Department of Physics, Chemistry and Biology

Master Thesis

Olfactory discrimination ability of spider monkeys

(*Ateles geoffroy*i) for the odours of ripe and unripe fruits

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LiTH-IFM- Ex--15/3011--SE

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Titel/Title:

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Sammanfattning/Abstract:

For frugivorous primates, the ability to discriminate between fruit odours, and of different degrees of ripeness in particular, should be important for an efficient food selection based on its nutritional value. It was therefore the aim of the present study to assess the ability of spider monkeys (*Ateles geoffroyi*), a frugivorous New World primate species, to discriminate between the odours of ripe and unripe fruits, and between odours representing different degrees of ripeness. A two-choice instrumental conditioning paradigm was used in which five spider monkeys were presented with odour mixtures mimicking different degrees of ripeness of two neotropical fruits, *Leonia cymosa* (*Violaceae*) and *Couma macrocarpa (Apocynaceae)*. The results showed that spider monkeys were able to discriminate between the ripe and unripe odours of these two fruits and also between the majority of their variations during the ripening process. This supports the notion that spider monkeys may use olfactory cues for food selection.

Nyckelord/Keyword:

Fruit odours, ripeness, spider monkeys, olfactory discrimination, seed dispersal, honest signals.

Content

1 Abstract

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2 Introduction

In tropical ecosystems, primates are one of the most important seed dispersal vectors (Chapman and Russo 2007, Corlett and Primack 2011). Due to the high diversity and quality of fruits, but also due to their spatial and temporal distribution, it is not an easy task for a primate to find which fruits are edible or optimal in terms of nutritional value. The way in which primates solve this task is still a poorly understood process (Dominy et al. 2006).

It has been reported that spider monkeys often sniff at the fruits they are inspecting, considering that visual cues such as colour might not give a clear sign of the maturity of the fruit (van Roosmalen 1985). It is presumed that the odour of a fruit serves as a signal of readiness for predation and seed dispersal, due to its systematic change across the ripening process (Rodriguez et al. 2013). Therefore, the ability to discriminate between fruit odours, and of different degrees of ripeness in particular, should be important for frugivorous species.

Interpreting the neuroanatomical features of primates such as relative size of olfactory brain structures (Stephan et al. 1988), or genetic features such as the number of functional olfactory receptor genes (Rouquier et al., 2000), has traditionally lead to the conclusion that primates have a poorly developed sense of smell (King and Fobes 1974, Walker and Jennings 1991). Nevertheless, there is a lack of physiological evidence of a positive correlation between allometric measures of neuroanatomical or genetic

features and olfactory performance (De Winter and Oxnard 2000, Schoenemann 2001).

It has been reported how some primate species use their sense of smell in tasks such as food identification and selection (Bolen and Green 1997, Ueno 1994), but also in social behaviours such as territorial defence (Mertl-Millhollen 1986), recognition of group members (Epple et al. 1993), establishment and maintenance of rank (Kappeler 1998), identification of sexual partners (Heymann 1998), and communication of reproductive status (Smith and Abbott 1998). The increasing reports on olfactory behavioural observations raises doubts with regard to the general belief that primates have only poor olfactory capabilities.

The use of psychophysical methods in new testing paradigms to assess olfactory performance in nonhuman primates (Laska and Hudson 1993, Hübener and Laska 2001, Laska et al. 2003), has demonstrated that spider monkeys, for example, are able to learn odours fast and have excellent long-term memory for odours (Laska et al. 2003). In addition, it has been demonstrated that they possess a high olfactory sensitivity for structurally related monomolecular substances, as seen for aliphatic esters (Hernandez Salazar et al., 2003), carboxylic acids (Laska et al. 2004), thiazoles (Laska et al. 2005), steroids (Laska et al. 2005,2006), alcohols and aldehydes (Laska et al. 2006), monoterpenes (Joshi et al. 2006, Laska et al. 2006), thiols and indols (Laska et al. 2007a), alkylpyrazines (Laska et al. 2009), amino acids (Wallén et al. 2012), "green odours" (Løtvedt et al. 2012), and sulfur-containing predator odours (Sarrafchi et al. 2013).

Therefore, the aim of the present study was to assess the ability of spider monkeys, a frugivorous New World primate species, to discriminate between the odours of ripe and unripe fruits, and between odours representing different degrees of ripeness. To this end, a food-rewarded operant conditioning paradigm was used, and the ability of the animals to discriminate between odour mixtures mimicking the composition of the odours of fruits of varying degrees of ripeness were tested. The results of the study allow us to draw first conclusions as to the ability of spider monkeys to use olfactory cues for food selection.

3   Material & methods

3.1   Animals

The study was carried out at the Field Station Pipiapan of the Universidad Veracruzana, near Catemaco, Veracruz, Mexico. Four adult females and one

male black-handed spider monkeys, *Ateles geoffroyi* (Figure 1). The animals were born in captivity and maintained at the Field Station. The male, Lorenzo, was 8 years old, and the females were, Frida 9 years old, Flaca 10 years old, Kelly 11 years old and Nanny 15 years old. The animals were kept in individual enclosures, situated next to each other, providing the possibility of social interaction between individuals. Attached to each enclosure were test cages of 80x50x50cm, which the animals had been trained to enter voluntarily when they were called. The animals had served in previous studies on olfactory performance and were thus familiar with the test procedure outlined below.
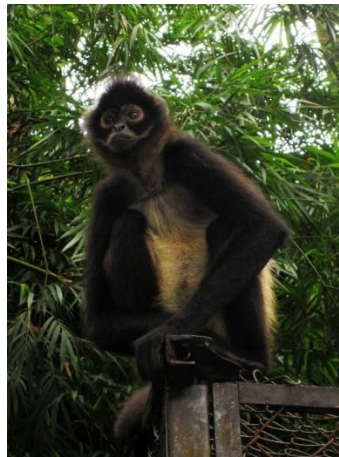

Figure 1. Black-handed spider monkey (Ateles geoffroyi).

