Original Research Article

High Prevalence of Cannabis Use Among Aka Foragers of the Congo Basin and Its Possible Relationship to Helminthiasis

CASEY J. ROULETTE,[1] MIRDAD KAZANJI,[2] SÉBASTIEN BREUREC,[2] AND EDWARD H. HAGEN[1]*
[1]*Department of Anthropology, Washington State University, Vancouver, WA*
[2]*Institute Pasteur, Bangui, Central African Republic*

Objectives: Little is known about cannabis use in hunter-gatherers. Therefore, we investigated cannabis use in the Aka, a population of foragers of the Congo Basin. Because cannabis contains anthelminthic compounds, and the Aka have a high prevalence of helminthiasis, we also tested the hypothesis that cannabis use might be an unconscious form of self-medication against helminths.

Methods: We collected self- and peer-reports of cannabis use from all adult Aka in the Lobaye district of the Central African Republic ($n = 379$). Because female cannabis use was low, we restricted sample collection to men. Using an immunoassay for Δ9-tetrahydrocannabinol-11-oic acid (THCA), a urinary biomarker of recent cannabis consumption, we validated cannabis use in men currently residing in camps near a logging road ($n = 62$). We also collected stool samples to assay worm burden. A longitudinal reinfection study was conducted among a subsample of the male participants ($n = 23$) who had been treated with a commercial anthelmintic 1 year ago.

Results: The prevalence of self- and peer-reported cannabis use was 70.9% among men and 6.1% among women, for a total prevalence of 38.6%. Using a 50 ng/ml threshold for THCA, 67.7% of men used cannabis. Cannabis users were significantly younger and had less material wealth than the non-cannabis users. There were significant negative associations between THCA levels and worm burden, and reinfection with helminths 1 year after treatment with a commercial anthelmintic.

Conclusions: The prevalence of cannabis use among adult Aka men was high when compared to most global populations. THCA levels were negatively correlated with parasite infection and reinfection, supporting the self-medication hypothesis. Am. J. Hum. Biol. 00:000–000, 2015.

INTRODUCTION

Cannabis is the world's most widely used illicit drug (Hall and Degenhardt, 2007), with a global prevalence of use estimated at 2.9–4.3% of all individuals between 15 and 64 years (UN, 2010). Research on cannabis use is limited, however, due to the potential legal and social consequences of admitting use. Consequently, little is known about cannabis use in the developing world (e.g., Degenhardt and Hall, 2012). Even less is known about cannabis use among the many small-scale populations scattered throughout remote parts of the developing world, such as in the Congo Basin. Here we investigate patterns of cannabis use in a population of foragers of the Congo Basin. This is one of the first systematic studies of cannabis use in a hunting-gathering population, and the first, to our knowledge, to validate use with a biomarker. It is also one of the first studies to explore the relationship between substance use and intestinal helminth infection, two of the developing world's great health problems.

Cannabis

Cannabis spp. are indigenous to Asia (Hillig, 2005), where they have been used as fiber, food, oil, medicine, and intoxicants since prehistory. They belong to the small family Cannabaceae, which also includes another socially and economically important species, *humulus* (hops) (Clarke and Merlin, 2013). Cannabis contains the psychoactive cannabinoid, Δ9-tetrahydrocannabinol (THC), as well as hundreds of other chemicals, including more than 60 cannabinoids (Huestis, 2007; Turner et al., 1980). *Cannabis sativa* is the most widely distributed of the cannabis species today, due in large part to its important economic role as a source of fiber.

Arrival of cannabis in Africa

It is uncertain when cannabis was brought to Africa. Schultes (1970) argues that cannabis arrived in Africa as early as 4000–3000 BP, but du Toit (1980), using linguistic, historical, and archaeological evidence, concludes that cannabis arrived via Moslem sea traders from the Indian subcontinent around the 1st century AD. It then spread west and south via the Arab caravan trade (du Toit, 1976). Philips (1983, p. 308), however, argues that Islamic Arabs were not involved and that even if cannabis was in Africa prior to European colonization, it was likely not smoked until the 17th century. Today, the prevalence of cannabis use in Africa is estimated at 5–12.5%, with the highest rate in West and Central Africa, although data are very scarce (UN, 2013).

Cannabis use among foragers of the Congo Basin

Foragers of the Congo Basin, also called "pygmies," are a large and diverse population of hunter-gatherers comprising at least 15 ethnolinguistic groups, of which the most well studied are the Aka, Baka, Efe, and Mbuti

Additional Supporting Information may be found in the online version of this article.

Contract grant sponsor: State of Washington Initiative Measure No. 171.

*Correspondence to: Edward H. Hagen, 14204 Northeast Salmon Creek Avenue, Vancouver, WA 98686. E-mail: edhagen@wsu.edu

Received 26 January 2015; Revision received 24 February 2015; Accepted 6 May 2015
DOI: 10.1002/ajhb.22740
Published online 00 Month 2015 in Wiley Online Library (wileyonlinelibrary.com).

(Bahuchet, 2014). These foragers are characterized by a preference for forest life, polyphonic music, an association with farmer populations, and are generally peaceful and egalitarian with marked gender equality. Most are transitional foragers in that they spend part of the year camped near villages to work in the farmers' fields, and the rest of the year in the forest hunting and gathering. When camped near the villages, they trade labor and forest products for agricultural foods, clothes, salt, axes and knives, and psychoactive substances like alcohol, tobacco, and cannabis (Hewlett, 2014).

Foragers of the Congo Basin smoke a variety of psychoactive substances, including commercial cigarettes, locally grown tobacco, cannabis, and native plants. It is probable that the foragers of the Congo Basin have always known of plants with psychoactive properties, such as the forest plant *medeaka* smoked by the Mbuti (Schebesta, 1941), which might be a species of *Mitragyna* (Rätsch, 2005), as well the forest plant *motunga* (*Polyalthia suaveolens*, Annonaceae, a.k.a. *Greenwayodendron suaveolens* Verdc., Annonaceae; Engl. and Diels), which is smoked by the Aka (Roulette et al., 2014) and Baka (Oishi and Hayashi, 2014).

Although the Mbuti say that they have smoked cannabis since the beginning of time (Hallet, 1975, p. 409), there is little evidence for forager use prior to European colonization. Ethnographically, elephant hunting specialists have been described as the heaviest users of cannabis (Hewlett, 1977; Schebesta, 1933), which might indicate that it was adopted at the height of the ivory trade, in the 19th century (Hewlett, 1977).

Linguistic evidence suggests that cannabis spread from eastern Africa west into the interior of the Congo Basin. For example, *bangui*, the common forager word for cannabis, is related to the popular east African root word, *mbangi*; and the west Congo Basin Baka word, *njama* (Oishi and Hayashi, 2014), and the east Congo Mbuti word, *djemu*, are both similar to the word, *njemu*, which is used by the Nyamwezi, an east African Bantu group (Hewlett, 1977).

Ethnographic descriptions of cannabis use among foragers of the Congo Basin are limited, and those that exist are often contradictory. Turnbull (1968, 1976), for instance, rarely mentions cannabis use among the Mbuti, whereas Schebesta (1933, 1936) notes its frequent use. Schebesta (1933) mentions that cannabis gives archers the "power" to kill elephants, but that it also makes them "dazed and trembling all over" (Schebesta, 1936, p. 214). In one of the only reviews of cannabis use among pygmies of the Congo Basin, Hewlett (1977) notes that cannabis plays important sociocultural and ecological functions and that it is associated with motivational effects. As he states, they "smoke to increase their courage on a hunt, dance better, increase their vital force, or to increase their work capacity when working for Europeans or village people" (Hewlett, 1977, p. 10). However, Turnbull (1983) notes that cannabis can also be detrimental to hunting (and mating) success. Bailey (1991) focuses on the negative economic impact of smoking cannabis (and tobacco) among Mbuti foragers, noting that Mbuti smokers are more likely to trade labor and goods for cannabis and tobacco, thus decreasing their overall material wealth.

Despite these few ethnographic descriptions, to our knowledge, there are no systematic studies of cannabis use among foragers of the Congo Basin. Thus, the current extent of cannabis use in these populations is unknown.

