

ECOLOGY

Fruit scent as an evolved signal to primate seed dispersal

Omer Nevo^{1*}, Diary Razafimandimby², Juan Antonio James Jeffrey^{1,3}, Stefan Schulz⁴, Manfred Ayasse¹

The tremendous diversity of floral and fruit traits is, to a large extent, a set of adaptations that promote plant reproduction through animal pollinators and seed dispersers. Yet, it is still unknown whether fruit scent is a by-product of fruit maturation or an evolved communication channel with animal mutualists. We show that in species that specialize on seed dispersal by lemurs—an olfactorily oriented primate—fruits increase scent production and change their chemical composition significantly more than sympatric species whose seeds are largely dispersed by birds. We further show that lemurs use these shifts in fruit scent to identify ripe fruits. These results show that fruit scent is an evolved communication system that facilitates animal-plant mutualism.

INTRODUCTION

Most woody tropical angiosperms produce fleshy fruits and rely on animals for seed dispersal (1). Fleshy fruits have evolved independently in more than half of extant angiosperm families (2), indicating that the need to offer an attractive reward to seed dispersers exerts a strong selection pressure on fruit traits. However, the degree to which fruit traits should be attributed to selection pressures exerted by seed dispersers is still debated. Fleshy fruits come in a tremendous diversity of sizes, shapes, and colors (3). Rather than being randomly distributed across taxa, their traits tend to be correlated and often go hand in hand with dispersal by only a share of the frugivore community (4, 5). The dispersal syndrome hypothesis postulates that fruit traits are selected to match the dietary requirements and sensory capacities of their primary seed dispersal vectors (4, 6). This view has been challenged by studies that emphasized the roles of phylogenetic inertia (7) and abiotic factors (8) as the major factors driving fruit trait evolution. However, more recent comparative studies have addressed these issues and suggest that a substantial portion of the variance in fruit traits can be explained by selection pressures exerted by seed dispersers (5, 9–11). This is not surprising given the extensive evidence for similar patterns in flower evolution, which, besides the various constraints of many floral traits, are the result of selection by animal pollinators (12, 13).

Fruit traits that have been attributed to selection by seed dispersers include, among others, size (4, 10, 14), shape (15), and location and presentation on the branch (16). In addition, fruit color has been shown to respond to selection pressures by visually oriented frugivores, primarily birds (9, 11, 13). Fruit color has also been suggested to be an honest signal that allows birds to assess a fruit's nutrient content (11). Fruit scent has long been suggested to play a similar role in mediating the interaction between plants and olfactorily oriented seed dispersers. Nevertheless, this hypothesis has only rarely been tested, possibly because rigorous analysis of fruit scent is substantially more complicated than fruit color. It has been shown that

bats rely on the scent of fig fruits (genus *Ficus*) to identify those that are ripe (17). Two studies compared bat-dispersed figs to those that rely on birds, which tend to be more vision- and less olfaction-oriented because of their excellent color vision. Figs of species that rely on bats produce more scent (5) and, in a model system of three species, only bat-dispersed species show a significant change in fruit scent upon ripeness (18). Another set of studies on a small model system indicated that, in the Neotropics, only primate-dispersed fruits show a shift in their scent profiles upon ripeness (19) and that primates can use the scent of ripe fruits to identify them (20). However, these works were limited to small model systems or focused on a single genus. Thus, the question whether fruit scent can be considered an evolved signal to facilitate the communication between plants and animals remains open.

Here, we examine whether fruit scent evolved as a signal to seed dispersers in 30 plant species of 15 families in Ranomafana National Park, Madagascar. As a group, the most important seed dispersers in Madagascar are the endemic lemurs (21). Contrary to most other tropical systems, frugivorous birds are rare, and the system is divided between a large group of lemur-dispersal specialists and a minority of species that are fully or partially dispersed by passerine birds (21). Many lemurs are nocturnal or cathemeral, and most or all individuals in all species are dichromatic, that is, red-green color blind (22). At the same time, they have relatively large main olfactory bulbs (23) and routinely use chemical cues for intraspecific communication (24), and some species have been shown to prefer more odorous fruits (25). It would thus appear that lemurs rely more on olfaction and less on vision during fruit selection. Like other primates, lemurs probably do not track the source of a scent to locate fruit crops, and the main function of olfaction in food acquisition is the identification that an individual fruit in a patch is ripe (movie S1) (23). Plants benefit from signaling that an individual fruit is ripe because (i) it reduces waste by allowing animals to identify and ignore unripe fruits and (ii) animals should be selected to prefer fruits that are easy to exploit; thus, a conspicuous display can be selected by increased seed dispersal over time. For these reasons, our main prediction is that the fruits of species that specialize on lemur seed dispersal emit scents that can be used by the lemurs to recognize their ripeness (19, 20, 26). All plant tissues, including unripe fruits, emit chemicals and thus have a scent, but we expect that to make ripe fruit more conspicuous in these species, ripe fruits would show a substantial

¹University of Ulm, Institute of Evolutionary Ecology and Conservation Genomics, Ulm, Germany. ²Department of Zoology and Animal Biodiversity, Faculty of Sciences, University of Antananarivo, Antananarivo, Madagascar. ³Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269, USA. ⁴Technische Universität Braunschweig, Institute of Organic Chemistry, Braunschweig, Germany. *Corresponding author. Email: omer.nevo@evolutionary-ecology.de

shift away from unripe fruits in quantity (amount) and quality (chemical composition) of scent.