The experiments outlined here comply with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication no. 86-23, revised 1985) and with current Mexican laws on animal welfare. The experiments were performed according to a protocol approved by the ethical board of the Federal Government of Mexico's Secretariat of Environment and Natural Resources (SEMARNAT; Official permits no. 09/GS-2132/05/10).

3.2 The odorants

The chemical composition of the odour of the fruits of two neotropical plants consumed by primates, *Leonia cymosa* (*Violacea*e) (Figure 2a) and *Couma macrocarpa* (*Apocynaceae*) (Figure 2b) was obtained by the German Primate Center (DPZ) in Göttingen, Germany, using a gas chromatography and crossing the calculated retention index with published mass spectra to identify the compounds. It was found that both fruits differed markedly between their ripe and unripe odour compositions. This was true both for the

odours emanating from intact fruits and for the odours emanating from the pulp of the open fruits (Tables 1 and 2).

Figure 2. a. Leonia cymosa fruits, ripe (left) and unripe (right).
b. Couma macrocarpa fruits, ripe (left) and unripe (right).

Table 1. Chemical composition of the odour of Leonia cymosa, provided by the German Primate Center (DPZ).

	Leonia cymosa			
Composition	Ripe intact	Unripe intact	Ripe open	Unripe open
Acetophenone	5.33%	18.10%	0.10%	1.07%
Benzaldehyde	12.67%	8.61%	0.74%	16.48%
trans -Cadina-1,4-diene	0.58%	0.26%	0.02%	0.66%
α-Copaene	39.30%	15.63%	0.72%	28.51%
Cumene	0.98%	29.94%	0.13%	5.71%
para-Cymene	2.34%	3.04%	0.24%	3.14%
para-Cymenene	0.00%	0.00%	2.38%	0.17%
α-Funebrene	0.40%	9.94%	0.04%	1.73%
Myrcene	0.00%	0.00%	0.30%	2.65%
allo-Ocimene	0.98%	0.00%	4.41%	4.17%
(E)-β-Ocimene	5.25%	0.00%	71.56%	2.70%
(Z)-β-Ocimene	0.00%	0.00%	0.51%	1.03%
neo-allo-Ocimene	0.00%	0.00%	1.59%	0.19%

Table 2. Chemical composition of the odour of Couma macrocarpa, provided by the German Primate Center (DPZ).

	Couma macrocarpa			
Composition	Ripe intact	Unripe intact	Ripe open	Unripe open
Benzaldehyde	0.71%	0.07%	6.12%	0.44%
E-Caryophyllene	16.2%	4.14%	22.39%	2.35%
α-Copaene	46.9%	41.67%	25.18%	50.17%
para-Cymene	0.26%	1.63%	0.34%	3.89%
Ethyl salicylate	1.05%	0.00%	2.56%	0.00%
(E,E)-α-Farnesene	2.23%	10.91%	1.14%	5.85%
(E)-4,8-Dimethyl-1,3,7-nonatriene	0.72%	1.33%	1.76%	6.30%
α-Humulene	1.48%	0.65%	2.25%	0.30%
Limonene	1.81%	15.40%	1.84%	10.72%
Methyl salicytate	2.35%	0.01%	8.72%	0.47%
Myrcene	1.61%	3.54%	1.53%	2.84%
2E, 6Z-Nonadeinal	0.11%	0.00%	2.50%	0.03%
Nonanal	1.39%	0.84%	3.45%	0.56%
2Z-Nonen-1-al	0.52%	0.00%	6.91%	0.10%
(E)-β-Ocimene	4.60%	0.76%	3.77%	1.02%
Sabinene	0.09%	1.51%	0.01%	1.26%
α-Terpinene	0.00%	0.43%	0.01%	0.93%
γ-Terpinene	0.54%	1.03%	0.03%	1.16%
Linalool	0.98%	0.13%	2.10%	3.18%

Couma macrocarpa had very different odour profiles for the unripe and ripe conditions already in the intact fruits, whereas *Leonia cymosa* only in opened fruits. Therefore, it was decided to test for the conditions of intact Couma macrocarpa and both intact and open Leonia cymosa.

Odour mixtures mimicking the composition of the odours of ripe and unripe fruits, and odour mixtures mimicking different degrees of ripeness were prepared and tested for discriminability. The mixtures were prepared using diethyl phthalate as a solvent. See Table 10 in Appendix for further information about the chemicals used.

3.3   Behavioural test

The spider monkeys were tested using a food-rewarded instrumental conditioning paradigm (see Laska et al. 2003 for details) in which the animals were simultaneously presented with two options, one correct and one incorrect. When the monkeys chose the correct option, they were immediately food-rewarded. If the incorrect option was chosen, the reward was withheld. The apparatus used (Figure 3) consisted of a metal bar 50 cm long and 6 cm wide, which had two PVC cube-shaped boxes attached to it at a distance of 22 cm between each other. Each box was equipped with a hinged metallic lid that hung 2 cm down in the front of the container. From the centre of the front part of the lid, a pin of 3 cm length extended. This pin served as a lever that the animals used to open the lid. On top of each lid there was a metal clip attached that held a 70 x 10 mm absorbent paper strip, which was impregnated with 20 µl of odorant. These paper strips extended approximately 3 cm into the cage when the apparatus was presented to the animals to sniff at them.

Figure 3. Apparatus used for the experiments.

The box that held the paper strip impregnated with the odorant considered as the rewarded stimulus (S+) was baited with a food reward consisting of a Kellogg's Fruit Loop cereal, while the box holding the strip impregnated with the odorant considered as the unrewarded stimulus (S-) was empty. During the development of this method, it was thoroughly investigated whether the smell of the food reward could help the monkey when choosing the correct box, but it was found not to be the case (unpublished data).

The monkeys were presented with the apparatus and sniffed both paper strips as much as they liked until they decided to open one of the boxes (Figure 4). After the decision, the apparatus was removed out of sight of the monkeys and prepared for the next presentation. In case a monkey tried to open a box without sniffing both paper strips, the apparatus was immediately removed from the test cage and reintroduced after a short time-out of approximately ten seconds until the animal sniffed both options.

