

Decaying toxic wood as sodium supplement for herbivorous mammals in Gabon

Yuji IWATA^{1,2}#, Yoshihiro NAKASHIMA³#, Sayaka TSUCHIDA²), Pierre Philippe Mbehang NGUEMA⁴), Chieko ANDO¹), Kazunari USHIDA²)* and Juichi YAMAGIWA¹)

¹)Kyoto University, Graduate School of Science, Kitashirakawa, Kyoto 606–8502, Japan

²)Kyoto Prefectural University, Graduate School of Life and Environmental Sciences, Shimogamo, Kyoto 606–8522, Japan

³)Nihon University, College of Bioresource Science, Kameino, Fujisawa, Kanagawa 252–0880, Japan

⁴)Institute des Recherches en Ecologie Tropicale, BP 13354, Libreville, Gabon

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ABSTRACT. African rainforest harbors herbivores at high density. However, because plants and soils typically lack in some essential minerals, rainforest is not always a suitable habitat for herbivores. How they fulfill the mineral requirements is therefore an important question to animal ecology and conservation. Although large marshes, called ‘bais’, are often mentioned as efficient mineral-resource, little information on other sodium resources has still been available. Our laboratory works and field surveys found that a peculiar item, decaying wood stumps of *Anthostema aubryanum*, played as a major sodium resource for herbivores in Moukalaba-Doudou National Park, Gabon. When *A. aubryanum* is alive, the sodium content of its bark is low and its latex is toxic. Sodium is accumulated in decaying stumps (mean=1,343 mg/kg dry matter). Eight herbivores visited stumps to ingest the dead wood. Fecal sample analysis revealed that western lowland gorillas, a species most-frequently using the stumps, consumed large amount of the dead wood as regular food. Our findings suggest that decaying *A. aubryanum* is critical sodium-resources and is a key species for herbivores in our study area. Importance of the *A. aubryanum* may be particularly large there, because it is a limited sodium-rich material that is available year round. Our study site is known as the site where the densities of several herbivores are among the highest at Central Africa. The relatively high herbivores density in our study site may partly depend on decaying *A. aubryanum* as sodium resources.

KEY WORDS: *Anthostema aubryanum*, decaying wood, herbivores, sodium resource, toxic plant

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In African rainforest, large herbivores (including frugivores/folivores), such as elephants and gorillas, occur sympatrically and constitute a major component of the mammalian biomass [27]. Sodium availability directly influences the distribution of herbivores and regulates their population size [3, 26], but rainforest is typically sodium-deficient habitats because tropical plants and soils are generally lower in nutrients than temperate plants are [8, 25], and heavy rainfall readily leached minerals from the ecosystems [16]. How these large herbivores fulfill the sodium requirements is therefore an interesting and important question to animal ecology and conservation, but has not still been well-elucidated one. Our study site seemingly lacks large sodium-resources, although sodium-rich (mean=823 mg/kg dry matter, range=339–1,293 mg/kg, see Supplementary Table 1) *Sacoglottis gabonensis* fruits are seasonally available. Previous studies have reported that herbivores in Central Africa have specific mineral resources [6]. Especially, large marshland, called “bai”, covered with year-round herbaceous vegetation supplies important mineral resources, such as mineral rich

aquatic herbs, soil and water, for herbivores [4, 7, 10, 19]. For example, western gorillas in Ndoki forest in Republic of the Congo consumed the root of *Hydrocharis* sp., which showed higher sodium concentration (1,080 mg/kg) than other herbs year-round in “bais” [13].

Here, we report that decaying wood stumps of a particular toxic plant are critical sodium resources for herbivores in Moukalaba-Doudou National Park (PNMD), Gabon, where lacks “bai”. This toxic plant, once dead, supports among the highest densities of herbivores, such as duikers [12], gorillas [20] and elephants [24], in Central Africa.

