

Supporting Information

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SI Text

Bertam Palm Census. We directly observed the length of the period during which ripe pollen was exposed in 18 inflorescences with male and 10 inflorescences with hermaphroditic flowers. The period between the staminate anthesis of male flowers and the staminate anthesis of hermaphroditic flowers could be directly observed in seven inflorescences. We directly observed the temporal spacing between staminate and pistillate anthesis in hermaphroditic flowers of 14 inflorescences. Of the long phase of nectar production sometimes only the begin or only the end was recorded. Therefore, scheme and goal of data collection corresponded with “survival analysis,” e.g., in biomedical research with staggered entry of subjects into study and occurrence of a defined event often not observed (“censored data”). We used Kaplan-Meier Product Limit Estimation techniques to determine the lengths of the nectar production periods from uncensored and censored data from 48 inflorescences with male and 34 inflorescences with hermaphroditic flowers (1, 2). We used a similar procedure to calculate the period between staminate anthesis of the male flowers and the onset of nectar production in the hermaphroditic flowers ($n = 27$). Subtraction of the median period of nectar production in hermaphroditic flower buds from the measured period between the staminate anthesis of male flowers and the staminate anthesis of hermaphroditic flowers yielded the length of the intermediate period between floral rewards available during the male and hermaphroditic stages of an inflorescence.

Identification of the Yeast Community. For identification of the yeast community samples were collected aseptically and streak-inoculated sequentially onto two YM agar plates (glucose 1%, peptone 0.5%, yeast extract 0.3%, malt extract 0.3%, 2% agar) supplemented with 50 mg/liter chloramphenicol. Plates were examined daily for 1 week, and representative colonies were purified. Yeast species were characterized physiologically by using genetic standard methods (Table S1) (3, 4). Representative cultures are preserved at the Department of Biology, University of Western Ontario.

Specificity of the Biosensor for Nectar Ethanol. The accuracy of the device is $\pm 2\%$ (determined at 138 mg/liter ethanol); imprecision is $<5\%$. The biosensor is not specific for ethanol, but responds to methanol, propanol, and isopropanol. Trace amounts of other alcohols may be present in mixed yeast fermentations, but their total content is two orders of magnitude less than ethanol (5). For all practical purposes, therefore, the measured alcohol content of nectar equates to ethanol content. Further robust support for ethanol being the primary alcohol involved in the described ecological relationship is provided by measurements of the metabolite EtG in the hair of mammals specific for ethanol metabolism. We excluded the possibility that the biosensor interfered with substances in bertam palm nectar other than alcohols by measuring nectar samples before and after adding defined volumes of ethanol and detected no odd responses.

Video Monitoring of Inflorescences. Inflorescences were picked at random from five different subplots separated by ≤ 200 m with the goal of monitoring visits by mammals at two inflorescences each per subplot for ≥ 4 whole nights (achieved: 4–14). The 200-m distance between subplots was chosen to reduce bias toward features of certain individual animals or certain social networks in calculated average visitation rates. To ensure that a

recording session lasted for a complete day (sunrise to sunset) or night without changing tapes or missing visits we recorded in time-lapse mode (automatically recording for 2 s every 30 s). Visits at inflorescences with nectar production invariably lasted longer than the 30-s intervals between recordings. Cameras were capable of recording periods of up to 14 h on tape in time-lapse mode. Cameras were repeatedly placed at the same inflorescence within a period 47–1 nights before staminate anthesis. If staminate anthesis occurred before the 4-night minimum was achieved we switched to another inflorescence in the same subplot. The total number of inflorescences video-monitored was 27. To reduce autoreplication the two cameras were never set together in the same subplot. Post hoc tests showed no bias toward monitoring frequently visited inflorescences longer than less frequently visited inflorescences (Pearson's correlation $r = -0.2$, $P = 0.447$). The proportion of inflorescences receiving visits is the proportion of all target inflorescences pooled together. We calculated the average number of visits per inflorescence per night as a grand mean from the means of each subplot. Ten inflorescences from the five subplots were surveyed one night each in interval mode during staminate anthesis. At best, little floral rewards were present during pistillate anthesis resulting in mammals shortening their visits to inflorescences. These shorter visits were more difficult to catch on tape recordings in interval mode. We nevertheless recorded nine visits by murids and four by slow lorises during 21 camera nights, showing that small mammals are very likely depositing pollen on receptive stigmas.