Figure 4. Black-handed spider monkey performing the olfactory discrimination experiment using the two-choice apparatus.

Each monkey performed three sessions per day consisting of ten trials each. In five of the ten trials comprising a session, the right box was baited, while in the other five trials, the left one was baited. The order for the "correct" and "wrong" sides was pseudo-randomized, with the limitation that the same box should not be baited more than three times in a row.

After every session, the apparatus was thoroughly cleaned with 96% ethanol to ensure that no traces of odorants were left.

The ability to discriminate between the odours of ripe and unripe fruits was assessed by assigning one odour mixture (e.g. that mimicking the ripe fruit odour) as the rewarded stimulus (S+) or "correct" option, and several other odour mixtures (representing different degrees of unripe fruit odours) as the unrewarded stimulus (S-) or "wrong" option.

3.4 Experimental program

The layout of the experiment was conceived in two phases, a first one in which the ability to discriminate between the ripe and unripe odours was tested, and if that was the case, followed by a second phase in which variations of the unripe odorant were tested against the ripe one, to check if the animals are also able to discriminate derived odours of those, and try to

identify the chemical/s responsible for that discrimination.*a) Ability to discriminate between ripe and unripe fruit odours*

The odour mixtures mimicking the odours of ripe *Leonia cymosa* and *Couma macrocarpa* fruits were used as rewarded stimuli (S+) and tested against their unripe counterparts as unrewarded stimuli (S-).

b) Ability to discriminate between ripe and partially ripe fruit odours

The odour mixtures mimicking the odours of ripe *Leonia cymosa* and *Couma macrocarpa* fruits were again used as rewarded stimuli (S+). The odour mixtures mimicking their unripe counterparts were then made partially more similar to the ripe version by systematically replacing the concentration of one or more of its components as found in the unripe fruit for that found in the ripe fruit.

The first experiment with each fruit odour was performed using anethole diluted at 1:500 as the unrewarded stimulus (S-), used also in previous experiments (Laska et al. 2003) and therefore familiar for the animals, as a training method for the monkeys in order to associate the ripe odours as the rewarded stimuli (S+). Once the animals reliably discriminated between these odours with a percentage of correct choices higher than chance level (>70%), it was considered that they had learned to associate the fruit odour as the rewarded stimulus (S+) and to distinguish the rewarded stimulus, and the rest of experiments was then performed.

In the case of *Leonia cymosa* intact, however, the monkeys failed to discriminate between the ripe odour and the odour of anethole 1:500. Therefore, water was used as the unrewarded stimulus (S-) instead.

Table 3. Experiments with Couma macrocarpa intact.

Couma macrocarpa intact		
(S+)		(S-)
Ripe	*vs*	Anethole 1:500
Ripe	*vs*	Unripe
Ripe	*vs*	Unripe + E-Caryophyllene at ripe level
Ripe	*vs*	Unripe + α-Copaene at ripe level
Ripe	*vs*	Unripe + Ethyl salicylate at ripe level
Ripe	*vs*	Unripe + Methyl salicylate at ripe level
Ripe	*vs*	Unripe + Nonen-1-al at ripe level
Ripe	*vs*	Unripe + Aromatic[1] compounds at ripe levels
Ripe	*vs*	Unripe + Sesquiterpene[2] compounds at ripe levels
Ripe	*vs*	Unripe + Monoterpene[3] compounds at ripe levels
Ripe	*vs*	Unripe + Aldehyde[4] compounds at ripe levels
Ripe	*vs*	Unripe + Monoterpene[3] + Sesquiterpene[2] compounds at ripe levels
Ripe	*vs*	Unripe + Aromatic[1] + Aldehyde[4] compounds at ripe levels

[1]*Aromatic: para-cymene + ethyl salicylate + methyl salicylate*
[2]*Sesquiterpenes:* α*-copaene +* α*-humulene*
[3]*Monoterpenes: limonene + myrcene + (E)-*β*-ocimene + sabinene + γ-terpinene*
[4]*Aldehydes: nonanal + nonen-1-al*

Table 4. Experiments with Leonia cymosa open.

Leonia cymosa open		
(S+)		(S-)
Ripe	*vs*	Anethole 1:500
Ripe	*vs*	Unripe
Ripe	*vs*	Unripe + (E)-β-Ocimene at ripe level
Ripe	*vs*	Unripe + α-Copaene at ripe level
Ripe	*vs*	Unripe + Cimenene at ripe level
Ripe	*vs*	Unripe + Benzaldehyde at ripe level
Ripe	*vs*	Unripe + Acetophenone at ripe level
Ripe	*vs*	Unripe + Aromatic[1] compounds at ripe levels
Ripe	*vs*	Unripe + α -Copaene + (E)-β-Ocimene at ripe levels
Ripe	*vs*	Unripe + (E)-β-Ocimene +Aromatic[1] compounds at ripe levels

[1]*Aromatic: acetophenone + benzaldehyde + cumene + para-cymene + para-cymenene*

Table 5. Experiments with Leonia cymosa intact.

Leonia cymosa intact		
(S+)		(S-)
Ripe	*vs*	Anethole 1:500
Ripe	*vs*	Water
Ripe	*vs*	Unripe

Every monkey performed six sessions of 10 trials for each stimulus combination, from which the first three sessions were considered training and only the last three were considered for the statistical analysis.

3.5 Data analyses

For each individual animal, the percentage of correct choices from 30 trials per stimulus combination was calculated. Correct choices consisted of the animal both rejecting the box bearing the unrewarded stimulus (S-) by failing to open it, and identifying the box bearing the rewarded stimulus (S+) by opening it to obtain the food reward. Conversely, errors consisted of animals opening the box bearing the S- or failing to open the box bearing the S+. Significance levels were determined by calculating binomial z-scores corrected for continuity (Siegel and Castellan, 1988) from the number of correct and false responses for each individual animal and condition. All tests were two-tailed and the alpha level was set at 0.05. Interindividual differences were analysed with Kruskal-Wallis and ANOVA tests, using the software IBM SPSS Statistics v.22.

