Coloured and Toxic Nectar: Feeding Choices of the Madagascar Giant Day Gecko, Phelsuma grandis

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Abstract

Coloured nectar is a rare phenomenon best known from islands and insular habitats. Islands are also known for lizard pollination, where coloured nectar potentially acts as a visual cue to attract pollinators, advertising the sweet reward. However, nectar may also contain secondary metabolites with toxic or deterrent effects. The aim of this study was to determine which factors are important as artificial nectar choice determinants to the Madagascar giant day gecko, Phelsuma grandis, an island pollinator: artificial nectar colour, artificial nectar colour saturation, artificial nectar conspicuousness and/or the presence of the alkaloid nicotine. Coloured artificial nectar and the darkest artificial nectar colour saturation were found to be important visual cues for the geckos, while the contrast between artificial nectar and petal colour was not. Geckos were deterred only by high nicotine concentrations (1000 lM in 0.63 M sucrose) and may even prefer low nicotine concentrations to sucrose-only solutions. Given their overall fondness for sugar solutions, Madagascar giant day geckos are likely to be important pollinators of Malagasy plant species that produce enough nectar to attract them, and plants with coloured nectar and/or secondary metabolites may have evolved those traits to attract the geckos in particular.

The role of lizards as seed dispersers and pollinators is relatively unknown among animal-plant interactions. Most reports of nectar consumption by lizards are from islands. Lizards may reach relatively high densities on islands as a result of low predation levels, and insect pollinators are often scarce on islands (reviewed in Olesen & Valido 2003). Consequently, it has been suggested that insectivorous lizards have expanded their diets to include fruit and nectar; this niche broadening has led to their playing unusual roles as pollinators and seed dispersers (Eifler 1995; Traveset & Saez 1997; Olesen & Valido 2003; Kaiser-Bunbury et al. 2009).

Another feature of plant-animal interactions on islands is the presence of coloured nectar (Hansen et al. 2007a). Although the information is insufficient, this floral trait appears to be more common in circumstances where invertebrate pollination is limited, such as insularity, high altitudes and where vertebrate pollinators are abundant; these correlates are not necessarily mutually exclusive (Hansen et al. 2007a). In addition to being a visual cue to vertebrate pollinators, it has been suggested that coloured nectar acts as an honest signal, where the flower advertises the strength and presence of its reward: the more saturated the colour of the nectar (i.e. the darker it is) the stronger the signal (Olesen et al. 1998; Johnson et al. 2006; Hansen et al. 2007a).

A large variety of nectar colours has been found to occur in nature (Hansen et al. 2007a). Plants that are known or suspected to be pollinated by lizards (*Trochetia boutoniana, T. blackburniana* and *Nesocodon mauritianus*) have (human-perceived) coloured nectar in the clear to red range and bell-shaped flowers (Olesen et al. 1998; Hansen et al. 2007a). Coloured nectar usually contrasts well with petals from a human perspective, except in T. boutoniana which has the same flower and nectar colour (red) (Olesen et al. 1998; Hansen et al. 2007a). Birds have been shown experimentally to prefer coloured nectar to clear nectar (Collias & Collias 1968; Johnson et al. 2006; Zhang et al. 2012). This is most likely due to the signal strength of coloured nectar (Johnson et al. 2006; Hansen et al. 2007a; Zhang et al. 2012) and the birds' visual capacities (Schaefer et al. 2004).

Coloured nectar is often associated with secondary metabolites, such as non-protein amino acids, phenolic compounds or alkaloids, and in fact, these substances may be the cause of the colour (Hansen et al. 2007a). The nectar of the suspected gecko-visited *N. mauritianus* contains aurones, products of flavonoid biosynthesis (Olesen et al. 1998). Polyphenolic compounds (mainly flavonoids) that have been found in the leaves of *T. boutoniana* and *T. blackburniana* (Lai Fang et al. 2002; Puchooa & Venkatasamy 2005) could potentially enter their nectar. Nectar has been observed to be deterrent or poisonous to many animals, including ants, butterflies, bees and birds (Janzen 1977; Stephenson 1981; Hagler & Buchmann 1993; Johnson et al. 2006). Secondary compounds in nectar may be beneficial in discouraging unwanted visitors (usually nectar thieves) (Adler 2000), while pollinators may be undeterred and even attracted to the nectar (Rhoades & Bergdahl 1981; Stephenson 1981; Singaravelan et al. 2005; Kessler et al. 2012). Johnson et al. (2006) found that the dark brown nectar of the South African *Aloe vryheidensis* acts as a filter by warning nectar robbers of its unpalatability, while also attracting efficient pollinators.

Alkaloids are bitter-tasting, nitrogen-containing compounds that are widely distributed in the plant kingdom (Facchini 2001) and commonly function in herbivore and plant–pathogen defences (Wink 1999). The response of pollinators to nectar alkaloids is dose dependent, and low concentrations of caffeine and/or nicotine have been recorded as attractive to honeybees and hummingbirds (Singaravelan et al. 2005; Kessler et al. 2012). Deterrence due to unpalatability has been proposed to increase the probability of cross-pollination by increasing visitation frequency and decreasing visitation length (Kessler & Baldwin 2006; Irwin & Adler 2008). Nicotine has been used as a model alkaloid in taste tests because of its bitter taste. It is one of the few alkaloids that has been quantified in nectar (Kessler et al. 2012) and can have a toxic or repellent effect (e.g. Tadmor-Melamed et al. 2004; Singaravelan et al. 2005; Kessler et al. 2012). Lizards are able to detect alkaloids in their diet via smell and taste (Schall 1990; Cooper et al. 2002). Whiptail lizards (*Cnemidophorus*) avoided quinine-treated solutions after tongue-flicking and responded negatively after quinine ingestion by opening their mouths widely and scraping them against the ground (Schall 1990).

The Indian Ocean day geckos *(Phelsuma,* Gekkonidae) are among the better known lizard flower visitors and pollinators (Hansen et al. 2006). The Madagascar giant day gecko *Phelsuma grandis* is a colourful, omnivorous lizard that is endemic to the island of Madagascar (D'Cruze et al. 2009). *Phelsuma* geckos have pure-cone retinas containing oil droplets with peaks from violet to red, enabling excellent vision during the day (Taniguchi et al. 1999; Roll 2000). Flower visitation observations indicate that day geckos *(Phelsuma ornata* and *P. cepediana)* visit flowers with coloured nectars in the range of yellow to red (Hansen et al. 2007a). *Phelsuma ornata* (a day gecko from Mauritius) has been found to have a preference for coloured artificial nectar (Hansen et al. 2006). *Phelsuma ornata* is a common endemic pollinator for many plant species on Ile aux Aigrettes (a small island off the coast of Mauritius), including the endemic *Gastonia mauritiana, Lomatophyllum tormentorii* and *Dracaena concinna.* On Mauritius, *P. ornata* and *P. cepediana* pollinate *T. boutoniana* and *T. blackburniana* (Nyhagen et al. 2001; Olesen et al. 2002; Hansen et al. 2006). It is not yet known whether *P. grandis* visits flowers in the wild and, unfortunately, not enough is known about the nectar chemistry of Malagasy plants.

