mott, editor]. New York: John Wiley & Sons Ltd.
- Kokwaro JO (1976) *Medicinal Plants of East Africa*. Nairobi, Kenya: General Printers Ltd.
- Koshimizu K, Ohigashi H, Huffman MA, Nishida T & Takasaki H (1993) Physiological activities and the active constituents of potentially medicinal plants used by wild chimpanzees of the Mahale Mountains, Tanzania. *International Journal of Primatology* **14**, 345–356.
- Kupchan SM, Hemingway RJ, Karim A & Werner D (1969) Tumor inhibitors XLVII. Vernodaline and vernomygdin, two new cytotoxic sesquiterpene lactones from *Vernonia amygdalina* Del. *Journal of Organic Chemistry* **34**, 3908–3911.
- Kyriazakis I, Oldham JD, Coop RL & Jackson F (1994) The effect of subclinical intestinal nematode infection on the diet selection of growing sheep. *British Journal of Nutrition* **72**, 665–677.
- McCann C (1932) A cure for tapeworm. *Journal of the Bombay Natural History Society* **36**, 282–284.
- Messner EJ & Wrangham RW (1996) *In vitro* testing of the biological activity of *Rubia cordifolia* leaves on primate *Strongyloide* species. *Primates* **37**, 105–108.
- Moore M (1979) *Medicinal Plants of the Mountain West*. Santa Fe, NM: Museum of New Mexico Press.
- Moutsamboté J, Yumoto T, Mitani M, Nishihara T, Suzuki S & Kuroda S (1994) Vegetation and list of plant species identified in the Nouabalé-Ndoki Forest, Congo. *TROPICS* **3**, 277–293.
- Muanza DN, Dangala NL & Mpay O (1993) Zairean medicinal plants as diarrhea remedies and their antibacterial activities. *African Study Monographs* **14**, 53–63.
- Murakami A, Ohigashi H & Koshimizu K (1994) Possible anti-tumor promoting properties of traditional Thai foods and some of their active constituents. *Asia Pacific Journal of Clinical Nutrition* **3**, 185–191.
- Murakami A, Ohigashi H & Koshimizu K (1996) Anti-tumor promotion with food phytochemicals: A strategy for cancer chemoprevention. *Bioscience, Biotechnology and Biochemistry* **60**, 1–8.
- Nelson GS (1960) Schistosome infections as zoonoses in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **54**, 301–314.
- Neuwinger HD (1996) *African Ethnobotany: Chemistry, Pharmacology, Toxicology*. London: Chapman & Hill.
- Nfi A, Ndi C, Bayemi PH, Njwe R, Tchoumboue J, Njakoi H, Mopoi, N, Njakoi M & Django S (1999) The anthelmintic efficacy of some indigenous plants in the Northwest province of Cameroon. *Revue D Elevage et de Medecine Veterinaire des Pays Tropicaux* **52**, 103–106.
- Nishida T & Uehara S (1983) Natural diet of chimpanzees (*Pan troglodytes schweinfurthii*): Long term record from the Mahale Mountains, Tanzania. *African Study Monographs* **3**, 109–130.
- Nyazema NZ (1987) Medicinal plants of wide use in Zimbabwe. In *Medicinal and Poisonous Plants of the Tropics*, pp. 36–43 [AJM Leeuwenburg, compiler]. Wageningen: Pudoc Wageningen.
- Ogilvie GH (1929) Bison eating bark. *Journal of the Bombay Natural History Society* **33**, 706–707.
- Ohigashi H (1995) Plants used medicinally by primates in the wild and their physiologically active constituents. *Report to the Japanese Ministry of Science, Education and Culture for 1994 Grant-in-Aid for Scientific Research* (No. 06303012). Tokyo: Japanese Ministry of Science, Education and Culture.
- Ohigashi H, Huffman MA, Izutsu D, Koshimizu K, Kawanaka M, Sugiyama H, Kirby GC, Warhurst DC, Allen D, Wright CW, Phillipson JD, Timmon-David P, Delmas F, Elias R & Balansard G (1994) Toward the chemical ecology of medicinal plant-use in chimpanzees: The case of *Vernonia amygdalina* Del. A plant used by wild chimpanzees possibly for parasite-related diseases. *Journal of Chemical Ecology* **20**, 541–553.
- Ohigashi H, Jisaka M, Takagaki T, Nozaki H, Tada T, Huffman MA, Nishida T, Kaji M & Koshimizu K (1991) Bitter principle and a related steroid glucoside from *Vernonia amygdalina*, a possible medicinal plant for wild chimpanzees. *Agricultural and Biological Chemistry* **55**, 1201–1203.
- Ohigashi H, Sakai Y, Yamaguchi K, Umezaki I & Koshimizu K (1992) Possible anti-tumor promoting properties of marine algae and in vitro activity of wakame seaweed extract. *Bioscience, Biotechnology and Biochemistry* **56**, 994–995.
- Okunji CO & Iwu MM (1988) Control of schistosomiasis using Nigerian medicinal molluscicides. *International Journal of Crude Drug Research* **26**, 246–252.
- Oloke JK, Kolawole DO & Erhun WO (1988) The antibacterial and antifungal activities of certain components of *Afromomum melegueta* fruits. *Fitoterapia* **LIX**, 384–388.
- Page JE, Balza FF, Nishida T & Towers GHN (1992) Biologically active diterpenes from *Aspilia mossambicensis*, a chimpanzee medicinal plant. *Phytochemistry* **31**, 3437–3439.
- Page JE, Huffman MA, Smith V & Towers GHN (1997). Chemical basis for medicinal consumption of *Aspilia* (Asteraceae) leaves by chimpanzees: a re-analysis. *Journal of Chemical Ecology* **23**, 2211–2225.
- Palgrave KC (1983) *Trees of Southern Africa*. 2nd revised ed. Cape Town, South Africa: Struik Publisher.
- Phillips R (1981) *Mushrooms and Other Fungi of Great Britain and Europe*. London: Pan.
- Rausch R (1954) Studies on the helminth fauna of Alaska. XXI. Taxonomy, morphological variation, and ecology of *Diphyllobothrium ursi* n. sp. provis. on Kodiak Island. *Journal of Parasitology* **40**, 540–563.
- Rausch R (1961) Notes on the black bear, *Ursus americanus* Pallas, in Alaska with particular reference to dentition and growth. *Zeitschrift für Säugetierk* **26**, 77–107.
- Riesenberg SH (1948) Magic and medicine in Ponape. *Southwest Journal of Anthropology* **4**, 406–429.
- Rodriguez E, Aregullin M, Nishida T, Uehara S, Wrangham R, Abramowski Z, Finlayson A & Towers GHN (1985) Thiarubrine A, a bioactive constituent of *Aspilia* (Asteraceae) consumed by wild chimpanzees. *Experientia* **41**, 419–420.
- Rodriguez E & Wrangham RW (1993) Zoopharmacognosy: the use of medicinal plants by animals. In *Recent Advances in Phytochemistry*. vol. 27, *Phytochemical Potential of Tropic Plants*, pp. 89–105 [KR Downum, JT Romeo and H. Stafford, editors]. New York: Plenum Press.
- Sengputa S (1981) Adaptive significance of the use of margosa leaves in nests of house sparrows *Passer domesticus*. *Emu* **81**, 114–115.
- Smolenski SJ, Silinis H & Farnsworth NR (1974) Alkaloid screening. V. *Lloydia* **37**, 506–536.
- Sugiyama Y & Koman J (1992) The flora of Bossou: Its utilization by chimpanzees and humans. *African Studies Monographs* **13**, 127–169.
- Swain T (1978) Plant-animal coevolution: A synoptic view of the Paleozoic and Mesozoic. In *Biochemical Aspects of Plant and Animal Coevolution. Phytochemical Society of Europe Symposia Series* no. 15, pp. 3–19 [JB Harbourn, editor]. London: Academic Press.
- Toft CA, Aeschlimann A & Bolis L (1991) *Parasite-Host Associations; Coexistence or Conflict?* Oxford: Oxford Science Publications.
- Toubiana R & Gaudemer A (1967) Structure du vernolide, nouvel ester sesquiterpique isolé de *Vernonia colorata* (Structure of vernolide, new sesquiterpene ester isolate of *Vernonia colorata*). *Tetrahedron Letters* **14**, 1333–1336.
- Tutin CEG, White LJT, Williamson EA, Fernandez M & McPherson G (1994) List of plant species identified in the northern part of the Lopé Reserve, Gabon. *TROPICS* **3**, 249–276.
- Wakelin D (1996) *Immunity to Parasites – How Parasitic Infections are Controlled*. 2nd ed. Cambridge: Cambridge University Press.
- Waterman PG (1984) Food acquisition and processing as a function of plant chemistry. In *Food Acquisition and Processing in Primates*, pp. 177–211 [DJ Chivers, BNA Wood and A Bilsborough, editors]. New York: Plenum Press.
- Watt JM & Breyer-Brandwinjk MG (1962) *The Medicinal and Poisonous Plants of Southern and East Africa*. Edinburgh: E. and S. Livingstone Ltd.
- Wilson E (1962) *Aubrey's Brief Lives*, p. xix [OL Dick, editor]. Ann Arbor, MI: Ann Arbor Paperbacks.
- Wink M, Hofer A, Billfinger M, Englert E, Martin M & Schneider D (1993) Geese and dietary allelochemicals – food palatability and geophagy. *Chemoeology* **4**, 93–107.
- Wrangham RW (1995) Relationship of chimpanzee leaf-swallowing to a tapeworm infection. *American Journal of Primatology* **37**, 297–303.
- Wrangham RW & Goodall J (1989) Chimpanzee use of medicinal leaves. In *Understanding Chimpanzees*, pp. 22–37 [PG Heltne and LA Marquardt, editors]. Cambridge, MA: Harvard University Press.
- Wrangham RW & Nishida T (1983) *Aspilia* spp. leaves: A puzzle in the feeding behavior of wild chimpanzees. *Primates* **24**, 276–282.
- Wrangham RM, Rogers ME & Isabiry-Basuta G (1993) Ape food density in the ground layer in Kibale Forest, Uganda. *African Journal of Ecology* **31**, 49–57.
- Yumoto T, Yamagiwa J, Mwanza N & Maruhashi T (1994) List of plant species identified in Kahuzi-Biega National Park, Zaire. *TROPICS* **3**, 295–308.

