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SEASONAL ANOINTMENT WITH MILLIPEDES IN A WILD PRIMATE: A CHEMICAL DEFENSE AGAINST INSECTS?

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Abstract—Members of a wild group of wedge-capped capuchin monkeys (*Cebus olivaceus*) intentionally anoint themselves with millipedes (*Orthoporus dorsovittatus*). Chemical analysis revealed these millipedes secrete two benzoquinones, compounds known to be potently repellent to insects. We argue that the secretion that rubs off on the monkeys in the course of anointment provides protection against insects, particularly mosquitoes (and the bot flies they transmit) during the rainy season. Millipede secretion is so avidly sought by the monkeys that up to four of them will share a single millipede. The anointment must also entail risks, since benzoquinones are toxic and carcinogenic. We suggest that for capuchins the immediate benefits of anointment outweigh the long-range costs.

Key Words—Capuchin monkey, millipede, benzoquinone, anointment, chemical defense, insect deterrent, carcinogen.

INTRODUCTION

Several anecdotal reports have called attention to the possible medicinal use of organic materials by animals, particularly nonhuman primates (Newton, 1991;

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Wrangham, 1995; Huffman et al., 1997). In most cases, the specific substances sought by the animals remain uncharacterized. One study had indicated that leaves of certain plants ingested by chimpanzees possess antibiotic properties (Rodriguez et al., 1985), but subsequent work failed to support this claim (Page et al., 1997). We report here that wedge-capped capuchin monkeys (*Cebus olivaceus*) intentionally anoint themselves with certain millipedes (*Orthoporus dorsovittatus*), for the apparent purpose of coating themselves with the benzoquinone-containing defensive secretion of the latter.

Wild wedge-capped capuchins were observed by some of us (X.V., J.G.R.) to anoint or "fur-rub" themselves with a single millipede at a time. Capuchins encounter millipedes in the rainy season during daily foraging, frequently along branches, in termite mounds, and in crowns of palm trees. Millipedes also occur inside furled leaves, under peeling bark, and inside decaying tree stumps and fallen branches.

Millipedes, like many other arthropods, emit defensive secretions when attacked. A diversity of compounds has been identified from these fluids, including hydrogen cyanide, quinazolinones, benzoquinones, and cresols (Eisner et al., 1978; Fairhurst, 1993). Our objective was to characterize the components of the secretion of *O. dorsovittatus* and to determine, insofar as possible, the function of the anointing behavior.

METHODS AND MATERIALS

Wedge-capped capuchins have been studied since 1977 at Fundo Pecuario Masaguaral, a 7800-ha cattle ranch and private reserve in the llanos of central Venezuela (8°34′N, 67°35′W) (Robinson, 1986; Srikosamatara, 1987; O'Brien, 1990, 1991). Monkeys are individually identified by age–sex class, pelage, and facial characteristics. Group home ranges (200–300 ha) overlap extensively in a semideciduous gallery forest characterized by a canopy height of 14–22 m and two permanent water courses, the Caño Caracol and Guárico River (Robinson, 1986). The mean annual rainfall (1450 mm) occurs mostly between May and October and, together with poor drainage, results in extensive seasonal flooding (Troth, 1979). In turn, the resulting standing water generates explosive outbreaks of mosquitoes that are very active in daytime as well as at night.

Mosquitoes are more than merely annoying because they are vectors of New World parasitic bot flies, which in the maggot stage cause subcutaneous cysts in mammalian hosts (Catts, 1982). The effect on nonhuman primates is severe debilitation and risk of secondary infection of the open sores left by the maggots on emergence. For example, in mantled howler monkeys (*Alouatta palliata*) in Panama, bot fly parasitism is correlated with an increase in host mortality during the middle to late rainy season (Milton, 1996). Capuchins at Fundo Pecuario

Masaguaral also are inflicted with frequent bot fly parasitization (X.V., personal observation).