At the same time, fruits that do not specialize on olfactorily oriented lemurs and receive dispersal services from passerine birds, which, along with a lower number of olfactory receptor genes compared to lemurs (27, 28), have excellent color vision (22, 29) and tend to rely on visual cues (5, 9, 11), are expected to emit scents like all plant tissue. However, crucially, ripe fruits of these species are not expected to be under selection to be olfactorily conspicuous relative to unripe fruits. This expectation would parallel the observation that flowers that are bird-pollinated primarily rely on visual cues and emit only trace amounts of scent (30). Thus, the second prediction is that the difference in the scent between ripe and unripe fruits would be significantly smaller than it is in lemur-specialist species. This would indicate that the shift in scent profiles in lemur specialists is not merely an inevitable by-product of fruit maturation but a trait that characterizes only species that specialize on seed dispersal by lemurs.

Finally, the hypothesis that fruit scent evolved to signal ripeness requires that lemurs rely on fruit scent when selecting fruits and that increased olfactory conspicuousness of ripe fruits drives heavier reliance on olfaction when selecting fruits. Thus, a third prediction is that there is a positive correlation between the distinctiveness of the scent of ripe fruits and the lemurs' tendency to use their sense of smell to identify them.

To test the predictions that (i) fruit scent changes upon ripeness in lemur-dispersed species and (ii) the scent change is greater than that in species that rely partially or exclusively on birds for the dispersal of their seeds, we collected 434 unripe and 428 ripe fruits from 90 plants of 30 species (tables S2 and S3). On the basis of published literature, we classified these species as one of two dispersal syndromes: lemur specialists ($n = 19$ species) and bird-mixed ($n = 11$ species). The former are dispersed exclusively by lemurs, while the latter are dispersed by birds, either exclusively or with some dispersal by lemurs. We sampled the scent of ripe and unripe fruits using the semistatic headspace technique and analyzed them using gas chromatography and mass spectrometry (MS) (see Materials and Methods). To identify differences attributable primarily to the ripening process,

we used a paired design in which ripe and unripe fruits of the same individual tree were compared. In so doing, we were able to eliminate much of the potential noise that may originate from individual differences or differences in abiotic conditions. To test the prediction that lemur reliance on olfaction is correlated with fruit olfactory conspicuousness (iii), we quantified the sniffing behavior of nine wild red-bellied lemurs (*Eulemur rubriventer*) while feeding on fruits of seven plant species.

RESULTS AND DISCUSSION

We identified and quantified 389 volatile organic compounds (VOCs), primarily terpenoids, aromatic compounds, and fatty acid derivatives such as aldehydes, carboxylic acids, alcohols, and aliphatic esters. Terpenoids were common in both ripe and unripe fruits across the system, but aliphatic esters were found almost exclusively in the ripe fruits of several of the lemur-specialist species (tables S1 to S3).

To test whether ripe fruits of lemur-specialist species increase their scent production more than those of bird-mixed species, we calculated a scent increase ratio for the fruits of each plant ($n = 90$) by dividing the amount of scent produced by a single ripe fruit by that of a single unripe fruit. We used a generalized linear mixed model (GLMM) in which dispersal syndrome (lemur specialist or bird-mixed) was the sole fixed factor and species was a random factor. The full model was statistically significant compared to the null model, which did not include syndromes [$\chi^2(1) = 4.2, P = 0.04$]. Fruits of lemur-specialist species increase their scent emission upon ripeness significantly more than those of bird-mixed species (GLMM: $P = 0.049$). To verify the robustness of this result, we also calculated the mean scent increase ratio in each of the 30 species (Fig. 1A). The median scent increase ratio was 0.88 in bird-mixed species, indicating that the amount of scent emitted by ripe and unripe fruits is similar. In contrast, the median scent increase ratio was 2.31 in lemur-specialist fruits, indicating a more than twofold increase in scent production in ripe fruits. The increase in scent production in ripe fruits was significantly higher in lemur-specialist species (two-tailed Welch two-sample t test, log-transformed data: $t = -2.45, df = 25.94, P = 0.02$).