Cannabis versus helminth infection

Human behavioral ecologists have rarely investigated psychoactive substance use (see Dudley, 2000; Hill and Chow, 2002; Lende and Smith, 2002). In non-human animals, including many vertebrate, mammalian, and primate species, there is increasing evidence that consumption of toxic plants is a form of self-medication against pathogens (Forbey et al., 2009; Huffman, 1997; Suárez-Rodríguez et al., 2013; Villalba et al., 2014; Wrangham and Nishida, 1983). Interestingly, all major human recreational plant drugs—coffee, tea, tobacco, cannabis, and betel nut—are toxic to helminths. Indeed, nicotine, the principal psychoactive compound in tobacco as well as arecoline, the psychoactive compound in betel nut, have been widely used as commercial anthelmintics (Hammond et al., 1997). This raises the possibility that human "recreational" substance use might be an unconscious form of self-medication against helminths and other macroparasites (Hagen et al., 2009; Roulette et al., 2014; Rodriguez and Cavin, 1982; Wyatt, 1977).

The global disease burden of helminth infections is greater than that of malaria and tuberculosis and is associated with anemia, growth stunting, protein-calorie under nutrition, fatigue, and poor cognitive development (Hotez et al., 2008). Most foragers of the Congo Basin are heavily parasitized (Froment, 2014; Lilly et al., 2002; Roulette et al., 2014), similar to other hunter-gatherer populations (Hurtado et al., 2008).

Cannabis contains anthelminthic compounds, and therefore, its use might protect against parasitic infection. *C. sativa*, for example, has been used as a pest repellent and pesticide as well as a companion crop to help control plant nematodes (McPartland, 1997). Aqueous extracts of *C. sativa* are, indeed, toxic to plant nematodes (Mukhtar et al., 2013). Cannabis extracts also have demonstrated activity against human pathogens and parasites. All five major cannabinoids (cannabidiol, cannabichromene, cannabigerol, THC, and cannabinol) have antibacterial activity against *Staphylococcus aureus*, a coccal bacterium often found in the human respiratory tract and on the skin (Appendino et al., 2008). Crude extracts of *C. sativa* leaves also cause paralysis and death of the intestinal trematode *Fasciolopsis buski*, and the effect is more lethal than the commercial flukicide, Oxyclozanide (Roy and Tandon, 1997).

Study aims

This study had two aims. First, using self-reports and a urinary biomarker, we wanted to provide a definitive prevalence of cannabis use for one forager population of the Congo Basin: Aka residing in the Lobaye district of the Central African Republic (CAR). Our previous research on the prevalence of tobacco use among the Aka found that 95% of Aka men smoked tobacco (Roulette et al., 2014), compared to ~17% of men in sub-Saharan Africa and 31% of men globally (Ng et al., 2014). This raised the possibility that the prevalence of Aka men's cannabis use might also be high. In contrast, less than 15% of Aka women self-reported smoking tobacco, and only 5% were smokers according to their levels of cotinine, a nicotine metabolite (Roulette et al., 2014). This resembled sex differences in substance use seen in other developing countries (e.g., Ng et al., 2014; Degenhardt et al., 2008).

We also wanted to determine if variation in cannabis use was associated with variation in: wealth, as previously reported (Bailey, 1991); acculturation; Aka social roles, such as traditional healers and camp leaders (elephant hunting is now very rare); residential proximity to a large village; and age and sex, as seen in other populations (Degenhardt et al., 2008).

Our second aim was to test the hypothesis that psychoactive drug use, in part, is a form of self-medication against helminths. Therefore, we investigated the relationship between cannabis use and helminth infections. In our previous study on Aka tobacco use, Roulette et al. (2014) found that, in Aka men, (1) the heaviest tobacco smokers had the lowest worm burden; (2) treatment with albendazole, a commercial anthelmintic, reduced tobacco smoking (or increased nicotine metabolism) compared to placebo controls; (3) after treatment, salivary cotinine levels were negatively correlated with helminth reinfection 1 year later; and (4) individuals with cytochrome P450 alleles that slowed nicotine metabolism had lower worm burden compared to those with normal metabolizing alleles.

Here we attempt to replicate results (1) and (3) for cannabis [we did not have funding to replicate (2) or (4)]. Specifically, our first study investigated the cross-sectional relationship between cannabis use and worm burden. Worm burden should be negatively correlated with Δ9-tetrahydrocannabinol-11-oic acid (THCA) concentrations. Our second study was longitudinal and explored cannabis use versus helminth reinfection. Among individuals whose helminth infections have been treated with a commercial anthelmintic in year 1, higher levels of cannabis use should limit reinfection with helminths in year 2.

MATERIALS AND METHODS

This study was approved by the Washington State University Institutional Review Board (IRB) and the IRB of the Medical School of the University of Bangui, CAR. Informed consent was obtained from all participants. Consent was oral, and not written, for two reasons. First, the Aka are not literate. Second, cannabis is illegal in CAR, although in rural villages this law is only lightly enforced. Oral consent helped ensure that privacy would not be breached. Both IRBs approved oral consent, and consent was documented by a local translator. Permission to conduct this research was obtained from the Mistere de l'Eduation Nationale, de l'Enseignement superieur et de la Recherche, CAR.

Study population and participant recruitment

The Aka (also called BaAka, Biaka, and Bayaka) are a group of hunter-gatherers (forest foragers) residing in the western Congo Basin. They number about 30,000, but live in small camps (approximately 6–12 adult Aka per camp) scattered throughout southwestern CAR and the northern part of the Republic of the Congo (Hewlett, 1996).

Participants were recruited from three regional subpopulations along the Lobaye River in CAR with a combined Aka population of 164 adult men and 215 adult women residing in 36 camps. Aka camps are located on trails radiating out from the main logging road into the forest. All participants resided in camps within ~1 km of the main logging road.

We first surveyed cannabis and tobacco use in all men and women in all camps. Not everyone in each camp was present at the time of the survey. According to self-report and peer report, 70.9% of males and 6.1% of females used cannabis, for a total prevalence of 38.6%. Due to the low percentage of female cannabis users, we restricted our remaining data collection to men.

We recruited all adult men who were currently residing in camp to participate in the study ($n = 62$). We did not prescreen these participants for cannabis or tobacco use, health complaints, or any other criteria. Most of these men (54) had been treated with albendazole 1 year ago.

Specimen collection

Using provided kits, all 62 male participants were asked to provide stool specimens every other day for about 6 days, for a total of 3 years—two worm specimens each, as well as three saliva and urine samples. Stool was collected using ParaPak vials with formalin. Participants were instructed to provide 2–5 ml of saliva immediately upon waking and before smoking their first cigarette. Urine was collected in sterile collection cups and then pipetted into 5-ml centrifuge tubes. Urine and saliva were stored at −20°C in a solar-powered freezer and then shipped to the Bioanthropology Laboratory at Washington State University (WSU) Vancouver for analysis.

A subsample of the albendazole-treated men ($n = 23$) had also provided one to three stool samples 2 weeks after treatment (see Roulette et al., 2014 for details); these men comprised the sample for the longitudinal reinfection study reported here. We refer to these immediate post-treatment stool samples as the "year 1 baseline" samples, s1–s3, and to the new samples collected specifically for this study as "year 2 samples," s4–s6.

Interview

All male participants were interviewed, using a semi-structured questionnaire (Bernard, 2006; Schensul et al., 1999) to assess the sociodemographic characteristics of cannabis use and to control for potential confounds in the cross-sectional and reinfection studies. The surveys produced 14 demographic variables in six domains: kombeti, nganga, wealth, acculturation, subpopulation, and age. Kombeti are the head of camps and are the oldest male member of each camp (1 = kombeti, 0 = not kombeti). Nganga are traditional healers (1 = nganga, 0 = not nganga). The "wealth score" was the sum of self-reported number of clothes, watches, flashlights, radios, and batteries owned. The "acculturation score" was the sum of self-reported attendance in school as a child and as an adolescent, church attendance, and preference for living in the village versus the forest (yes = 1, no = 0). Subpopulation was either coded as belonging to one of the three regional subpopulations or as village (the largest subpopulation) versus non-village (the other two, smaller, subpopulations). Because Aka do not keep track of age, age was estimated by an Ngandu research assistant who had lifelong associations with these Aka populations.

