

Department of Physics, Chemistry and Biology

Master Thesis

Taste responsiveness for food-associated
sugars in the black-and-white ruffed lemur
(*Varecia variegata*).

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The mechanisms underlying the evolution of taste perception across the animal kingdom continues to be a subject of inquiry. Black-and-white ruffed lemurs (*Varecia variegata*) are considered to be highly frugivorous and presumably rely on sweet taste perception when selecting food. Currently, no data on the taste responsiveness for naturally occurring food-associated sugars in the black-and-white ruffed lemur are available. Thus, the aim of this study was two-fold: (1) to determine the ruffed lemurs' taste preference thresholds for sucrose, fructose, glucose, maltose, and lactose (2): To determine the relative preference of the lemurs for these five saccharides. Testing was carried out using two-bottle preference tests of short duration in three captive black-and-white ruffed lemurs. Taste preference thresholds were found to be 25-50 mM for sucrose, 25-50 mM for fructose, 75 mM for glucose, 50 mM for maltose and 50 mM for lactose. Furthermore, the lemurs significantly preferred sucrose over all other saccharides and fructose over glucose, maltose and lactose when presented at equimolar concentrations of 50 mM 100 mM and 200 mM, respectively. The lemurs' taste sensitivity falls into the same range as that reported in other primates and the order of relative preference is similar to that in humans and the majority of the other primates tested so far. The notion of a possible positive correlation between the degree of frugivory and taste sensitivity was not supported by the findings of this study.

Nyckelord/Keyword:

Taste preference thresholds, sweet taste, primates

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1 Abstract

The mechanisms underlying the evolution of taste perception across the animal kingdom continues to be a subject of inquiry. Black-and-white ruffed lemurs (*Varecia variegata*) are considered to be highly frugivorous and presumably rely on sweet taste perception when selecting food. Currently, no data on the taste responsiveness for naturally occurring food-associated sugars in the black-and-white ruffed lemur are available. Thus, the aim of this study was two-fold: (1) to determine the ruffed lemurs' taste preference thresholds for sucrose, fructose, glucose, maltose, and lactose (2): To determine the relative preference of the lemurs for these five saccharides. Testing was carried out using two-bottle preference tests of short duration in three captive black-and-white ruffed lemurs. Taste preference thresholds were found to be 25-50 mM for sucrose, 25-50 mM for fructose, 75 mM for glucose, 50 mM for maltose and 50 mM for lactose. Furthermore, the lemurs significantly preferred sucrose over all other saccharides and fructose over glucose, maltose and lactose when presented at equimolar concentrations of 50 mM, 100 mM and 200 mM, respectively. The lemurs' taste sensitivity falls into the same range as that reported in other primates and the order of relative preference is similar to that in humans and the majority of the other primates tested so far. The notion of a possible positive correlation between the degree of frugivory and taste sensitivity was not supported by the findings of this study.

2 Introduction

The mechanisms underlying the evolution of taste perception across the animal kingdom continues to be a subject of inquiry and has been a subject of debate (e.g. why many animals show a preference for sweet taste; see Ramirez 1990, Simmen & Hladik 1998). It is however reasonable to assume that taste perception has been, and still is, adaptive for animals when assessing nutritional content in foods (Drewnowski et al. 2012, Le Magnen 1985) and in detecting potential noxious or toxic substances in potential foods (Hladik et al. 2002, Simmen et al. 1999a). At the same time, the mutualistic relationship between seed-bearing plants and seed-dispersers and pollinators raises the possibility that taste

perception in animals has also at least to some degree, co-evolved with the radiation of flowering plants (Hladik 1993).

Primates display a large variety of dietary specializations, ranging from e.g. strictly leaf-eating colobus monkeys (*Colobus guereza spp.*) to omnivorous humans (*Homo sapiens*). Gummivorous as well as insectivorous diets are also common among other primate species, e.g. the marmosets (genus *Cebuella*) and tarsiers (family *Tarsiidae*). As such, primates are a particularly suitable order of mammals for comparative studies of differences in the perception of, and responsiveness to taste stimuli between species. Carbohydrates, such as sugars, have a major metabolic importance for many primates (Kare & Brand 1986, Leonard & Robertson 1992) and it would thus be advantageous for a primate to possess the ability to perceive these easy metabolizable foodstuffs (Hladik et al. 2002, Schmidt et al. 2010).