Michael A. Huffman · Satoshi Hirata

An experimental study of leaf swallowing in captive chimpanzees: insights into the origin of a self-medicative behavior and the role of social learning

Received: 8 August 2002 / Accepted: 17 October 2003 / Published online: 23 January 2004
© Japan Monkey Centre and Springer-Verlag 2004

Abstract Chimpanzees in the wild swallow the rough hispid leaves of certain plant species as a means of physically expelling intestinal parasites. A plant with such a leaf texture was introduced in 36 trial sessions to a captive group of 11 healthy adult chimpanzees to investigate the possible origin and acquisition of leaf swallowing behavior. One male (housed separately from the group during testing) and one female, both captive born, spontaneously exhibited the behavior on their first trial without prior opportunity to observe others with this plant. Six other chimpanzees on their first trial displayed a phobic response to these leaves and rejected them entirely, while another two chewed and swallowed the leaves in a normal way. Four individuals eventually exhibited the behavior, after having approached and closely observed the leaf swallowing of the first female to exhibit the behavior in the group. Four of the six individuals that initially avoided the leaves never overcame their phobia toward this plant and were not in proximity to a chimpanzee performing leaf swallowing during test sessions. Individuals born to wild chimpanzee mothers were no more likely to perform the behavior than captive-reared group mates. These results suggest that the acquisition of this behavior is based in part on a propensity to fold and swallow rough, hispid leaves, but that the acquisition and spread of leaf swallowing within a group is likely to be socially influenced. This study provides support for the hypothesis that leaf swallowing originated in the

wild from opportunistic feeding behavior and was later passed down in the form of a self-medicative behavioral tradition.

Keywords Feeding · Social tolerance · Behavioral tradition · Self-medication

Introduction

Attention was first brought to leaf swallowing behavior by Wrangham and Nishida (1983) when they pointed out the significance of this ingestive behavior as unlikely to be that of providing any nutritional value. They noticed a pattern for the occurrence of folded, undigested leaves of *Aspilia* species in the dung of chimpanzees at both Gombe and Mahale. Currently, leaf swallowing is documented in the greatest detail in chimpanzees at four study sites in East Africa (Mahale, Gombe, Kibale, and Budongo), where its use is associated with the expulsion of adult intestinal nematodes and or cestode proglottids (Wrangham 1995; Huffman et al. 1996; Huffman and Caton 2001). Leaf swallowing has since been noted to occur in at least 22 social groups of chimpanzees, bonobos, and lowland gorillas at 13 study sites across Africa (Huffman 2001). The 34 different plant species selected at these sites vary in life form (herb, vine, shrub, and tree), but they all share the common property of being rough and hispid (Huffman 1997).

The widespread occurrence of leaf swallowing and this universal criterion for selecting rough, bristly leaves suggests a behavioral predisposition for leaf swallowing in all African great apes. There is also evidence to suggest that transmission within the group occurs at least in part by some form of social learning (Huffman and Hirata 2003). Observations from the wild suggest that individuals' first exposure to leaf swallowing and other forms of self-medicative behavior occurs at an early age, not when ill themselves, but by observing the behavior of close family members or associates that are ill.

Electronic Supplementary Material Supplementary material is available in the online version of this article at <http://dx.doi.org/10.1007/s10329-003-0065-5>

M. A. Huffman (✉)
Primate Research Institute, Kyoto University,
41-2 Kanrin, Inuyama, Aichi 484-8506, Japan
E-mail: huffman@pri.kyoto-u.ac.jp

S. Hirata
Great Ape Research Institute,
Hayashibara Biochemical Laboratories, Inc., Okayama, Japan

Chimpanzees as young as 1 year of age closely watch this behavior and have been seen to attempt immediately thereafter to perform it on their own with varied success, regardless of health condition (Huffman and Seifu 1989; Huffman and Wrangham 1994). At this stage, it is most likely treated as one more element of the mother's foraging repertoire that engages the infant's interest.

We do not expect that the behavior is first acquired because of any understanding of its self-medicative function, rather, that the self-medicative aspects of use are likely to be learned over time if the appropriate selective forces (e.g. parasite infection) are present in the habitat in question. A combination of learning the context of use from watching others and positive feedback from any perceived personal relief from physical discomfort that leaf swallowing may assist in providing the ingestor is predicted to occur. Presently, the details of this underlying mechanism and how long it takes to acquire the behavior in its self-medicative context is unclear. It is extremely difficult to collect such evidence from the field given that these traditions appear to be already well established in all groups where leaf swallowing is known to occur (Huffman 2001). Furthermore, as the behavior itself occurs at low frequencies in the wild due to strong seasonality linked with periods of parasite reinfection (Huffman et al. 1996; Huffman 1997), the acquisition process of the behavior by new individuals is difficult to follow precisely under natural conditions.

There is a range of possibilities for how leaf swallowing behavior started and how individuals come to swallow leaves instead of simply chewing them. At one extreme, animals may have an innate tendency to select appropriate properties in plants when ill, so that the role of social context is local enhancement. That is, naive individuals may have their attention drawn to areas with plant species used by others (Huffman and Wrangham 1994). At the other extreme, animals may have a propensity to swallow certain items without chewing, but due to unfamiliarity with rough bristly leaves in their daily diet, must somehow learn that such leaves can be ingested before they are able to learn about any possible benefit from doing so. Thus, initial acquisition by young or naive individuals, like the proposed origin of this behavior itself, may occur in the context of opportunistic

feeding behavior that is later molded over time through further individual experience and association of its practice with illness in other group members. To elucidate some of these important questions regarding the intrinsic nature and acquisition process of leaf swallowing behavior, we conducted a study on a socially living group of captive chimpanzees.

Methods

The study was conducted between 9 October and 11 November 1997 on a group of 11 (3 males, 8 females) adult chimpanzees used in cognitive studies at Kyoto University's Primate Research Institute in Inuyama, Japan (Table 1). Among the 6 individuals born in Africa, all but 2 of the oldest (Gon, Puchi) were brought into captivity and reared from the age of 1 year. The remaining 5 individuals were captive born and or human reared in Japan or Europe. The veterinary staff routinely examines the chimpanzees. None of the subjects displayed symptoms of parasite infection during the study period.

From 1996 up to the time this study was conducted in 1997, the chimpanzees had daily access to an environmentally enriched outdoor enclosure containing 63 species (390 individual plants) of trees and shrubs, a flowing stream, and an 8-m-high multi-tiered tower (Ochiai and Matsuzawa 1998, 1999). Prior to the experiments described below, their exposure to plants in the outdoor compound was limited to these 63 known species and a few local grass species that spontaneously took root. None of the plants in the compound resembled the texture of plants used for leaf swallowing by chimpanzees in the wild.

The test plant species used in this study, *Helianthus tuberosus* (Compositae), was selected because the leaves are virtually identical in texture to species such as *Aspilia mossambicensis*, *Lippia plicata*, and *Ficus exasperata* used by chimpanzees at Mahale, Gombe, and elsewhere. No member of the study group had been exposed to this or other species resembling it in leaf texture prior to testing. *H. tuberosus* grows semi-wild between June and November along roadsides, in open lots, and in some gardens in the neighborhood of the Institute. Fresh branches were collected within 20 min prior to testing and placed in water to maintain their freshness. Native to North America, *H. tuberosus* is a nontoxic plant whose leaf and stem are often used as cattle fodder. Introduced to Japan over 100 years ago, the tubers are a delicacy consumed by people in some rural areas of the country. The plant is known as "kiku imo" in Japanese and "earth apple" in English.

In total, 36 trial sessions were conducted on the chimpanzee colony, with a minimum of 3 sessions per individual. A branch of 29–40 cm in length with 18–40 leaves and occasionally 1–8 flowers was given to an individual, selected randomly over the study period. It was possible to call an individual chimpanzee to the wall of the enclosure and drop a branch down to it from the observation

Table 1 Leaf swallowing test subjects at the Primate Research Institute (PRI)

Name	Sex	Age ^a	Arrived at PRI (age at arrival)	Origin (date of birth)
Gon	M	31	30.1.79 (12)	West Africa, pet in Japan (1966, month unknown)
Puchi	F	31	30.1.79 (12)	West Africa, pet in Japan (1966, month unknown)
Reiko	F	31	3.7.68 (1.6)	West Africa (December 1966)
Mari	F	21	30.1.78 (1.5)	West Africa (June 1976)
Akira	M	21	30.1.78 (1.5)	West Africa (June 1976)
Ai	F	21	10.11.77 (1)	West Africa (October 1976)
Pendesa	F	20	12.11.79 (2.7)	Japan Monkey Centre (2 February 1977)
Chloe	F	16	28.1.85 (5)	Paris Zoo (13 December 1980)
Popo	F	15		PRI ^b (7 March 1982) daughter of Puchi–Gon
Reo	M	15		PRI ^b (18 May 1982) son of Reiko–Gon
Pan	F	13		PRI ^b (7 December 1983) daughter of Puchi–Gon