For each trail, we also recruited three adults to peer rate the frequency of cannabis use of all participants who resided on that trail (0 = less than average, 1 = more than average). We then summed those three ratings to create a peer-rated frequency of use score, which ranged from 0 to 3.

THCA and cotinine assays

THCA is a metabolic product of THC. It is predominantly excreted in urine as the ester-linked glucuronide conjugate (Foltz, 2007). THCA is a superior biomarker to THC because it can be detected for several weeks following a single dose of THC (Agurell et al., 1986; Ellis et al., 1985) and it is more soluble and absorbs less onto the walls of storage containers (Rogers et al., 1978).

Nicotine, the principal psychoactive alkaloid in tobacco, has a half-life of 2–3 hours whereas cotinine, the principal metabolite of nicotine, has a half-life of approximately 17 hours. Cotinine is thus a superior biomarker of recent nicotine exposure (Benowitz, 1996).

Urine and saliva were assayed in the Bioanthropology Laboratory at WSU Vancouver using THCA and cotinine enzyme-linked immunosorbent assay kits, respectively, according to the manufacturer's (Salimetrics and Immunalysis) protocols. THCA and cotinine concentrations were the mean of s4–s6.

Semiquantification of worm burden and reinfection score

Worm burden was estimated by counting helminth eggs in stool. Stool samples were analyzed by the Institute Pasteur in Bangui, CAR. Three species of geohelminths were assessed for worm burden score: *Trichuris trichiura* (roundworm); *Ascaris lumbricoides* (giant roundworm); and hookworm (including both *Ancylostoma duodenale* and *Necator americanus* because their eggs cannot be distinguished). Different techniques for counting eggs have different advantages and disadvantages, so we estimated egg counts per-species using three methods: (1) a wet smear that was microscopically examined for helminth eggs; (2) the merthiolate iodine formaldehyde (MIF) concentration technique (Blagg et al., 1955); and (3) the Kato method (Komiya and Kobayashi, 1966). Worm burden comprised the average number of eggs on each ×40 microscope field. There were thus three measurements for each of the three helminth species (hookworm, whipworm, *Ascaris*), for a total of nine measurements per stool sample, which were averaged to produce the sample worm burden score. The MIF technique is the most sensitive at detecting eggs, so this count was weighted by a factor of 2. The average was then adjusted for the volume of stool examined. A worm reinfection score was computed by subtracting the mean of year 1 baseline worm burden scores (s1–s3) from the mean of year 2 worm burden (s4–s6).

Data analysis

Statistics were computed with R version 3.1.0 (2014-04-10) for Mac. The worm burden score was an average of egg counts and was overdispersed. Therefore, we modeled worm burden using negative binomial generalized linear models (GLMs) and generalized additive models (GAMs) with a log link. Cannabis smoker status was dichotomous and was modeling using logistic regression. Among smokers, we modeled log (THCA) with ordinary least squares (OLS) regression. All models were evaluated using standard diagnostics, such as residual plots used to assess homogeneity and independence, and to identify possible outliers. We used the corrected Akaike information criterion (AICc) for model selection (Burnham and Anderson 2004). Sample size for the reinfection study was small, limiting our ability to use control variables. THC concentrations were log transformed in some of the figures for visual clarity. Data will be available at https://thedata.harvard.edu/dvn/dv/wsuv-bioanthro-lab.

TABLE 1. Descriptive statistics

	N	Min	Max	Median	Mean	SD
Age (years)	61	18.0	60	35.0	36.70	10.00
THCA (ng/ml)	62	1.3	4,100	370.0	663.00	830.00
Cotinine (ng/ml)	61	0.0	980	240.0	301.00	270.00
Worm burden score	62	0.0	14	2.1	3.27	3.10
Wealth score	56	1.0	8	2.0	2.27	1.40
Acculturation score	56	0.0	4	2.0	2.21	0.89

RESULTS

Of our 62 participants, 42 resided in camps near a large village, and 12 and 8 resided near one of two smaller outlying villages; 18 were kombeti (camp leaders); and 12 were nganga (healers). Descriptive statistics for worm burden, THCA and cotinine concentrations, age, wealth, and acculturation are presented in Table 1.

THCA versus self- and peer-reported cannabis use

THCA concentrations were bimodally distributed, with 67.7% ($n = 42$) of scores above the conventional cutoff of 50 ng/ml THCA (SAMHSA, 2008) and the rest below the cutoff (Supporting Information Figure S1). In comparison, 60.7% of the sample self-reported as cannabis users. All self-reported cannabis users had THCA concentrations above the 50 ng/ml cutoff, whereas 19 of 24 self-reported non-cannabis users (79.2%) had THCA concentrations below the 50 ng/ml cutoff (Supporting Information Figure S2). Self-reported non-cannabis users had a median THCA concentration of 8.3 ng/ml, and self-reported cannabis users had a median THCA concentration of 832 ng/ml. Unless stated otherwise, in the remainder of the study cannabis smoker status is defined by THCA concentration > 50 ng/ml. THCA values below the cutoff represent either second hand exposure to cannabis smoke or less recent use of cannabis.

Peer-rated frequency of use ranged from 0 to 3. Non-cannabis smokers were reliably distinguished from cannabis smokers by the raters: only 1 of the 14 participants with a peer-rating of 0 had THCA concentrations above 50 ng/ml, whereas only 2 of the 42 participants with a rating of 1 or greater had THCA concentrations below 50 ng/ml. Among the peer-rated smokers, there was a marginally significant trend for THCA levels to increase with higher peer ratings of use ($Z = 1.29$, $P = 0.099$).

THCA versus demographic and social role variables

Cannabis smokers (THCA > 50 ng/ml) were younger than nonsmokers (mean age = 33.6 vs. 43.1, respectively), and had lower wealth scores (mean wealth = 1.92 vs. 2.95, respectively). A logistic regression model of cannabis smoker status versus the demographic variables (age, wealth, acculturation, village) found that both age and wealth were independent, significant negative predictors (the other demographic variables were not significant predictors alone or in combination with other variables and were, therefore, omitted). See Figure 1 and Table 2.

Of the social roles, ngangas were not significantly more or less likely to be cannabis smokers ($X^2 = 2.14$, $P = 0.14$). Kombeti were significantly less likely to be cannabis smokers than other men but this was due to a confound

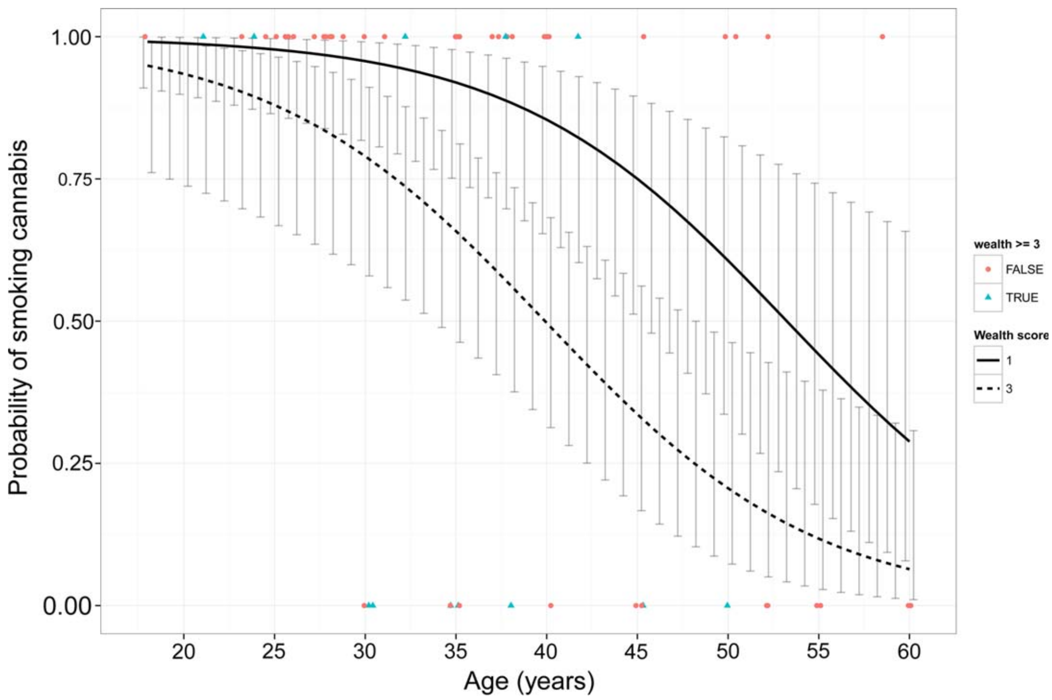

Fig. 1. Probability of smoking cannabis as a function of age and wealth (1 = low, 3 = high). Bars are 95% confidence intervals. See Table 2 for model coefficients and indices of fit. Wealth = 3 was chosen as "high" wealth because although wealth scores ranged from 1 to 8, 78.6% of men had scores less than 3. To display overlapping points, a small amount of jitter was added to age. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

with age. "Kombeti" literally means "older brother," and they are typically the oldest male in camp. After controlling for age in a logistic regression model, kombeti status was no longer a significant predictor of smoker status (test not reported).