Taste sensitivity in animals is usually quantified as taste preference thresholds, defined as the lowest concentration at which an animal displays a significant preference for a taste stimulus when tested against a blank stimulus, e.g. tap water (Simmen et al. 1999a). These thresholds are most often assessed using a single motivation, two-alternative choice test (Kirkden & Pajor 2006), e.g. a two-bottle preference test. The resulting taste preference thresholds are widely used to investigate gustatory abilities in primates (Simmen & Hladik 1998). It is important to keep in mind that preference thresholds are a conservative approximation of neural sensory detection thresholds, although 'behavioral' taste preference thresholds have been shown to be comparable with electrophysiologically determined taste thresholds in some cases (e.g. Hellekant et al. 1993, Ogawa et al. 1972).

The perception of and responsiveness to sweet taste has previously been tested behaviorally in a wide range of non-human primate species (e.g. Glaser et al. 1996; Laska 1996; Nofre et al. 1996; Simmen & Hladik 1998; Simmen et al. 1999a). Comparative studies of sugar taste sensitivity using two-bottle preference tests show notable differences among species of primates (Laska et al. 2001, Simmen & Hladik 1998), raising questions if these differences in taste sensitivity are due to phylogenetic relatedness, similarities in the characteristics of diets or allometric relationships (Simmen et al. 1999a, Simmen et al. 1999b, Simmen & Hladik 1998). Sweet stimuli have also been shown to vary in their stimulating efficiency; for example in humans, saccharin is up to 700 times less concentrated than sucrose at their corresponding threshold level (Pfaffmann et al. 1971). Unfortunately, there is still sparse information on this perceived 'relative sweetness' of naturally occurring

soluble sugars across the mammalian class, inferred from the animals' preference for one sugar over another when presented at equal concentrations.

To this date, there are no current data on the taste responsiveness for naturally occurring food-associated sugars, such as sucrose or fructose in the prosimian black-and-white ruffed lemur (*Varecia variegata*). Ruffed lemurs are highly frugivorous and presumably rely on sweet taste perception when selecting food (Britt 2000). Furthermore, given the proposed positive correlation between body mass and taste sensitivity for sucrose and fructose in primates (Simmen & Hladik 1998), it would be of interest to assess the taste response of *V. variegata* as it is the largest species of the extant *Lemuridae* family (Britt 2000).

Thus, the aim of this study was two-fold: (1) to determine taste preference thresholds for sucrose, fructose, glucose, maltose, and lactose in ruffed lemurs (2): To determine the relative preference of ruffed lemurs for these five saccharides.

3 Material & methods

3.1 Animals

Testing was carried out using three male black-and-white ruffed lemurs (*V. variegata*) (fig.1), in this study referred to as *Subject* (S) 1, 2 and 3. Two of the individuals were 11 years of age and one was 19 years of age, all thus being adult ruffed lemurs at the time of the study. This all-male and captive-bred group was housed at Kolmården Wildlife Park, Sweden, in a 117 m³ indoor exhibit with access to a 100 m² outdoor island. Testing was performed in a smaller holding cage adjacent to the indoor exhibit with two connecting gates. Additionally, the cage could be divided into three compartments in which the animals were tested separately to avoid competition and distraction. All three subjects were trained to voluntarily enter the test cage and were completely accustomed to the procedure described below. The subjects were fed fresh fruit and vegetables once per day at approximately 0800 h. Commercial primate chow pellets and water were provided ad libitum.

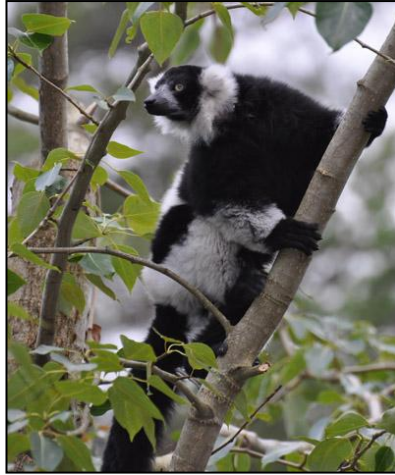


Figure 1: One of the studied black-and-white ruffed lemurs, courtesy of Kolmården Wildlife Park.

3.2 Societal and ethical aspects

The experiments reported here comply with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication no. 86-23, revised 1996) and also with current Swedish laws. The results of this study could aid in the development of suitable husbandry methods related to diet in captive lemurs, as diet-related problems such as obesity can be common among captive ruffed lemurs (Schwitzer and Kaumanns 2001).

3.3 Taste stimuli

The taste stimuli used in this study were all obtained from Sigma-Aldrich Sweden AB, Stockholm, Sweden. The stimuli were sucrose (CAS 57-50-1), fructose (CAS 57-48-7), glucose (CAS 50-99-7), maltose (CAS 6363-53-7) and lactose (CAS 63-42-3).