The forest of PNMD is characterized by the abundance of *Anthostema aubryanum* (Euphorbiaceae) [23]. Logging business was conducted between the 1960s and the 1980s in PNMD, but *A. aubryanum* was worthless as lumber. Then, *A. aubryanum* trees were not logged and still abundantly exist in PNMD. One single decayed stump of *A. aubryanum* could only last for several weeks after the first bit by herbivores. It is confirmed by direct observation that herbivores in PNMD feed on large amount of decaying wood of *A. aubryanum* (local name: Nzanzala). Especially, western lowland gorillas (*Gorilla gorilla gorilla*) showed high attachment to decaying *A. aubryanum* (see Fig. 1 and Supplementary Video). Interestingly enough, *A. aubryanum* was only species that herbivores consumed decaying wood, although *A. aubryanum* latex is toxic and caustic which deter animals from feeding on live wood; in fact, local people sometime use small amount of this latex as purgative [14].

Some researchers reported that decaying wood of par-

*CORRESPONDENCE TO: USHIDA, K., Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Shimogamo Sakyo-ku, Kyoto 606–8522 Japan. e-mail: k_ushida@kpu.ac.jp

#These authors contributed equally to this work.

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Fig. 1. Gorillas rushed to feed on decaying *Anthostema aubryanum* stump.

ticular tree plays an important roll for great apes as sodium resource [15, 18]. Therefore, we hypothesized that decaying stumps of *A. aubryanum* might be critical sodium resources not only for great apes but also for other herbivorous animals in our study site. To test this hypothesis, we conducted the following three researches. First, we performed chemical analyses on wood bark and latex of *A. aubryanum*, and confirm that *A. aubryanum* has high sodium concentrations. Second, we revealed which species ingest decaying *A. aubryanum* by using camera traps in the field. Third, we analyzed the frequency of appearance of *A. aubryanum* debris in fecal samples of gorillas to confirm that decaying *A. aubryanum* wood was regular food material for gorillas in PNMD.

MATERIALS AND METHODS

Study site: Our field study site, PNMD, covers an area of 5,028 km², and its southwestern boundary abuts the Atlantic Ocean. The study area covers about 120 km² in the southeastern part of the park at an altitude of 50–800m. This area typically experiences 2 seasons: the rainy season from October to May and the dry season from June to September. Mean annual rainfall was 1,777 mm (range: 1,583–2,163 mm). The mean monthly minimum and maximum temperatures varied from 21.3 to 24.1°C and 29.3 to 33.7°C, respectively. Seasonal fluctuations in fruit production are distinct in our study site. Fruit production is peaked between December and February and is the bottom between April and July (see Supplementary Fig. 2).

Collection of wood bark, latex samples and fruits: Live

wood bark with cambium (ca. 3~5 × 5~10 × 1 cm) was collected from *A. aubryanum* (local name: nanzala), *Ficus* sp. (divevengui rouge), *Ficus* sp. (divevengui blanc), *Ficus* sp. (ditsanda), *Milicia excelsa* (kambala), *Mitragyna ciliata* (tobu), *Myrianthus arboreus* (mububa) and *S. gabonensis* (musuga) in February 2012. Decaying bark with cambium and a surface portion of sapwood (ca. 3~5 × 5~10 × 1 cm thick) were collected from *Anthonotha macrophylla* (ibora; dingandu), *Berlinia macrophylla* (ibora), *Diospyros manii* (kingu nzibu), *Klainedoxa gabonensis* (mugoma), *M. excelsa* (kambala), *M. cecropioides* (dibala), *Nauclea diderrichii* (ivala kumu) and *S. gabonensis* (musuga) found adjacent to decaying stumps of *A. aubryanum* in August 2012.

Samples of bark with a surface portion of sapwood (ca. 3~5 × 5~10 × 2 cm) were taken from every decaying *A. aubryanum* stump found along streams within the study site in November 2011 and August 2012. These samples were placed in plastic bags with silica gel for transportation. Samples of latex were collected from two live *A. aubryanum* trees in February 2012. The liquid latex (10 ml) was collected and preserved in 30 ml of ethanol to avoid decomposition.

Following fruits were subjected to mineral analysis; *S. gabonensis*, *M. cecropioides*, *Cissus dinklagei* and *Aframomum giganteum*. Three fruits species, except for *S. gabonensis*, were top 3 fruits which were contained at the highest frequency in fecal samples collected from August 2004 to July 2008 (*M. cecropioides*=64.2% of fecal samples, *C. dinklagei*=32.3% and *A. giganteum*=26.4%). We collected intact fruits from the ground in July and August 2012. The fruits were seemingly freshly fallen from the tree, and some of them were actually showed signs of feeding of gorillas.