We then investigated, among cannabis smokers, whether our demographic variables predicted log THCA levels (using OLS). According to AICc scores, no model with any combination of demographic variables outperformed an intercept-only model (results not reported). In addition, using permutation tests, neither ngnanga ($Z = -1.21$, $P = 0.23$) or kombeti status ($Z = 1$, $P = 0.32$) was associated with THCA levels among cannabis smokers. Finally, among cannabis smokers, cotinine levels were positively rank correlated with THCA levels (r_s=0.36, $P = 0.02$).

Worm burden versus demographic and social role variables

Of the 62 participants, 3 (4.8%) had no evidence of helminth infection, 20 (32.3%) were infected with at least one species of helminth, 22 (35.5%) were infected with two species of helminths, and 17 (27.4%) were infected will all three species of helminths. By species, 56 (90.3%) participants were infected with hookworm (including both *Ancylostoma duodenale* and *Necator americanus*), 31 (50%) were infected with *T. trichiura*, and 28 (45.2%) were infected with *A. lumbricoides*.

Most participants had low worm burden scores, but a few had high scores (Supporting Information Figure S3). We investigated worm burden scores versus our demographic and social role variables using permutation tests, correlation tests, and negative binomial GLMs and GAMs with a log link. There were no convincing associations between worm burden and any of these variables either alone or in combination. Although there was a negative rank correlation between worm burden and age, as seen in other populations, it was only marginally significant ($r_s = -0.23$, $P = 0.076$). In addition, there was a significant, u-shaped relationship between acculturation and worm burden in a GAM, but inspection revealed it was due to high worm burden in two individuals with the minimum acculturation score (0), and three individuals with the maximum acculturation score (4). For the large majority of participants with intermediate acculturation scores (1–3), the relationship was essentially flat.

Worm burden versus THCA

To test if THCA was a negative predictor of worm burden after controlling for potentially confounding variables, we first computed the same GAM as we did for the tobacco versus helminths study in Roulette et al. (2014), except that here we included THCA concentration. This model thus fit worm burden in all participants as a function of village, wealth, acculturation, age, cotinine, and THCA. We found significant negative effects but they were not curvilinear, negating the need to use a GAM. We, therefore, fit several GLMs with the negative binomial family and log link, and used AICc for model selection. Model 1 (Table 3) best approximates the GAM model used in Roulette et al. (2014), but model 2 (Table 3, Figure 2), which does not include cotinine, wealth, or acculturation, had the lowest AICc score. Because some data were missing for some variables, we used multiple imputations

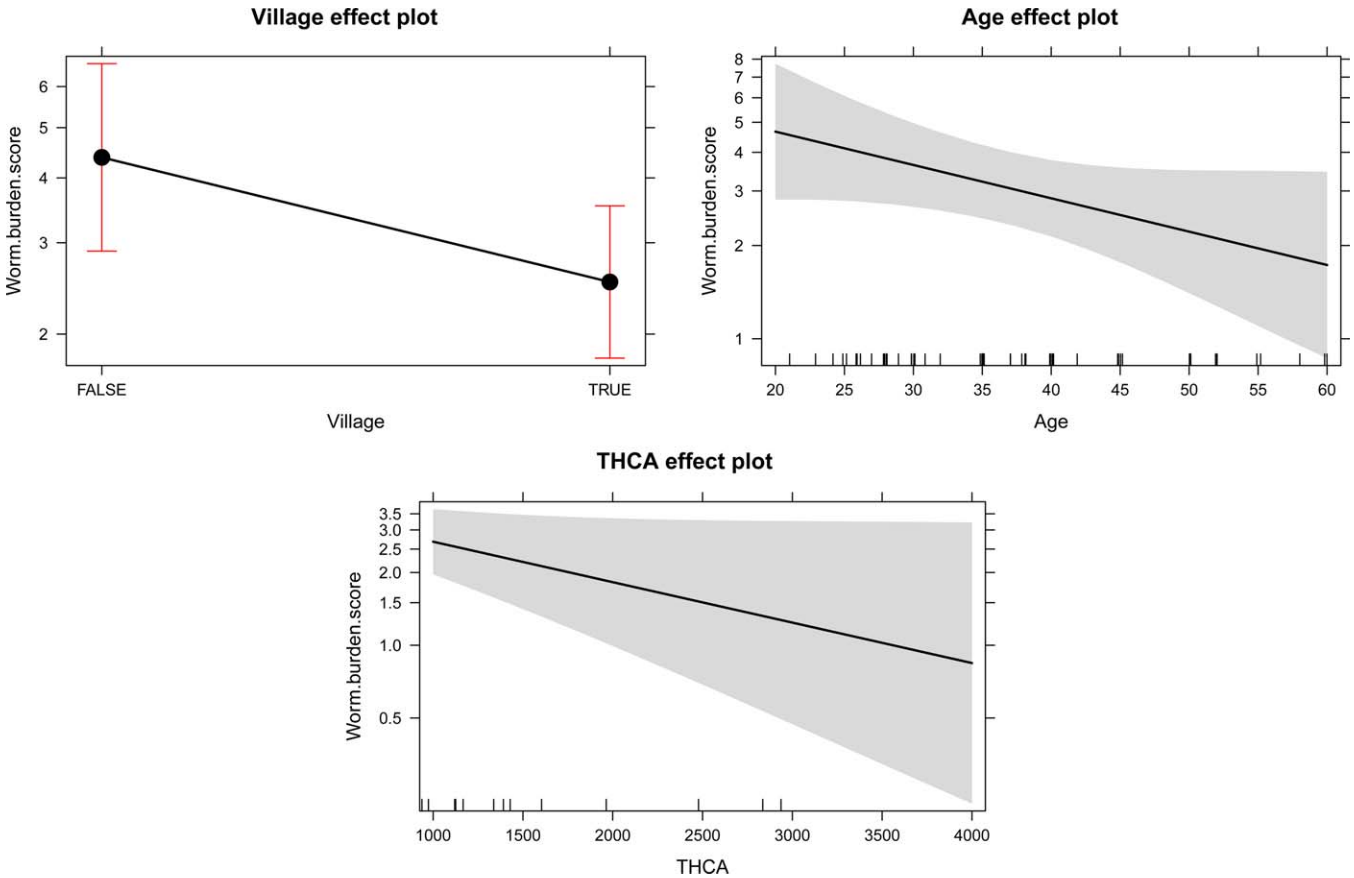

Fig. 2. Effect plot of worm burden versus THCA, village, and age (model 2, Table 3). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 2. Logistic regression model of cannabis smoker status (THCA > 50 ng/ml) versus age (year) and material wealth score

	Dependent variable
	Smoker
Age	−0.134***
	(0.040)
Wealth	−0.89**
	(0.315)
Constant	8.000***
	(2.109)
Observations	56
Log likelihood	−24.630
Akaike Inf. Crit.	55.270

$n = 56$. Likelihood ratio test of model significance: X2 = 22, df = 2, $P = 1.3 \times 10^{-5}$. Null deviance: 71.7 on 55 df. Residual deviance: 49.3 on 53 df. Comparing predicted versus observed smoker status, and using cutoff = 0.50, the sensitivity (true positives) was 92% and the specificity (true negatives) was 63%. The area under the receiver operating curve (AUC), corrected for bias using 1,000 bootstrap replications (Harrell, 2001), was AUC = 0.85.
$^{**}P < 0.01$; $^{***}P < 0.001$.

to test whether imputing missing data would make a difference in the coefficients and standard errors, but it did not (results not shown). Among cannabis smokers, peer-reported frequency of use was negatively rank correlated with worm burden, though only marginally significantly ($r_s = -0.28$, $P = 0.052$).