4 Results

4.1 General discrimination performance

Regarding the performance of the monkeys as a group in discriminating the odour of *C.macrocarpa* intact ripe from other stimuli, with 12 of the 13 tested stimulus combinations, the average percentage of correct choices was higher than 70.0%, and therefore, significantly different from chance at $p<0.05$. With 11 of the 13 stimulus combinations, the animals even scored an average of more than a 76.6% correct choices, corresponding to $p<0.01$. Thus, the monkeys were clearly able to distinguish the odour of *C.macrocarpa* intact ripe from unripe, but also from almost all variations of this odour when modifying compounds so that they became partially similar to the ripe one. Only when changing the concentration of methyl salicylate or sesquiterpenes compounds (α-copaene + α-humulene) from their unripe

to their ripe values, two individuals failed to significantly discriminate between the two odorants (Table 6).

Table 6. Performance of the five black-handed Spider monkeys as a group in discriminating the odour of *Couma macrocarpa* intact ripe from mixtures mimicking different degrees of ripeness of this fruit, represented by the mean percentage (± SD) of correct choices.

Couma macrocarpa intact ripe (S+)			
		Number of individuals	
(S-)	Mean ± SD	p>0.05	p<0.05
Anethole 1:500	78.7 ± 12.2	1	4
Unripe	82.0 ± 6.1	0	5
Unripe + E-Caryophyllene at ripe level	82.0 ± 5.6	0	5
Unripe + α-Copaene at ripe level	78.0 ± 8.0	1	4
Unripe + Ethyl Salicylate at ripe level	80.0 ± 8.5	0	5
Unripe + Methyl Salicylate at ripe level	68.0 ± 6.9	2	3
Unripe + Nonen-1-al at ripe level	71.3 ± 3.8	1	4
Unripe + Aromatic[1] compounds at ripe levels	80.7 ± 7.2	0	5
Unripe + Sesquiterpene[2] compounds at ripe levels	84.0 ± 17.5	2	3
Unripe + Monoterpene[3] compounds at ripe levels	80.7 ± 10.4	0	5
Unripe + Aldehyde[4] compounds at ripe levels	77.3 ± 8.6	1	4
Unripe + Monoterpenes[3] + Sesquiterpene[2] compounds at ripe levels	79.3 ± 8.0	1	4
Unripe + Aromatic[1] + Aldehyde[4] compounds at ripe levels	78.7 ± 9.3	1	4

[1]*Aromatic: para-cymene + ethyl salicylate + methyl salicylate*
[2]*Sesquiterpenes: α-copaene + α-humulene*
[3]*Monoterpenes: limonene + myrcene + (E)-β-ocimene + sabinene + γ-terpinene*
[4]*Aldehydes: nonanal + nonen-1-al*

For all 13 stimulus combinations of *C.macrocarpa*, either all five animals, or at least a majority of animals succeeded in discriminating an odour pair above chance level (p<0.05). With 11 of the 13 stimulus combinations, the majority of individuals even scored more than a 76% correct choices (p<0.01). In the combination of *C.macrocarpa* intact ripe vs nonen-1-al at ripe level only one individual, Kelly, reached more than a 76.6% of correct

choices, corresponding to p<0.01, and vs *C.macrocarpa* intact unripe + methyl salicylate, no individual reached this criterion and only 2 individuals achieved more than a 70% of correct choices, corresponding to p<0.05 (Figure 5).
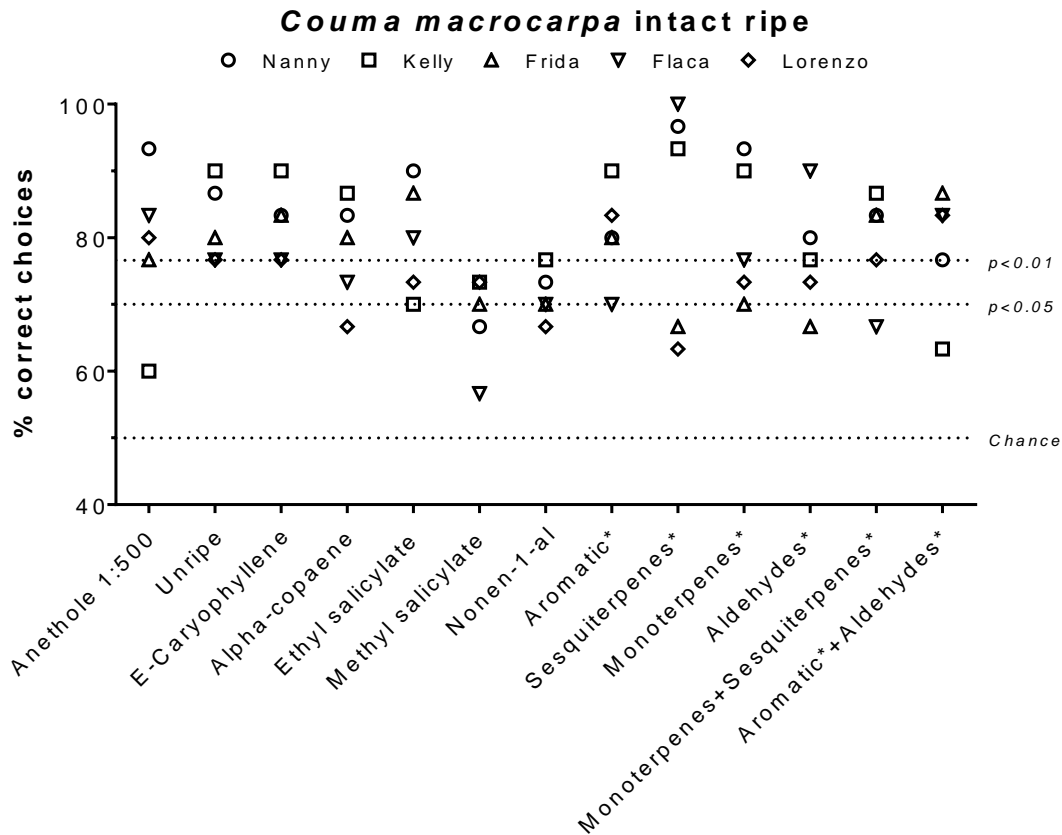