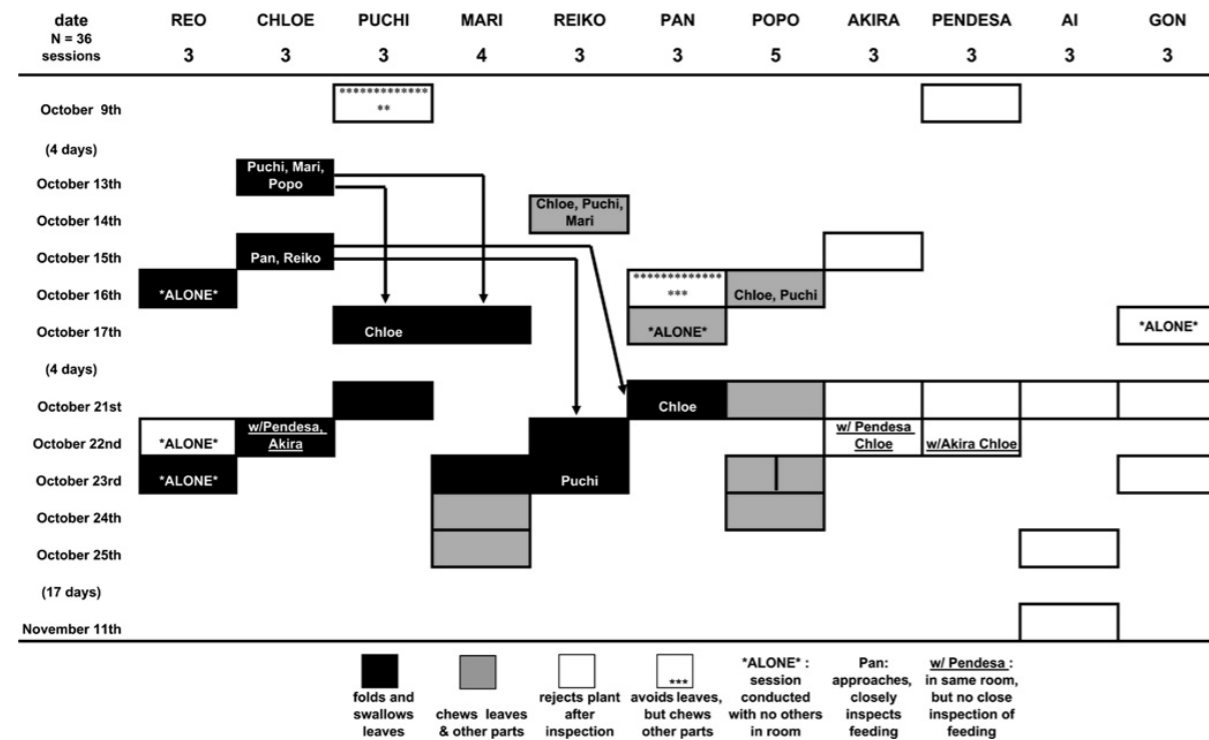
^a Age at the time of experiments in October 1997

^b Artificial insemination, hand reared

point. Normally tests were conducted on individuals in a social group setting in the outdoor enclosure ($n=28$) with all or most members present. Three test sessions, however, were conducted with a sub-group of 3 individuals in a smaller holding room; one plant was introduced and the 3 interacted consecutively with the same plant. During the entire study period, adult male Reo was kept in a separate enclosure from the main group during the day, with occasional access to his mother or other female companions. During testing Reo was always alone in all 3 of his trial sessions. Due to the physical separation of his enclosure from the main outdoor enclosure, Reo had no opportunity to see other individuals during their sessions and vice versa. The old adult male Gon and adult female Pan were also tested alone on one occasion each. None of the subjects had access to the test plant species outside of these trial sessions.

Observations were recorded by the authors using 8-mm video and by handwritten notes. All feeding behaviors and behavioral interactions between the focal animal and others in the group were recorded. To obtain complete behavioral sequences of a subject's response to the plant material and interactions with others, observations were continued for 5 min after the focal individual no longer possessed test plant material. There was no stealing or disruption by others while the plant was in the possession of the focal subject. Because it was impossible to retrieve a plant from the enclosure if it was abandoned or retrieved by another, the behavior of the new possessor and interactions with all others was recorded as a new trial session. In eight cases, an onlooker picked up all of the remaining plant material after the original focal subject freely discarded it. Because of this, no plant material was left in the enclosure after the end of the final trial session of any given day.

Fig. 1 Observed responses of chimpanzees in trial sessions with novel plant stimuli (*Helianthus tuberosus* L.). Solid lines connecting boxes denote putative social learning network between Chloe (leaf swallowing "demonstrator") and individuals that first displayed leaf swallowing behavior in subsequent trial sessions



To independently verify our assessment of leaf swallowing in the trial sessions, individuals were separated from the rest of the group when they were brought inside for the night and put singly into a night sleeping room when day-to-day management and ongoing research protocol allowed. The following morning, after these individuals were rejoined with the group, feces in their sleeping rooms were inspected.

Results

Verification of leaf swallowing behavior

Folding and swallowing of leaves became the major mode of ingestion for 6 of the 11 individuals at some point during the study (Fig. 1). The behavior was similar to that observed for chimpanzees in the wild (LS-Wild ESM1, LS-Chloe ESM2), supporting the notion of a species-wide propensity to perform this behavior in chimpanzees. Leaves were deliberately folded, using a combination of lips, palate, and tongue, while a leaf still attached to the stalk was slowly drawn into the mouth. The behavior was distinct from normal feeding as chewing action was overtly absent and the leaves were deliberately put into the mouth one at a time. Of those 6 individuals that leaf swallowed, there was no significant difference in the number of African born (3/6) versus captive born (3/5) subjects (Fisher's exact test, two-tailed, $P=1.000$, NS) to exhibit this behavior.

Results of the 11 next-morning follow-up dung inspections are presented in Table 2. All individuals selected for the follow-up inspections were observed

Table 2 Results of dung inspection the day after individual observation sessions. Underlined subjects were observed to swallow leaves during the trial session

u>

Date	Subject	Parts ingested the previous day	Contents of dung
10 October	Puchi	Stalk, flowers	No leaf trace
14 October	<u>Chloe</u>	Leaves, flowers	3 folded leaves
15 October	<u>Reiko</u>	Leaves, flowers	No leaf trace
16 October	<u>Chloe</u>	Leaves, flowers	10 leaves
18 October	<u>Puchi</u>	Stalk, leaves, flowers	2 leaf fragments
18 October	<u>Mari</u>	Flowers, leaves	No trace
22 October	<u>Pan</u>	Stalk, leaves	1 leaf
22 October	<u>Puchi</u>	Stalk bark, leaves, flowers	No leaf trace
23 October	<u>Reiko</u>	Stalk bark, leaves, flowers	6 leaves
24 October	<u>Mari</u>	Stalk, leaves	No leaf trace
24 October	<u>Reiko</u>	Stalk, leaves	6 leaves

to have ingested some or all parts of the plant during the test session in question. Eight of these cases were of individuals directly observed to exhibit leaf-swallowing-like behavior during the session. The following morning, in 6 of these 8 cases, dung was found to contain one to six folded, nondigested *H. tuberosus* leaves. No whole or partial undigested leaves were found in the 3 cases in which individuals were only observed to chew leaves and or other plant parts.

Individuals' first response to rough hispid leaves

The first response to the leaves varied from individual to individual. Chloe and Reo, two captive-born individuals, spontaneously displayed leaf-swallowing-like behavior in their first session without the benefit of observing it in other group members. Chloe consistently displayed this pattern as her major mode of ingestion during all three of her test sessions. Mari also folded and swallowed a portion of the leaves on the branch given to her on her first (and second) session. She did so after having closely observed Chloe fold and swallow leaves 4 days earlier (Fig. 1).

The typical first response to the plant for six other individuals was an almost phobic rejection of the rough hispid leaves (Akira, Pendesa, Ai, Gon, Puchi, and Pan). Four of these individuals consistently rejected the entire plant throughout the study, while the other two discarded all of the leaves but ate other plant parts (Pan, Puchi). In contrast, two other individuals, (Reiko and Popo) chewed and ate the leaves in all of her subsequent sessions. She displayed no aversion whatsoever, rapidly consuming everything (Fig. 1).

Leaf swallowing and social interactions among individuals during the trial session

Leaf swallowing was observed in 10 of the 31 trial sessions conducted in a group setting. Individuals in possession of *H. tuberosus* attracted the attention of others in 5 of these sessions (Fig. 1). Puchi, Mari, Reiko, Pan, and Popo approached within close proximity (< 1 m) to, and intently inspected, Chloe while she exhibited leaf

swallowing during her 1st and 2nd trial sessions. This group of females also approached and closely inspected one another when leaves and other parts were being eaten (Fig. 1), showing their mutually tolerant social relationships. With the exception of Popo, these females all subsequently exhibited leaf swallowing behavior themselves for the first time 2–9 days after having observed Chloe perform leaf swallowing behavior. During this study, Chloe was the first and only possible model of leaf swallowing for these four females (Fig. 1). Chloe approached closely and intently watched the first instance of leaf swallowing exhibited by Reiko and Pan.

Due to other ongoing research Ai and Pendesa were frequently absent when group-setting test sessions were conducted in the outdoor enclosures. During ten sessions outdoors in which leaf swallowing was observed, Ai was absent during seven and Pendesa during six sessions. Akira, Gon, Ai, and Pendesa were never observed to approach and inspect the feeding behavior of others in the possession of *H. tuberosus*, whether they were leaf swallowing or chewing leaves or other parts of the plant. These four individuals consistently rejected *H. tuberosus* in all of their test sessions.