To investigate why cotinine was not a significant negative predictor of worm burden in year 2 data, contrary to results from year 1 data (Roulette et al. 2014), we examined the relationship separately in each subpopulation. Whereas THCA was negatively associated with worm burden in all subpopulations, cotinine was negatively associated in the largest subpopulation and in one smaller subpopulation, but positively in the other small population. Also, our sample size here was about one-third of that in Roulette et al. (2014).

Reinfection study

The reinfection study involved a subsample of the cross-sectional sample that included all participants with year 1 baseline worm burden scores (s1–s3, $n = 23$). Summary statistics for the reinfection sample did not differ from the total sample, except for mean cotinine, which decreased slightly (301 ng/ml vs. 241 ng/ml). Most men had been reinfected with helminths: 60.9% had positive delta worm burden scores (year 2 burden minus year 1 burden) and 39.1% had negative scores.

Delta worm burden was significantly negatively rank correlated with THCA concentrations ($r_s = -0.43$, $P = 0.02$) (Figure 3). Our ability to control for potential confounds was limited due to the small sample size. Keeping in mind that use of multiple regression with such a

TABLE 3. Negative binomial GLM models of worm burden score with log link, n = 56. (1) Model that best approximates model in Roulette et al. (2014). (2) Lowest AICc value of a priori models

	Dependent variable	
	Worm burden score	
	(1)	(2)
Village	−0.69**	−0.55*
	(0.24)	(0.24)
Wealth	0.15	
	(0.08)	
Acculturation	−0.22	
	(0.14)	
Age	−0.03*	−0.02*
	(0.01)	(0.01)
THCA	−0.0003*	−0.0004*
	(0.0002)	(0.0002)
Cotinine	0.0002	
	(0.0005)	
Constant	3.04***	2.66***
	(0.67)	(0.52)
Observations	56	56
Log likelihood	−121.50	−124.20
Theta	3.02* (1.18)	2.44** (0.86)
AIC	257.10	256.40

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

small sample is questionable, we centered and scaled all variables so that no intercept would be estimated, saving one degree of freedom. We then fit two multiple regression models of reinfection. The first aimed to test if THCA and cotinine had independent effects on reinfection (Table 4, model 1). The second aimed to test if THCA had an effect on reinfection controlling for age, another potential confound (Table 4, model 2). THCA was a significant negative predictor in both models as were cotinine and age. The AICc of model 2 was lower (55.8) than that of model 1 (60.8).

DISCUSSION

This, to our knowledge, is the first biomarker-validated study of cannabis use in a hunting-gathering population, and also the first to explore the relationship between the use of cannabis, which is toxic to helminths, and intestinal helminth infection. We first discuss the patterns and sociodemographic characteristics of Aka cannabis use. We then discuss the results of the cross-sectional and longitudinal worm burden studies.

Cannabis use among Aka: High rate in men and low rate in women

According to prospective population-based cohorts and/or retrospective studies, an estimated 3.9% of the global population aged 15-64 used cannabis in 2011 (i.e., used at least once in the last year), with the highest rate in West and Central Africa (12.4%) (UN, 2013). In Africa as a whole, 7.5% of the population aged 15–64 years used cannabis in 2011. The highest lifetime prevalence is 42.4% in the United States (Degenhardt et al., 2008).

Based on our demographic survey of self- and peer-reported cannabis use in the entire population, 38.6% of all adolescent and adult Aka were current cannabis smokers, and most of these were males: the adolescent and adult male prevalence was 70.9%. Using THCA biomarker data, and a cut-off of 50 ng/ml, 67.7% of men in our study were classified as cannabis smokers. The THCA levels of the cannabis smokers were comparable to, though somewhat higher than, the THCA levels of chronic cannabis smokers in the West (cf. Lowe et al., 2009). The prevalence of cannabis use among Aka men is indeed exceptionally high.

Previously, we found that the prevalence of tobacco use among Aka men is also high: 95% of adult Aka men smoked tobacco (validated with a cotinine assay; Roulette et al., 2014), whereas the highest nation-level male tobacco smoking prevalence rates are, e.g., Timor-Leste (61.1%) and Indonesia (57%), Russia (51%), and China (45.1%) (Ng et al., 2014). Thus, cannabis and tobacco smoking rates among Aka men exceed the highest nation-level prevalence rates.

Aka adolescent and adult women's cannabis smoking prevalence was 6.1%. The low rate relative to men is similar to that seen in other developing countries, although Aka women's use might still be high compared to other African women (Degenhardt et al., 2008). The prevalence tobacco smoking among Aka women was 5% (Roulette et al., 2014), compared to a median national female prevalence of tobacco smoking in sub-Saharan Africa of 1.95% and 6.2% globally (Ng et al., 2014). Regarding tobacco, Aka women explain that, although they can smoke if they want, they avoid it because it harms the fetus. Interestingly, this seemed to be an indigenous cultural model rather than a Western medical model, because the fetal problems included "making the baby black" and "making the fetus cough." For tobacco and cannabis, they also said that smoking is for men, and that they simply did not like it because it makes them sick (Roulette et al., under review). Tobacco is a teratogen, and cannabis is associated with low birth weight and mild developmental abnormalities (Hall and Degenhardt, 2009). Hagen et al. (2013) argue that the large sex differences in substance use, particularly in the developing world, might be a consequence, in part, of women's avoidance of toxic and teratogenic substances during the childbearing years.

There is a notable association between tobacco use and cannabis use (Agrawal & Lynskey, 2009). In the US population aged 12 and older, for example, 90% of cannabis users reported being a cigarette smoker at some point during their life (compared to 57.9% of cigarette smokers who reported ever using cannabis) (Agrawal et al., 2012). Among the Aka, there was a positive correlation between cotinine and THCA. However, based on self-reports, very few participants actually preferred to mix tobacco and cannabis together. This contrasts with earlier reports that the Mbuti and Aka often mixed cannabis with tobacco before smoking it (Hewlett, 1977).

The high prevalence of Aka men's use of cannabis and tobacco is remarkable considering their high cost in this population. Aka men earn about US$ 0.50 per day. A cannabis or tobacco cigarette costs about US$0.10, and Aka men report smoking one-to-three cigarettes per day. In many cases, they obtain cigarettes in trade for labor or forest products, or via sharing with other Aka. Nevertheless, about half of Aka "wages" are used to obtain cannabis or tobacco (Roulette et al., under review). This could explain the negative association we found between cannabis smoking and wealth (Table 2 and Figure 1). It also accords with a study of Efe foragers in the eastern Congo that found a negative relationship between smoker status and material wealth (Bailey, 1991). Studies in Western populations have also found an inverse relationship

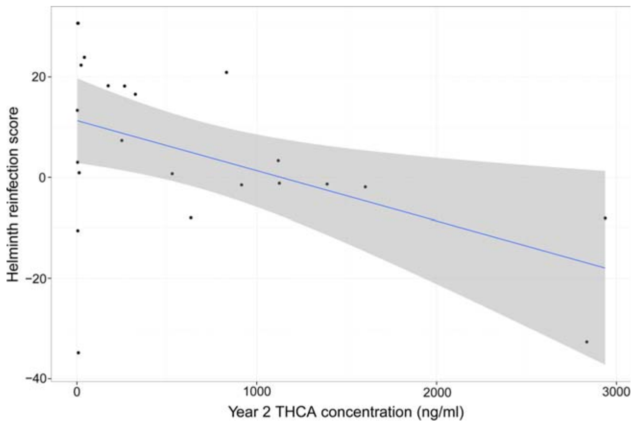

Fig. 3. Helminthes reinfection score versus THCA concentration. Line fit by linear regression. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 4. OLS models of helminth reinfection score. (1) Reinfection versus THCA and cotinine (AICc=60.8); (2) reinfection versus THCA and age (AICc = 55.8)

	Dependent variable	
	Scale (delta worm)	
	(1)	(2)
Scale (THC)	−0.400*	−0.725***
	(0.178)	(0.170)
Scale (cotinine)	−0.359†	
	(0.175)	
Scale (age)		−0.488**
		(0.152)
Observations	23	23
R^2	0.372	0.494
Adjusted R^2	0.312	0.446
Residual SE (df = 21)	0.811	0.728
F Statistic (df = 2; 21)	6.210**	10.250***

†P= 0.053; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

between socioeconomic status (SES) and cannabis use, although the direction of causality might be that low SES is a cause of use (Daniel et al., 2009).