3.4 Procedures

Taste preference thresholds and the relative preferences for the tested sugars were assessed using a two-bottle preference test of short duration (Richter and Campbell 1940). Tests were performed twice per day, once in the morning and the second time in the evening, approximately 1h before and 7h after the animals were fed. Each test comprised of two trials (for a total of four trials per individual and day) in which each animal was allowed 1 min to drink from a pair of simultaneously presented graduated bottles with metal drinking spouts. The rather short duration was chosen to reduce postingestive effects on taste perception. The bottles were filled with appropriate taste stimuli and/or tap water before each set of trials and the recording of individual consumption of

liquid was done after each set of trials. Each pair of stimuli was presented for a total of ten times per individual, with the positions of the bottles pseudo-randomized to counter possible position bias. Saccharide solutions in all experiments were prepared 24 h before use to allow mutarotation. The order of the tested saccharides was the same for all individuals ((1) sucrose, (2) fructose, (3) glucose, (4) maltose, (5) lactose).

3.4.1 Experiment 1 – Determination of taste preference thresholds

To determine preference threshold values, the animals were given the choice between tap water and a predetermined concentration of sugar solution prepared with tap water. The tested concentrations for all saccharides ranged between 10 mM up to 200 mM (fig. 2) and testing did not follow a strictly descending or ascending order of concentrations. Instead, the testing sequence was pseudo-randomized in order to keep up the animals' motivation to cooperate. The lowest concentration of a saccharide that was significantly preferred over tap water was considered as the preference threshold value.

3.4.2 Experiment 2- Assessment of relative preference

The relative preference for the five saccharides was assessed using the same method as outlined above. However, in this experiment the lemurs were given the choice between two saccharide solutions (prepared with tap water) presented at equimolar concentrations. All possible binary combinations of the five saccharides, i.e. ten combinations, were tested. To assess whether relative preferences were stable at different concentrations, three series of tests were performed at 50, 100 and 200 mM, respectively.

3.5 Data analysis

For each animal and combination of stimuli, the amount of liquid consumed from each bottle was recorded and then summed for the ten trials. The consumed amount of each taste stimulus (summed over ten trials) was then converted to percentages, relative to the total amount of liquid consumed for both bottles (over ten trials), and 66.7% of a consumed liquid was regarded as the criterion of preference. A more liberal (i.e. lower) percentage could be used to infer a preference, although we regard consumption of more than two-thirds of the total amount of liquid consumed to be a reasonable and biologically relevant value of preference. Additionally, two-tailed binomial tests (Siegel and Castellan 1988) were performed and only when an animal reached the criterion of 66.7%, and consumed more of a preferred liquid in at least 8

of the 10 trials (binomial test, $p < 0.05$) was the preference regarded as a significant preference. Thus, the used definition of a taste preference threshold in this study was the lowest tested concentration of a taste stimulus at which an animal met both criteria mentioned above. These rather conservative criteria were chosen to reduce the risk of false positives and also for comparability purposes, as the same criteria have been used in previous studies on other species using the same method (Laska 1994, 1997, 2000; Laska et al. 1998, 1999a).