*Figure 5. Performance of five black-handed Spider monkeys in discriminating between the odour of Couma macrocarpa intact ripe and mixtures mimicking different degrees of ripeness of this fruit. Each data point represents the percentage of correct choices from 30 decisions per animal. The horizontal lines indicate chance level (at 50 %), and two levels of criterion (at 70.0 %, corresponding to p<0.05, and at 76.6 %, corresponding to p<0.01), respectively. (*Aromatic: para-cymene + ethyl salicylate + methyl salicylate; Sesquiterpenes: α-copaene + α-humulene; Monoterpenes: limonene + myrcene + (E)-β-ocimene + sabinene + γ-terpinene; Aldehydes: nonanal + nonen-1-al).*

With all 10 stimulus combinations of *L.cymosa* open ripe versus another stimuli, the average percentage of correct choices was higher than 76.6%, and therefore, significantly different from chance at p<0.01. Only when changing the levels of (E)-β-ocimene or the combination of α-copaene + (E)-β-ocimene to ripe levels in the unripe mixture, one individual, failed to significantly discriminate between the odours. Nevertheless, on average the monkeys were able to distinguish the odour of *L.cymosa* open ripe from

unripe, but also from variations of this odour when modifying compounds so that they became partially similar to the ripe one (Table 7).

Table 7. Performance of the five black-handed Spider monkeys as a group in discriminating the odour of Leonia cymosa open ripe from mixtures mimicking different degrees of ripeness of this fruit, represented by the mean percentage (± SD) of correct choices.

	Leonia cymosa open ripe (S+)		
		Number of individuals	
(S-)	Mean ± SD	p>0.05	p<0.05
Anethole 1:500	82.7 ± 10.1	0	5
Unripe	84.7 ± 6.5	0	5
Unripe + (E)-β-Ocimene at ripe level	78.7 ± 11.9	1	4
Unripe + α-Copaene at ripe level	94.0 ± 6.0	0	5
Unripe + Para-Cymenene at ripe level	91.3 ± 6.5	0	5
Unripe + Benzaldehyde at ripe level	92.7 ± 5.7	0	5
Unripe + Acetophenone at ripe level	92.7 ± 4.3	0	5
Unripe + Aromatic[1] compounds at ripe levels	92.7 ± 8.6	0	5
Unripe + α-Copaene + (E)-β-Ocimene at ripe levels	80.0 ± 13.3	1	4
Unripe + (E)-β-Ocimene + Aromatic[1] compounds at ripe levels	92.0 ± 6.1	0	5

[1]*Aromatic: acetophenone + benzaldehyde + cumene + para-cymene + para-cymenene*

All five individuals achieved a percentage of correct choices significantly higher than chance level (p<0.05) when discriminating *L.cymosa* open ripe from the other stimuli (Figure 6). In fact, in 7 out of 10 stimuli combinations, all 5 individuals performed more than a 76.6% of correct choices, corresponding to a p<0.01. However, in the combinations of *L.cymosa* open ripe vs unripe + (E)-β-Ocimene at ripe level, and vs α-copaene + (E)-β-ocimene at ripe levels, one individual (Nanny and Frida respectively) did not reach a 70% of right choices, and therefore no significant difference from chance level.
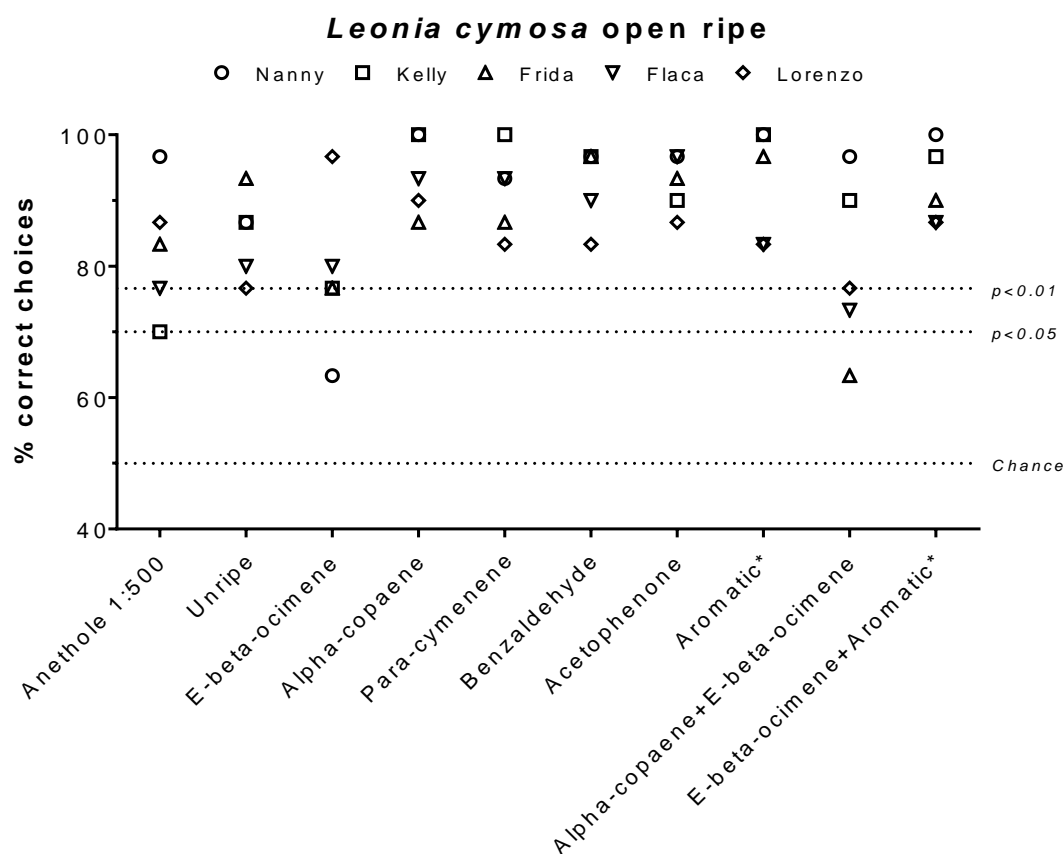
*Figure 6. Performance of five black-handed Spider monkeys in discriminating between the odour of Leonia cymosa open ripe and mixtures mimicking different degrees of ripeness of this fruit. Each data point represents the percentage of correct choices from 30 decisions per animal. The horizontal lines indicate chance level (at 50 %), and two levels of criterion (at 70.0 %, corresponding to p<0.05, and at 76.6 %, corresponding to p<0.01), respectively. (*Aromatic: acetophenone + benzaldehyde + cumene + para-cymene + para-cymenene).*