Aka men who smoked cannabis were significantly younger than those who did not, which is in agreement with national and cross-national studies that have found that cannabis use rates peak in young adulthood and then decline as people age, enter the workforce, get married, and/or have children (Bachman et al., 1997; Degenhardt and Hall, 2012). Alternatively, it is possible the high male prevalence of cannabis use is a relatively recent phenomenon: over 30 years ago, Hewlett (1977) noted that most Aka men “rarely” smoke cannabis. Thus, the relative youth of Aka cannabis users might be a cohort effect.

The cause of high, almost universal, smoking among Aka men is unclear. Speculatively, it might be due to a lack of effective social restrictions on smoking cannabis and tobacco, combined with several population-specific benefits. According to most foragers of the Congo Basin, smoking increases strength, vital force, courage, and warmth (Hewlett, 1977). It is also possible that villagers, who control access to these substances, encourage Aka dependency on cannabis and tobacco to obtain and secure labor (see e.g., Jankowiak and Bradburd, 1996, 2003). Aka women also expressed a preference for husbands who smoke, which could encourage male smoking (Roulette et al., under review). Finally, if smoking does help control helminthiasis, the high rate of helminth infections among the Aka, combined with their lack of access to commercial anthelmintics, could unconsciously encourage high consumption of more readily available anthelmintics.

Cannabis versus helminths

Most men (95.2%) were infected with helminths, the majority (62.9%) with two or more species, similar to findings of earlier studies (Froment, 2014; Lilly et al., 2002; Roulette et al., 2014).

Worm burden was significantly negatively correlated with THCA, which is consistent with the chemotherapeutic hypothesis of drug use (e.g., Roulette et al., 2014). With THCA in the model, cotinine was no longer a significant predictor of worm burden, contrary to our earlier study of tobacco vs. helminths (Roulette et al., 2014). However, our sample size here was only about one-third that of the previous study. Inspection of the relationship between cotinine and worm burden by subregion in the current study showed the predicted negative relationship in the largest and smallest subregions (albeit with wide confidence intervals for the small subregion).

We found support for the chemoprophylaxis hypothesis: of those who were treated with a commercial anthelmintic in year 1, THC concentrations in year 2 were significantly negatively associated with reinfection levels. Furthermore, whereas Roulette et al. (2014), using the same sample, found that cotinine concentrations were significantly negatively correlated with worm reinfection, here we found that THC and cotinine were both independently and significantly negatively correlated with reinfection. However, a model that controlled for age instead of cotinine had a lower AICc. The sample size of the reinfection study was small ($n = 23$), so the multiple regression results must be treated with caution.

The Aka do not associate smoking cannabis or tobacco with antiparasitic properties. Cannabis and tobacco are also not used medicinally as anthelmintics. This contrasts with the indigenous plant, *motunga*, which is smoked recreationally, and also consumed in a tea to treat parasitic infections (Roulette et al., 2014). Whereas there are numerous medicinal uses of *motunga*, there are relatively few uses of cannabis and tobacco, perhaps because, compared to *motunga*, cannabis and tobacco use by the Aka is relatively recent. However, cannabis is consumed in a tea to treat yellow fever and tobacco is placed on the skin to treat an unidentified skin infection.

Candidate self-medication mechanisms

Although the conventional view is that drug abuse impairs immunity, thus increasing susceptibility to infection (Friedman et al., 2003), if recreational drug use is explained (at least in part) by the drugs’ antiparasitic properties, this would suggest that the immune system plays a key role in regulating drug use. Indeed, there is increasing evidence that central immune signals like toll-like receptor 4 and interleuken 15 mediate use of tobacco, opiates, and alcohol (Blednov et al., 2011, 2012; Hutchinson et al., 2012; Liu et al., 2009). Complex effects of THC on cellular and humoral immunity have also been observed in animal and cell experiments (Cabral, 2002; Melamede, 2002; Sofia et al., 1973).

Future research on the relationship between cannabis and tobacco use and worm burden should determine whether the transition to smoking in adolescence is mediated by worm burden, and whether cannabis users treated for worms with commercial anthelmintics decrease cannabis use relative to placebo controls, as was found for Aka tobacco use (Roulette et al., 2014).

Limitations

Our observational research design could not establish causation. Moreover, although we were able to control for some confounds, it is possible that unmeasured variables, or perhaps sampling variation (type I error), were responsible for the effects we found. This is a particular concern for the reinfection study, which had a small sample size that limited the use of controls. One of our important controls, age, could not be measured accurately because Aka do not know their ages.

For the cross-sectional study of THCA versus worm burden, although most of our participants had been treated with albendazole 1 year before, a few had not. We added a dummy variable indicating treatment status to our model, and the coefficients, standard errors, and *P*-values of our predictor variables were essentially the same (results not reported). In addition, studies have found a nonlinear relationship between fecal egg counts and number of worms in the host (perhaps due to density dependence, i.e., increased competition among worms for host resources results in fewer eggs per worm; Medley and Anderson 1985; Sithithaworn et al., 1991), which means our worm burden score, based on egg counts, only approximates actual worm burden.

THC and other cannabinoids in cannabis are lipophilic and bind readily to body fat. Consequently, and unlike nicotine (which is rapidly eliminated from the body), these compounds accumulate in body fat and other tissues, slowly rediffusing to plasma. Hence, they continue to be present in urine for an extended period—days to weeks—after the last consumption of cannabis. This might account for the seemingly stronger negative impact of cannabis on worm burden relative to tobacco.

In addition, urinary levels of THCA do not decrease monotonically in time after last exposure (Grotenhermen, 2003). This means that THCA levels have a variable relationship to recent cannabis smoking behavior. Other sources of uncontrolled variation include common polymorphisms in metabolic enzymes that alter rates of elimination.

Although cannabis has been shown to be toxic to plant and human parasitic helminths in vitro, the responsible compounds have not yet been identified (Mukhtar et al., 2013; Roy and Tandon, 1997). Further, it is not known if cannabis is toxic to any of the three helminth species measured here or if the levels of THC and other cannabis toxins consumed by smoking cannabis would be effective against any human parasite in vivo.

Finally, we did not validate the low female cannabis smoking prevalence with urinary THCA. We note, however, that there was a close correspondence between men's self-reported cannabis use and their THCA levels, and Roulette et al. (2014) found that, if anything, Aka women's self-reports overstated their tobacco use relative to that inferred from their cotinine levels.

CONCLUDING REMARKS

Sixty-seven percent of Aka men were recent cannabis users, a very high rate relative to other populations, especially considering the high cost of cannabis in this population. Similar to other populations, Aka male cannabis users were younger than non-cannabis users and tended to smoke more tobacco. Like an earlier study, we found a negative relationship between cannabis use and material wealth, but no relationship to acculturation or regional subpopulation. The low female prevalence of cannabis use corresponds to the low rates seen in other developing countries.

Most Aka men were infected with one or more species of helminths. We found a negative relationship between men's THCA levels and worm burden, and between THCA levels and reinfection with helminths 1 year after treatment with the commercial anthelmintic albendazole. It is worth considering that the exceptionally high prevalence of cannabis and tobacco use among Aka men is linked to their exceptionally high rate of helminthiasis and relative lack of access to commercial anthelmintics (Roulette et al., 2014). More generally, these results provide further evidence of a link between parasite infection and drug use, two of the world's great health problems, and highlight the need for more research on the high rate of male substance use in this population.