Time Spent on Consuming Nectar. We first calculated the average daily activity period as the time between the median observed onset of activity (movement away from the sleeping site) and the median observed end of activity (entering of the sleeping site). Petailed treeshrews were active on average for 615 min per night; slow lorises were active on average for 648 per night (6). During each sighting of an animal we recorded the first behavior seen as an instantaneous observation (7). We recorded feeding on nectar of the bertam palm when the animals were clearly seen active on nectar-producing inflorescences of the bertam palm probing flowers with the head. Animals of the two species that were regularly followed habituated quickly and could be observed without causing obvious disturbance sometimes at distances <5 m. We observed focal animals from the ground by using a 4.5 V-head lamp or torchlight and binoculars. Close-range observations and video-recordings with infrared light sources confirmed that the animals' probing of flowers coincided with them licking off exuded nectar with their tongues.

We determined the time individual slow lorises spent consuming nectar of the bertam palm from observational data on instantaneous observations of intensively radio-tracked animals. To avoid possible bias from irregular sampling intervals we included only instantaneous feeding observations on any animal that were separated by at least the time required by an animal to cross the length of an average home range in traveling speed. Observations separated by at least this time, therefore, were considered independent. The proportion of nectar licking from the total number of independent instantaneous observations was used to calculate the daily time spent licking nectar per individual. The times slow lorises spent on nectar were recalculated from previously published data using the previously outlined methods (8) but only six individuals from high-density bertam

palm areas. The total number of independent instantaneous feeding observations per slow loris was 52 ± 34 SD.

In contrast to slow lorises that could be observed in all vertical forest zones, clear observations of behavioral details such as feeding in much smaller and more agile pentailed treeshrews were more likely at bertam palm inflorescences close to the ground than in emergent trees that the animals climbed. To avoid overestimating feeding on bertam palm nectar it was necessary to modify the calculation procedure for pentailed treeshrews. We first calculated the time spent in bertam palms from the total number of independent locational records. Independent records were separated by >50 min as 50 min was the time required to cross the length of an average home range in traveling speed. We then calculated the proportion of nectar licking from the total number of independent instantaneous observations in bertam palms and multiplied both figures to obtain a good estimate of the time pentailed treeshrews spent on consuming nectar. We omitted feeding data on the first observed pentailed treeshrew from quantitative analyses because behavioural sampling methods were not yet established during this period. For the remaining seven pentailed treeshrews the total number of independent locational records per individual was 71 ± 45 SD; the number of independent instantaneous observations in bertam palms per individual was 17 ± 8 SD.

Choice of Our Food Model. Our intention was to estimate ingested alcohol doses, because absorption and digestion kinetics are unknown and therefore blood alcohol levels are much harder to estimate. We developed a model of nectar ingestion based on the assumption of proportionality between variables, which is commonly made in modeling exposure to environmental toxicants (9). Although reasonably simple, the model does not seem to consist of fewer variables than the number of dimensions of the real system, thus avoiding two pitfalls of ecological extrapolation. One problem is the exclusion of key processes in simple models resulting in unknown prediction biases. The other is an increase in estimation and measurement error of complex approaches (10). Further confirmation that our food model is realistic was provided by calculating the mean daily ingested nectar volume using a similar formula (total ingested volume = $T \times ER \times LT \times EC$, where T was the time spent on ingesting bertam palm nectar per day, ER was the rate at which flower buds are emptied during

visits, LT was the lapse time to the last mammalian visit, EC was the renewal rate of encountered nectar crop at a single bud, see the description of our other food model in *Materials and Methods* for comparison) with the same data sets. The mean daily volume ingested by pentailed treeshrews is 38 ml. This is in line with estimated volumetric capacities and a mean “grams-of-food-intake-per-kilogram-of-body-weight” for small mammals and small birds living on nectar (11). We have stated that, assuming alcohol metabolism similar to human, a pentailed treeshrew would be intoxicated approximately every third day. This statement is based on a simplified 1D view on consumption patterns. Feeding patterns and the pattern of alcohol consumption are likely to differ within and between individuals.