Here, we present descriptions of the anointment process, as observed in capuchins of a single focal group (Main Group) from: (1) May 1978 through July 1979 [51 anointment bouts, recorded in the course of systematic behavioral sampling (1051 to 1420 observation periods across all individuals per month)]; (2) January to July 1995 [19 bouts, *ad libitum* sampling (626–2418 min of observation per month)]; and (3) February to July 1998 [8 bouts, *ad libitum* sampling (777 to 2491 minutes of observation per month)]. Each observation period in systematic behavioral sampling consisted of a slow scan sample of the group, in which each member's first behavior lasting at least 5 sec was recorded (Altmann, 1974; Robinson, 1986). In *ad libitum* sampling, anointment bouts were recorded whenever observed during group contact. In both sampling methods, a bout was defined as a single observed record of one or more capuchins using a millipede for self- or group anointment for at least 5 sec.

Anointment by Capuchins. Upon finding a millipede, capuchin monkeys typically rub it vigorously against the back and roll over it, while intermittently taking it in the mouth and slowly withdrawing it again. During mouthing they drool copiously and their eyes appear to glaze over. Anointment bouts may last over 2 min. Up to 10 bouts have been noted to be undertaken by the group in one day; on two occasions, an individual was observed anointing itself four times in a single day. Both male and female capuchins, including individuals of all age classes and mothers with newborn infants, take part in the anointment. The youngest individual observed anointing itself was an infant female.

Anointment often involves the sharing of one millipede by multiple capuchins. Of a total of 87 recorded bouts, 40 (or 46%) involved sharing. Individuals approach the user and attempt to remove the millipede from its hand, and if unsuccessful may wipe their body against the user. They may also wipe their tail repeatedly against the user, and then against themselves. The resulting writhing cluster of monkeys can consist of up to four individuals. We believe that it is inevitable that the monkeys become topically wetted with secretion in the course of this behavior. Moreover, we feel that since the behavior may involve no more than body-against-body rubbings, rather than the obligatory handling of the millipede itself, the intent of the action is specifically the acquisition of secretion.

Sharing a millipede creates an unprecedented setting for affiliative behavior among certain age–sex classes that normally avoid interactions, as for example females and unrelated immature males (O'Brien, 1991). In addition, dominance relationships based on agonistic interactions do not seem to determine which individuals participate in the millipede-sharing. Overall, the collective anointment appears to proceed without competitive friction.

Taxonomic Identification. We are indebted to Richard Hoffman (Virginia

Museum of Natural History, 1001 Douglas Avenue, Martinsville, Virginia 24112) for identifying *O. dorsovittatus*. The millipede belongs to the order Spirostreptida, whose members produce a defensive secretion containing benzoquinones (Eisner et al., 1978). The fluid is produced by segmental glands that open along the sides of the body and may be ejected in copious quantity in response to even mild disturbance.

Chemical Analysis. Six live *O. dorsovittatus*, including 3 individuals that had been used by capuchins 33, 48, and 56 days beforehand, were "milked" of secretion by tapping their bodies and wiping up with pieces of filter paper the dark fluid that oozed in substantial quantity from their flanks. The papers were extracted with dichloromethane, and the extracts were analyzed by gas chromatography–mass spectrometry (GC-MS). Apparatus and experimental conditions were as previously described (Attygalle et al., 1993).