The aims of this study were to determine the importance of nectar colour, conspicuousness and nectar colour saturation to the Madagascar giant day gecko and how these animals respond to nectar containing the alkaloid nicotine. We predicted that the geckos would prefer coloured to clear nectar, and that nectar conspicuousness and colour saturation would be additional determinants of nectar colour preference. We further predicted that geckos would prefer low nectar nicotine concentrations over sugar-only solutions, but would be deterred by high nicotine concentrations. Coloured nectar, as well as an increase in nicotine concentration, was expected to increase feeder visitation frequency and decrease visitation length.

Methods

Animal Housing and Care

We habituated seven unrelated, captive-bred *P. grandis* geckos (body mass 33.39 ± 1.36 g, mean ± SE) for two weeks to experimental conditions. They were kept in a controlled environment room at 25°C on a 12D:12L cycle (lights-on at 07h00). Sunrise and sunset were simulated with 15 min of dimmed light before the onset of the light and dark phases. We housed the geckos in individual Perspex" boxes (38 **x** 21 **x** 15 cm), which were permeable to all wavelengths (Heiling & Herberstein 2004), with breathing slits in the lid, a small plastic plant and a white paper-towelling substrate. Ultraviolet (UV) lights (Exo-Terra Repti Glo 5.0 UV linear fluorescent bulbs (T8), Mansfield, MA, USA) were placed above all the cages to enable geckos to maintain skeletal health (Townsend & Cole 1985). We conducted experiments in the home cages to avoid the effects of variable housing conditions (Cooper & Habegger 2000). We isolated the animals visually, using cardboard dividers between cages and one-way glass in front of the cages to limit disturbance from humans (Cooper 1998). The maintenance diet (beige in colour) consisted of 1 tsp T-Rex^{H} day gecko powder (T-Rex Ltd., Chula Vista, CA, USA), 1 tsp honey, 1 tsp Purity fruity custard delight (Johannesburg, South Africa), 2 tsp Aviplus® parrot handrearing formula (Avi Products, Durban, South Africa), **%** tsp calcium powder and small crickets. We provided water and the maintenance diet *ad lib.,* but removed them during experiments. We periodically weighed and examined the geckos to assess their body condition and overall health. Surgical gloves were used when handling the geckos, owing to the sensitivity of their dermis (Raxworthy et al. 2007).

Artificial Nectar Colour Preference

We tested colour preference in pairwise feeding experiments using red, yellow and clear artificial nectar (0.63 M sucrose). We used red and yellow food colouring (Robertsons", Unilever, Cape Town, South Africa) to colour the solutions (3.3 **|il** food colouring per 1 ml sugar solution), which were presented to the geckos in 1.5 ml Eppendorf tubes. The food colouring did not alter the nutritional value of the sugar solutions and was scentless to a human nose. We filled the tubes with 1.65 ml of solution to supply as much test solution to the gecko as possible without spillage occurring. The position of the two Eppendorf tubes (placed 2.5 cm apart) was randomised on the first day of testing and then reversed on the second day to correct for possible side bias, resulting in two trials for each colour pair combination. We tested each gecko only twice to avoid learning and to keep them naive to coloured artificial nectar. We started trials 3 h after simulated sunrise (i.e. at 10h00), and we terminated them when a gecko's consumption had lowered the artificial nectar level to the 1 ml mark on the Eppendorf® tube (the level that its tongue could still comfortably reach) or after 6 h. Geckos were given their maintenance diet for one day between trials, then starved for one day before test days (to ensure that geckos fed from the test solution). Each of the seven geckos received all colour pairs, the order of which was randomly determined.

Earlier studies have used first choice as an index of colour preference (Hansen et al. 2006; Schaefer et al. 2006; Zhang et al. 2012). In addition to first choice, we recorded food intake, visitation frequency and visitation length to compare these feeding parameters as indices of colour preference. The Eppendorf tube that the gecko approached first to within 1.5 cm of the opening was noted, representing the first choice of that gecko (Hansen et al. 2006). For each visit to the test solutions, we recorded the solution that the gecko approached and how long it drank. We used these measurements to determine the visitation frequency (the number of feeder visits per solution as a proportion of the total number of visits to both feeders for each colour pair) and visitation length (the time spent feeding at each solution taken as a proportion of the total time spent feeding at both solutions). We weighed the solutions before and after each test to determine food intake (±0.1 mg; Mettler Toledo AG-64, Microsep Ltd, Johannesburg, South Africa). Intake was corrected for evaporation of the sugar solutions, measured by placing colour pairs in empty cages during trials. Food intake for a particular artificial nectar solution is presented as a percentage of the total intake.

Colours of the solutions were spectrometrically determined using a Libra® S12 UV/Visible Spectrophotometer (Biochrom Ltd., Cambridge, England), to compare the colours of the solutions to those used in other studies and to nectar colours found in nature (e.g. Schmidt & Schaefer 2004; Schaefer et al. 2006). We do not directly know the photopigment characteristics of *P. grandis;* however, the closely related *P. madagascariensis longinsulae* has photopigments similar to the 'blue' (467 nm peak) and 'green' (521 nm peak) visual pigments of *Gekko gekko,* and *P. m. longinsulae* may possibly see in the UV range (300-400 nm) (Taniguchi et al. 1999; Roll 2000). Furthermore, *Phelsuma* species have oil droplets in their retina, which have 'almost uniformly high transmissions at wavelengths of 350-700 nm' (Roll 2000). Due to the aforementioned, red (all saturations), yellow and clear sugar solutions were each measured in the 300-700 nm colour spectrum range with 20 nm intervals.

Effect of Petal Colour

To test for artificial nectar conspicuousness, we repeated the artificial nectar colour preference test, but added coloured petals (Hansen et al. 2006). Six geckos were used as one was omitted due to illness. Artificial flowers were constructed using four cardboard petals (0.6 mm thick) taped onto the Eppendorf© tubes with white tape to simulate a white corolla (Hansen et al. 2006). The flowers were 2.5 cm in diameter. We used the same petal colours as Hansen et al. (2006) (red, yellow, white, green and blue) with the addition of orange petals. Petal colours were tested on separate days in random order. Two trials for each artificial nectar and petal colour combination were conducted per gecko to account for side bias. To keep the geckos motivated for repeated trials in one day, we filled flowers with only 1 ml of 0.63 M sucrose solution with red, yellow or clear colouring. This volume allowed the geckos to view the solution without consuming substantial amounts. First choice was only recorded if the gecko had viewed the solution from above before its approach. We did not measure the reflectance of the cardboard petals, nor how different the artificial nectar and petal colours were from the gecko's perspective: the aim of the experiment was to offer varying levels of nectar and petal contrasts, thus it was not important for the petal and nectar colours to match exactly.