ACKNOWLEDGMENTS

The authors thank Nicaise Molende for assistance in the field, and the Aka for generously agreeing to participate in our study. The authors declare no conflicts of interest.

LITERATURE CITED

Agrawal A, Budney AJ, Lynskey MT. 2012. The co-occurring use and misuse of cannabis and tobacco: a review. Addiction 107:1221–1233.

Agrawal A, Lynskey MT. 2009. Tobacco and cannabis co-occurrence: does route of administration matter? Drug Alcohol Depend 99:240–247.

Agurell S, Halldin M, Lindgren JE, Ohlsson A, Gillespie H, Hollister L. 1986. Pharmacokinetics and metabolism of Δ-1-tetrahydrocannabinol and other cannabinoids with emphasis on man. Pharmacol Rev 38:21–43.

Appendino G, Gibbons S, Giana A, Pagani A, Grassi G, Stavri M, Smith E, Rahman MM. 2008. J Nat Prod 71:1427–1430. doi:10.1021/np8002673

Bachman JG, Wadsworth KN, O'Malley P, Johnston L, Schulenberg J. 1997. Smoking, drinking and drug use in young adulthood: the impacts of new freedoms and responsibilities. Mahwah, NJ: Lawrence Erlbaum.

Bahuchet S. 2014. Cultural diversity of African pygmies. In: Hewlett BS, editor: Hunter-gatherers of the Congo basin: cultures, histories and biology of African Pygmies. New Brunswick, NJ: Transaction Publishers. p 1–30.

Bailey R. 1991. The behavioral ecology of Efe pygmy men in the Ituri Forest, Zaire. Anthropological Papers, No. 86. Ann Arbor, MI: Museum of Anthropology, University of Michigan.

Benowitz NL. 1996. Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiol Rev 18:188–204.

Bernard HR. 2006. Research methods in anthropology: qualitative and quantitative approaches, 4th ed. Lanham, MD: AltaMira Press.

Blagg W, Schloegel EL, Mansour NS, Khalaf GI. 1955. A new concentration technic for the demonstration of protozoa and helminth eggs in feces. Am J Trop Med Hyg 4:23–28.

Blednov YA, Benavidez JM, Geil C, Perra S, Morikawa H, Harris RA. 2011. Activation of inflammatory signaling by lipopolysaccharide produces a prolonged increase of voluntary alcohol intake in mice. Brain Behav Immun 25:S92–S105.

Blednov YA, Ponomarev I, Geil C, Bergeson S, Koob GF, Harris RA. 2012. Neuroimmune regulation of alcohol consumption: behavioral validation of genes obtained from genomic studies. Addict Biol 17:108–120.

Burnham KP, Anderson DR. 2004. Multimodel inference: understanding AIC and BIC in model selection. Sociol Methods Res 33:261–304.