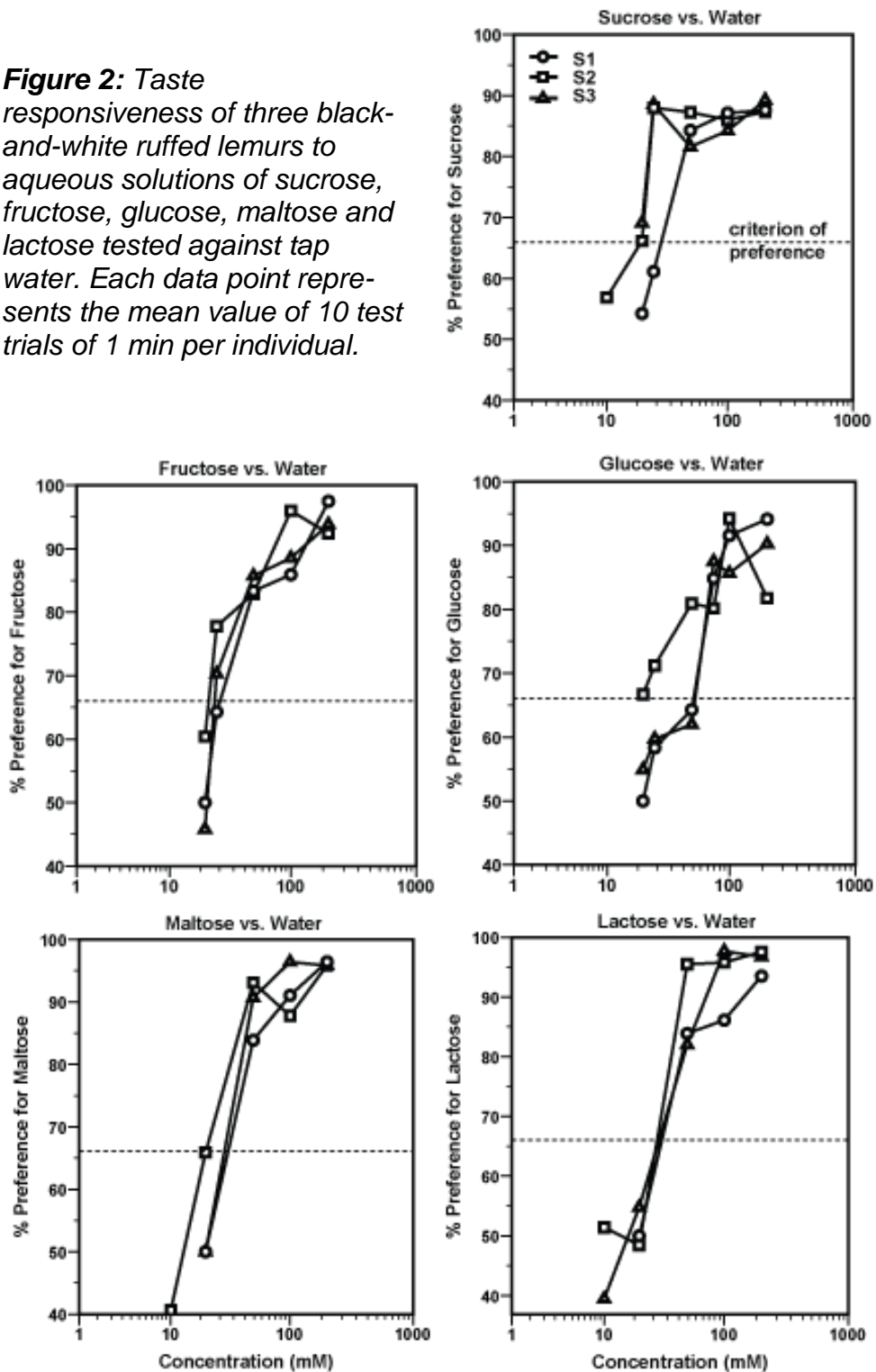
Preliminary analysis of the data indicated no systematic differences in neither preference nor the amount of consumed liquid between the first and second presentation of the day in either of the two experiments. In experiment 2, inter-individual variability in preference scores for individual saccharides was remarkably low as can be inferred from low standard deviations. Therefore, the data in assessing relative preferences were combined for all three individuals and presented as group means with standard deviations.

4 Results

4.1 Experiment 1 – Determination of taste preference thresholds

Figure 2 shows the taste responsiveness of the three lemurs to various concentrations of sucrose, fructose, glucose, maltose and lactose when tested against tap water. Taste preference thresholds were found to be 25-50 mM for sucrose, 25-50 mM for fructose, 75 mM for glucose, 50 mM for maltose and 50 mM for lactose. Preference thresholds for sucrose and fructose revealed a degree of inter-individual variability, with one or two individuals scoring a lower threshold value than the other individual(s). Specifically, *S(subject)2* and *S3* showed a preference threshold for sucrose at 25 mM while *S1* scored a threshold value of 50 mM. For fructose, *S2* scored a threshold value of 25 mM while *S1* and *S3* showed a higher threshold at 50 mM, with *S3* only fulfilling criterion one at 25 mM. Worth noting is *S2*'s apparent preference ($>66.7\%$ consumed liquid) for glucose solution over tap water at the concentrations at 20, 25 and 50 mM (fig. 2). However, these results did not fulfill criterion two at these concentrations (binomial test, $p > 0.05$).

Figure 2: Taste responsiveness of three black-and-white ruffed lemurs to aqueous solutions of sucrose, fructose, glucose, maltose and lactose tested against tap water. Each data point represents the mean value of 10 test trials of 1 min per individual.