Artificial Nectar Colour Saturation

To test whether colour saturation influenced artificial nectar preference, we used three different shades of red artificial nectar in a pairwise feeding setup. We concentrated and diluted the red artificial nectar used in the previous experiments ten times to obtain the lightest and darkest artificial nectar saturations, respectively. Based on the results of the artificial nectar colour preference experiments, first choice can be used as a proxy to infer preference. Thus, only first choice was noted for both petal colour and artificial nectar colour saturation experiments, after which solution sides were switched and the test repeated. By measuring only first choice, we were able to test all artificial nectar colour saturation combinations in one day for each of the six geckos.

Nicotine in Artificial Nectar

In pairwise preference experiments, six geckos were given a choice between a pure 0.63 M sucrose solution and one of the same concentration but containing nicotine (0.5, 3, 6, 15, 30, 60, 125, 250, 500 or 1000 lM; (–)Nicotine, Ref. N3876, Sigma Aldrich, Munich, Germany). The procedure was the same as for the colour preference experiments discussed above, in terms of experimental design and protocol, and all four feeding parameters were measured. However, the effects of nicotine on these geckos were not known before this experiment, so we tested the lowest nicotine concentrations first, followed by the higher ones. We spectrometrically checked solutions to determine if nicotine-containing solutions were visible in the UV range.

Data Analysis

Data were tested for normality (Kolmogorov-Smirnov) and homogeneity of variance (Bartlett's X^2). Data from the first and second day of testing for each colour and nicotine pair tested were pooled for each gecko, resulting in a comparison between seven or six geckos for each pairwise feeding trial combination. To test for differences between colour and nicotine pairs, we performed two-tailed, dependent (paired sample) f-tests on intake, visitation frequency and visitation length. To perform a GLMM/GLM for any data set, the explanatory variable needs to have continuous data (Zuur et al. 2009). Our explanatory variables for first choice were either Day 1 and Day 2 or Gecko 1-6/7, both categorical variables, so the most appropriate statistical test to use was either a chi-square or binomial test, and our sample size was too small for a chi-square test. For the binomial test, the choice of each gecko for each artificial nectar colour pair was analysed as a separate event, resulting in the violation of the independency assumption for binomial tests. We tested side bias by comparing the intake of the colour/nicotine treated solution on Day 1 (treated solution on the right) to Day 2 (treated solution on the left). Side bias was not tested for the effect of petal colour nor the colour saturation experiments as intake was not measured, but was controlled for by swapping the solutions during each colour trial. We conducted statistical tests using STATISTICA 9.0 (StatSoft®) and SPSS Statistics 17.0 (binomial test only). Significance level was $p < 0.05$; values are presented as means ± SE.

Results

Coloured Artificial Nectar

Geckos preferred red to clear artificial nectar for all feeding parameters measured: intake, visitation frequency, visitation length ($t(6) > 3.3$, $p < 0.02$) and first choice (z = —2.2, $p = 0.01$) (Fig. 1). Even though there was no significant preference between yellow and clear artificial nectar for intake, visitation frequency, visitation length ($t(6) > 1.2$, $p > 0.26$) and first choice ($z = 0.8$, $p = 0.79$), yellow artificial nectar appears to be preferred to clear artificial nectar (Fig. 1). The preference for coloured artificial nectar (data for red and yellow artificial nectar combined) to clear artificial nectar was significant for intake, visitation frequency, visitation length ($t(13) > 2.9$, $p = 0.01$) and first choice (z = —1.8, $p = 0.04$). The red artificial nectar had an absorption maximum (1_{max}) of 520 nm, the yellow artificial nectar 420 nm, and the clear artificial nectar had no absorbance spectrum.

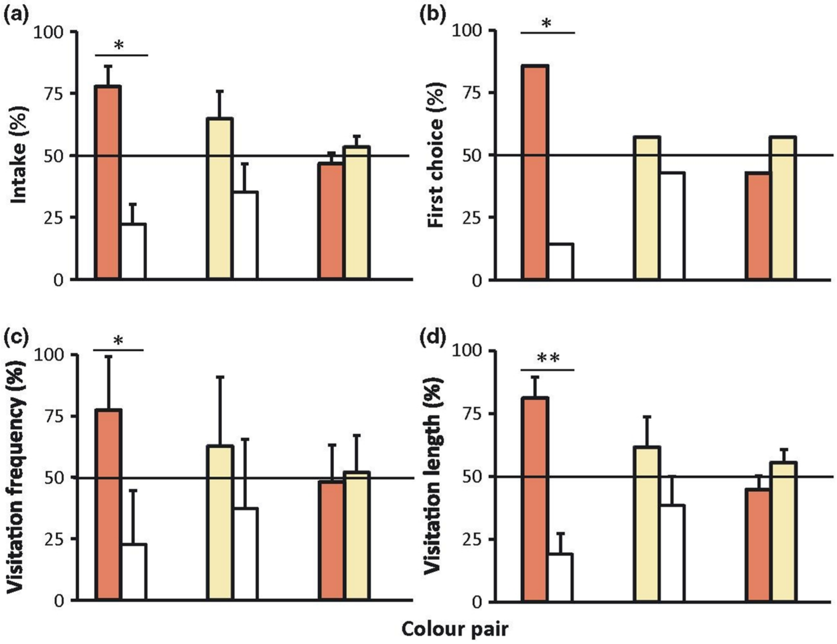

Fig. 1: Artificial nectar colour preference of seven Phelsuma grandis geckos (means + SE). Red artificial nectar is an important visual attractant, shown by (a) intake, (b) first choice, (c) visitation frequency and (d) visitation length. Statistical results derive from t-tests for dependent samples for intake, visitation frequency and visitation length, while a binomial test was performed on first choice; * $p < 0.05$, ** $p < 0.01$. A horizontal line crossing at 50% indicates no preference

The choice of red artificial nectar, however, became insignificant in combination with red petals or any other petal colour (z = —0.2, p > 0.39) (Fig. 2). Yellow artificial nectar too was not preferred given any of the petal colours (z = —1.0, p > 0.15). Overall though, coloured artificial nectar tended to be chosen before clear artificial nectar for all petal colours combined (z = —1.4, p = 0.08). In the artificial nectar colour saturation experiments, geckos chose the dark red artificial nectar before the light red artificial nectar (z = —2.5, p < 0.01), with the light red artificial nectar having a A,$_{max}$ of 520 nm, and the dark red artificial nectar absorbing all the light from 300 to 580 nm. In addition to the absorption maxima, the red and light red artificial nectar solutions had peaks at 300 nm, indicating that these solutions (along with dark red) were also visible in the UV range. Although not significant, geckos tended to prefer the darker artificial nectar over the lighter one for all pairwise combinations (z = —1.0, p > 0.15). Geckos did not show a side bias in the coloured artificial nectar experiments (coloured pairs *(t(6)* < 1.7, p > 0.80); coloured *vs.* clear (t(13) = 0.4, p = 0.70)). Absorbance spectra for red, light red, dark red and yellow artificial nectars have been supplied as supporting information; red, light red and dark red artificial nectar solutions varied in saturation. All statistical results have been provided as supporting information.