Discussion

Possible modes of acquisition and the origin of leaf swallowing

This is the first ever attempt to investigate leaf swallowing under semi-controlled conditions. Bearing in mind the limited sample size and management restraints on observational procedure, some basic aspects of the nature of leaf swallowing and insights into the possible modes of acquisition of this behavior by chimpanzees can be considered. The spontaneous performance of leaf swallowing by two individuals during their first trial, in the absence of a leaf swallowing "demonstrator," shows that chimpanzees may have a propensity to fold and swallow rough hispid leaves. This supports evidence from the wild that the behavior can emerge without inter-group social contact in geographically isolated populations or subspecies (sec. Huffman and Hirata 2003). However, unlike their counterparts in the wild that often exhibit this behavior when ill and/or infected with

parasites (Wrangham 1995; Huffman et al. 1996), these captive individuals were parasite free and showed no visible signs of ill health when they performed the behavior. From this we conclude that folding and swallowing of leaves is not an innate response to parasite infection. Indeed, the initial response for more than half of the subjects was a phobic avoidance of these leaves or the entire plant. Our results support the current hypothesis regarding the origin of leaf swallowing behavior (Huffman and Wrangham 1994), which proposes it may have originated in the feeding context of a novel plant item. It is suggested here that the leaf's rough hispid texture encourages the peculiar mode of ingestion, that is, folding and swallowing leaves whole.

Our results also support observations in the wild that the acquisition and spread of leaf swallowing within a group is at least in part socially influenced, rather than being solely the product of individual trial-and-error learning. It is clear that the behavior was not acquired by every individual in this study via the same mode of learning. Excluding Chloe and Reo, who seemingly acquired leaf swallowing behavior spontaneously, the other individuals that later exhibited the behavior may indeed have used multiple modes of learning over the period of acquisition (see Visalberghi and Frigaszy 1990). Among the individuals that eventually exhibited the behavior during our study, social tolerance among them in the feeding context (see van Schaik et al. 1999) is considered to have been an important facilitator of exposure to leaf swallowing, and thus opportunities for social learning. As suggested for object manipulation in Japanese macaques (Huffman and Quiatt 1986) and foraging-related tool-use acquisition in orangutans (van Schaik 2003), here too, interpersonal social networks and social tolerance could be important factors in the transmission of leaf swallowing behavior. This is further supported by the fact that those individuals that never approached and observed another chimpanzee leaf swallowing never overcame their phobia of the plant's rough, hispid leaves. All individuals that eventually exhibited leaf swallowing behavior did so only after having seen the behavior being exhibited by Chloe, the first individual in the social group observed to display folding and swallowing of *H. tuberosus* leaves spontaneously.

Future studies

Controlled studies of self-medicative behavior in parasitized organisms are rare and limited to mice (Vitazkova et al. 2001) and invertebrates (Karban and English-Loeb 1997). There are obvious ethical reasons why we chose not to conduct a controlled study on chimpanzees in the context of parasite infection. We have shown here that there are important things to be learned without such invasive methods. Given the fact that bonobos and lowland gorillas too are known to habitually exhibit leaf swallowing behavior in the wild (Huffman 1997), the

propensity for folding and swallowing of rough hispid leaves is expected to be a shared trait of all African great apes. It follows then that the self-medicative function of leaf swallowing behavior must be a secondary adaptation of a feeding response brought about by leaf texture, raising the question as to how the self-medicative context of leaf swallowing emerged independently across Africa.

Further refined, noninvasive testing of captive chimpanzee groups naive to rough hispid leaves is being planned. Similar controlled and noninvasive studies regarding the acquisition process of leaf swallowing and other self-medication in the African great apes and other primates is strongly encouraged. Greater attention should be paid to the social networks of group individuals to grasp greater detail of the possible mode(s) of social learning involved in the transmission of such behaviors within the group (e.g. White and Burgman 1990; Ginsberg and Young 1992; Coussi-Korbel and Frigaszy 1995; Whitehead 1997). Such studies are expected to increase our understanding of the possible origins of self-medicative behaviors and the role social learning may play in their maintenance in nature.

Acknowledgements We wish to give our sincere thanks to Testuro Matsuzawa, Professor of the Section of Language and Intelligence (PRI) and Professor Iver Iversen of Northern Florida State University, for their encouragement and support in conducting this study. We are indebted to the primate health care staff of the Center for Human Evolution Modeling Research for their assistance and day-to-day care of the chimpanzees. The present research was partially financed by Grants 07102010, 12301006, and 10CE2005 from the Ministry of Education, Science, Sports, and Culture, Japan. We thank our anonymous referees and Jean-Baptiste Leca for their insightful comments and criticisms of the manuscript in its final stages. Last but not least, we thank the chimpanzees for their patience and cooperation, without which this study would not have been possible.

References

- Coussi-Korbel S, Frigaszy DM (1995) On the relationship between social dynamics and social learning. *Anim Behav* 50:1441–1453
- Ginsberg JR, Young TP (1992) Measuring association between individuals or groups in behavioural studies. *Anim Behav* 44:377–379
- Huffman MA (1997) Current evidence for self-medication in primates: a multidisciplinary perspective. *Yearb Phys Anthropol* 40:171–200
- Huffman MA (2001) Self-medicative behavior in the African great apes: an evolutionary perspective into the origins of human traditional medicine. *BioScience* 51:651–661
- Huffman MA, Caton JM (2001) Self-induced increase of gut motility and the control of parasitic infections in wild chimpanzees. *Int J Primatol* 22:329–346
- Huffman MA, Hirata S (2003) Biological and ecological foundations of primate behavioral traditions. In: Frigaszy DM, Perry S (eds) *The biology of tradition: models and evidence*. Cambridge University Press, Cambridge, pp 267–296
- Huffman MA, Quiatt D (1986) Stone handling by Japanese macaques (*Macaca fuscata*): implications for tool use of stone. *Primates* 27:413–423
- Huffman MA, Seifu M (1989) Observations on the illness and consumption of a medicinal plant *Vernonia amygdalina* by a wild chimpanzee in the Mahale Mountains, Tanzania. *Primates* 30:51–63

- Huffman MA, Wrangham RW (1994) Diversity of medicinal plant use by chimpanzees in the wild. In: Wrangham RW, McGrew WC, deWaal FB, Heltne PG (eds) *Chimpanzee cultures*. Harvard University Press, Cambridge, Mass., pp 129–148
- Huffman MA, Page JE, Sukhdeo MVK, Gotoh S, Kalunde MS, Chandrasiri T, Towers GHN (1996) Leaf-swallowing by chimpanzees: a behavioral adaptation for the control of strongyle nematode infections. *Int J Primatol* 72:475–503
- Karban R, English-Loeb G (1997) Tachinid parasitoids affect host plant choice by caterpillars to increase caterpillar survival. *Ecology* 78:603–611
- Ochiai T, Matsuzawa T (1998) Planting trees in an outdoor compound of chimpanzees for an enriched environment. In: Hare VL, Worley KE (eds) *Proceedings of the third international conference on environmental enrichment. The Shape of Enrichment*, San Diego, Calif., pp 355–364
- Ochiai T, Matsuzawa T (1999) Environmental enrichment for captive chimpanzees (*Pan troglodytes*): introduction of climbing frames 15 m high. *Primate Res* 15:289–296
- Schaik CP van (2003) Local traditions in orangutan and chimpanzees: social learning and social tolerance. In: Frigaszy DM,

- Perry S (eds) *The biology of tradition: models and evidence*. University of Cambridge Press, Cambridge, pp 297–328
- Schaik CP van, Deaner RO, Merrill MY (1999) The conditions for tool use in primates: implications for the evolution of material culture. *J Hum Evol* 36:719–741
- Visalberghi E, Frigaszy DM (1990) Food washing behaviour in tufted capuchin monkeys, *Cebus apella*, and crab eating macaques, *Macaca fuscicularis*. *Anim Behav* 40:829–836
- Vitazkova SK, Long E, Paul A, Glendinning JI (2001) Mice suppress malaria infection by sampling 'bitter' chemotherapy agent. *Anim Behav* 61:887–894
- White FJ, Burgman MA (1990) Social organization of the pygmy chimpanzee (*Pan paniscus*): multivariate analysis of intracommunity associations. *Am J Phys Anthropol* 83:193–201
- Whitehead H (1997) Analysing animal social structure. *Animal Behav* 53:1053–1067
- Wrangham RW (1995) Relationship between chimpanzee leaf swallowing to a tapeworm infection. *Am J Primatol* 37:297–303
- Wrangham RW, Nishida T (1983) *Aspilota* spp. leaves: a puzzle in the feeding behavior of wild chimpanzees. *Primates* 24:276–282

Descriptive urological record of chimpanzees (*Pan troglodytes*) in the wild and limitations associated with using multi-reagent dipstick test strips

Kaur T, Huffman MA. Descriptive urological record of chimpanzees (*Pan troglodytes*) in the wild and limitations associated with using multi-reagent dipstick test strips. J Med Primatol 2004; 33:187–196. Blackwell Munksgaard, 2004

Abstract: Ten urine chemistry parameters were measured on 74 voided urine samples from 34 wild chimpanzees (*Pan troglodytes*). Multi-reagent urine dipstick tests were performed and results determined using colorimetric scales. Urine pH measured between 8 and 9 units in 91% of the chimpanzees. Test pads detected protein, erythrocytes, leukocyte esterase activity, and nitrites, ketones and bilirubin in 47, 32, 29, and <10% of the chimpanzees, respectively. No apparent association between positive test results for blood in adult females and reproductive status was found. Overall, 17 of the 34 chimpanzees had positive urine test results for protein, hemoglobin, erythrocytes, leukocytes, nitrites, ketones, and/or bilirubin. Dipstick urinalysis alone is an unreliable method for assessing health and physiological status of wild chimpanzees. However, if combined with other diagnostics it could prove to be a valuable health-monitoring tool. Limitations associated with this methodology need to be considered when interpreting urinary dipstick test results.

Introduction

In both human and veterinary medicine, it is not uncommon to use rapid urine screening tests to help assess the health and physiological status of a patient. Rapid urine screening can be performed using a variety of methodologies, including microscopic, enzymatic, filtration, and automated procedures [19]. They can provide a simple, quick, and relatively inexpensive method to measure certain indicators of health and disease [17, 19, 24]. For example, rapid urine screens are used to detect indicators of urinary tract infection (UTI) (i.e. leukocytes and nitrites), measure urine-specific

gravity and protein, and identify physiological conditions such as glucosuria and ketonuria.

