Wild Blonde Capuchins (*Sapajus flavius*) Perform Anointing Behaviour Using Toxic Secretions of a Millipede (*Spirobolida: Rhinocricidae*)

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Abstract
Defensive secretions of millipedes are remarkable for containing toxic quinones known to efficiently repell hematophagous arthropods. Here we show that Endangered blonde capuchin monkeys make use of such secretions. We (i) describe the anointing behavior performed by the monkeys (ii) identify the millipede species used in the process (iii) describe the volatile chemical composition of its secretion. The blonde capuchin monkeys selectively searched for millipedes hidden under the ground. We observed three bouts of anointing behavior, performed by 13 individuals of all age classes (from adults to independent infants), both solitary (1 event) and socially (10 events). The only millipede species used by the monkeys is an undescribed species of the genus *Poecilocrius* (*Spirobolida, Rhinocricidae*). The volatile chemical composition of the secretions was predominantly comprised of a mixture of benzoquinones and hydroquinones. The social nature of the behavior and time of the observations (mosquito season), suggest that social bonding and mosquito avoidance is linked to the anointing behavior of the monkeys.

Keywords Diplopoda · Benzoquinones · Neotropical Primate · Anointing Behavior · Cebidae

Introduction

A wide range of mammals and birds perform anointing behavior (Brockie 1976; Bowler et al. 2015; Morozov 2015; Xu et al. 1995) by rubbing foreign substances with strong odor on their body (Lynch Alfaro et al. 2012). The behavior is performed both solitarily, whereby one individual rubs the substance on its body parts, and socially when several individuals simultaneously take part in the activity (Baker 1996; Lynch Alfaro et al. 2012). Studies suggest several reasons for anointing including medicinal, sexual, communication and social functions (Bowler et al. 2015). Wild captive capuchin monkeys (Cebidae) use millipede secretions in anointing behavior (Lynch Alfaro et al. 2012), with some evidence of seasonal patterns to deter mosquitoes (Valderrama et al. 2000; Weldon et al. 2003) and social anointing to enhance intraspecific bonding (Bowler et al. 2015).

The defensive secretions of some millipede species contain high concentrations of benzoquinones and hydroquinones, natural organic compounds known for their antimicrobial (Stanković et al. 2016) and deterrent (Blum 1981; Shear 2015) properties. Used in animal chemical warfare, they can be particularly harmful to mammals, causing contact dermatitis, chemical burns and even cancer (Cardoso and Haddad Júnior 2003; Haddad Júnior et al. 2015; Shpall and Frieden 1991; Valderrama et al. 2000). Curiously, Bobo people of
Burkina Faso population consume millipedes after boiling and drying (Enghoff et al. 2014). Among several species of non-human primates and birds, millipede secretions are believed to be used as an antoin for protection against mosquitoes, ectoparasites and even for alleged self-medication (e.g. Birkinshaw 1999; Bowler et al. 2015; Jefferson et al. 2014; Peckre et al. 2018; Valderrama et al. 2000; Weldon et al. 2003; Zito et al. 2003). Thus, the immediate benefits of consumption or smearing of millipede secretions seem to somehow compensate the costs involved (Valderrama et al. 2000). For instance, considering recent yellow fever outbreaks in Brazil and the deadly consequences for several primate populations in the Southeast of the country (e.g. Almeida et al. 2014; Dorigatti et al. 2017; Fernandes et al. 2017; Klitting et al. 2018; Possas et al. 2018), using millipede secretions as antoin protection against mosquito bites might prove advantageous.

Blonde capuchin monkeys (Sapajus flavius) are social arboreal primates endemic to Northeast Brazil (Fialho et al. 2014; Medeiros et al. 2019), where their habitat is under constant fragmentation and destruction (ICMBio 2011; Valença Montenegro et al. 2020). Our aim was to describe antoin behavior using millipedes in a long-term monitored population of blonde capuchins. We also aimed to identify the millipede species used by the monkeys and the composition of its volatile defensive secretion.

Methods and Materials

Study Site, Animals and Observations Since August 2010, we have been periodically monitoring a population of blonde capuchin monkeys in a 936 ha fragment of primary and secondary Atlantic Forest located at Mataraca, in the State of Paraiba, Brazil (06°29’.902’S; 34°58’.704’W). Since the early 1970s, the area has been under constant deforestation and reforestation processes due to mining activity. The most recent census in 2018 revealed 163 individuals, but we believe there are more individuals in the area. The blonde capuchins at the study site show fission-fusion dynamics, where a large group splits into smaller groups throughout the day (Medeiros et al. 2019). We conducted ad libitum observations (Altmann 1974) along with our systematic observations of the animals to register unusual behaviors such as the one described in the present study.