RESULTS

Figure 1 illustrates the distribution of monthly rainfall and incidence of anointment bouts in 1978, 1995, and 1998, respectively. Incidence of bouts per month for 1978, expressed as bouts per period of observation, were calculated as the ratio of total number of bouts for the month, over the total number of periods that the group was subjected to behavioral observation (in other words, behavioral sampling) for that month. Bouts received during *ad libitum* sampling in 1978–1979 were not included here. Incidence of bouts per month for 1995 and 1998, expressed as bouts per minute of observation, was calculated as the ratio of total number of bouts observed *ad libitum* in that month to the total time in minutes that the group was observed. The monthly incidence of anointment behavior correlated positively with the monthly distribution of rainfall in 1978 (Spearman rank correlation coefficient, $P = 0.004$) and 1995 ($P = 0.006$). The association was not significant in 1998, as the incidence of bouts remained high while rainfall decreased in the last month of sampling, although the level of standing water remained high. Monthly distribution of rainfall and levels of standing water in turn are predictors of mosquito abundance. The period between January and March is marked in particular by a lack of standing water and few mosquitoes. Behavioral observations from December 1986 to March 1988 with the same capuchin group corroborated that anointing occurs only during the rainy season (T. O'Brien, personal communication).

Monkeys made use of millipedes of both sexes and varying sizes. Fifteen millipedes that were collected after they were used for anointment (Figure 2A) had the following dimensions: 0.4–0.7 cm in width ($N = 14$), 9.0–10.5 cm in length ($N = 3$), and 3.11–3.97 g in mass ($N = 3$). Although capuchins are frugivores–insectivores, they did not ingest or even invariably kill the mil-

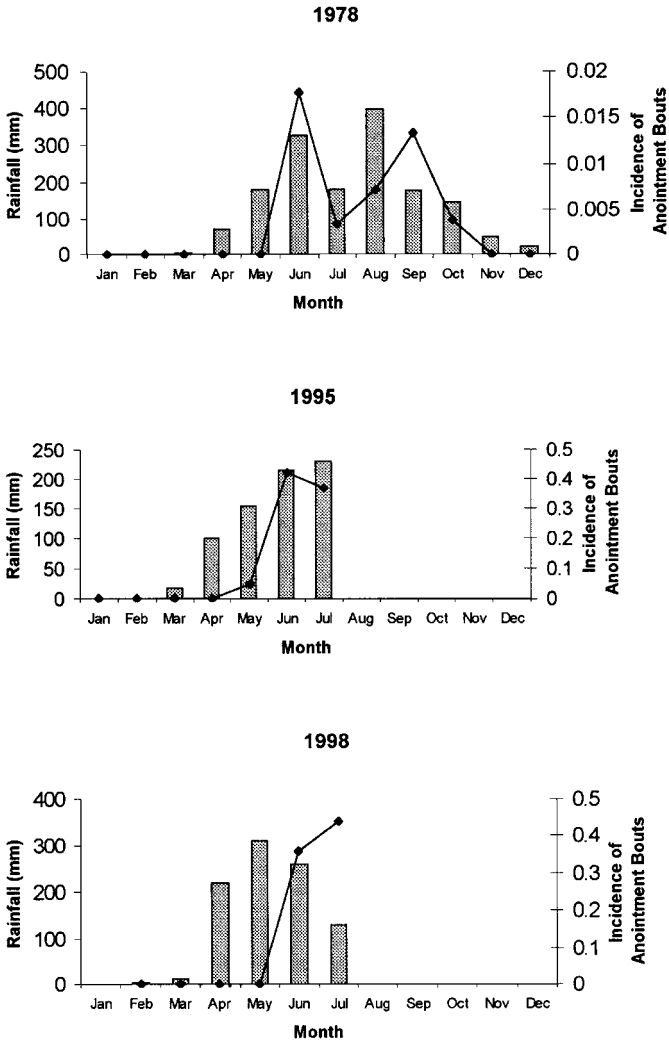

FIG. 1. Incidence of anointment, plotted as a function of month of year and precipitation. Incidence for 1978 is given as number of anointment bouts witnessed per period of observation. Incidences for 1995 and 1998 are given as number of bouts noted per minute of observation.

lipedes they used. Of the 15 collected millipedes, 7 had been torn apart, 4 had been decapitated (Figure 2B), 2 bore bite marks but survived (Figure 2C), and 2 survived intact. Small millipedes seemed more prone to be torn apart by the