Nicotine in Artificial Nectar

Geckos tolerated low nicotine concentrations, but showed partial deterrence at 500 **(J**M *(t(5)* = —2.1, p = 0.09), and their intake was significantly reduced at a concentration of 1000 |^M *(t(5)* = —4.4, p = 0.01) (Fig. 3a). The same pattern was seen for visitation length where geckos spent significantly less time eating from the 1000 *M* nicotine concentration than from the pure sugar solution *(t(5)* = —6.9, p = 0.001) (Fig. 3b). Interestingly, visitation length tended to increase on 0.5 *M* nicotine *(t(5)* = 2.5, p = 0.06) (Fig. 3b). Nicotine presence had no effect on the first choice of a solution (z = —0.2, p > 0.4). Surprisingly, geckos did not alter their visitation frequency *(t(5)* < 2.1, p < 0.9). Learning did not take place, and there was no side bias, as our results showed no choice of a solution (z = —0.2, p > 0.4). Surprisingly, geckos did not alter their visitation frequency *(t(5)* < 2.1, p < 0.9). Learning did not take place, and there was no side bias, as our results showed no difference in intake between the two consecutive test days for any of the nicotine concentrations *(t(5)* < 2.5, p > 0.052). Some geckos shook their heads and gaped after eating from the 1000 ^M nicotine solution. Nicotine-containing solutions were not visible in the UV range and did not change the absorbance spectrum of the solutions. All statistical results have been provided as supporting information.

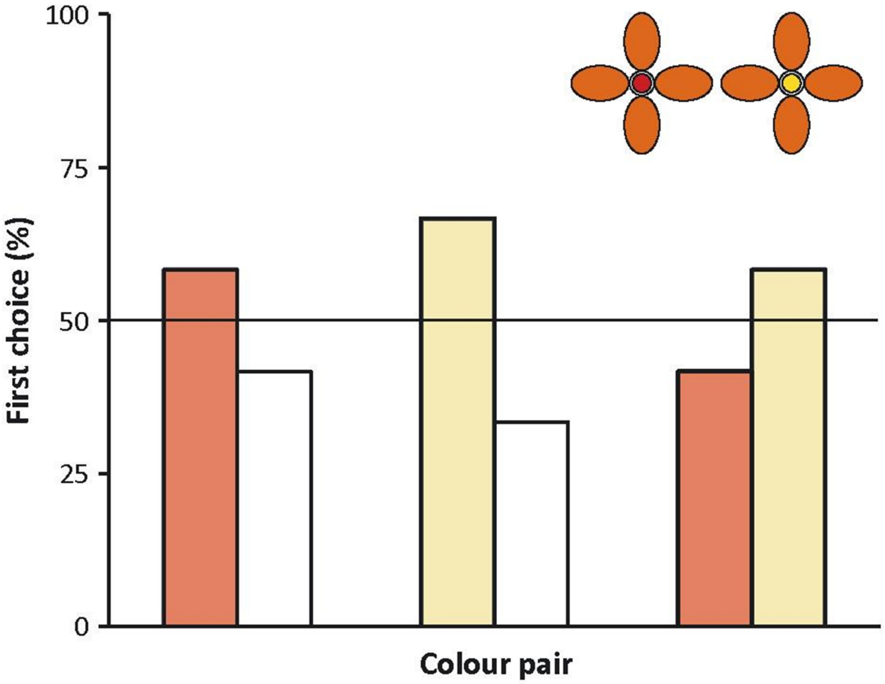

Fig. 2: First choice of six *Phelsuma grandis* geckos for different artificial nectar colour pairs surrounded by orange petals (means). The same pattern was seen for all petal colours tested (red, yellow, orange, white, blue and green). Nectar conspicuousness is not an important nectar selection determinant (binomial test; p > 0.15).

Discussion

Coloured nectar has been proposed to act as an honest signal by advertising the presence of a reward and its size (Olesen et al. 1998; Hansen et al. 2007a). Madagascar giant day geckos showed a strong preference for red artificial nectar. Although there was no significant difference in preference between yellow and clear artificial nectars, yellow artificial nectar is seemingly preferred according to all variables measured, indicating that a larger sample size may show significance. Coloured artificial nectar as a floral attractant to geckos is not surprising as these animals are sensitive to colourful environments (Taniguchi et al. 1999). The geckos used in this experiment were naive to coloured artificial nectar (and coloured food), indicating that the preference for coloured artificial nectar is innate.

Hansen et al. (2006) conducted a similar experiment in the field with *P. ornata,* using artificial flowers taped to the trunks of trees. Using first choice only as an indication of preference, they found a significant preference for both yellow and red artificial nectar to clear artificial nectar.

Because first choice may indicate initial attraction rather than overall preference, we additionally measured visitation frequency, visitation length and food intake. Our results show that not only did the geckos choose the red artificial nectar first more often, but also that they consumed more of it and ate from it for longer. This confirms that first choice can indeed be used as a proxy to infer preference for red coloured artificial nectar.

Our results for artificial nectar conspicuousness differ from those of Hansen et al. (2006), who found that *P. ornata* chose red artificial nectar first with all petal colours except red. In our experiment, red artificial nectar was no longer preferred by *P. grandis* when coloured petals were attached to the feeders. Because there was no difference between the first and second days of testing in the nectar colour preference experiment (the only time geckos were allowed to consume the coloured solutions) for any of the colour pairs, geckos did not learn that the sugar concentrations of the solutions were similar. Perhaps, petal colour is important in attracting the gecko from a distance (advertising its position), and nectar colour advertises the reward to the gecko at close range. In our experiments, geckos knew where the feeders were, which could explain why artificial nectar conspicuousness was not important, as compared to Hansen's study which was carried out in the field, on geckos that were native to coloured nectar but not, of course, to coloured flowers. Alternatively perhaps, the colour of nectar alone is more important