Millipede Species identification

The specimens (three males and five females) were identified by using the specific literature for the group such as Attens (1943), Schubert (1962a, b), Hoffman (1980) Hoffman et al. (1996), Marek et al. (2003), and Rodrigues et al. (2018). The specimens are stored at the Coleção Zoológica da Universidade Federal de Mato Grosso (CZUFMT) under the following accession numbers: CZUFMT (1804) four females and CZUFMT (1805) one female and three males.

Sampling and Chemical Analysis of Volatile Millipede Defensive Secretions Fragments of millipedes, discarded by the monkeys after antoin, were collected (n = 9) to determine the species used. Live specimens (n = 8) were also collected in the field and kept in a rearing terrarium (50 × 50 × 50 cm) for ca. 48 h. These live specimens were used to collect volatile defensive secretions under controlled laboratory conditions. Sampling was conducted through dynamic headspace extraction from specimens (n = 4) that were disturbed to induce the release of secretion while enclosed in PET film oven bags (Bratschlauch, Melitta GmbH, Germany), from which scented air was drawn for 5 min by a battery-operated vacuum pump (model G 12/01 EB, ASF Thomas, Inc., Germany) at a constant flow rate of ca. 200 mL min⁻¹. The air enriched with the volatiles was trapped in miniaturised quartz glass tubes containing adsorbent polymer (3 mg), consisting of a 1:1 mixture of Tenax™ TA (80/100 mesh, Supelco, Bellefonte, PA, USA) and Carbopack™ X (20/40 mesh, Supelco) (see Dötter et al. 2005 for details). A negative control drawn from an empty bag was collected to detect environmental contaminants. Following scent collection, the adsorbent traps were stored at -24°C until analysis.

The volatiles trapped in the microtubes were analysed by using a gas chromatograph linked to a mass spectrometer (GC—MS; Agilent 7890A™ gas chromatograph, Agilent 5975C Series MSD™ mass spectrometer), fitted with a thermal separation probe (Agilent G4381A) and equipped with a non-polar HP-5 ms column (Agilent J&W; 30 m x 0.25 mm d.i. 0.25 μm film thickness). Each sample was loaded into the probe in split mode (1:30) with the injector temperature set to 250°C. The oven temperature was kept at 40°C for 1 min, then increased at a rate of 6°C/min to 250°C, while the flow of the carrier gas (He) was 1.5 mL/min. MS source and quadrupole temperatures were kept at 230°C and 150°C, respectively, 70 eV EI-mass spectra were taken with a scanning speed of 1.0 scan/s from m/z 35–450. A homologous series of alkanes (C8 – C28) was used to determine linear retention indices of the volatiles (van den Dool and Kratz 1963). Target compounds were tentatively identified by comparing their mass spectra and retention indices with those of reference samples available from personal and commercial mass spectral libraries (FFNSC 2, NIST14 and Wiley Registry™ 9th edition), integrated to the software GCMSolution Version 4.11 (Shimadzu Corporation, Kyoto, Japan). Mass spectra of target compounds and their calculated retention indices were cross-referenced with data published by Attygalle et al. (1993), Vujišić et al. (2011), Bodner et al. (2016), and Makarov et al. (2017). Peak areas in the GC-MS-
chromatograms were integrated to determine the relative proportions of each compound.

**Results**

In the first observation of the anointing behavior among blonde capuchin monkeys, on April 20th 2018, five adult males were digging in the forest ground while actively searching for millipedes. After finding a millipede, the monkeys broke it using their hands and mouth and then rubbed and scratched the exuding secretions vigorously over the face and head, throat skin flap (males), hands and feet, tail and back (Fig. 1, Supplementary Material 1). On average, the animals spent a little more than a minute per session of anointing. Only adults were engaged in the anointing activity on this occasion, while juveniles sat close by as if observing and copying their postural behavior without using a millipede.