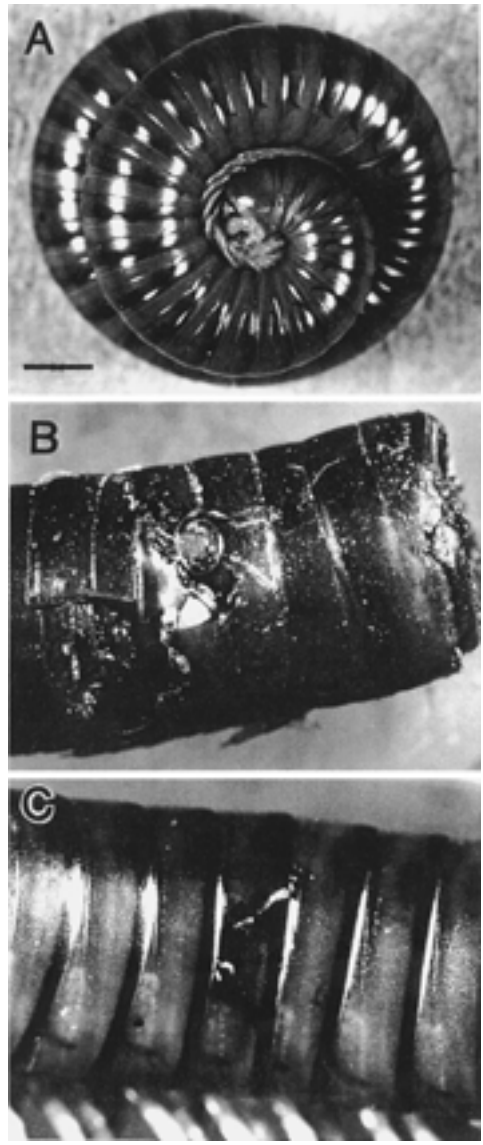

FIG. 2. Collected specimens of *Orthoporus dorsovittatus*, illustrating (A) an intact individual, (B) a close-up of an individual decapitated by a wedge-capped capuchin, and (C) a close-up of a puncture wound inflicted by a capuchin bite 33 days beforehand; the wound had healed and the millipede was fully viable (reference bar = 2 mm).

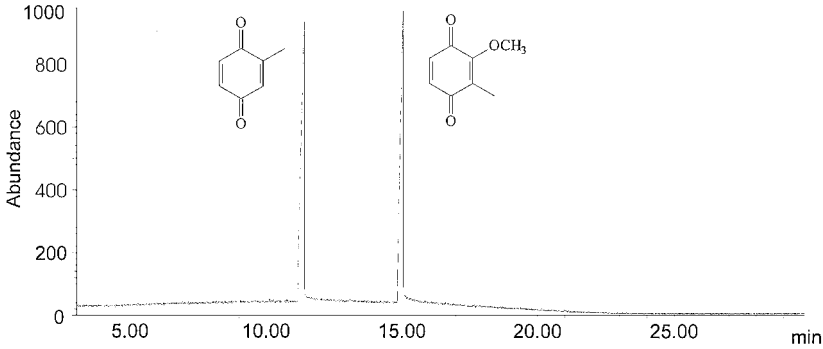

FIG. 3. Reconstructed gas chromatogram from GC-MS analysis of a dichloromethane extract of secretion from a male *Orthoporus dorsovittatus* (fused silica column, 30 m × 0.22 mm, coated with DB-5; oven temperature: 40°C for 4 min and raised 8°C/min to 270°C).

monkeys. Careful inspection of pieces of millipedes discarded by the monkeys showed none to be depleted of viscera, indicating that the monkeys derive no nutrient from the millipedes.

Composition of Secretion. The millipedes' secretion contains two volatile components, characterized as 2-methyl-1,4-benzoquinone and 2-methoxy-3-methyl-1,4-benzoquinone by GC-MS comparison with authentic samples (Figure 3). The ratio of the two quinones was about 1 : 1 in five of the samples (from 3 male millipedes, 1 female, and 1 unsexed immature); in the sixth sample (from 1 unsexed immature), the methoxyquinone predominated (1.3 : 1).