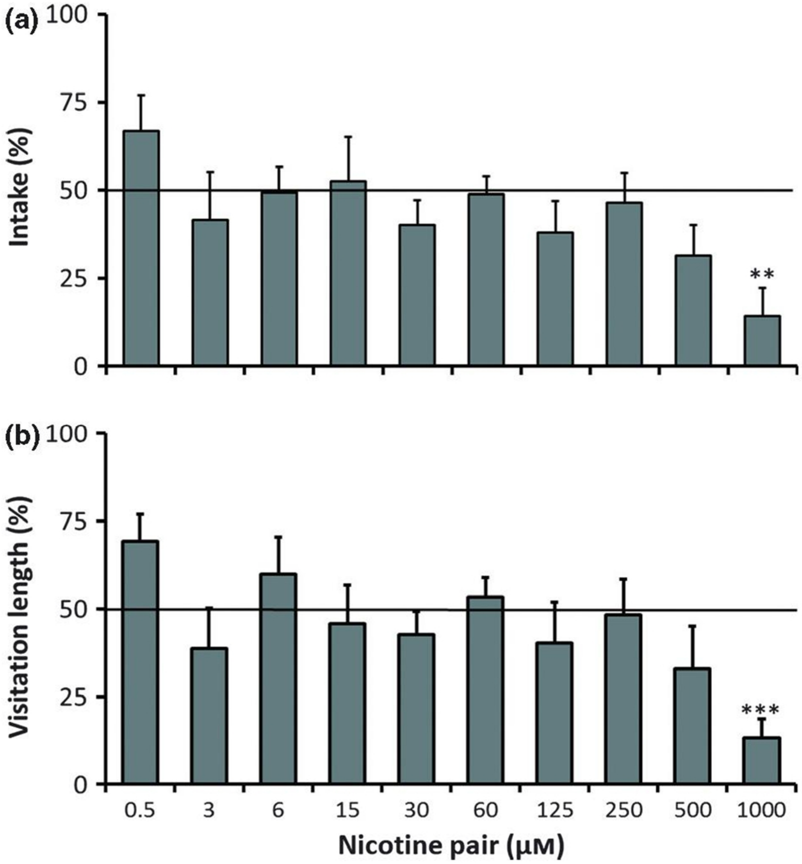

Fig. 3: Intake (a) and visitation length (b) of six Phelsuma grandis geckos offered ten nicotine concentrations in pairwise tests with pure sugar solutions (means + SE). Geckos were deterred only by the highest nicotine concentration and spent less time feeding from it. Statistical results derive from t-tests for dependent samples; ** $p < 0.01$, *** $p < 0.001$.

than its contrast. To confirm either of these speculations, tests should be performed on free-ranging geckos, or in a larger arena with random positioning of feeders.

Other than the contrast between nectar and petal colour, the signal strength of nectar (i.e. nectar colour saturation) may influence nectar choices. Geckos were more attracted to the darkest artificial nectar, suggesting that they perceived darker artificial nectar as offering a greater reward than lighter artificial nectar. Signal strength may be enhanced by either larger drops being formed on the plant's corolla, as a result of the nectar volume increasing when the nectar remains uneaten, or by nectar colour changes that occur over time, perhaps due to oxidation (Hansen et al. 2007a; Zhang et al. 2012), or evaporation (Nicolson & Nepi 2005; Zhang et al. 2012), which may indicate a higher reward as the sugar concentration increases. To the pollinator, this means that fewer flowers must be visited to acquire the same amount of energy, thereby reducing the cost of searching for food. The importance of nectar sugar concentration to pollinators was shown in a study by Cnaani et al. (2006), where bumblebees (Bombus impatiens) preferred smaller volumes of concentrated artificial nectar over larger volumes of dilute artificial nectar, even though the dilute solution ultimately offered a greater reward.

From the plant's perspective, advertising the energy-rich nectar reward with colour is beneficial as it increases the probability of successful pollination (Zhang et al. 2012). Islands have a depauperate insect pollinator fauna, and so, plants need to contend for vertebrate pollinators if they are to be pollinated (Olesen & Valido 2003; Hansen et al. 2007a; Kaiser-Bunbury et al. 2009). After successful attraction of pollinators, the plants may benefit from secondary metabolites in nectar that cause the pollinators to remove less nectar, leave the flower more quickly and return more frequently (Kessler & Baldwin 2006; Kessler et al. 2012). Zhang et al. (2012) showed that the unpalatable nectar of the reproductively inactive stages of Leucosceptrum canum (Labiatae) 'directed' birds to the palatable nectar of the reproductively active flowers. Furthermore, the antimicrobial properties of secondary metabolites may conserve the sugar content of exposed nectar (Cowan 1999; Hansen et al. 2007a). Antimicrobial benefits may also cause herbivores to exploit secondary metabolites for therapeutic purposes (Forbey et al. 2009).

The few studies that have investigated the response of lizards to plant secondary metabolites show that these animals are able to detect secondary metabolites prior to ingestion via olfactory cues and consequently avoid them. Schall (1990) treated tomato juice-soaked sponges with the alkaloid quinine (one part quinine to 1000 parts tomato juice (3 mM)) and presented them to free-ranging whiptail lizards (Cnemidophorus arubensis and C. murinus). The lizards were able to detect the quinine even though the tomato juice was believed to mask the odour and flavour of the alkaloid. In another study, C. murinus was unable to detect quinine (15 mM) through tongue-flicking when it was presented on a tile (Cooper et al. 2002). The geckos in our experiment did not tongue-flick towards the food source, but did pump their throats when approaching the test solution. This throat-pumping action, known as buccal pulsing, is a possible olfaction mechanism that can be likened to mammal sniffing (Dial & Schwenk 1996). Also, the concentration of quinine used in Schall's experiments was three times stronger than the highest nicotine concentration we used. Our lizards likely did not detect the nicotine via smell, as they rejected the highest nicotine concentration only after tasting it (or perhaps the sugar solution masked the smell of nicotine).

The sugar concentration could also explain why no preference pattern for nicotine was seen for first choice. Future studies should test a range of sugar concentrations containing nicotine to elucidate what effect sugar concentration may have on the ability of lizards to detect the presence of nicotine through smell. A high sucrose concentration was found to decrease the deterrent effect of the alkaloids gelsemine (Gegear et al. 2007) and nicotine (Köhler et al. 2012) on the artificial nectar consumption of bees. In our study, increased nicotine concentration also proportionally reduced both the amount of time a gecko spent eating from the artificial nectar and the amount it consumed. In the field, this behaviour could potentially result in an increased probability of outcrossing (Kessler & Baldwin 2006) as geckos would need to visit more flowers to compensate for energy deficits. As a psychoactive stimulant, nicotine may also be a reward offered by plants, thereby ensuring that these addicted pollinators visit time and time again (Singaravelan et al. 2005); a possible strategy employed by flowers in a competitive landscape, such as islands. Our results showed no difference in intake between the two consecutive test days for any of the nicotine concentrations but, to infer addiction, intake may need to be tested over a longer period of time.

Head-shaking and gaping occurred only when the geckos consumed the highest nicotine concentration, and no other adverse physiological effects were observed during or after experimentation. Many physiological mechanisms that decrease the potency of secondary compounds have been discovered (review in Dearing et al. 2005). Nicotine may also be metabolized and excreted (Yildiz 2004). Alkaloid-rich diets increase the excretion rate of insects (Wink & Theile 2002; Manson et al. 2010). In Palestine Sunbirds, Cinnyris oseous, dietary nicotine decreased gut transit time and sugar assimilation efficiency (Tadmor-Melamed et al. 2004). Future studies could investigate whether alkaloids have similar physiological effects in day geckos.

Geckos were remarkably tolerant to artificial nectar nicotine and were deterred only by a high concentration (1000 μM). The fact that these geckos were able to tolerate such a high concentration of a toxin warrants special attention. By having bitter nectar, plants could be trying to deter nectar robbers (Adler 2000; Johnson et al. 2006); so, to drink from these plants, the gecko may need to have a high tolerance. Generalist nectar-feeding birds are more tolerant to secondary metabolites in nectar than are specialists (Johnson et al. 2006; Lerch-Henning & Nicolson, in press); this may apply to other generalist pollinators as well.