Table 8 shows the performance of the spider monkeys as a group in discriminating the odour of *L.cymosa* intact ripe from other stimuli. All the individuals fulfilled the criterion of 76.6 % of correct choices (corresponding to a p<0.01 in the two-tailed binomial test), when discriminating *L.cymosa* intact ripe from water. However, they failed to discriminate *L.cymosa* intact ripe from unripe.

Table 8. Performance of the five black-handed Spider monkeys as a group in discriminating the odour of Leonia cymosa intact ripe from mixtures mimicking different degrees of ripeness of this fruit, represented by the mean percentage (± SD) of correct choices.

	Leonia cymosa intact ripe (S+)		
		Number of individuals	
(S-)	Mean ± SD	p>0.05	p<0.05
Water	80.0 ± 4.7	0	5
Unripe	60.7 ± 9.5	4	1

Only one individual, Nanny, reached more than a 76.6% of correct choices when discriminating *L.cymosa* intact ripe from water, while the other 4 individuals did not reach a 70% of correct choices, corresponding to a p<0.05, and therefore did not performed significantly different from chance level (Figure7).

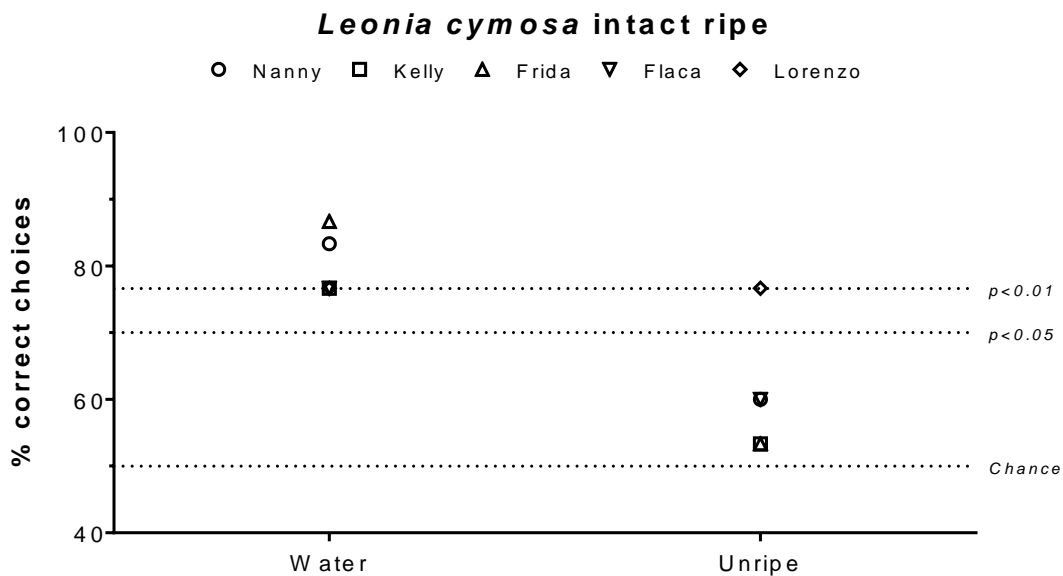*Figure 7. Performance of five black-handed Spider monkeys in discriminating between the odour of Leonia cymosa intact ripe and unripe. Each data point represents the percentage of correct choices from 30 decisions per animal. The horizontal lines indicate chance level (at 50 %), and two levels of criterion (at 70.0 %, corresponding to p<0.05, and at 76.6 %, corresponding to p<0.01), respectively.*

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4.2 Individual differences in discrimination performance

The average difference between the best- and poorest-performing animal in the percentage of correct choices was of 21.03 ± 7.50; 19.33 ± 8.58; 16.67 ± 9.43 % of correct choices for the stimulus combinations with *C.macrocarpa* intact, *L.cymosa* open and *L.cymosa* intact, respectively.

While Lorenzo was the most constant individual in his performances, with the smallest variation between best and worst performances, Frida was the least constant, with a maxim variation between her performances of a 23.6%. However, this interindividual variability was not significant (*C.macrocarpa intact* (H=8.45, 4 d.f., P=0.77); *L.cymosa* open (H=10.24, 4 d.f., P=0.37); *L.cymosa* intact (H=0.97, 4 d.f., P=0.91)).

Nanny was the best-performing animal with an average of 82.75 (±10.69) % correct choices, whereas the four other individuals, Kelly, Frida, Flaca and Lorenzo, scored similarly with 78.73 (±12.93), 77.86 (±8.37), 76.95 (±8.50) and 78.68 (±5.60) % of correct choices, respectively (Table 6). However, the difference in the mean performance between the five monkeys was not statistically significant [$F_{(244, 120)} = 2.03$, $p = 0.094$].

Table 9. Individual average difference between the best and the worst performance for all the odour pairs tested, represented by the mean percentage (± SD) of correct choices.