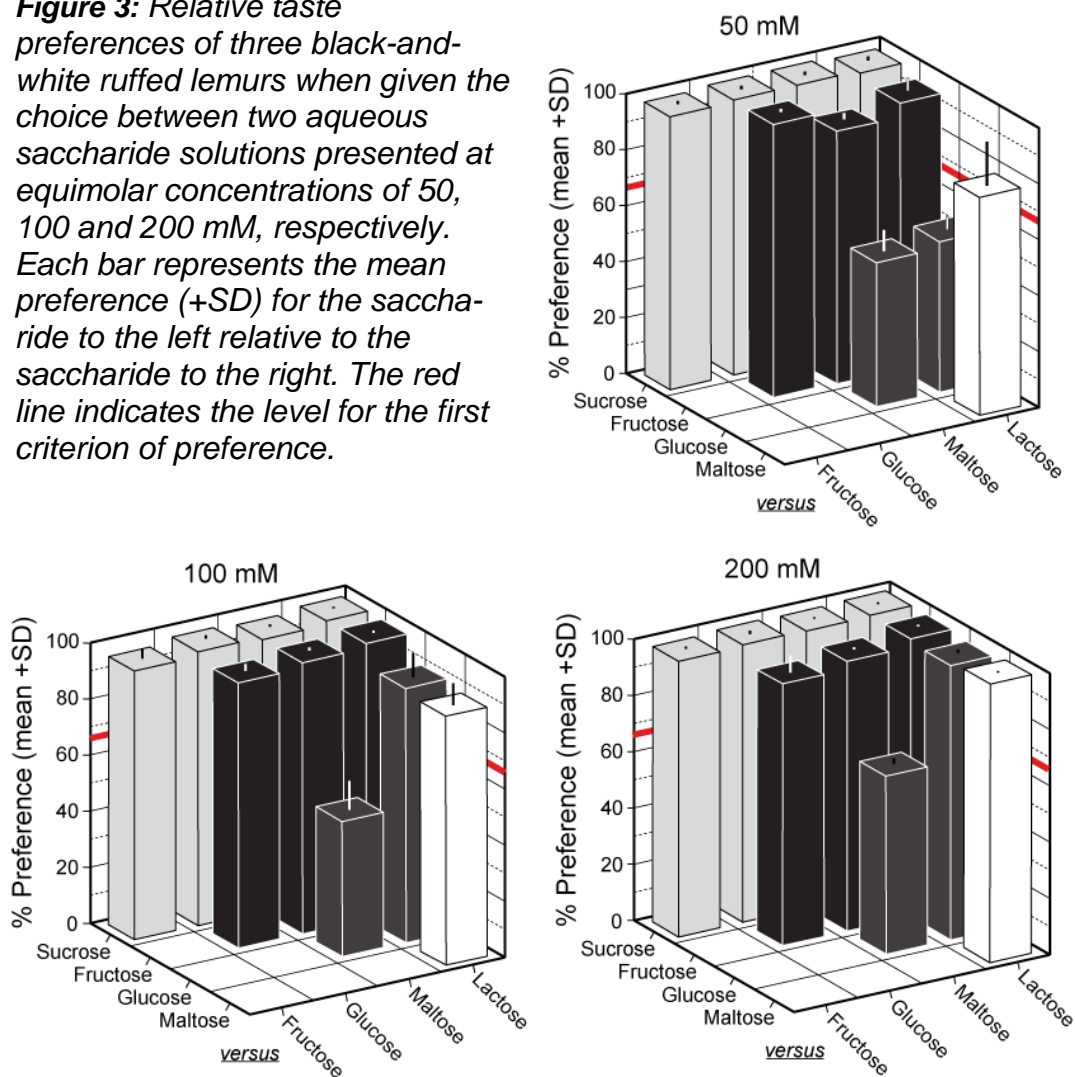


4.2 Experiment 2 - Assessment of relative preference

Figure 3 shows the mean preference (+SD) of the three lemurs when given the choice between two aqueous saccharide solutions presented at equimolar concentrations of 50, 100 and 200 mM, respectively. At all

three concentration levels, the lemurs significantly preferred sucrose over all other saccharides and fructose over glucose, maltose and lactose. However, tests between glucose, maltose and lactose did not show consistent preferences across the three tested concentration levels. For example, at the highest concentrations (100 mM and 200 mM) all three lemurs significantly preferred glucose over lactose and maltose over lactose. Only *S2* showed a significant preference for maltose over lactose at 50 mM while the other two lemurs showed non-significant tendencies to prefer maltose over lactose at this concentration. Worth noting is the lemurs' apparent preference for glucose over lactose. Here, all three individuals showed a preference for glucose over lactose at the two higher concentrations, but failed to show a preference at 50 mM. However, this result is not surprising as the lemurs' taste preference threshold for glucose was at 75 mM.

Figure 3: Relative taste preferences of three black-and-white ruffed lemurs when given the choice between two aqueous saccharide solutions presented at equimolar concentrations of 50, 100 and 200 mM, respectively. Each bar represents the mean preference (+SD) for the saccharide to the left relative to the saccharide to the right. The red line indicates the level for the first criterion of preference.



5 Discussion

The results of this study give a first conservative approximation of taste sensitivity for food-associated sugars in the prosimian *V. variegata*. Taste preference thresholds were found to be 25-50 mM for sucrose, 25-50 mM for fructose, 75 mM for glucose, 50 mM for maltose and 50 mM for lactose. Furthermore, the lemurs significantly preferred sucrose over all other saccharides and fructose over glucose, maltose and lactose when presented at equimolar concentrations.