Recent research has highlighted the role of Phelsuma species in pollinating endangered Indian Ocean endemics in Mauritius (Hansen et al. 2007b; Hansen & Müller 2009). *Phelsuma ornata* is an important generalist island pollinator whose demise could have a negative impact on the reproduction of certain plant species and ecosystem function (Olesen et al. 2002; Hansen et al. 2007b; Kaiser-Bunbury et al. 2009). Phelsuma grandis may also be an important generalist pollinator of endemic plants in Madagascar. By understanding and identifying the floral traits that affect interactions with gecko pollinators, we will be able to identify endemic plants that are potentially reliant on them for reproduction (Kaiser-Bunbury et al. 2009). To determine whether the floral traits tested in this study are ecologically relevant and adaptive, field experiments need to be conducted to study effects on plant fitness (Hansen et al. 2006). Currently, the abundance and distribution of day geckos are being threatened by anthropogenic habitat destruction, zebu overgrazing and the pet trade (D'Cruze et al. 2007, 2009). Field studies are a high priority for Madagascar and its surrounding islets, which have been identified as one of the top three biodiversity hotspots in need of conservation (Myers et al. 2000).

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Literature Cited

Adler, L. S. 2000: The ecological significance of toxic nectar. Oikos 91, 409—420.

Cnaani, J., Thomson, J. D. & Papaj, D. R. 2006: Flower choice and learning in foraging bumblebees: effects of variation in nectar volume and concentration. Ethology 112, 278—285.

Collias, N. E. & Collias, E. C. 1968: Anna's hummingbirds trained to select different colors in feeding. Condor 70, 273—274.

Cooper, W. E. 1998: Evaluation of swab and related tests as a bioassay for assessing responses by squamate reptiles to chemical stimuli. J. Chem. Ecol. 24, 841—866.

Cooper, W. E. & Habegger, J. J. 2000: Lingual and biting responses to food chemicals by some Eublepharid and Gekkonid geckos. J. Herpetol. 34, 360—368.

Cooper, W. E., Perez-Mellado, V., Vitt, L. J. & Budzinsky, B. 2002: Behavioral responses to plant toxins by two omnivorous lizard species. Physiol. Behav. 76, 297— 303.

Cowan, M. M. 1999: Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12, 564—582.

D'Cruze, N., Sabel, J., Green, K., Dawson, J., Gardner, C, Robinson, J., Starkie, G., Vences, M. & Glaw, F. 2007: The first comprehensive survey of amphibians and reptiles at Montagne des Francais, Madagascar. Herpetol. Conserv. Biol. 2, 87—99.

D'Cruze, N., Sabel, J., Dawson, J. & Kumar, S. 2009: The influence of habitat type and structure on the abundance of *Phelsuma madagascariensis grandis* (Gekkoninae) in northern Madagascar. Herpetol. Conserv. Biol. 4, 55—61.

Dearing, M. D., Foley, W. J. & McLean, S. 2005: The influence of plant secondary metabolites on the nutritional ecology of herbivorous terrestrial vertebrates. Annu. Rev. Ecol. Evol. Syst. 36, 169—189.

Dial, B. E. & Schwenk, K. 1996: Olfaction and predator detection in Coleonyx brevis (Squamata: Eublepharidae), with comments on the functional significance of buccal pulsing in geckos. J. Exp. Zool. 276, 415—424.

Eifler, D. A. 1995: Patterns of plant visitation by nectar-feeding lizards. Oecologia 101, 228—233.

Facchini, P. J. 2001: Alkaloid biosynthesis in plants: biochemistry, cell biology, molecular regulation, and metabolic engineering applications. Annu. Rev. Plant. Physiol. Plant. Mol. Biol. 52, 29—66.

Forbey, J. S., Harvey,A. L., Huffman, M. A., Provenza, F. D., Sullivan, R. & Tasdemir, D. 2009: Exploitation of secondary metabolites by animals: a response to homeostatic challenges. Integr. Comp. Biol. 49, 314—328.

Gegear, R. J., Manson, J. S. & Thomson, J. D. 2007: Ecological context influences pollinator deterrence by alkaloids in floral nectar. Ecol. Lett. 10, 375—382.

Hagler, J. R. & Buchmann, S. L. 1993: Honey bee (Hymenoptera: Apidae) foraging responses to phenolic-rich nectars. J. Kansas Entomol. Soc. 66, 223—230.

Hansen, D. M. & Müller, C. B. 2009: Invasive ants disrupt gecko pollination and seed dispersal of the endangered plant Roussea simplex in Mauritius. Biotropica 41, 202— 208.

Hansen, D. M., Beer, K. & Muller, K. B. 2006: Mauritian coloured nectar no longer a mystery: a visual signal for lizard pollinators. Biol. Lett. 2, 165—168.

Hansen, D. M., Olesen, J. M., Mione, T., Johnson, S. D. & Mu€ller, C. B. 2007a: Colored nectar: distribution, ecology, and evolution of an enigmatic floral trait. Biol. Rev. 82, 83—111.

Hansen, D. M., Kiesbüy, H. C., Jones, C. G. & Mu€ller, C. B. 2007b: Natural history miscellany: positive indirect interactions between neighbouring plant species via a lizard pollinator. Am. Nat. 169, 534—542.

Heiling, A. M. & Herberstein, M. E. 2004: Predator-prey coevolution: Australian native bees avoid their spider predators. Proc. R. Soc. Lond. B 271, S196—S198.

Irwin, R. E. & Adler, L. S. 2008: Nectar secondary compounds affect self-pollen transfer: implications for female and male reproduction. Ecology 89, 2207— 2217.

Janzen, D. H. 1977: Why don't ants visit flowers? Biotropica 9, 252.

Johnson, S. D., Hargreaves, A. L. & Brown, M. 2006: Dark, bitter-tasting nectar functions as a filter of flower visitors in a bird-pollinated plant. Ecology 87, 2709—2716.

Kaiser-Bunbury, C. N., Traveset, A. & Hansen, D. M. 2009: Conservation and restoration of plant-animal mutualisms on oceanic islands. Perspect. Plant Ecol. Evol. Syst. 12, 131—143.

Kessler, D. & Baldwin, I. T. 2006: Making sense of nectar scents: the effects of nectar secondary metabolites on floral visitors of *Nicotiana attentuata*. Plant J. 49, 840—854.

Kessler, D., Bhattacharya, S., Diezel, C, Rothe, E., Gase, K., Schottner, M. & Baldwin, I. T. 2012: Unpredictability of nectar nicotine promotes outcrossing by hummingbirds in *Nicotiana attenuata.* Plant J. 71, 529—538.

Kohler, A., Pirk, C. W. W. & Nicolson, S. W. 2012: Honeybees and nectar nicotine: deterrence and reduced survival versus potential health benefits. J. Insect Physiol. 58, 286—292.