The presence of bacteria in a urine specimen can occur from an infection or from colonization and contamination of the urine sample [19]. The enzyme nitrate reductase present in Gram-negative bacteria converts nitrates to nitrites. Enzymatic urine dipstick screens for the detection of nitrites has been reported to be highly specific (98%), yet not a very sensitive (<45%) indicator for the presence of Gram-negative bacteria [8]. In a study conducted in a human emergency department, investigators determined that urine dipstick tests were not reliable in excluding the presence of

Taranjit Kaur¹, Michael A. Huffman²

¹Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, USA, ²Section of Ecology, Primate Research Institute, Kyoto University, Aichi, Japan

Key words: dipstick test strips – health monitoring – multi-reagent urine dipsticks – physiological status – urinalysis – wild chimpanzees

Accepted 10 March 2004.

Taranjit Kaur, University Veterinarian, Director, Office of Animal Resources, Assistant Professor, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061, USA. Tel: (540) 231-6522; fax: (540) 231-6033; e-mail: taranjit@vt.edu

Funding: Taranjit Kaur was supported by the National Science Foundation under award nos 0196295 and 0238069. Michael A. Huffman was supported by the Monbusho International Scientific Research Program (award no. 63043017 03041046 to T. Nishida) and the Plant Sciences Research Foundation of the Faculty of Agriculture, Kyoto University.

Kaur and Huffman

bacteria or leukocytes in human urine samples [22]. However, studies conducted at two other human emergency departments using enzymatic testing on suspected cases of UTI concluded that the detection of leukocytes, as determined by leukocyte esterase activity (Leukocyte Esterase Dipstick Test; LE; Bio Dynamics, Indianapolis, IN, USA), blood or UTI (Chemstrip 10 UA dipstick; Boehringer-Mannheim Corp., Mannheim, Germany) proved to be a useful diagnostic tool for clinical decision making and directing subsequent culture efforts [10, 21]. In one of these studies use of the LE test for pyuria to determine the significance of low count bacteruria [10^3 – 10^5 Colony Forming Units (CFU)/ml], authors reportedly achieved maximum sensitivity (92–99%) and specificity (99.2–99.6%) for the diagnosis of UTI [21]. In the other study, microscopy reportedly added nothing to dipstick results when clinicians suspected conditions causing hematuria alone and subsequent findings did not prompt a change in case management [10].

Investigations of the diagnostic accuracy of dipstick leukocyte esterase and/or nitrite tests as predictors of UTI have been conducted. Results of leukocyte esterase and nitrite tests (index test) were compared with results obtained from use of quantitative culture as the reference test [7]. This study showed that the disjunctive pair (e.g. dipstick positive for nitrites or leukocyte esterase reactions, or both) was the most accurate index test, relative to either of these tests alone, or when both were positive together ($P < 0.0001$); and yet a negative urine dipstick test did not exclude the diagnosis of a UTI in patients at a high risk of contracting a UTI [7]. One study in hospital patients showed that when dipstick results were positive for either leukocyte esterase or nitrites as a marker of UTI, the sensitivity was 78%, specificity 75% and negative predictive value was 94%, with 22% false-negative results [27]. Another more recent study using urine microscopy results as the reference test determined that the leukocyte esterase tests cannot accurately detect pyuria in young febrile children [26]. It did not reduce the need for urine microscopy [26]. Thus, the authors concluded that it was not a reliable indicator for hospitalization in cases of probable UTI [26].

As urine can be collected in a non-invasive manner and without direct contact with the subject rapid urine assessments using the dipstick method potentially could be a useful diagnostic tool for evaluating the health and physiological status of orangutans and chimpanzees under field conditions [12, 13].

The purpose of this study was (i) to describe urinary profiles obtained from wild chimpanzees in

different age and gender categories by a rapid urine screening method using a multi-reagent dipstick, and (ii) to evaluate the potential merit of urine screening with multi-reagent dipsticks to assess health and physiological status of wild chimpanzees.

Methods and materials

Multiple urine samples were collected from individually identified chimpanzees in conjunction with an ongoing study of behavioral adaptations for the self-treatment of sickness using medicinal plants in the Mahale Mountains National Park, Western Tanzania. Seventy-four voided urine samples were collected from 34 different chimpanzees over a 4-month period between 1993 and 1994.

Small sterile polyethylene containers with suction action were used to collect urine off the surface of leaves, rocks and bare ground immediately after micturition by identified chimpanzees. All urine samples were tested using Urine Dipstick Test 9-RC for Urotron RL9 (Boehringer-Mannheim) for 10 markers: pH, protein, hemoglobin, red blood cells (RBCs), white blood cells (WBCs), nitrites, ketones, bilirubin, glucose, and urobilinogen. Multi-reagent dipstick tests were performed on unrefrigerated urine specimens within 5 hours post-collection. Results were determined visually using colorimetric scales provided by the manufacturer and not by the Urotron (an automated dipstick reader). In many cases, observations on behavior and reproductive status were recorded. In addition, stool consistency was noted and feces were examined for *Oesophagostomum stephanostomum*, *Trichuris* spp., and *Strongyloides fulleborni* using gross examination and the MacMaster's technique for egg counts using microscopy [6].

Negative and positive dipstick test pad results were tabulated for the 34 wild chimpanzees and 74 voided samples. In addition, observations were sorted based on chimpanzee age and gender categories [5] as follows: nine adult males (26 samples), 16 adult females (33 samples), three adolescent males (six samples), two adolescent females (three samples), one juvenile female (three samples), one infant male (one sample), and two infant females (two samples). In addition, results for adult female chimpanzees with and without perineal swelling were described separately. Qualitative data from positive test results were evaluated. Moreover, urine test pad results were tabulated in two-way contingency tables to assess the possibility of interactions when positive results were noted in more than one category in a given urine specimen. Finally, other external signs of ill

Urological record of wild chimpanzees

health and physiological status in chimpanzees with positive test pad results were summarized.

Results

Age and gender categories for the 34 chimpanzees under study are provided in Table 1. Most common multi-reagent test pad results for all 74 voided urine samples are given in Table 2. Positive urinary results for one or more analytes were observed in 17 of 34 wild chimpanzees (13/25 adults and 4/9 sub-adults). Qualitative data for all positive multi-reagent test pad results for the entire study population are provided in Table 3. Age and gender distributions of positive test results are described in Tables 4 (adults) and 5 (sub-adults). Table 6 shows the positive urine dipstick test results for 16 adult females during the presence and absence of noticeable perineal swelling.

Urine was collected from three adult females both during the absence and presence of perineal

Table 1. Number of chimpanzees (number of urine specimens) in study population by age and gender categories

Gender category	Age category				Totals
	Sub-adults				
	Adults	Adolescents	Juveniles	Infants	
Males	9 (26)	3 (6)	0	1 (1)	13 (33)
Females	16 (33)	2 (3)	1 (3)	2 (2)	21 (41)
Totals	25 (59)	5 (9)	1 (3)	3 (3)	34 (74)

Table 2. The most common urine dipstick test pad results from 74 samples collected from 34 wild chimpanzees

Urine chemistry parameters	Most common results		
	Test result ¹	Chimpanzees (%) n = 34 ¹	Samples (%) n = 74
pH	8-9	31/34 (91) ²	71/74 (96) ²
Protein	Negative (<6 mg/dl)	18/34 (53)	48/74 (65)
Hemoglobin	Negative (<10 Ery/ l)	26/34 (76)	57/74 (77)
RBC	Negative (<5 Ery/ l)	23/34 (68)	54/74 (73)
WBC	Negative (<10 Leuk/ l)	24/34 (71)	52/74 (70)
Nitrites	Negative (<0.05 mg/dl)	32/34 (94) ³	70/73 (96)
Ketones	Negative (<5 mg/100 dl)	33/34 (97) ^{2,4}	71/72 (99) ^{2,4}
Bilirubin	Negative (<0.5 mg/dl)	31/34 (91) ⁵	69/73 (95) ⁵
Glucose	Normal (<40 mg/dl)	34/34 (100)	74/74 (100)
Urobilinogen	Normal (<0.4 mg/dl)	33/34 (97) ⁶	73/73 (100) ⁶

¹If a given chimpanzee had one positive test result in any urine specimen, he/she was not included as a negative.

²One sample from one adult female had a pH of 5 and ketones. One sample from one adolescent male and one sample from one juvenile female had a pH of 7.

³No data on nitrites for one sample from one male adult chimpanzee.

⁴No data on ketones for two samples from one adult male chimpanzee.

⁵No data on bilirubin for one urine sample from one adult male chimpanzee.

⁶No data on urobilinogen for one sample from one adult male chimpanzee.

swelling. One of these females had hemoglobin, RBCs, and WBCs in the presence of perineal as well as in absence of perineal swelling. The other two females had no detectable hemoglobin, RBCs, and/or WBCs in their urine (Table 6).

Tables 7 and 8 show qualitative data for positive urine dipstick test results from adult females with and without perineal swelling, respectively. Positive urine multi-reagent test pad results for the three

Table 3. Qualitative data from positive urine dipstick test pad results from 74 samples collected from 34 wild chimpanzees

Chemistry parameter	Qualitative data		
	+1	+2	+3
Protein ¹	26	0	0
Hemoglobin ²	4	7	6
RBC ³	7	2	11
WBC ⁴	8	9	5
Nitrites ⁵	3	NA	NA
Ketones ⁶	1	NA	NA
Bilirubin ⁷	1	3	0

¹Protein: 0 = negative; +1 = <30 mg/dl; +2 = <100 mg/dl; +3 = <500 mg/dl.

²Hemoglobin: 0 = negative; +1 = 10; +2 = 50; +3 = 250 Ery/ l.

³Red blood cells: 0 = negative; +1 = 5-10 Ery/ l; +2 = 50 Ery/ l; +3 = 250 Ery/ l.