Fruits samples were air dried at 60°C and stored in desiccation until analysis.

Chemical analyses on wood bark, latex and fruits: Portions of samples were dried to a constant weight at 80°C and digested with nitric acid and perchloric acid following standard methods [21]. Metals (Mg, Ca, P, Fe, Cu, Zn, Mo, Cd, Ni, Co, Mn, Cr and Al) were analyzed in duplicate with inductively coupled Argon plasma spectrograph, and two macro-elements (Na and K) were analyzed in duplicate by atomic absorption as indicated elsewhere [22]. The latex in ethanol was well mixed, and subsamples were dried to a constant weight at 80°C before digestion. Comparison was made by Student *t*-test or Welch *t*-test for metal concentrations between decaying *A. aubryanum* and live *A. aubryanum* or decaying wood of other varieties.

Camera Trap: To determine the animal species that utilize the decaying stumps of *A. aubryanum*, and to estimate the frequency of visits and stay duration at decaying *A. aubryanum* stumps by these species, camera traps with an infrared triggering system (Bushnell Trophy Cam 2010, Bushnell Outdoor Product, Overland Park, KS, U.S.A.) were set up in front of five *A. aubryanum* stumps from November to December 2012. The focal stumps were selected from the decaying woods sampled for chemical analysis and located at least 50 m from each other. Cameras were installed on the nearest tree trunk at a height of ca. 70 cm. We used 'Video mode' of cameras, which takes 60-sec videos after the sensors are triggered and thus allows behavioral observations of animals. Medium and large-sized, ground-dwelling mammals (>1 kg) were targeted, and the species that consumed the decaying stumps from the videos were determined.

A video was considered to indicate a single visit by a given species, if the picture was obtained <30 min after the previous picture of that species. Mean stay duration (hr) of each animal species was estimated from the start time of the first video to the end time of the last video. Because it was impossible to recognize individuals for group-living animals (e.g. gorillas), visitation frequencies and stay durations as a group were estimated. Capture rates (the number of videos/100 camera-trapping days) and mean visit duration for each species at *A. aubryanum* sites were compared with the results from ten camera traps (as controls) located at 200-m intervals along the 2 km-transect. All the control sites were positioned within 1-km from the nearest *A. aubryanum* sites. For camera-trapping data, Wilcoxon rank sum tests were used to compare the capture rates and mean stay duration of each animal species at *A. aubryanum*.

Fecal sample analysis for Western Lowland Gorillas: We analyzed the fecal sample data to test the frequency of decaying *A. aubryanum* consumption by the western lowland gorilla group. Fresh fecal samples (<48 hr) were collected from the nest sites of a habituated gorilla group. Fecal samples were washed in water through 1-mm mesh sieves and dried in sunlight within 1 day of collection. Remnants were visually identified together with live observatory information. Remnants of decaying wood and live wood in the feces were easily distinguishable. Decaying wood in fecal samples was much smaller than that of living wood, because

bark of living wood split lengthwise but dead wood became fragments.

Fecal sample data at the same study period with camera trap are not available. However, we monitored diet of gorillas for 4 years (Aug, 2004–July 2008) and ascertained that is a highly consistent pattern of diet, with fruit consumption by gorillas peaking three months between December to February (See supplementary Fig. 1). We used the fecal samples collected between November and December, as the same months with camera trap, in 2004 to 2007 for the analysis.

RESULTS

Mineral concentration in decaying A. aubryanum and other foods: Concentrations of macro- and oligo-elements are summarized in Fig. 2 and Supplementary Table 1 in which the bark from decaying *A. aubryanum* was compared with bark from live *A. aubryanum*, other live wood species or other decaying wood species adjacent to decaying *A. aubryanum* stumps. Macro-elements, sodium, potassium, magnesium, calcium and phosphorus were detected in all samples. Among the oligo-elements, zinc, copper and iron were detected in all samples, while other minerals, such as molybdenum, cadmium, nickel, cobalt, manganese, chromium and aluminum, were detected only in trace amounts (see Supplementary Table 1). Therefore, these trace elements were omitted from consideration.