Lai Fang, N. R., Bahorun, T. & Khittoo, G. 2002: Chemo-systematics: a new source of evidence for the classification of the endemic flora of Mauritius. In: Proceedings of the Fifth Annual Meeting of Agricultural Scientists (Lalouette, J. A. & Bachraz, D. Y., eds). Food and Agricultural Research Council, Reduit, Mauritius, 3—4 May 2001.

Lerch-Henning, S. & Nicolson, S. W. (in press): Bird pollinators differ in their tolerance of a nectar alkaloid. J. Avian Biol., in press.

Manson, J. S., Otterstatter, M. C. & Thomson, J. D. 2010: Consumption of a nectar alkaloid reduces pathogen load in bumble bees. Oecologia 162, 81—89.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., de Fonseca, G. A. B. & Kent, J. 2000: Biodiversity hotspots for conservation priorities. Nature 403, 853—858.

Nicolson, S. W. & Nepi, M. 2005: Dilute nectar in dry atmospheres: nectar secretion patterns in *Aloe castanea* (Asphodelaceae). Int. J. Plant Sci. 166, 227—233.

Nyhagen, D. F., Kragelund, C, Olesen, J. M. & Jones, C. G. 2001: Insular interactions between lizards and flowers: flower visitation by an endemic Mauritian gecko. J. Trop. Ecol. 17, 755—761.

Olesen, J. M. & Valido, A. 2003: Lizards as pollinators and seed dispersers: an island phenomenon. Trends Ecol. Evol. 18, 177—181.

Olesen, J. M., Ronsted, N, Tolderlund, U., Cornett, C, Molgaard, P., Madsen, J., Jones, C. G. & Olsen, C. E. 1998: Mauritian red nectar remains a mystery. Nature 393, 529.

Olesen, J. M., Eskildsen, L. I. & Venkatasamy, S. 2002: Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. Divers. Distrib. 8, 181—192.

Puchooa, D. & Venkatasamy, K. 2005: A protocol for the isolation of DNA from *Trochetia boutoniana.* Int. J. Agric. Biol. 7, 82—85.

Raxworthy, C. J., Ingram, C. M., Rabibisoa, N. & Pearson, R. G. 2007: Applications of ecological niche modeling for species delimitation: a review and empirical evaluation using day geckos *(Phelsuma)* from Madagascar. Syst. Biol. 56, 907—923.

Rhoades, D. F. & Bergdahl, J. C. 1981: Adaptive significance of toxic nectar. Am. Nat. 117, 798—803.

Röll, B. 2000: Gecko vision- visual cells, evolution, and ecological constraints. J. Neurocytol. 29, 471—484.

Schaefer, H. M., Schaefer, V. & Levey, D. J. 2004: How plant-animal interactions signal new insights in communication. Trends Ecol. Evol. 19, 577—584.

Schaefer, H. M., Levey, D. J., Schaefer, V. & Avery, M. L. 2006: The role of chromatic and achromatic signals for fruit detection by birds. Behav. Ecol. 17, 784—789.

Schall, J. J. 1990: Aversion of whiptail lizards (Cnemidophorus) to a model alkaloid. Herpetologica 46, 34—39.

Schmidt, V. & Schaefer, H. M. 2004: Unlearned preference for red may facilitate recognition of palatable food in young omnivorous birds. Evo. Ecol. Res. 6, 919—925.

Singaravelan, N., Nee'man, G., Inbar, M. & Izhaki, I. 2005: Feeding responses of free-flying honeybees to secondary compounds mimicking floral nectars. J. Chem. Ecol. 31, 2791—2804.

Stephenson, A. G. 1981: Toxic nectar deters nectar thieves of *Catalpa speciosa*. Am. Midl. Nat. 105, 381—383.

Tadmor-Melamed, H., Markman, S., Arieli, A., Distl, M., Wink, M. & Izhaki, I. 2004: Limited ability of Palestine sunbirds *Nectarinia osea* to cope with pyridine alkaloids in nectar of tree tobacco *Nicotiana glauca*. Funct. Ecol. 18, 844—850.

Taniguchi, Y., Hisatomi, O., Yoshida, M. & Tokunaga, F. 1999: Evolution of visual pigments in geckos. Fed. Eur. Biochem. Soc. Lett. 445, 36—40.

Townsend, C. R. & Cole, C. J. 1985: Additional notes on requirements of captive whiptail lizards (Cnemidophorus), with emphasis on ultraviolet radiation. Zoo Biol. 4, 49—55.

Traveset, A. & Saez, E. 1997: Pollination of *Euphorbia dendroides* by lizards and insects: spatio-temporal variation in patterns of flower visitation. Oecologia 111, 241—248.

Wink, M. 1999: Plant secondary metabolites from higher plants: biochemistry, function and biotechnology. In: Biochemistry of Plant Secondary Metabolism, Annual Plant Reviews (Wink, M., ed.). Sheffield Academic, Sheffield, pp. 1—16.

Wink, M. & Theile, V. 2002: Alkaloid tolerance in *Manduca sexta* and phylogenetically related sphingids (Lepidoptera: Sphingidae). Chemoecology 12, 29—46.

Yildiz, D. 2004: Nicotine, its metabolism and an overview of its biological effects. Toxicon 43, 619—632.

Zhang, F. P., Cai, X. H., Wang, H., Ren, Z. X., Larson-Rabin, Z. & Li, D. Z. 2012: Dark purple nectar as a foraging signal in a bird-pollinated Himalayan plant. New Phytol. 193, 188—195.

Zuur, A. F., Ieno, E. N, Walker, N. J., Saveliev, A. A. & Smith, G. S. 2009: Mixed Effects Models and Extensions in Ecology with R. Springer, New York, pp. 193—260.

Supporting Information

Figure 1

Absorbance spectra of a) red, b) light red, c) dark red, and d) yellow artificial nectar.

Table 1

Statistical data analysis results for all experiments. Statistical results derive from *t*-tests for dependent samples for intake, visitation frequency and visitation length, while a binomial test was performed on first choice; * $p < 0.05$, ** $p < 0.01$ (statistically significant results are shown in bold). Geckos did not have a side bias (Intake % (Day 1, 2)).