DISCUSSION

The results point to a correlation between anointment frequency and monthly rainfall and hence mosquito abundance and provide strong indications that anointment spreads millipede secretion over the capuchins' fur. Although we have no direct evidence that capuchins are less vulnerable to insect attack following anointment, we propose that the capuchins do in fact put the millipede's secretion to defensive use. They anoint themselves exclusively during the rainy season, the precise time when they can be expected to be beleaguered by mosquitoes. Their purpose in anointing themselves, we would argue, is to acquire benzoquinones.

Previous studies have shown benzoquinones to be potent insect repellents (Peschke and Eisner, 1987). They also act as topical irritants. They have been shown, for instance, to induce scratching in cockroaches at a concentration as

low as 10^{-4} M (Eisner et al., 1978). The evidence is also strong that in the many arthropods that produce benzoquinones, including millipedes, carabid and tenebrionid beetles, earwigs, termites, cockroaches, and opilionids, the compounds serve as antiinsectan agents (Blum, 1981).

Of all arthropods that produce benzoquinones, millipedes are probably the richest source, given the multiplicity of their glands. Benzoquinones are discharged by millipedes in virtually pure form (Attygalle et al., 1993) and are persistent, vaporizing slowly by sublimation (Peschke and Eisner, 1987). *O. dorsovittatus* appears in no way to be unusual. As is typical for spirostreptoids, it proved capable of discharging secretion in quantity and did so only in response to direct body stimulation.

The capuchins could also benefit from the fact that benzoquinones have antimicrobial potency (Lauer et al., 1991; Stärk et al., 1991). The compounds could well act as disinfectants, both topically and orally.

The monkeys, of course, could also incur costs as a consequence of anointment, since benzoquinones are bioactive in negative ways as well. As we know from personal experience, benzoquinones are potently irritating to the eyes, painful to inhale, and noxious to the taste. One wonders, therefore, why capuchins intermittently mouth the animals. Do they thereby trigger accelerated glandular emission from the millipedes? We know, for instance, from observation of another *Orthoporus* (*O. punctilliger*, from Arizona) that a mere puff of human breath can cause the millipede to coil and begin ejecting secretion. One wonders whether the excess salivation that occurs in capuchins when they mouth millipedes is a reaction to the irritancy of the benzoquinones.

Several studies have demonstrated that benzoquinones are highly toxic and carcinogenic. The LD_{50} for benzoquinone in rats is 15.8 ± 5.7 mg/kg (Eisner et al., 1978). Individuals of certain tropical millipedes may contain as much as 350 mg benzoquinone (Fairhurst, 1993). El-Mofty et al. (1992) found that 33.6% of mice fed 1,4-benzoquinones developed tumors in the liver and spleen. Persistent exposure to these compounds could therefore have long-range ill effects on capuchins as well.

It is possible that the capuchins restrict their anointment behavior to the wet months because the millipedes themselves are only available to them at that time. It is certainly true that millipedes in other habitats—the "scrub" of central Florida is one example—remain in hiding except in the rainy season. Millipedes are highly susceptible to desiccation and may need to remain in shelter during the dry months in the Venezuelan llanos as well.

Capuchins are unusual in that they seek exposure to an animal that is generally shunned on account of its chemical defense. Remarkably, it may be precisely because of this defense that they appear to put the animal to use. The benefit that they reap from anointing themselves with *O. dorsovittatus* secretion is presumably immediate. They gain a coating of the millipede's defensive fluid, thereby

possibly acquiring the capacity to repel mosquitoes and avoid bot fly parasitization. Anointment could also entail risks, but we propose that in the real-life situation of the Venezuelan llanos, where insects can pose such a recurrent threat, being able to cope with insects may have been the factor that promoted the evolution of anointment behavior.

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