	Individual average performance (Mean % ± S.D.)		
	Couma macrocarpa intact	*Leonia cymosa* open	*Leonia cymosa* intact
Nanny	83.59 ± 8.55	93 ± 11.16	71.67 ± 16.5
Kelly	80.51 ± 11.21	90.67 ± 10.4	65 ± 16.5
Frida	76.92 ± 7.39	86.67 ± 10.3	70 ± 23.57
Flaca	77.18 ± 10.87	85.33 ± 7.89	68.33 ± 11.79
Lorenzo	74.36 ± 6.14	85 ± 5.93	76.67 ± 0

The largest difference in scores between animals in a given task was 36.67% with the combination of "*C.macrocarpa* intact ripe vs *C.macrocarpa* intact unripe + Sesquiterpenes at ripe level", where Flaca scored a 100% of correct choices, but Frida and Lorenzo did not reach a statistical significant level of correct choices. In contrast, there was only a 10% difference between the best- and poorest-scoring animal in the tests "*C.macrocarpa* intact ripe vs *C.macrocarpa* unripe + nonen-1-al at ripe level", "*L.cymosa* open ripe vs

L.cymosa unripe + acetophenone at ripe level" and "*L.cymosa* intact ripe vs water".

5   Discussion

The results of the present study show that spider monkeys (*Ateles Geoffroyi*) have a well-developed ability to discriminate between the ripe and unripe odorants of *C.macrocarpa* intact and *L.cymosa* open, and also between the majority of their variations tested. However, they failed to discriminate between the ripe and unripe odorants of *L.cymosa* intact.

In case of discrimination between the ripe and unripe odours, the unripe odorant was partially modified to make it more similar to the ripe odorant. This was designed in order to try to identify which chemical compound/s are responsible for the discrimination. That would be important to see which change in the chemical composition of the odour of the fruit during the ripening process is the responsible for the animal to detect that the fruit is ready to be consumed. However, the animals were able to discriminate between the modified unripe and ripe odorants as well. That might indicate that the variation of all chemical compounds in general is responsible for the conformation of the specific ripe and unripe odours, and single variations of a chemical or group of chemicals in the composition does not affect the discrimination of those odours. Nevertheless, it could also be possible that the chemical compound or group of compounds responsible for the discrimination was not one of the experimentally modified compounds and, therefore, more combinations should be tested in future experiments. Also it would be interesting to repeat the experiments with the odours of other fruits present in the diet of spider monkeys, in order to draw larger conclusions.

An aspect to take in consideration is that the odorants produced may not have been an exact reproduction of those found in the natural fruits. The chemical composition obtained with the gas-chromatography reflects those compounds that are present in higher concentrations, but other compounds might have been neglected. Also, during the preparation of the solutions, some high volatile compounds might have been slightly dispersed. Nonetheless, the odorants presented to the animals are presumed to be a good representation of the natural ones, and therefore, the results of this study should be good indicators on the discriminability of these fruit odours.

There was no significant interindividual variability in the results. Even though the sample size was only five individuals, it can be presumed that

their performance was due to the natural discriminatory abilities of this species, rather than individual capabilities.

Once more, the general olfactory capabilities of spider monkeys have been shown to be better than previously assumed. And this is true not just for food-related odours, but also for other odorants with a relevant role in other behavioural contexts, such as sulfur-containing predator odours (Sarrafchi et al. 2013), which would allow them to recognize and avoid predators, or carboxylic acids (Laska et al. 2004), which are body-borne compounds (Flood, 1985) present in primate vaginal odours (Matsumoto Oda et al. 2003), that may be relevant as social communication cues (Laska et al. 2004).

As Heymann (2006) already suggested, maybe it is time to leave behind the concept that primates are "microsmatic species", as they are still presented in many textbooks. Even though their neuroanatomical features are proportionally smaller than those of other mammals (Stephan et al. 1988, Rouquier et al. 2000), it is becoming clear that this does not correlate with their olfactory performance (De Winter and Oxnard 2000, Schoenemann 2001). Primates do not seem to have a poorly-developed sense of smell as it was previously thought (King and Fobes 1974, Walker and Jennings 1991), and recent studies demonstrate the importance of this sense in various behavioural contexts, such as foraging (Bolen and Green 1997, Ueno 1994), social communication (Mertl-Millhollen 1986, Epple et al. 1993) and reproduction (Heymann 1998, Smith and Abbott 1998, Kjeldmand et al 2011). Further research is necessary to corroborate the extent to which the olfactory capabilities of primates play a vital role in these species.

A previous study found that spider monkeys use olfactory, gustatory, tactile and visual cues to evaluate novel food (Laska et al. 2007b). Laska suggested that the olfactory cues might be particularly useful when learning about the palatability of novel food, and more specifically in the case of plants, where the odours have been shown to be generally species-specific (Knudsen et al. 1993, Linskens and Jackson 1997) and to change systematically during the maturation process (Brady 1987, Nursten, 1970).

The high sensitivity to food-associated odorants of spider monkeys seen in previous studies (Hernandez Salazar et al. 2003,Laska et al. 2004, Laska et al., 2005, Laska et al. 2005,2006, Laska et al. 2006, Joshi et al. 2006, Laska et al. 2006, Laska et al. 2007a, Laska et al. 2009, Wallén et al. 2012, Løtvedt et al. 2012), and the discriminative capabilities shown in the present study for the different odours of ripeness in fruits, suggest that the sense of smell

plays a vital role in the food selection process in this species, such as determination of the nutritional value of the fruits (Dominy 2004).

Fruit odour has long been speculated to signal ripeness to olfactory-guided dispersal agents (Howe and Westley 1986, 1988). Therefore, the ability to discriminate between fruit odours and their changes during the ripening process should be important for frugivorous species, since they serve as honest signal of readiness for predation and seed dispersal (Rodriguez et al. 2013).