Cabral G. 2002. Immune system. In: Grotenhermen F, Russo E, editors. Cannabis and cannabinoids: pharmacology, toxicology, and therapeutic potential. Binghamton, NY: Haworth Press. p 279–288.
Clarke R, Merlin M. 2013. Cannabis: evolution and ethnobotany. Oakland, CA: University of California Press.
Daniel JZ, Hickman M, Macleod J, Wiles N, Lingford-Hughes A, Farrell M, Araya R, Skapinakis P, Haynes J, Lewis G. 2009. Is socioeconomic status in early life associated with drug use? A systematic review of the evidence. Drug Alcohol Rev 28, 142–153.
Degenhardt L, Chiu WT, Sampson N, Kessler RC, Anthony JC, Angermeyer M, Bruffaerts R, de Girolamo G, Gureje O, Huang Y, Karam A,Kostyuchenko S, Lepine JP, Mora ME, Neumark Y, Ormel JH, Pinto-Meza A, Posada-Villa J, Stein DJ, Takeshima T, Wells JE. 2008. Toward a global view of alcohol, tobacco, cannabis, and cocaine use: findings from the WHO World Mental Health Surveys. PLoS Med 5:e141. doi:10.1371/journal.pmed.0050141
Degenhardt L, Hall W. 2012. Extent of illicit drug use and dependence, and their contribution to the global burden of disease. Lancet 379:55–70.
du Toit BM. 1976. Man and cannabis in Africa: a study of diffusion. Afr Econ Hist (1):17–35.
du Toit BM. 1980. Cannabis in Africa: a survey of its distribution in African and a study of cannabis use in multi-ethnic South Africa. Rotterdam: A. A. Balkema.
Dudley R. 2000. Evolutionary origins of human alcoholism in primate frugivory. Q Rev Biol 75:3–15.
Ellis GM Jr, Mann MA, Judson BA, Schramm NT, Tashchian A. 1985. Excretion patterns of cannabinoid metabolites after last use in a group of chronic users. Clin Pharmacol Ther 38:572–578.
Foltz RL. 2007. Mass spectrometric methods for determination of cannabinoids in physiological specimens. In: ElSohly MA, editor. Marijuana and the cannabinoids. Totowa, NJ: Humana Press. p 179–204.
Forbey JS, Harvey AL, Huffman MA, Provenza FD, Sullivan R, Tasdemir D. 2009. Exploitation of secondary metabolites by animals: a response to homeostatic challenges. Integr Comp Biol 49:314–328. doi:10.1093/icb/icp046
Friedman H, Newton C, Klein TW. 2003. Microbial infections, immunomodulation, and drugs of abuse. Clin Microbiol Rev 16:209–219.
Froment A. 2014. Human biology and the health of African rainforest inhabitants. In: BS Hewlett, editor. Hunter-gatherers of the Congo basin: cultures, histories and biology of African Pygmies. New Brunswick, NJ: Transaction Publishers. p. 117–164.
Grotenhermen F. 2003. Pharmacokinetics and pharmacodynamics of cannabinoids. Clin Pharmacokinet 42:327–360.
Hagen EH, Sullivan RJ, Schmidt R, Morris G, Kempter R, Hammerstain P. 2009. Ecology and neurobiology of toxin avoidance and the paradox of drug reward. Neuroscience 160:69–84.
Hagen EH, Roulette CJ, Sullivan RJ. 2013. Explaining human recreational use of 'pesticides': the neurotoxin regulation model of substance use vs. the hijack model and implications for age and sex differences in drug consumption. Front Psychiatry 4:142. doi: 10.3389/fpsyt.2013.00142
Hall W, Degenhardt L. 2007. Prevalence and correlates of cannabis use in developed and developing countries. Curr Opin Psychiatry 20:393–397.
Hall W, Degenhardt L. 2009. Adverse health effects of non-medical cannabis use. Lancet 374:1383-1391.
Hallet JP. 1975. Pygmy Kitabu. USA: Fawcett Crest Books.
Hammond JA, Fielding D, Bishop SC. 1997. Prospects for plant anthelmintics in tropical veterinary medicine. Vet Res Commun 21:213–228.
Harrell FE. 2001. Regression modeling strategies: with applications to linear models, logistic regression, and survival analysis. New York: Springer.
Hewlett BS. 1977. Notes on the Mbuti and Aka Pygmies of Central Africa. Master's Thesis (Anthropology). Chico, CA: California State University Chico.
Hewlett BS. 1996. Cultural diversity among African pygmies. In: Kent S, editor. Cultural diveristy among twentieth-century foragers: an African perspective. Cambridge: Cambridge University Press. p 215–244.
Hewlett BS, editor. 2014. Hunter-gatherers of the Congo basin: cultures, histories and biology of African Pygmies. New Brunswick, NJ: Transaction Publishers.
Hill EM, Chow K. 2002. Life history theory and risky drinking. Addiction 97:401–413.
Hillig KW. 2005. Genetic evidence for speciation in Cannabis (Cannabaceae). Genetic Resources Crop Evol 52:161–180.
Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J. 2008. Helminth infections: the great neglected tropical diseases. J Clin Invest 118:1311–1321.
Huestis MA. 2007. Human cannabinoid pharmacokinetics. Chem Biodivers 4:1770–1804.
Huffman MA. 1997. Current evidence for self-medication in primates: a multidisciplinary perspective. Am J Phys Anthropol 104(Suppl. 25):171–200.
Hurtado AM, Frey M, Hill K, Hurtado I, and Baker J. 2008. The role of helminthes in human evolution: implications for global health in the 21st century. In: Elton S, O'Higgins P, editors. Medicine and evolution: current applications, future prospects. New York: CRC Press. p 151e–178e.
Hutchinson MR, Northcutt AL, Hiranita T, Wang X, Lewis SS, Thomas J, van Steeg K, Kopajtic TA, Loram LC, Sfregola C, Galer E, Miles NE, Bland ST, Amat J, Rozeske RR, Maslanik T, Chapman TR, Strand KA, Fleshner M, Bachtell RK, Somogyi AA, Yin H, Katz JL, Rice KC, Maier SF, Watkins LR.. 2012. Opioid activation of toll-like receptor 4 contributes to drug reinforcement. J Neurosci 32:11187–11200.
Jankowiak W, Bradburd D. 1996. Using drug foods to capture and enhance labor performance: a cross-cultural perspective. Curr Anthropol 37:717–720.
Jankowiak W, Bradburd D, editors. 2003. Drugs, labor, and colonial expansion. Tucson, AZ: University of Arizona Press.
Komiya Y, Kobayashi A. 1966. Evaluation of Kato's thick smear technic with a cellophane cover for helminth eggs in feces. Jpn J Med Sci Biol 19:59–64.
Lende DH, Smith EO. 2002. Evolution meets biopsychosociality: an analysis of addictive behavior. Addiction 97, 447–458.
Lilly AA, Mehlman PT, Doran D. 2002. Intestinal parasites in gorillas, chimpanzees, and humans at Mondika Research Site, Dzanga-Ndoki National Park, Central African Republic. Int J Primatol 23:555–573.
Liu YZ, Pei YF, Guo YF, Wang L, Liu XG, Yan H, Xiong DH, Zhang YP, Levy S, Li J, Haddock CK, Papasian CJ, Xu Q, Ma JZ, Payne TJ, Recker RR, Li MD, Deng HW. 2009. Genome-wide association analyses suggested a novel mechanism for smoking behavior regulated by IL15. Mol Psychiatry 14:668–680.
Lowe RH, Abraham TT, Darwin WD, Herning R, Cadet JL, Huestis MA. 2009. Extended urinary Δ9-tetrahydrocannabinol excretion in chronic cannabis users precludes use as a biomarker of new drug exposure. Drug Alcohol Depend 105:24–32.
McPartland JM. 1997. Cannabis as repellent and pesticide. J Int Hemp Assoc 4:87–92. http://www.druglibrary.net/olsen/HEMP/IHA/jiha4210.html
Medley G, Anderson RM. 1985. Density-dependent fecundity in Schistosoma mansoni infections in man. Trans R Soc Trop Med Hyg 79:532–534.
Melamede R. 2002. Possible mechanisms in autoimmune diseases. In: Grotenhermen F, Russo E, editors. Cannabis cannabinoids: pharmacology, toxicology, and therapeutic potential. Binghamton, NY: Haworth Press. p 111–122.
Mukhtar T, Kayani MZ, Hussain MA. 2013. Nematicidal activities of *Cannabis sativa* L. and *Zanthoxylum alatum* Roxb. against *Meloidogyne incognita*. Industrial Crops Products 42:447–453.
Oishi T, Hayashi K. 2014. From ritual dance to disco: change in habitual use of tobacco and alcohol among the Baka hunter-gatherers of Southeastern Cameroon. Afr Study Monographs Suppl 47:143–163.
Ng M, Freeman MK, Fleming TD, Robinson M, Dwyer-Lindgren L, Thomson B, Wollum A, Sanman E, Wulf S, Lopez AD, Murray CJ, Gakidou E. 2014. Smoking prevalence and cigarette consumption in 187 countries, 1980-2012. JAMA 311:183–192. doi:10.1001/jama.2013.284692
Philips JE. 1983. African smoking and pipes. J Afr History 24:303–319.
Rätsch C. 2005. The encyclopedia of psychoactive plants: ethnopharmacology and its applications. Rochester, VT: Inner Traditions/Bear & Co.
Rodriguez E, Cavin C. 1982. The possible role of amazonian psychoactive plants in the chemotherapy of parasitic worms—a hypothesis. J Ethnopharmacol 6:303–309.
Rogers R, Crowl CP, Eimstad WM, Hu MW, Kam JK, Ronald RC, Rowley GL, Ullman EF. 1978. Homogenous enzyme immunoassay for cannabinoids in urine. Clin Chem 24:95–100.
Roulette CJ, Mann H, Kemp BM, Remiker M, Roulette JW, Hewlett BS, Kazanji M, Breurec S, Monchy D, Sullivan RJ, Hagen EH. 2014. Tobacco use vs. helminths in Congo basin hunter-gatherers: self-medication in humans? Evol Hum Behav 35:397–407. doi:10.1016/j.evolhumbehav.2014.05.005
Roy B, Tandon V. 1997. In vitro fluckicidal effect of leaf extract *Cannabis sativa* Linn. on the trematode *Fasciolopsis buski*. Indian J Exp Biol 35: 80–82.
SAMHSA. 2008. Mandatory guidelines for federal workplace drug testing programs. Federal Register 73:71858–71907. www.gpo.gov/fdsys/pkg/FR-2008-11-25/pdf/E8-26726.pdf
Schebesta P. 1933. Among Congo Pygmies. London: Hutchinson and Co.
Schebesta P. 1936. My Pygmy and Negro hosts. London: Hutchinson and Co.
Schebesta P. 1941. Die Bambuti-Pygmäen von Ituri, Vol. 2: Ethnographie der Ituri-Bambuti. Brussels: Goerge van Campenhout.

Schensul S, Schensul JJ, LeCompte MD. 1999. Essential ethnographic methods: observations, interviews, and questions. Walnut Creek, CA: AltaMira Press.
Schultes RE. 1970. Random thoughts and queries on the botany of cannabis. In: Joyce CRB, Curry SH, editors. The botany and chemistry of cannabis. London: J. & A. Churchill. p 11–33.
Sithithaworn P, Tesana S, Pipitgool V, Kaewkes S, Pairojkul C, Sripa B, Paupairoj A, Thaiklar, K. 1991. Relationship between faecal egg count and worm burden of Opisthorchis viverrini in human autopsy cases. Parasitology 102, 277–281.
Sofia RD, Nalepa SD, Harakal JJ, Vassar HB. 1973. Anti-edema and analgesic properties of Δ9-tetrahydrocannabinol (THC). J Pharmacol Exp Ther 186:646–55.
Suárez-Rodríguez M, López-Rull I, Garcia CM. 2013. Incorporation of cigarette butts into nests reduces nest ectoparasite load in urban birds: new ingredients for an old recipe? Biol Lett 9:20120931. doi:10.1098/rsbl.2012.0931
Turnbull CM. 1968. The forest people. New York: Touchstone.
Turnbull CM. 1976. Wayward servants: the two worlds of the African Pygmies. Westport, CT: Greenwood Press.
Turnbull CM. 1983. The Mbuti Pygmies: change and adaptation. New York: Holt, Rinehart, and Winston.
Turner CE, Elsohly MA, Boeren EG. 1980. Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. J Nat Prod 43:169–234.
UN. 2010. UN Office on Drugs and Crime. World drug report 2010. United Nations, New York. Available at www.unodc.org/documents/wdr/WDR_2010/World_Drug_Report_2010_lo_res.pdf. Accessed on December 11, 2014.
UN. 2013. UN Office on Drugs and Crime. World drug report 2013. United Nations, New York. Available at www.unodc.org/unodc/secured/wdr/wdr2013/World_Drug_Report_2014.pdf. Accessed on December 11, 2014.
Villalba JJ, Miller J, Ungar ED, Landau SY, Glendinning J. 2014. Ruminant self-medication against gastrointestinal nematodes: evidence, mechanism, and origins. Parasite 21:31 doi:10.1051/parasite/2014032
Wrangham, RW, Nishida T. 1983. Aspilia spp. leaves: a puzzle in the feeding behavior of wild chimpanzees. Primates 24:276–282.
Wyatt GB. 1977. Health in Melanesia. In: Winslow JH, editor. The Melanesian environment. Canberra, Australia: National University Press. p 459–462.