Deleterious Alcohol Effect Benchmark. The choice of $r = 0.55$ (for women) for input into Widmark’s formula instead of $r = 0.68$ (for men) and $\beta = 0.017$ (moderate drinkers) instead of $\beta = 0.02$ (heavy drinkers) (12) gives a low benchmark compared with alternative settings. For comparison, a heavily alcohol drinking man after 12 h drinking would reach an identical BAC with an intake dose of 2.0 g/kg. However, input data in the MC simulation came from animals of both sexes. Even for males and all other mentioned concerns set aside, our benchmark does not give a false impression of risky drinking as driver BACs of 0.05 g/dl already correlate with a doubled risk of crashing (13). In addition, drinking has health risks other than inebriation that should be minimized by natural selection, e.g., it is cell damaging.

Alterations of the risk to reach harmful BACs due to inter-specific differences in the distribution factor r and the metabolic rate β are neglected in this calculation. First, because they are unknown and, second, because they are the predicted evolutionary result of the alcohol related selective pressures that we intend to measure. Rate of overall metabolism in both pentailed treeshrews and slow lorises are much lower than predicted from their mass (8, 14). Standard corrections like allometric scaling are therefore unfruitful for achieving a realistic β value. Intuitively, very low overall metabolic rates should mean very low β values and higher risks to achieve the benchmark dose. However, we have neither attempted any downward correction of β , because Wiens *et al.* (8) have argued for a possible nonintuitive causal link between a low overall metabolism and very effective specific detoxification pathways, making guesses at specific enzyme activities from overall metabolic rates also unfruitful.

- Kaplan EL, Meier P (1958) Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 53:457–481.
- Pollock KH, Winterstein SR, Buck CM, Curtis PD (1989) Survival analysis in telemetry studies: The staggered entry design. *J Wildl Manage* 53:7–15.
- Yarrow D (1998) in *The Yeasts: A Taxonomy Study*, eds Kurtzman CP, Fell JW (Elsevier, Amsterdam), pp 77–100.
- Marinoni G, Lachance MA (2004) Speciation in the large-spored *Metschnikowia* clade and establishment of a new species, *Metschnikowia borealis* comb nov. *FEMS Yeast Res* 4:587–596.
- Engan S (1981) in *Brewing Sciences*, ed Pollock JRA (Academic, London), Vol 2, pp 98–105.
- Wiens F, Zitzmann A (2003) Social structure of the solitary slow loris *Nycticebus coucang* (Lorisidae). *J Zool (London)* 261:35–46.
- Altmann J (1974) Observational study of behavior: Sampling methods. *Behaviour* 49:227–267.
- Wiens F, Zitzmann A, Hussein NA (2006) Fast food for slow lorises. Is low metabolism related to secondary compounds in high-energy plant diet? *J Mammal* 87:790–798.
- Sample BE, Aplin MS, Efrogmson RA, Suter GWI, Welsh CJ (1997) *Methods and Tools for Estimation of the Exposure of Terrestrial Wildlife to Contaminants* (Environmental Science Division, Oak Ridge National Laboratory, Oak Ridge, TN).
- Peters DPC, Herrick JE (2004) Strategies for ecological extrapolation. *Oikos* 106:627–636.
- Warrington PD (2001) Animal Weights and Their Food and Water Requirements (Water Management Branch, Environment and Resource Division, Ministry of Environment, Lands and Parks, Government of British Columbia, Canada).
- Gullberg RG, Jones AW (1994) Guidelines for estimating the amount of alcohol consumed from a single measurement of blood alcohol concentration: Re-evaluation of Widmark’s equation. *Forensic Sci Int* 69:119–130.
- Zador PL, Krawchuk SA, Voas RB (2000) Alcohol-related relative risk of driver fatalities and driver involvement in fatal crashes in relation to driver age and gender: An update using 1996 data. *J Stud Alcohol* 61:387–395.
- Whittow GC, Gould E (1976) Body temperature and oxygen consumption of the pentailed tree shrew (*Ptilocercus lowii*). *J Mammal* 57:754–756.



Movie S1. A pentailed treeshrew probing an inflorescence of the bertam palm and licking nectar off flower buds.

[Movie S1 \(MOV\)](#)



Movie S2. A slow loris probing an inflorescence of the bertam palm and licking nectar off flower buds.