Table 1 compares the taste preference thresholds for food-associated sugars in nonhuman primates. Here, primate taste preference thresholds for the five common saccharides obtained using two bottle preference tests (with the exception of the human data which were obtained using psychophysical methods) can be seen. The thresholds for all five saccharides determined in *V. variegata* fall into the range of thresholds reported in other non-human primates (between 3-330 mM for sucrose, 15-75mM for fructose, 20-330 mM for glucose, 10-90 mM for maltose and 10-250 mM for lactose) and humans (10, 40, 80, 31, 72 mM, respectively) studied to date (see table 1 for references).

Table 1 –Taste preference thresholds (in mM) for sucrose, fructose, glucose, maltose and lactose in primates.

Species	Sucrose	Fructose	Glucose	Maltose	Lactose	Ref
Prosimian primates:						
<i>Varecia variegata</i>	25-50	25-50	75	50	50	This study
<i>Eulemur coronatus</i>		21±10				Simmen & Hladik (1998)
<i>Eulemur fulvus</i>	9±1	22,5±8,5				Simmen & Hladik (1998)
<i>Eulemur macaco</i>	8±0,5	14±7				Simmen & Hladik (1998)
<i>Eulemur mongoz</i>	125					Glaser (1986)
<i>Hapalemur simus</i>	17,5±2,5	18,5±7,5				Simmen & Hladik (1998)
<i>Hapalemur griseus</i>		16±9,5				Simmen & Hladik (1998)
<i>Phaner furcifer</i>	65±35					Simmen & Hladik (1998)
<i>Microcebus murinus</i>	167	44-51				Glaser (1986), Simmen et al. (1999b)
<i>Microcebus coquereli</i>	90±10					Simmen & Hladik (1998)
<i>Cheirogaleus major</i>	50					Simmen & Hladik (1998)
<i>Cheirogaleus medius</i>	143					Glaser (1986)
<i>Propithecus verreauxi</i>	52,5±7,5					Simmen & Hladik (1998)
<i>Loris tardigradus</i>	50					Glaser (1986)
<i>Nycticebus coucang</i>	330					Glaser (1986)
<i>Galago senegalensis</i>	66					Glaser (1986)
Platyrrhine primates:						
<i>Ateles geoffroyi</i>	3	15	20	20	10	Laska et al. (1996)
<i>Saimiri sciureus</i>	10	40	90	90	100	Laska (1996)
<i>Saguinus midas niger</i>	66	66	330		250	Glaser (1986)

<i>Saguinus fuscicollis</i>	50					Glaser (1986)
<i>Saguinus oedipus</i>	125	16±6				Glaser (1986), Simmen & Hladik (1998)
<i>Cebuella pygmaea</i>	33	50	100		125	Glaser (1986)
<i>Callithrix jacchus</i>	25	29,5±2,5				Glaser (1986), Simmen (1994)
<i>Callithrix geoffroyi</i>		41±10				Simmen & Hladik (1998)
<i>Callithrix argentata</i>		19,5±7,5				Simmen & Hladik (1998)
<i>Leontopithecus rosalia</i>		19,5±7,5				Simmen & Hladik (1998)
<i>Leontopithecus chrysomelas</i>		21,5±9,5				Simmen & Hladik (1998)
<i>Callimico goeldii</i>		31±4				Simmen & Hladik (1998)
<i>Cebus apella</i>	8±3					Simmen & Hladik (1998)
<i>Aotus trivirgatus</i>	17					Glaser (1986)
Catarrhine primates:						
<i>Macaca nemestrina</i>	10	20	20	10	30	Laska (2000)
<i>Macaca mulatta</i>	6					Glaser (1986)
<i>Macaca radiata</i>	10			10		Sunderland & Sclafani (1988)
<i>Papio hamadryas anubis</i>	10	20	25	20	20	Laska et al. (1999a)
<i>Cercopithecus pygerythrus</i>	11					Glaser (1986)
<i>Cercopithecus nictitans</i>	11					Glaser (1986)
<i>Pongo pygmaeus</i>		15±5				Simmen & Charlot (2003)
<i>Pan troglodytes</i>		45±5				Simmen & Charlot (2003)
<i>Gorilla gorilla</i>		75±5				Simmen & Charlot (2003)
<i>Homo sapiens*</i>	10	40	80	31	72	Dunkel & Hofmann (2009), van Germert (2011).