Figure 1

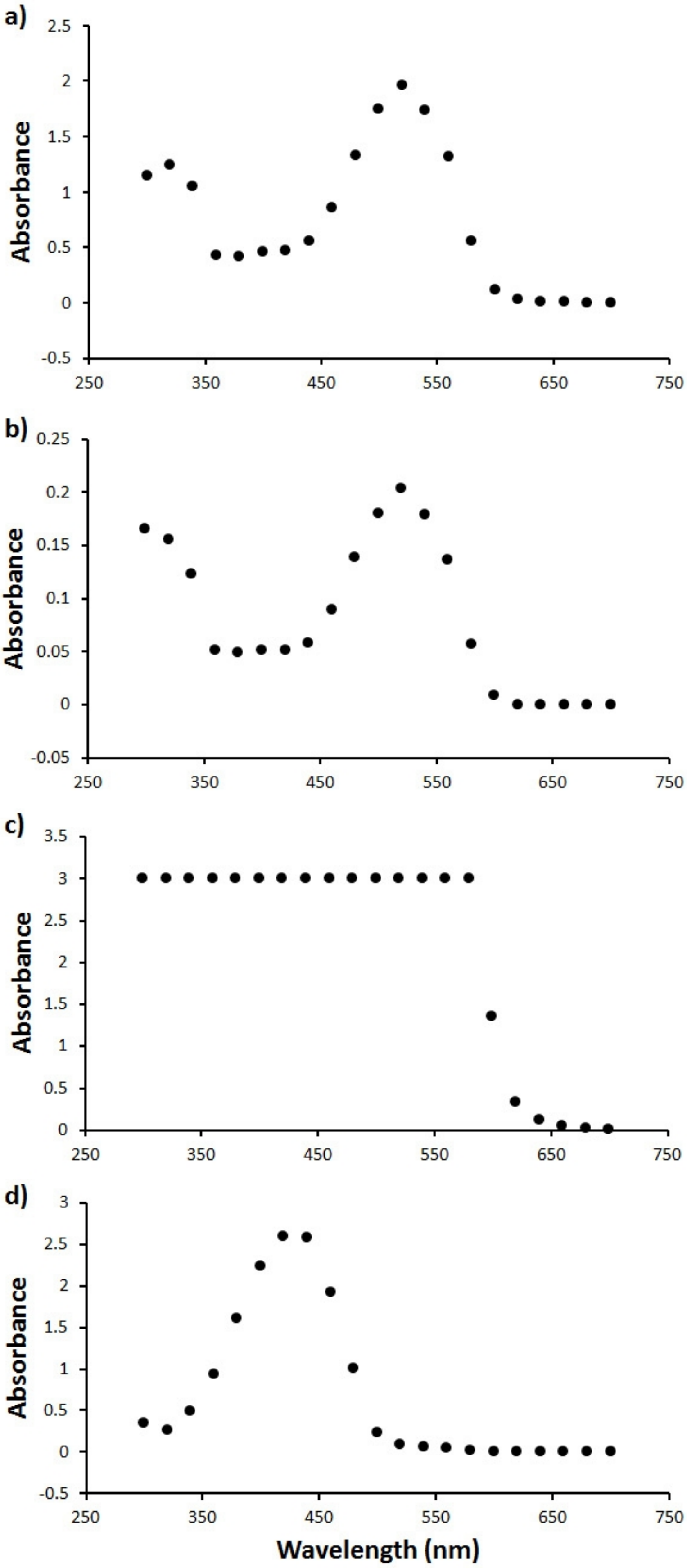
a)
Absorbance
2.5
2
1.5
1
0.5
0
-0.5
250
350
450
550
650
750
b)
Absorbance
0.25
0.2
0.15
0.1
0.05
0
-0.05
250
350
450
550
650
750
c)
Absorbance
3.5
3
2.5
2
1.5
1
0.5
0
250
350
450
550
650
750
d)
Absorbance
3
2.5
2
1.5
1
0.5
0
-0.5
250
350
450
550
650
750
Wavelength (nm)

Table 1

Test	First choice		Visitation frequency			Visitation length			Intake %			Intake % (Day 1,2)		
	z	p	df	*t*	p	df	*t*	p	df	*t*	p	df	*t*	p
Red vs. clear	-2.2	**0.013**	6	3.3	**0.017**	6	4.1	**0.007**	6	3.4	**0.014**	6	-0.3	0.795
Yellow vs. clear	0.8	0.791	6	1.2	0.276	6	1.0	0.364	6	1.2	0.263	6	-0.3	0.797
Red vs. yellow	0.8	0.791	6	-0.3	0.745	6	-1.1	0.332	6	-0.8	0.480	6	1.7	0.150
Coloured vs. clear	-1.8	**0.036**	13	3.0	**0.011**	13	2.9	**0.012**	13	3.1	**0.009**	13	0.4	0.697
0.5 vs. clear	0.7	0.774	5	1.3	0.263	5	2.5	0.058	5	1.6	0.163	5	1.8	0.133
3.0 vs. clear	0.7	0.774	5	-0.9	0.411	5	-1.0	0.366	5	-0.6	0.562	5	2.5	0.052
6.0 vs. clear	-0.2	0.388	5	1.1	0.331	5	1.0	0.376	5	0.2	0.876	5	0.9	0.414
15 vs. clear	0.7	0.774	5	0.1	0.905	5	-0.4	0.711	5	0.6	0.579	5	1.4	0.211
30 vs. clear	-0.2	0.388	5	-1.0	0.354	5	-1.1	0.310	5	-1.4	0.227	5	1.8	0.128
60 vs. clear	3.4	1.000	5	1.0	0.386	5	0.6	0.590	5	-0.2	0.850	5	0.4	0.705
125 vs. clear	0.7	0.774	5	-0.4	0.695	5	-0.8	0.435	5	-1.3	0.236	5	1.1	0.332
250 vs. clear	0.7	0.774	5	2.1	0.085	5	-0.2	0.876	5	-0.4	0.699	5	0.7	0.544
500 vs. clear	0.7	0.774	5	0.2	0.863	5	-1.4	0.215	5	-2.1	0.089	5	0.1	0.961
1000 vs. clear	3.4	1.000	5	-0.5	0.628	5	-6.9	**0.001**	5	-4.4	**0.007**	5	0.5	0.646
Nicotine vs. clear	1.4	0.927	5	0.6	0.558	5	-1.6	0.174	5	2.4	0.062	5	0.9	0.394
Blue petals red vs. clear	0.7	0.774												
Blue petals yellow vs. clear	0.7	0.774												
Blue petals red vs. yellow	3.4	1.000												
Green petals red vs. clear	-0.2	0.388												
Green petals yellow vs. clear	-1.0	0.146												
Green petals red vs. yellow	0.7	0.774												
Orange petals red vs. clear	0.7	0.774												
Orange petals yellow vs. clear	-0.2	0.388												
Orange petals red vs. yellow	0.7	0.774												
Red petals red vs. clear	0.7	0.774												
Red petals yellow vs. clear	0.7	0.774												
Red petals red vs. yellow	-0.2	0.388												
White petals red vs. clear	-0.2	0.388												
White petals yellow vs. clear	3.4	1.000												
White petals red vs. yellow	0.7	0.774												
Yellow petals red vs. clear	3.4	1.000												
Yellow petals yellow vs. clear	0.7	0.774												
Yellow petals red vs. yellow	0.7	0.774												
All petals red vs. clear	-1.1	0.125												
All petals yellow vs. clear	-0.2	0.410												
All petals red vs. yellow	0.5	0.724												
All petals colour vs. clear	-1.4	0.080												
Light vs. medium	-0.2	0.388												
Medium vs. dark	-1.0	0.146												
Light vs. dark	-2.5	**0.006**												