Mean sodium concentrations recorded in decaying *A. aubryanum* stumps were 1,343 mg/kg dry matter (range=357–4,318 mg/kg) with a relatively high variation and the highest of all the samples analyzed (Fig. 2 and Supplementary Table 1). Similar trends were observed for magnesium and phosphorus; decaying *A. aubryanum* had the highest levels of all the samples analyzed. However, decaying *A. aubryanum* did not necessarily have the highest levels of potassium and calcium. *A. aubryanum* dead wood also did not show higher concentrations of zinc, iron and copper than the other samples.

Latex from live *A. aubryanum* (n=2) contained ca. 199 mg (293 and 104) Na/ml, ca. 11,988 mg (14,332 and 9,644) K/ml, ca. 494 mg (417 and 517) Mg/ml, 117 mg (132 and 104) Ca/ml and 173 mg (184 and 161) P/ml. Although the sample size was small (n=2), the variation between two samples seems to be small in comparison with dead *A. aubryanum*.

The fruits were seemingly freshly fallen from the tree on the ground, and some of them were actually showed signs of feeding of gorillas. Among them, fruits of *S. gabonensis* showed higher Na concentration (mean=823 mg/kg dry matter, range=339–1,293 mg/kg) than other fruits (from 0 to 70 mg/kg) (Supplementary Table 1).

Frequency of animal visiting to A. aubryanum stumps: During the study period, our camera traps captured eight species, including *Cephalophus* spp., at decaying wood sites (Fig. 3a and 3b). All animal species captured at decaying wood sites were herbivorous species. In contrast, insectivores/carnivores were never observed to consume decaying *A. aubryanum* stumps. The species that visited most frequently was the blue duiker (*Philotomba monticola*), followed by

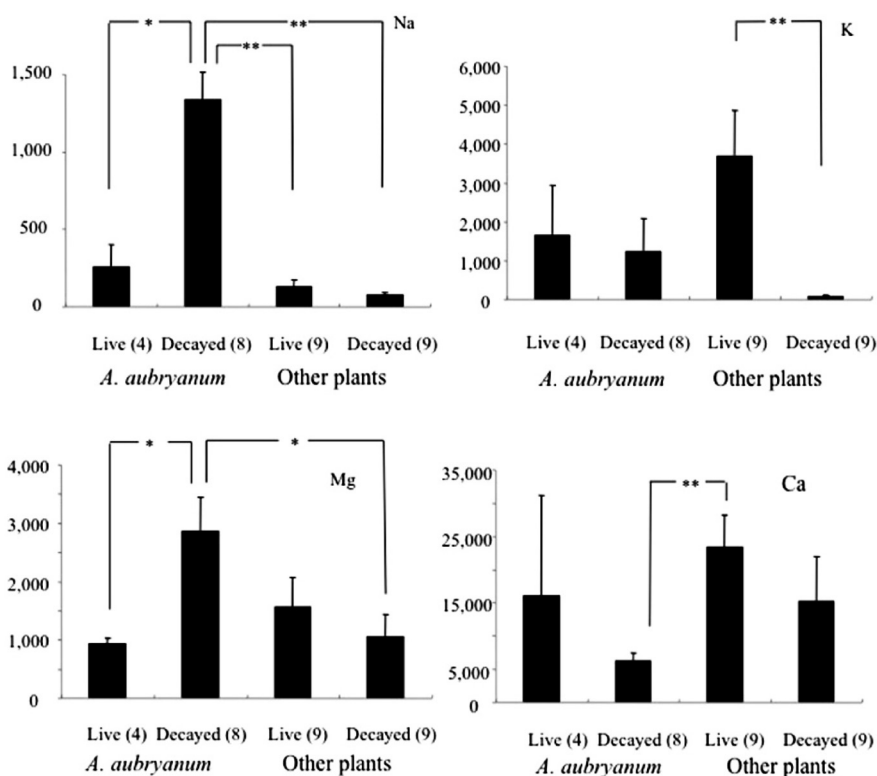


Fig. 2. Comparison of the mineral content (mg/kg dry matter) in live and decayed wood of *Anthostema aubryanum* and other plants. The numbers in parentheses are the number of samples. ** $P < 0.01$, * $P < 0.05$.

gorillas, yellow-backed duiker (*Cephalophus silvicultor*) and chimpanzees (*Pan troglodytes troglodytes*) (Fig. 3a). Blue duikers, gorillas, yellow-backed duikers, chimpanzees and red-capped mongabey (*Cercocebus torquatus*) stayed significantly longer at decaying *A. aubryanum* sites than at control sites (Fig. 3b). Our camera trap results show that two species of great apes visited *A. aubryanum* stumps significantly more frequently (Fig. 3a) and stayed longer than at control sites (Fig. 3b). These results indicate that great apes were strongly attracted to decaying *A. aubryanum*.