5.1 Taste preference threshold for sucrose and fructose

Among prosimian primates, *V. variegata* exhibits thresholds for sucrose and fructose that places it in the lower midrange of values, supporting the previous observation that species of the Lemuridae family generally respond to lower concentrations of these two sugars compared to other prosimian families (Bonnaire & Simmen 1994, Simmen & Hladik 1998). Compared to other primates in the Lemuridae family (e.g. the brown lemur, *Eulemur fulvus* and black lemur, *Eulemur macaco*), *V. variegata* has a comparatively high threshold for sucrose, second only to the mongoose lemur (*Eulemur mongoz*). However, the reliability of the data for the mongoose lemur has been questioned (Simmen & Hladik 1998). As taste sensitivity for sucrose and fructose has been argued to be positively correlated with body mass in primates (Simmen & Hladik 1998), the relatively low sensitivity in *V. variegata* for sucrose is surprising as it is the largest species of the extant Lemuridae (Britt 2000). However, the two tested species of the *Hapalemur* family, *H. simus* and *H. griseus*, show comparable taste sensitivity towards fructose (table 1)

despite the distinct weight differences (2500 grams and 850 grams [Simmen & Hladik 1998], respectively). This could indicate that factors other than body mass may underlie between-species differences in sweet-taste sensitivity, as suggested by Simmen & Hladik (1998).

5.2 Taste preference threshold for glucose, maltose and lactose

Unfortunately, there are no current data on taste preference thresholds for glucose, maltose or lactose in other prosimian species to this date. When comparing the obtained thresholds for glucose in *V. variegata* with platyrrhine and catarrhine species, the black-and-white ruffed lemur places in the higher midrange with a threshold value slightly lower than that for humans. An overall midrange position is also seen for maltose and lactose, although compared to platyrrhine monkeys, *V. variegata* has a rather low threshold (i.e. high sensitivity) for glucose, maltose and lactose. Tilden & Oftedal (1997) reported a lactose content of 7.7g/100ml (corresponding concentration of 225 mM) in the milk of ruffed lemurs, which is well above the threshold found in this study and thus should be readily perceptible for *V. variegata*.

5.3 Relative preference between sugars

Table 2 shows the relative preferences for individual saccharides in *V. variegata* as well as in other non-human primates and the rat (*Rattus norvegicus*) obtained using the same or a similar method as in this study. In humans, relative sweetness was assessed using psychophysical methods.

Table 2 –The relative preference for sucrose, fructose, glucose, maltose and lactose in primates and the rat.

<i>V. variegata</i>	sucrose > fructose > glucose = maltose ≥ lactose	Present study
<i>S. sciureus</i>	sucrose > fructose > glucose ≥ maltose ≥ lactose	Laska (1997)
<i>At. geoffroyi</i>	sucrose > fructose > glucose ≥ lactose ≥ maltose	Laska et al. (1998)
<i>H. sapiens</i>	sucrose > fructose > maltose ≥ glucose ≥ lactose	Pfaffmann et al. (1971); Tinti and Nofre (2001)
<i>M. nemestrina</i>	maltose > sucrose > glucose ≥ fructose ≥ lactose	Laska (2000)
<i>Rattus norvegicus</i>	maltose > sucrose = glucose > lactose	Richter and Campbell (1940)
<i>R. norvegicus</i>	maltose > sucrose > glucose = fructose	Sclafani and Mann (1987)

Two conclusions can be drawn from the obtained results from experiment 2. First, the order of relative preference coincides roughly with the order of sensitivity obtained from experiment 1, indicating that at least for sucrose and fructose, taste preference thresholds may have some relation to the perceived attractiveness of the sugar (i.e. the most preferred sugar has the lowest taste preference threshold). Second, the findings of experiment 2 are in accordance with the results from three out of the four

other primates tested so far (table 2), placing sucrose as the most attractive saccharide relative to the other tested sugars. The exception is the pig-tailed macaque (*Macaca nemestrina*), showing a relative preference for maltose over all other sugars, similar to rats and other rodents e.g. gerbils, spiny mice and hamsters (Feign & Sclafani 1987; Sclafani & Mann 1987). *V. variegata* has very similar relative preferences as squirrel monkeys, *S. sciureus* and Geoffroy's spider monkey, *Ateles geoffroyi*, which are other primates that rely heavily on fruits in their diet (Laska 1999b). This trend among primates of sucrose being preferred over other food-associated saccharides is in contrast to the pattern found in the rodent species tested so far. In rats, for example, maltose is the most preferred saccharide at low concentrations, followed by sucrose (Sclafani & Mann 1987). This apparent dichotomy in sucrose versus maltose as the most preferred saccharide in primates and rodents could be attributed to the species' specific diets. So in rats and other rodents for example, the ability to perceive maltose at low concentrations would likely be beneficial for the animal when selecting and feeding on starch-heavy foods. The same case could be made for the pigtail macaque, which incorporates a high proportion of starchy plants in its diet (Laska 2000). The high affinity of rodents and macaques towards starch-derivatives such as maltose points towards specialized taste receptors for starch derived polysaccharides in these species (Laska et al. 2001, Sclafani & Mann 1987, Sunderland & Sclafani 1988).