⁴White blood cells: 0 = negative; +1 = 10-25; +2 = 75; +3 = 500 Leuk/ l.

⁵Nitrites: 0 = negative; +1 = positive 0.05 mg/dl.

⁶Ketones: 0 = negative; +1 = positive (5 mg/dl).

⁷Bilirubin: 0 = negative, 1 = +(0.5 mg/dl); 2 = ++; 3 = +++.

Kaur and Huffman

Table 4. Gender distribution of positive urine dipstick results on 59 samples from 25 adult chimpanzees

Urine chemistry parameters	Adult males		Adult females	
	Chimps n = 9 (%)	Samples n = 26 (%)	Chimps n = 16 (%)	Samples n = 33 (%) ¹
Protein	3 (33)	6 (23)	9 (56)	16 (48)
Hemoglobin	2 (22)	5 (19)	6 (38)	12 (36)
RBC	3 (33)	6 (23)	7 (44)	13 (39)
WBC	3 (33)	6 (23)	5 (31)	14 (42)
Nitrites	1 (11)	2 (8)	1 (6)	1 (3)
Ketones	0	0	1 (6)	1 (3)
Bilirubin	1 (11)	1 (4)	2 (13)	3 (9)

¹Number of specimens is provided for informational purposes only (as it is possible that the number of specimens collected from a single individual may distort results).

adult males and four sub-adults are shown in Tables 9 and 10, respectively.

Of these 17 individuals with positive dipstick test results, eight were tested for gastrointestinal

parasites with seven testing positive. All seven also had abnormal feces and/or flatulence, with the exception of one sub-adult male, in which no data was available (Table 11). Many of these chimpanzees also had obvious external signs of disease, such as respiratory signs, gastrointestinal signs, and/or malaise (Table 11).

Discussion

Ten urine chemistry parameters (pH, protein, hemoglobin, RBCs, WBCs, nitrites, ketones, bilirubin, glucose, and urobilinogen) were measured on 74 voided urine samples from 34 wild chimpanzees. Of the 34 chimpanzees, 50% (13/25 adults and 4/9 sub-adults) had positive urine dipstick test pad results for one or more analytes, most notably protein, hemoglobin, RBCs, and WBCs.

Ninety-one percent of the chimpanzees (96% of the samples) had very alkaline urine, with urine pH measuring between 8 and 9 units (Table 2). Only

Table 5. Gender distribution of positive urine dipstick results on 15 samples from nine sub-adult chimpanzees

Urine chemistry parameters	Adolescent males and females		Adolescent, juvenile and infant males		Adolescent, juvenile and infant females	
	Chimps n = 5 (%)	Samples n = 9 (%)	Chimps n = 4 (%)	Samples n = 7 (%)	Chimps n = 5 (%) ¹	Samples n = 8 (%) ¹
Protein	3 (60)	3 (33)	3(75)	3(43)	1 (20) ²	1 (13) ²
RBC	0	0	0	0	1 (20) ³	1 (13) ³
WBC	1 (20)	1 (11)	1(25)	1(14)	1 (20) ³	1 (13) ³

¹Results are from two different samples from the same juvenile female.

²Trace protein (<30 mg/dl) detected from this juvenile female.

³Red blood cells (+1 = 5-10 Ery/ l) and white blood cells (+1 = 10-25) occurred in the same sample from this juvenile female.

Table 6. Gender distribution of positive urine dipstick results on 33 samples from 16 adult female chimpanzees with and without noticeable perineal swelling

Urine chemistry parameter	All adult females		With perineal swelling		Without perineal swelling	
	Chimps n = 16 (%) ¹	Samples n = 33 (%)	Chimps n = 7 (%) ¹	Samples n = 11 (%) ²	Chimps n = 12 (%) ^{1,3}	Samples n = 22 (%) ²
Protein	9 (56)	16 (48)	2 (29)	3 (27)	7 (58)	13 (59) ²
Hemoglobin	6 (38)	12 (36)	3 (43) ¹	3 (27) ²	4 (33) ¹	9 (41) ²
RBC	5 (31)	14 (42)	3 (43) ¹	3 (27) ²	5 (42) ¹	10 (45) ²
WBC	7 (44)	13 (39)	3 (43) ¹	3 (27) ²	3 (38) ^{1,3}	11 (61) ²
Nitrites	1 (6)	1 (3)	0	0	1 (13) ³	1 (6) ²
Ketones	1 (6)	1 (3)	1 (14)	1 (9)	0	0
Bilirubin	2 (13)	3 (9)	0	0	2 (25)	3 (17)

¹Three adult female chimpanzees were tested both during the presence and absence of perineal swelling. Leukocyte esterase activity, red blood cells and hemoglobin were detected in one adult female with and without perineal swelling (chimpanzee no. 7). No leukocyte esterase activity, red blood cells or hemoglobin were detected in the other two adult females during times of perineal swelling and no perineal swelling.

²Number of samples is provided for informational purposes only (as it is possible that the number of specimens collected from one individual (chimpanzee no. 7) may distort results).

³One adult female had leukocyte esterase activity and nitrites in one of her urine specimens (chimpanzee no. 7).

Urological record of wild chimpanzees

Table 7. Qualitative data for four adult female chimpanzees (seven samples) with perineal swelling and positive urine dipstick test results

ID no. ¹	pH	Protein (mg/dl)	WBC (Leuk/ l)	RBC (Ery/ l)	Hemoglobin (Ery/ l)	Ketones (mg/dl)
4	8	<30	75	5–10	10	Negative
	9	<30	Negative	Negative	Negative	Negative
5	5	<30	Negative	Negative	Negative	5
	8	Negative	Negative	Negative	Negative	Negative
	8	Negative	Negative	Negative	Negative	Negative
6	9	Negative	10–25	50	50	Negative
7 ¹	9	Negative	500	250	250	Negative

¹Urine was collected from one adult female (chimpanzee no. 7) during both the presence and absence of perineal swelling.

Table 8. Qualitative data for seven adult female chimpanzees (16 samples) without perineal swelling and positive urine dipstick test

ID no. ¹	pH	Nitrite (mg/dl)	Protein (mg/dl)	WBC (Leuk/ l)	RBC (Ery/ l)	Hemoglobin (Ery/ l)	Bilirubin (mg/ml)
7 ¹	9	Negative	<30	500	250	250	Negative
	9	0.05	<30	75	250	250	Negative
	9	Negative	<30	10–25	250	50	Negative
	9	Negative	<30	Negative	250	250	Negative
8	9	Negative	<30	Negative	5–10	Negative	Negative
	9	Negative	<30	Negative	Negative	Negative	Negative
10	9	Negative	<30	500	250	50	Negative
	9	Negative	<30	75	5–10	10	Negative
11	9	Negative	<30	Negative	5–10	10	Negative
12	8	Negative	<30	75	250	250	>0.5
	9	Negative	<30	500	Negative	Negative	Negative
	9	Negative	<30	10–25	Negative	Negative	>0.5
	9	Negative	Negative	10–25	Negative	Negative	Negative
	9	Negative	Negative	500	5–10	10	Negative
	9	Negative	Negative	75	Negative	Negative	Negative
	9	Negative	<30	Negative	Negative	Negative	Negative
13	8	Negative	<30	Negative	Negative	Negative	Negative

¹Urine was collected from one adult female (chimpanzee no. 7) during both the presence and absence of perineal swelling.

Table 9. Qualitative data for three adult male chimpanzees (seven samples) with positive urinary profiles

ID no.	pH	Nitrite (mg/dl)	Protein (mg/dl)	WBC (Leuk/ l)	RBC (Ery/ l)	Hemoglobin (Ery/ l)	Bilirubin (mg/ml)
1	9	Negative	<30	75	250	50	Negative
2	8.5	Negative	Negative	10–25	5–10	Negative	Negative
	9	ND	<30	Negative	Negative	Negative	Negative
3	9	0.05	<30	75	250	250	ND
	9	Negative	<30	10–25	50	50	Negative
	9	Negative	<30	75	250	50	Negative
	9	0.05	<30	75	250	50	Positive

ND, no data.

three chimpanzees had a pH of less than 8 units. Two sub-adults (one adolescent male and one juvenile female) had a pH of 7, and one adult female had a pH of 5, as well as ketones in her urine (Table 2). A normal pH range from 4.5 to 9 units has been reported in captive chimpanzees using dipstick test methods (J. Fontenot, pers. comm.; M. Martino, pers. comm.; C. Snider, pers. comm.). The method of urine sample collection and storage can influence urinalysis results [18]. Voided urine samples are frequently contaminated

with cells, bacteria and other debris located in the distal urethra or genital tract, and/or on skin and hair [18]. In this study, urine samples were collected off the surface of leaves, rocks, and bare ground so environmental contamination has to be taken into account although the urine was transferred into sterile specimen containers. In addition, in this study, specimens were stored at ambient temperature without preservatives for up to 5 hours. Some bacteria may multiply at a rate that doubles their numbers every 45 minutes [18]. *In vitro*

Kaur and Huffman

Table 10. Qualitative data for four sub-adult chimpanzees (six samples) with positive urinary profiles.