[Movie S2 \(MOV\)](#)

Table S1. Principal yeast species from samples of bertam palm nectar ($N = 70$), in decreasing order of frequency

Yeast species	Fermentative ability
<i>Pichia</i> cf. <i>membranifaciens</i>	+
<i>Schizosaccharomyces japonicus</i>	+
<i>Hanseniaspora valbyensis</i>	+
<i>Zygorhizula</i> cf. <i>florentinus</i>	+
<i>Trichomonascus</i> cf. <i>petasosporus</i> 1	+
<i>Trichomonascus</i> cf. <i>petasosporus</i> 2	+
<i>Saccharomyces cerevisiae</i>	+
<i>Candida</i> cf. <i>salmanticensis</i>	+
<i>Candida</i> cf. <i>thailandica</i>	+
<i>Pichia galeiformis</i>	-
<i>Candida</i> cf. <i>drosophilae</i>	-
<i>Candida</i> cf. <i>guilliermondii</i>	+
<i>Arthroascus fermentans</i>	+

The insertion of "cf" indicates a new species with affinities to the species whose name is given, as determined by rDNA sequencing. Only species that were present in three or more samples are listed.

Table S2. Description of MC distributions used in the food model

Symbol, unit	Monte Carlo Input Distributions (MC)	
	Pentailed treeshrew	Slow loris
<i>DE</i> , mg/kg/day	Output	Output
<i>C</i> , vol/vol	Lognormal (0.49; 0.44) Data on 100 measurements (nectar droplets from five flowers each from 20 inflorescences). Distribution shape χ^2 -tested.	Lognormal (0.49; 0.44) Data on 100 measurements (nectar droplets from five flowers each from 20 inflorescences). Distribution shape χ^2 -tested.
<i>T</i> , min/day	Normal (138;53;0) Assumed shape. Mean and SD of seven individuals.	Normal (86;55;0) Assumed shape. Mean and SD of six individuals.
<i>ER</i> , buds/min	Normal (3.8;0.8;0) Assumed shape. Mean and SD of visits to four inflorescences.	Normal (3.3;0.4;0) Assumed shape. Mean and SD of visits to five inflorescences.
<i>LT</i> , min	Normal (115;40;0) Assumed shape. Weighted mean and standard deviation of 62 monitored visits in 4 areas within the study site.	Normal (197;104;0) Assumed shape. Weighted mean and standard deviation of 29 monitored visits in 5 areas within the study site.
<i>EC</i> , μ l/min/bud	Beta (0.62;4.14;0.03;3.1) Data on 67 interval measurements obtained from 32 flower buds from eight inflorescences. Distribution shape χ^2 -tested.	Beta (0.62;4.14;0.03;3.1) Data on 67 interval measurements obtained from 32 flower buds from eight inflorescences. Distribution shape χ^2 -tested.
<i>BM</i> , kg	Normal (0.047; 0.004) Data on 12 nonpregnant adult and subadult animals of both sexes from the study area. Distribution shape χ^2 -tested.	Normal (0.684; 0.091;0.35; 0.9) Data on 24 nonpregnant adult and subadult animals of both sexes from the study area. Distribution shape χ^2 -tested. Upper bound value for the largest captured animal by F.W. in West-Malaysia. Lower bound reflects developmental shift infant->subadult (1).
<i>ED</i> , mg/ μ l	0.783 Value assumed fix (for 27°C) Reference: AlcoDens 1.0 (Katmar Software)	0.783 Value assumed fix (for 27°C) Reference: AlcoDens 1.0 (Katmar Software)

Distribution notations are: Beta (alpha1, alpha2, lower bound, upper bound); Lognormal (arithmetic mean, arithmetic SD); Normal (arithmetic mean, arithmetic SD, lower bound, upper bound). The MC approach assumes known dependency structures between variables. We defined dependencies between all paired variables in the model as independent except for an inverse correlation (correlation coefficient set at -1) between *EC* and *C*. This inverse correlation incorporates the empirical finding of diminishing ethanol content of nectar on the buds' surface with time (Fig. 2).

1. Wiens F, Zitzmann A (2003) Social structure of the solitary slow loris *Nycticebus coucang* (Lorisidae). *J Zool (London)* 261:35–46.