![Sequence of anointing behavior](image)

**Fig. 1** A sequence of the anointing behavior performed by an adult male blonde capuchin monkey in a fragment of Atlantic Rain Forest at Paraiba State, Northeast Brazil. (a) a male is looking for a millipede; (b) handling; (c) rubbing between hands; (d) sniffing; (e) biting; (f) rubbing and scratching the secretions over the face; (g) rubbing the secretions over the head; (h) rubbing the secretions over the back; (i) rubbing the secretions on the tail.
Table 1 Volatile chemical composition (min-max relative amounts of each compound) of the defensive secretion of *Poecilocricus* sp. (Spirobolidae, Diplodota), obtained by dynamic headspace sampling (n = 4)

<table>
<thead>
<tr>
<th>RI</th>
<th>Relative amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoquinones</td>
<td></td>
</tr>
<tr>
<td>920</td>
<td>1,4-benzoquinone *</td>
</tr>
<tr>
<td>1016</td>
<td>2-methyl-1,4-benzoquinone</td>
</tr>
<tr>
<td>1180</td>
<td>2-methoxy-3-methyl-1,4-benzoquinone</td>
</tr>
<tr>
<td>Hydroquinones</td>
<td></td>
</tr>
<tr>
<td>1346</td>
<td>2-methylhydroquinone</td>
</tr>
<tr>
<td>1424</td>
<td>2-methoxy-3-methylhydroquinone</td>
</tr>
<tr>
<td>Unidentified compounds</td>
<td></td>
</tr>
<tr>
<td>1055</td>
<td>mz 123,43,54,39,94 *</td>
</tr>
<tr>
<td>1119</td>
<td>mz 69,123,39,137,110 *</td>
</tr>
</tbody>
</table>

Compounds are listed according to compound class and gas chromatographic linear retention index (RI; HP-5 ms phase), tr = compounds present in less than 0.1% of total amounts. *Compounds present in only one of the analysed samples.

Discussion

Anointing behavior with millipedes by blonde capuchin monkeys at Mataraca was first observed through *ad libitum* observations followed by ca. eight years of monitoring. Systematic observations of the individuals were then carried out until August 2019, during which we recorded the behavior on additional occasions. Long-term field studies allow a better understanding of the interactions between animals and their habitats (Kappeler et al. 2012). The behavioral posture used by the monkeys to anoint themselves was similar to that observed in other primates, including several species of capuchin monkeys (e.g. Lynch Alfaro et al. 2012). Red-fronted lemurs, *Eulemur rufifrons*, for example, also rub broken millipedes vigorously over their tails and other body parts (Peckre et al. 2018). Owl monkeys, *Aotus* spp, perform millipede anointment socially and even share fragments of millipedes during the bouts (Jefferson et al. 2014). It is still unclear why blonde capuchin monkeys use millipede secretion, however, considering the time of the year when the events were observed (i.e. wet season) and their social nature, it is likely related to mosquito avoidance and social bonding.

*Poecilocricus* Schubart 1962a, b is a Brazilian monotypic millipede genus of the large Neotropical family Rhinocricidae which currently encompasses ca. 605 species (Roskov et al. 2019). Even though it is the first record of the genus in being selectively used by primates, closely related *Anadenobolus* spp. have been documented as in use for fur anointment by owl monkeys in captivity (Zito et al. 2003). The chemical composition of the *Poecilocricus* secretion is fairly similar to those previously documented for various millipede species (reviewed by Shear 2015), and both *p*-toluquinone and 2-methoxy-3-methyl-1,4-benzoquinone have been shown to be directly associated with triggering anointment behavior in different species of capuchin monkeys (Weldon et al. 2003). In Brazil, quinones released by Rhinocricidae are involved in many human accidents causing skin lesions (De Capitani et al. 2011; Neto et al. 2014). Thus, animals using millipedes must counterbalance the obvious risks by careful handling techniques, which may be transferred at least in part through social learning (Fragazy and Perry 2003). In our first observation, we only saw adults anointing themselves with the millipedes, while the youngsters seemed to watch and copy the behavior of the adults, suggesting that learning might be involved in transfer of the behavior. Nevertheless, further studies will be needed to confirm social learning.

Because the described anointing behavior was only observed during the wet season, when disease vector species of mosquitoes are most abundant at the study site (personal observation), we assume that it could play a role in the deterrence of these insects as demonstrated in other studies with primates (Valderrama et al. 2000; Weldon et al. 2003). Weldon et al. (2003) showed that *p*-toluquinone and 2-methoxy-3-methyl-
1,4-benzoquinone deter contact and feeding by Yellow fever mosquitoes (*Aedes aegypti*), which along with Asian tiger mosquitoes (*A. albopictus*) are the most important vectors of viral hemorrhagic fevers in the Atlantic Forest domain. By using millipede secretions, the blonde capuchin monkeys of the Matacará population potentially avoid mosquito-borne diseases, including the deadly and rapidly spreading yellow fever in Brazil.

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