ID no.	pH	Protein (mg/dl)	WBC (Leuk/ l)	RBC (Ery/ l)	Hemoglobin (Ery/ l)
14	8	<30	Negative	Negative	Negative
	9	Negative	10–25	Negative	Negative
15	9	<30	Negative	Negative	Negative
16	9	<30	Negative	Negative	Negative
17	9	Negative	10–25	5–10	Negative
	9	<30	Negative	Negative	Negative

Table 11. Summary of obvious external signs of non-health observed in nine of the 17 chimpanzees with positive urine dipstick test results by age and gender categories

ID no. and age/gender category	Gastrointestinal signs			
	Parasites	Feces	Respiratory signs	General health
1 AM	<i>O. stephanostomum</i> , <i>Trichuris</i> spp.	Diarrhea	Coughing; congestion	Malaise; thin
2 AM	<i>O. stephanostomum</i> , <i>Trichuris</i> spp., <i>S. fulleborni</i>	Flatulence	Nasal discharge	ND
3 AM	<i>Trichuris</i> spp.	Diarrhea; passing worms, leaves	ND	Malaise; passing <i>O. stephanostomum</i> out penis
4 AFS	ND	Flatulence	Coughing	Slept late
7 AFS	<i>O. stephanostomum</i>	Frequent soft stools	ND	Active
7 AF	<i>O. stephanostomum</i>	Diarrhea	Blocked nasal passage	Ill, malaise, slept late in day bed; returned after long absence
9 AF	ND	Freq stools, small amount; flatulence	Sneezing	Subdued; lactating
12 AF	<i>O. stephanostomum</i> , <i>Trichuris</i> spp., <i>S. fulleborni</i>	Vomiting diarrhea, flatulence; passing worms, leaves, undigested food	Picking, blowing nose	Malaise, frequents day bed, frequent rests, frequent rests in day bed; left group ill; thin with rib showing; forages/travels
14 SM	<i>S. fulleborni</i>	ND	ND	Ingesting medicinal plant (<i>Aspilla</i> leaves)
15 AdM	<i>S. fulleborni</i> , <i>Trichuris</i> spp., <i>O. stephanostomum</i>	Diarrhea, passing worms	ND	ND

AM, adult male; AFS, adult female with perineal swelling; AF, adult females without perineal swelling; SM, sub-adult male; AdM, adolescent male; ND, no data.

proliferation studies of bacteria have reported an increase of over 100-fold following collection of a contaminated urine sample maintained at room temperature (1000 bacteria/ml increases to 128,000/ml in 3.5 hours) [18]. *In vivo* or *in vitro* contamination of urine samples with urease-producing bacteria (primarily *Staphylococci* and *Proteus* spp.) reportedly increases urine pH [18]. However, this does not rule out the possibility that *in vivo* factors may be contributing to alkalinization of urine. For instance, dietary factors, pregnancy, and disease (i.e. UTIs with urease-producing bacteria, respiratory or metabolic alkalosis and vomiting) can also lead to considerable increases in urine pH [18].

The urological record of the study population showed that 44% of the chimpanzees, or a total of

16 of the 17 chimpanzees with positive urine dipstick test results, had protein in the urine (see Tables 4–7, 9 and 10). In terms of age categories, dipstick test pads detected protein in 13 (76%) adults when compared with four (24%) sub-adults. Table 3 shows the qualitative data, and in all cases only trace amounts of protein were detected (<30 mg/dl). Enzymatic urine dipstick testing is sensitive to albumin, and pathological proteinuria will usually produce persistent values above 30 mg/dl [4, 9]. In this study, trace amounts of protein in urine did not appear to be associated with obvious external signs of illness, such as gastrointestinal parasites, respiratory signs and/or malaise. Although inconclusive, it appears unlikely that detection of trace amounts of protein represent clinical significance. More importantly, these

findings cannot be interpreted independent of urine pH, as alkaline urine does cause lysis of RBCs (both *in vivo* and *in vitro*) and can lead to false-positive proteinuria, as well as false-positive hemoglobinuria (F. Almy, pers. comm.) [18].

Hemoglobinuria was detected in 24% of the chimpanzees and in 23% of the specimens (Table 2). In terms of age and gender categories, hemoglobinuria was detected in 22% (3/9) and 38% (6/16) of the adult males and adult females, respectively (Table 4). Tables 3 and 7–10 show the qualitative data for urinary profiles from all chimpanzees with positive multi-reagent dipstick test pad results. In many cases, both proteinuria and hemoglobinuria were detected in the same samples, and in all cases, those samples had a pH of 8–9 units (Tables 7–10). Controlled studies using automated analysis have demonstrated that, even when urine samples are refrigerated for 24 hours, urine protein values increase after storage [2]. This is in contrast to other analytes, including glucose and ketones, which proved to be stable under similar conditions [2].

In general, multi-reagent dipstick test pads detected erythrocytes in 32% of the chimpanzees and 27% in urine specimens (Table 2). In 67% of the cases, obvious external signs of disease were also present (Tables 7–11). In regard to adult females, 43% (3/7) with perineal swelling and 42% (5/12) of females without perineal swelling had RBCs in their urine (Table 6). Under the field study conditions described herein, dipstick positive results for RBCs are not a useful indicator for detecting menses in adult females chimpanzees. Of the four adult females with perineal swelling and positive urinary profiles, three had hemoglobin and RBCs (Table 7). Of the seven adult females without perineal swelling and positive urinary profiles, five had RBCs and four of those five had hemoglobin in their urine (Table 8). Voided urine specimens were collected from three adult females during both the presence and absence of perineal swelling. One of these chimpanzees (chimpanzee no. 7) had RBCs in her urine during the presence and absence of perineal swelling, while the other two adult females had no detectable RBCs or hemoglobin in their urine during either time (Tables 6–8). But, low numbers of RBCs in voided urine is not unexpected and is often observed in the urine of normal subjects (F. Almy, pers. comm.) [18]. In fact, normal chimpanzee urine has been reported to have up to 100 Ery/high power field using the urine dipstick method (M. Martino, pers. comm.). It is also important to note that RBCs decrease due to their disintegration in alkaline urine (F. Almy, pers. comm.) [18]. Thus, given the

aforementioned information a positive urine dipstick test result for RBCs and/or hemoglobin does not seem to be a reliable indicator of disease and/or physiological status in chimpanzees.

In addition to RBCs, urine dipsticks test pads detected WBCs in the urine of chimpanzee no. 7 during both the presence and absence of perineal swelling. In regard to the other two adult females tested during the presence and absence of perineal swelling, as with RBCs, no WBCs were detected (Tables 6–8). Thus, as pyuria is consistent with host injury, this finding suggests that the detection of blood (either RBCs and/or hemoglobin) in conjunction with WBCs in voided urine samples of adult female chimpanzees may be associated with unhealthy conditions, such as a UTI, vaginitis, and/or trauma suffered during mating [23].

In urine specimens collected from normal captive chimpanzees, microscopy on urinary sediment confirms the absence of WBCs (M. Martino, pers. comm.). In our study population, urine from adult females with perineal swelling (43%) and without perineal swelling (38%) had evidence of leukocyte esterase reactions (Table 6). These findings suggest that the detection of WBCs in the voided urine of female chimpanzees may not be an indicator of pathology.

Dipstick test strips detect the presence of leukocytes in urine specimens by measuring leukocyte esterase reactions. In human beings, there have been conflicting reports on the validity of using dipstick test pad results as a reliable indicator of pyuria. In two studies conducted in human emergency departments, using dipstick urinalysis on suspected cases of UTIs, investigators concluded that the detection of leukocytes, as determined by leukocyte esterase activity, or hematuria, proved to be a reliable diagnostic tool [10, 21]. Other studies in human beings have found that a positive chemical reagent strip (i.e. leukocyte esterase, protein, hemoglobin, and/or nitrite) can be effectively used as a diagnostic screening test [24]. In addition, investigators summarized the predictive value of positive dipstick leukocyte esterase and/or nitrite tests (the index test) as predictors of UTI as defined by quantitative culture (the reference test) [7]. This analysis revealed that the dipstick positive results for nitrite or leukocyte esterase, or both were the most accurate index test, relative to either of these tests alone or when both were positive together ($P < 0.0001$) [7]. However, more importantly the authors of this study went on to state that in many clinical settings the posterior probability of UTI given a negative dipstick is too high to exclude it [7]. Another study conducted in a human emergency department determined that urine

dipstick tests were not reliable in excluding the presence of leukocytes and bacteria in human urine samples [22]. In addition, it has also been reported that urine dipstick test pads have low sensitivities (<90%) for the detection of leukocyte esterase activity [18, 19]. These studies all suggest that a negative urine dipstick test does not exclude the diagnosis of a UTI in patients at a high risk of contracting a UTI [7]. As such, it has been recommended that test pads not be used alone as a screen for UTIs [19]. Not only is there evidence of poor sensitivity, but also for poor specificity as microbial peroxidases can cause false-positive leukocyte esterase reactions (F. Almy, pers. comm.; K. Zimmerman, pers. comm.). Thus, the accuracy of urine dipstick positive results for urinary nitrites and leukocytes is questionable.

As pyuria is an indicator for the presence of host injury, confirmation of pyuria by microscopy serves to differentiate infection from bacterial colonization or contamination when bacteria and/or nitrites are detected in a urine sample [23, 24]. Thus, pyuria together with 10^2 coliforms/ml is a better predictor of UTI than either 10^5 or 10^2 CFU/ml without pyuria [19, 20].

Another consideration when evaluating multi-reagent urinary dipstick test strips is product validation studies in the species of choice. For example, urine dipstick test strips have proven to be unreliable in cats (false-positive results in the absence of pyuria) and insensitive in dogs (false-negative results even in the presence of pyuria) (F. Almy, pers. comm.) [18]. No documentation for validation studies in veterinary subjects or in non-human primates on the 9-RC for Urotron[®] RL9 (Boehringer-Mannheim) test strips used in this study could be located by the authors.

Urine dipstick tests on two of the wild chimpanzees tested positive for nitrites, which is indicative of bacteruria in several other species. These same two chimpanzees also had *c.* 75 Leuk/1 according to the urine dipstick test pad results. As shown in Tables 8, 9, and 11, both of these chimpanzees (one adult male and one adult female) were reportedly ill (chimpanzees nos 3 and 7). The adult male (chimpanzee no. 3) had gastrointestinal parasites (*Trichuris* spp.). In addition, he was observed passing *O. stephanostomum* through his penis and had malaise. He died a few weeks later. The adult female (chimpanzee no. 7) tested positive for fecal *O. stephanostomum*, had dark, runny diarrhea, and other external signs of illness (respiratory signs and malaise). However, as both dipstick leukocyte esterase and nitrite tests are poor indicators of bacteruria in domestic animals and human beings, it would be premature to

suggest the possibility of UTIs (F. Almy, pers. comm.) [18]. That is, although the urinary nitrite test pads are highly specific (*c.* 98%), they are not a sensitive indicator (*c.* 45%) of the presence of Gram-negative bacteria [8].