5.4 “Frugivory hypothesis “

As with the proposed evolutionary relationship between trichromatic vision and frugivory (e.g. Osorio et al. 2004), taste sensitivity for sugars has been proposed to be connected to the degree of frugivory among primates. According to this notion, species with a more frugivorous diet exhibit a higher taste sensitivity compared to species with less fruit in their diets (Laska et al. 1999b, Hladik & Simmen 1996, Simmen 1994). The reasoning behind this idea is that an animal having a high sensitivity for soluble sugars would perceive a wider range of potential foodstuffs as more palatable than an animal with low taste sensitivity for sugars. This increased foraging efficiency would be of importance especially for Malagasy frugivores as their food sources are generally scattered over large home-ranges and have asynchronous fruiting phenology (Balko & Underwood 2005; Herrera et al. 2011), entailing a high energy requirement from the individual foraging for them.

The taste preference thresholds of *V. variegata* obtained in this study are well below the concentrations of soluble sugars (mainly sucrose, fructose and glucose) found in tropical fruits (Food Standards Agency 2002),

indicating that these sugars should play a role in the taste sensation by *V. variegata* while feeding on fruits. Thus it is reasonable to assume that *V. variegata* uses sweetness as a criterion for food selection. Indeed, Schwitzer & Kaumanns (2000) showed that *V. variegata* exhibits a preference for foodstuffs with high sugar content, albeit in non-experimental conditions. According to this “frugivory hypothesis”, the black-and-white ruffed lemur should have a high taste sensitivity for sugars since their diet consists of up to 92% of fruits, making it one of the most frugivorous primates (Britt 2000). However, the results of the present study do not lend support to this prediction as both the less frugivorous (Dew and Wright 1998, Ross 1992) and closely related, brown and black lemur have lower thresholds for sucrose and fructose than *V. variegata*.

However, *V. variegata* shows taste preference thresholds of similar magnitude across all five sugars, with small variation among the obtained taste thresholds between sugars (fig. 2). This is in contrast to e.g. the common squirrel monkey, *Saimiri sciureus* or the black-handed tamarin, *Saguinus midas niger*, which both show a large (nearly ten-fold in *S. sciureus*) difference between the highest and lowest thresholds for the tested sugars (table 1). Considering the forest ecology of Madagascar, having similar and relatively low taste preference thresholds for naturally occurring sugars can be argued to be potentially adaptive to the black-and-white ruffed lemur. The fruiting phenology in Madagascar is rather different compared to the fruiting patterns of other rain forests (Wright 1999). For example, the peak production of fruits is on average three months shorter than the peak fruit production in Amazonian (Stevenson 2005) and African (Chapman 2005, Gautier-Hion et al. 1985) forests, coupled with a prolonged season with few trees in fruit (Overdorff, 1993). Being one of the few obligate frugivorous lemurs in Madagascar (Dew & Wright 1998), it would thus be conceivable that *V. variegata* might have difficulty in acquiring food resources during certain periods of the year. In these scenarios, it would seem beneficial for *V. variegata* to be able to perceive a wider array of potential energy-containing foods (as a consequence of relatively low taste preference thresholds for many food-associated sugars) as palatable during scarce food availability. *V. variegata* does show rather unique life-history traits among primates, e.g. the use of infant nests and fast development of young that could be a response towards erratic and uncertain food supply (Balko & Underwood 2005).

At the same time it is important to consider the possibility that phylogenetic relatedness might also affect taste sensitivity (Laska et al

1999a). For example, Old World and New World primates have been shown to differ in their ability to perceive artificial sweeteners e.g. aspartame and thaumatin (Nofre et al. 1996). The influence of scaling and allometric effects on taste sensitivity (Simmen & Hladik 1998) is also important to be considered when analyzing between-species differences in sweet-taste sensitivity.

6 Conclusions and future directions

The black-and-white ruffed lemur was found to have rather similar thresholds across all the tested sugars, fitting well into the range of threshold values reported in other primates. *V. variegata* was also found to prefer sucrose (followed by fructose) over all other tested sugars, which is similar to the results from other primate species. The notion of a possible positive correlation between the degree of frugivory and sweet-taste sensitivity was not supported by the findings of this study. However, more effort is needed to develop the list of tested species as well as the list of tested sugars to enable more robust comparative analyses. Even though the results of this study should be considered preliminary due to a small sample size, the obtained taste preference performances of *V. variegata* are well needed considering the sparse data that currently exists on taste sensitivity for sugars in prosimians.

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