Decaying A. aubryanum as regular food material for gorillas: In total, 704 fecal samples (monthly mean=88, range=40–161) were analyzed (Fig. 4). Debris from decaying *A. aubryanum* wood was found in 28.9% of the fecal samples. During the study period, only December 2007 showed low (6%) proportion of *A. aubryanum* occurrence in fecal samples. In contrast, *A. aubryanum* occurrence rates were more than 10% in other seven months. Especially, the proportions of fecal samples containing *A. aubryanum* in November 2004 and November 2006 were more than 40%. Thus, it can be said that *A. aubryanum* dead wood is one of a regular food for western lowland gorillas in PNMD.

DISCUSSION

A. aubryanum wood as sodium resource for herbivores: Our chemical analysis confirmed that decaying woods of *A.*

aubryanum have high cations, especially sodium concentrations was much higher than those of other live/dead trees or fruits (Fig. 2 and Supplementary Table 1). Thus, decaying wood stump of *A. aubryanum* is an important mineral resource for diverse herbivores in PNMD. Herbivores should selectively ingest it to compensate specifically for a shortfall in sodium. To our knowledge, this is the first report indicating the tight relationship between herbivores and a particular plant species whose latex is toxic and caustic when it is alive. When *A. aubryanum* is alive, the sodium content of the bark of this tree is low, although its latex contains cations at relatively high concentrations. Latex produced by Euphorbiaceae is known to carry higher quantities of cations [2]. The higher cation content of latex may contribute to cation accumulation in decaying *A. aubryanum* wood. But, latex of *A. aubryanum*, as a component of live plant, contained potassium as the major cation (Supplementary Table 1). Other mechanisms would be involved in sodium accumulation in dead *A. aubryanum*.

Difference of selectivity of A. aubryanum among mammals: We detected clear difference of selectivity of *A. aubryanum* consumption among mammals inhabiting in PNMD. These differences of decaying *A. aubryanum* consumption reflected the difference of dietary feature or ecological conditions among them. We did not detect carnivores and omnivores visiting to *A. aubryanum* stumps, because they can acquire sodium from animal food. On the contrary, all