When compared with the culture method rapid urine screens are relatively insensitive to nitrite detection when microbial counts are below 10^5 CFU/ml of urine [11]. It has been reported that most false-negative results obtained with chemical reagent test strips have been associated with nitrites, i.e. bacteruria [24]. In this study, urine dipstick test results showed that the presence of nitrites was rare and occurred in only one female (chimpanzee no. 7) and one male urine specimen (chimpanzee no. 3) (Tables 8 and 9). Thus, it appears that exposure of these urine specimens to non-aseptic surfaces (leaves, rocks, and bare ground) did not lead to false-positive results suggestive of bacteruria. It is important to also realize that negative dipstick test results can be due to the presence of urinary ascorbic acid which can decrease the sensitivity of the nitrate reductase reactions [8]. Vitamin C is water soluble and therefore, there is the possibility that dietary factors influence dipstick test pad results for nitrites. All specimens were negative for ketones (<5 mg/100 ml acetoacetate) with the exception of one female with a urine pH of 5 units. Bilirubin was detected in three chimpanzees (four urine samples), and glucose and urobilinogen were not detected.

Spectrum bias is a term used when diagnostic tests have different sensitivities or specificities in patients with different manifestations of the disease for which the test is intended [16]. Spectrum bias may also vary test results when using the urine dipstick method. Both leukocyte esterase and nitrite urine dipstick tests are susceptible to spectrum bias [16]. In wildlife research this seems particularly important because species signalment and history often remain unknown as opposed to controlled laboratory studies. Subject history, age, diet, food availability, and other factors such as symptomatology should also be taken into account when interpreting urine dipstick test pad results [3, 14, 15, 18, 19, 25]. Finally, factors such as specimen collection methods, specimen preservation and time of urine production, micturition, and collection may alter urine composition and hence, influence test strip results [18]. Thus, the dipstick urinalysis alone should not be considered a reliable method to evaluate health or physiological status in wild chimpanzees.

In general, the data suggested that, in adult males ($n = 9$), there were possible associations

Urological record of wild chimpanzees

between the presence of parasites and normal resting patterns, and the presence of parasites and diarrhea (Table 11). Positive test results for RBCs were noted in the urine of three adult males with gastrointestinal parasites and diarrhea (*O. stephanostomum*, *Trichuris* spp., and/or *S. fulleborni*); and in three other adult males with gastrointestinal parasites and diarrhea (*O. stephanostomum* and/or *S. fulleborni*) urine test results were not positive for RBCs or hemoglobin. However, when using the dipstick test alone, it is not possible to determine whether the presence of RBCs in these voided urine specimens is due to normal specimen contamination from bloody diarrhea and/or an abnormal urinary finding. Although data suggest that there may be an association between parasites and chimpanzee behavior and obvious external signs of disease [1], we can only speculate at this time to the causative relationship (Table 11).

In conclusion, multi-reagent urine dipstick test strips are a simple, quick (60–120 s) and relatively inexpensive (*c.* 0.59/dipstick) method to measure certain indicators of health and physiological status. As such this method could serve as a useful, supplementary, non-invasive in-field screening tool for evaluating the health and physiological status of chimpanzees and other wildlife species. However, as this descriptive study demonstrates, limitations associated with this methodology need to be considered when making observations and interpreting the results. The value of the dipstick urinalysis is considered to be directly proportional to diagnostic skills in both observation, as well as interpretation [18]. As in the case of this study, under field conditions multi-reagent urine dipstick test results would be based on observations and comparisons using colorimetric scales. Subjective results typically have a greater margin of error than results obtained when using automated dipstick analysis (i.e. Urotron) (F. Almy, pers. comm.) [18]. In addition, the selection of urine dipstick products should be made based on validation studies specifically for the species of interest. Limitations associated with this methodology need to be considered when interpreting urinary dipstick test results. Thus, we conclude that dipstick urinalysis alone is not a reliable method to evaluate health or physiological status in wild chimpanzees. However, if combined with other diagnostics, it could prove to be a valuable health monitoring tool.

Acknowledgements

The authors wish to thank Dr Yasu Suzuki for translating the Boehringer-Mannheim Test 9-RC for Urotron package

insert from Japanese to English; and Drs Kurt Zimmerman and Tripp Almy for their review and expert comments regarding Veterinary Clinical Pathology.

References

- ALADOS CL, HUFFMAN MA: Fractal long-range correlations in behavioral sequences of wild chimpanzees: a non-invasive analytical tool for the evaluation of health. *Ethology* 106:105–116, 2000.
- FROOM P, BIEGANIEC B, EHRENREICH Z, BARAK M: Stability of common analytes in urine refrigerated for 24 h before automated analysis by test strips. *Clin Chem* 46:1384–1386, 2000.
- GRAHAM JC, GALLOWAY A: The laboratory diagnosis of urinary tract infection. *J Clin Pathol* 54:911–919, 2001.
- HINBERG IH, KATZ L, WADDELL L: Sensitivity of in vitro diagnostic dipstick tests to urinary protein. *Clin Biochem* 11:62–64, 1978.
- HIRAIWA-HASEGAWA M, HASEGAWA T, NISHIDA T: Demographic study of a large-sized group of chimpanzees in the Mahale Mountains, Tanzania: a preliminary report. *Primates* 25:401–413, 1984.
- HUFFMAN MA, GOTOH S, TURNER LA, HAMAI M, YOSHIDA K: Seasonal trends in intestinal nematode infections and medicinal plant use among chimpanzees in the Mahale Mountains, Tanzania. *Primates* 38:111–125, 1997.
- HURLBUT TA, LITTENBERG B: The diagnostic accuracy of rapid dipstick tests to predict urinary tract infection. *Am J Clin Pathol* 96:582–588, 1991.
- JAMES GP, PAUL KL, FULLER JB: Urinary nitrite and urinary-tract infection. *Am J Clin Pathol* 70:671–678, 1978.
- JANSEN BS, LUMSDEN JH: Sensitivity of routine tests for urine protein to hemoglobin. *Can Vet J* 26:221–223, 1985.
- JOU WW, POWERS RD: Utility of dipstick urinalysis as a guide to management of adults with suspected infection or hematuria. *South Med J* 91:266–269, 1998.
- KELLOGG JA, MANZELLA JP, SHAFFER SN, SCHWARTZ BB: Clinical relevance of culture versus screens for the detection of microbial pathogens in urine specimens. *Am J Med* 83:739–745, 1987.
- KNOTT CD: Monitoring health status of wild orangutans through field analysis of urine. *Am J Phys Anthropol (Suppl)* 22:139–140, 1996.
- KNOTT CD: Field collection and preservation of urine in orangutans and chimpanzees. *Trop Biodiversity* 4:95–102, 1997.
- KNOTT CD: Changes in orangutan caloric intake, energy balance, and ketones in response to fluctuating fruit availability. *Int J Primatol* 19:1061–1078, 1998.
- KUNIN CM, WHITE LV, HUA TH: A reassessment of the importance of 'low-count' bacteriuria in young women with acute urinary symptoms. *Ann Intern Med* 119:454–460, 1993.
- LACHS MS, NACHAMKIN I, EDELSTEIN PH, GOLDMAN J, FEINSTEIN AR, SCHWARTZ JS: Spectrum bias in the evaluation of diagnostic tests: lessons from the rapid dipstick test for urinary tract infections. *Ann Intern Med* 117:135–140, 1992.
- ONESON R, GROSCHEL DH: Leukocyte esterase activity and nitrite test as a rapid screen for significant bacteriuria. *Am J Clin Pathol* 83:84–87, 1985.

Kaur and Huffman

- OSBORNE CA, STEVENS JB: *Urinalysis: A Clinical Guide to Compassionate Patient Care*. Leverkusen, Germany: Bayer AG, 1999.
- PEZZLO M: Detection of urinary tract infections by rapid methods. *Clin Microbiol Rev* 1:268–280, 1988.
- PEZZLO MT, WETKOWSKI MA, PETERSON EM, DELAMAZA LM: Detection of bacteriuria and pyuria within 2 minutes. *J Clin Microbiol* 21:578–581, 1985.
- PFALLER M, RINGENBERG B, RAMES L, HEGEMAN J, KOONTZ F: The usefulness of screening tests for pyuria in combination with culture in the diagnosis of urinary tract infection. *Diagn Microbiol Infect Dis* 6:207–215, 1987.
- PROPP DA, WEBER D, CIESLA ML: Reliability of a urine dipstick in emergency department patients. *Ann Emerg Med* 18:560–563, 1989.
- STAMM WE: Measurement of pyuria and its relation to bacteriuria. *Am J Med* 75(Suppl. 1B):53–58, 1983.

- WENZ B, LAMPASSO JA: Eliminating unnecessary urine microscopy. Results and performance characteristics of an algorithm based on chemical reagent strip testing. *Am J Clin Pathol* 92:78–81, 1989.
- WOOLHANDLER SR, PELS J, BOR DH, HIMMELSTIEN DU, LAWRENCE RS: Dipstick urinalysis screening of asymptomatic adults for urinary tract disorders. I. Hematuria and proteinuria. *J Am Med Assoc* 262:1214–1219, 1989.
- YUEN SF, NG FN, SO LY: Evaluation of accuracy of leukocyte esterase testing to detect pyuria in young febrile children: prospective study. *Hong Kong Med J* 7:5–8, 2001.
- ZAMAN Z, BORREMANNS A, VERHAEGEN J, VERBIST L, BLANCKAERT N: Disappointing dipstick screening for urinary tract infection in hospital patients. *J Clin Pathol* 51:471–472, 1998.