C.macrocarpa has a stronger odour, and thus a stronger signal, in the husk of the fruit than in the pulp, whereas *L.cymosa* presents a stronger odour, and thus signal, in the pulp of the fruit (unpublished data). Husk signalling involves the synthesis of volatile compounds for a constant signalling, with a high metabolic cost, as the odorants are diffusing away from the surface of the fruit and thus have to be permanently replaced (Gershenzon 1994), but is considered to be an efficient method for olfactory detection by seed dispersers during the search phase (Hodgkison et al. 2007, 2013, Borges et al. 2008; Lomáscolo et al. 2010, Valenta et al. 2013). However, primates are presumed to not rely on their sense of smell for foraging (Bicca-Marques and Garber 2004), but only in the food selection phase (Laska et al. 2007b, Hiramatsu et al. 2009, Melin et al. 2009). Even though the main dispersal vector of *C.macrocarpa* are primates (Culot et al, 2009), fruits of this genus are also dispersed by bats (Miller and Miller 2002, Lobova et al. 2009), which rely on chemosensation in the food-search phase (Thies et al. 1998).

Signalling in the pulp is a metabolically less costly strategy because the volatile chemicals are stored inside the fruit and thus do not need a continuous synthesis and replacement (Gershenzon 1994). Furthermore, when primates remove a fruit, they do not necessarily consume it, but rather proceed with a series of visual, tactile and chemosensory examinations (Dominy et al. 2006) before making that decision. Discarding a fruit after inspection implies a waste of seeds (Howe 1980). Thus, it is in the interest of the plant to provide a positive signal at this stage too. Moreover, signalling in an overly conspicuous manner may attract predators such as insects, that can cause serious seed loss (Andersen 1988). Since *L.cymosa* is only dispersed by primates (Pfrommer 2009), pulp signalling is an exclusive targeted system that may avoid predators and involves a lower metabolic cost.

This supports the "dispersal syndrome" hypothesis (van der Pijl 1982, Janson 1983, Howe and Westley 1988), which postulates that plant species evolved

to specialize on a certain frugivore guild (e.g. birds or primates) and hence their fruits are adapted to the physical, nutritional and sensory capacities of their respective dispersal vectors. This hypothesis has been questioned due to several properties attributed to selection pressures by frugivores being easily explained by common ancestry (Jordano 1995) or abiotic factors (Bollen et al. 2005), but recent studies have shown that at least some of the variation in the fruit traits can be attributed to selection by frugivores as originally hypothesized (Flörchinger et al. 2010, Lomáscolo and Schaefer 2010, Lomáscolo et al. 2010, Schaefer and Ruxton 2011, Schaefer et al. 2014).

Therefore, it seems that *C.macrocarpa* and *L.cymosa* could have evolved to signal ripeness so that their main dispersers can detect and select their fruits, with the odour being an honest signal under the costly signalling paradigm (Zahavi and Zahavi 1997). This would be in the same line as the notion of a shared evolutionary history of primates and angiosperms (Sussman et al. 2013).

5.1 Conclusions

The findings of this study show that spider monkeys have a well-developed sense of smell to discriminate between the ripe and unripe odours of fruits and the majority of their variations during the ripening process. This supports the notion that spider monkeys may use olfactory cues for food selection.

5.2 Societal & ethical considerations

Research with captive animals always raises controversy, not only due to welfare aspects, but also because some scientists defend that the results obtained by this kind of research might be biased from what is real in the wild.

However, we have to be realistic and accept that there are many questions that cannot be answered only by studies on wild animals. This study is a clear example, because the behavioural observations of wild spider monkeys just give us some insight on their foraging habits, but cannot provide detailed physiological information behind those processes. We could speculate about the meaning of monkeys sniffing the fruit before consuming it, but we would not be able to know if they are really perceiving any odour signal, and if so, if that information is of any relevance in their choices.

With further research, the data obtained with this study could provide us with new insights into the mechanisms of food choice and foraging in primates,

more specifically in spider monkeys, and this information could be useful for a better conservations of these species and its habitats.

Therefore, when it comes to the conservation of wildlife, information obtained by research in captive animals can be key to achieve a successful protection and survival of the species.

However, it is vital that the animals should be kept in adequate conditions to provide a good welfare, and the experiments have to follow all protocols stated by the laws.

All the experiments undertaken in this study comply with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication no. 86-23, revised 1985) and with current Mexican laws on animal welfare, and were performed according to a protocol approved by the ethical board of the Federal Government of Mexico's Secretariat of Environment and Natural Resources (SEMARNAT; Official permits no. 09/GS-2132/05/10).

6 Acknowledgement

7 References

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8   Appendix

Table 10. Information about the chemical compounds used: Common name, CAS (Chemical Abstract Service) registry numbers, purity percentage and laboratory of origin.

Common name	CAS	Purity	Origin
Acetophenone	98-86-2	≥98%	Sigma Aldrich, Germany
Benzaldehyde	100-52-7	≥98%	Sigma Aldrich, Germany
(*E*-) Caryophyllene	87-44-5	80%	Dragon, China
Cumene	98-82-8	98%	Sigma Aldrich, Germany
Diethyl phthalate	84-66-2	≥99%	Sigma Aldrich, Germany
D-limonene	5989-27-5	98%	Sigma Aldrich, Germany
Ethyl salicylate	118-61-6	99%	Sigma Aldrich, Germany
E-β-ocimene	3779-61-1	90% + isomers	Dragon, China
α-Humulene	6753-98-6	96%	Sigma Aldrich, Germany
Linalool	78-70-6	97%	Sigma Aldrich, Germany
Methyl salicytate	119-36-8	99%	Sigma Aldrich, Germany
Myrcene	123-35-3	≥90%	Sigma Aldrich, Germany
Nonanal	124-19-6	≥95%	Sigma Aldrich, Germany
Sabinene	3387-41-5	75%	Sigma Aldrich, Germany
Trans-2-nonenal	18829-56-6	97%	Sigma Aldrich, Germany
α-copaene	138874-68-7	70%	ACC Corporation, CA, US
γ-Terpinene	99-85-4	97%	Sigma Aldrich, Germany