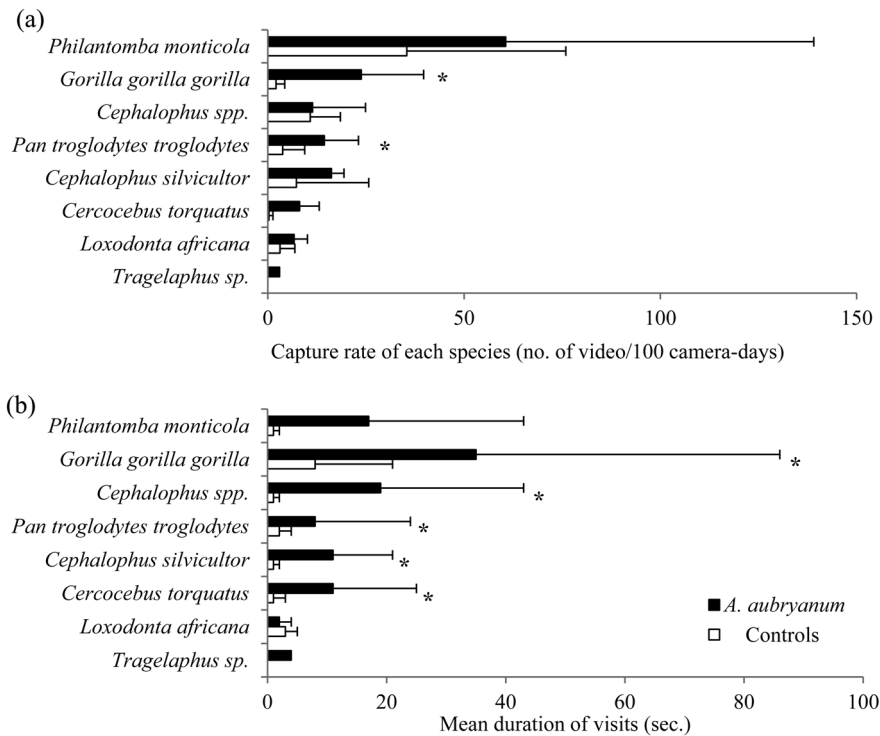


Fig. 3. Capture rate (a) and stay duration (b) of herbivorous species (N of video/100 camera-days, Mean \pm SD) at decaying wood of *A. aubryanum* wood sites (n=5) and at control sites (n=10). * $P < 0.05$.

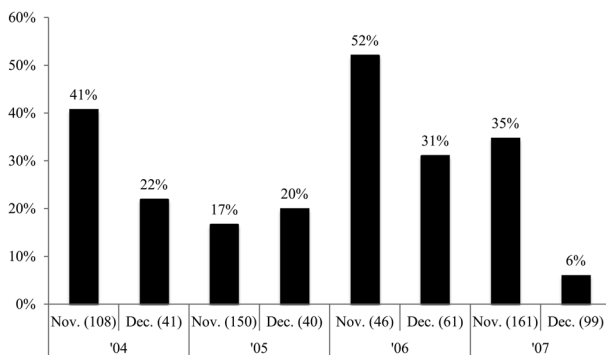


Fig. 4. Proportions of fecal samples of *Gorilla gorilla gorilla* containing *A. aubryanum* debris in November to December from 2004 to 2007. Numbers in parentheses are total number of fecal samples collected in each month.

mammals captured by camera trapping were limited to forest herbivores. Because sodium concentration of regular diet for herbivores is low, so herbivores consumed *A. aubryanum* as sodium resource in PNMD. We detected at least two duikers, blue duikers and yellow-backed duikers, visited with high frequency (more than 10 captures/ 100 camera-days), but duration of staying was much shorter than gorillas (Fig. 3b). Duikers' short retention time at decaying stumps was to decrease the possibility to be found by their predator, such

as leopards.

Our results indicate that *A. aubryanum* decaying wood is the critically important sodium resource for gorillas. Gorillas showed the second highest frequency and the longest duration of visit (Fig 3a and 3b). Fecal sample analysis showed that *A. aubryanum* is one of a regular food for gorillas. Chimpanzees also showed significantly higher frequency and longer duration to visit *A. aubryanum* stumps than control sites, but gorillas showed higher frequency and longer duration than chimpanzees. Great apes are known that their main diet consists of fruits and leaves, but previous reports showed that chimpanzees occasionally hunt other animals or fishing ants or termites [9]. In contrast, gorillas do not hunt other animals, and frequency of insect consumption is low in PNMD (5% of fecal samples; Iwata *et al.* unpublished data). The longest duration of staying at *A. aubryanum* decaying wood by gorillas might be caused by lack of predator for them. Furthermore, chimpanzees have fission-fusion group structure and forage in smaller parties or alone [5], but large body-sized gorillas are group living animal, and our research group has more than 20 individuals [1]. Thus, it should take long duration for gorillas that plural individuals consume enough amount of decaying wood, because of higher feeding competition than chimpanzees.

Herbivores which inhabiting in the forest which lacks "bai" have alternative mineral resources. In Petit Loango, Gabon, elephant often used the coastal habitat for the consumption of salt-coated vegetation [11]. In Lopé National

Park, Gabon, western lowland gorillas depend on sodium-rich *Marantochloa cordifolia* [17]. Although further chemical analysis on other food items is necessary, *A. aubryanum* showed almost the same level of sodium concentration with *Hydrocharis* sp. which is an important sodium resource for gorillas in Ndoki forest (*Hydrocharis* sp.: 1,080 mg/kg vs. *A. aubryanum*: 1,343 mg/kg). *A. aubryanum* which abundantly exists in PNMD might play an important role for herbivores as sodium resource, which is available year-round for herbivores in our study site.

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