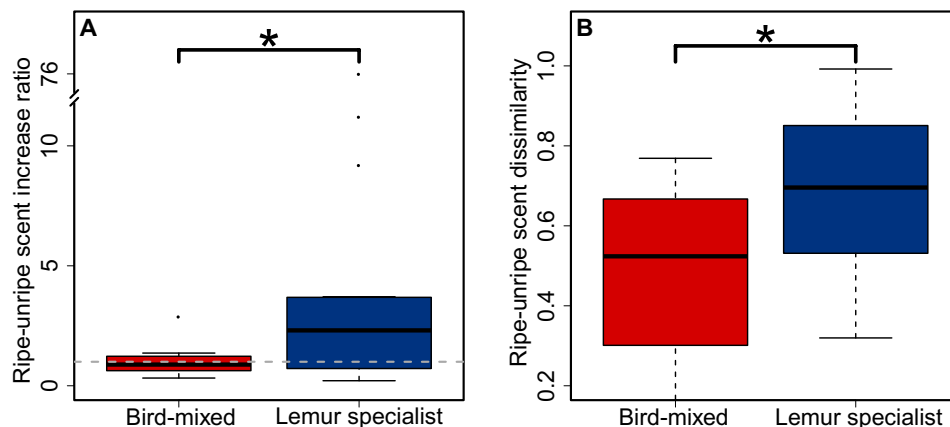


Fig. 1. Shifts in fruit scent upon ripeness. (A) Scent increase ratio: changes in overall VOC emission upon ripeness. y axis is the ratio between the overall VOC emission of ripe and unripe fruits in a given species. The dashed line marks a ratio of 1, that is, no difference in emission between ripe and unripe fruits. Data presented are raw data, while statistical tests were conducted on log-transformed data to comply with the assumptions of the models. (B) Ripe-unripe scent dissimilarity: changes in chemical composition of fruit scent upon ripeness. Dissimilarity was calculated as the Bray-Curtis dissimilarity index based on relative amounts of 389 VOCs. Asterisks denote significance at $\alpha < 0.05$ in both a GLMM on $n = 90$ individuals and a two-tailed two-sample Welch t test on $n = 30$ species.

We then examined whether lemur-specialist species change the chemical composition of their scent upon ripeness more than bird-mixed species. For each ripe-unripe fruit pair from a single individual, we calculated the Bray-Curtis dissimilarity index based on the relative amounts of 389 VOCs and analyzed the results as above. The use of relative amounts means that these results are fully independent of the changes in scent amount described above. The GLMM was significant compared to the null model, which did not include dispersal syndrome [$\chi^2(1) = 5.3, P = 0.02$]. The model indicates that in lemur-specialist species, the dissimilarity in fruit scent between ripe and unripe fruits is significantly higher than it is in bird-mixed species (GLMM: $P = 0.028$). To verify the robustness of the analysis, we also calculated the mean ripe-unripe chemical dissimilarity for each of the 30 species (Fig. 1B). Median Bray-Curtis dissimilarity indices between ripe and unripe fruits were 0.7 in lemur-specialist species and 0.52 in bird-mixed species. Ripe-unripe scent dissimilarity was significantly higher in lemur-specialist species (two-tailed Welch two-sample t test: $t = -2.32, df = 19.92, P = 0.03$). Notably, the VOCs present in ripe fruits of lemur-specialist and bird-mixed plant species did not show any clustering; that is, lemur specialists have not converged to use the same VOCs (Fig. 2). This pattern is in agreement with the prediction that as long as the function of ripe fruit scent is to signal only the ripeness of a fruit, the specific chemicals used are of lesser importance as long as they are sufficiently different from those emitted by conspecific unripe fruits (26).

We further examined the relationship between scent increase ratio and ripe-unripe scent dissimilarity, under the assumption that species that are selected to offer olfactorily conspicuous fruits would score high on both variables. In the bird-mixed dispersal syndrome,

there was no relationship between the two variables (linear model: $P = 0.94$), whereas in the lemur-specialist syndrome, there was a strong correlation between the two ($P < 0.01$; fig. S1). This finding implies that whereas there is little investment in olfactory signals in the bird-mixed syndrome, among species that specialize on seed dispersal by lemurs, some variation in the degree of investment in olfactory signaling exists, and an increase in the quantity of the scent signal is associated with a larger shift in its chemical composition.

These results cannot be attributed to common ancestry. First, lemur-specialist and bird-mixed species are fully intermingled phylogenetically (Fig. 2). Second, neither the scent increase ratio nor the ripe-unripe chemical dissimilarity indices showed any phylogenetic signal (scent increase ratio: Pagel's lambda $< 0.001, P = 1$; chemical dissimilarity: Pagel's lambda = 0.09, $P = 1$). Last, even these low rates of phylogenetic signal overestimate the similarities between sister taxa, as both scent increase ratio and chemical dissimilarity are statistical constructs that summarize a complex matrix of 389 VOCs. For example, two sister taxa may score similarly on either of the indices and thus appear to be phylogenetically conserved, but emit different VOCs that are produced by distinct biochemical pathways. This similarity can hardly be the result of phylogenetic conservatism. To further evaluate the importance of phylogeny in determining ripe fruit scent, we conducted a cluster analysis based on Bray-Curtis distances between ripe fruits of the 30 species in our model system. The resulting dendrogram is highly different from the phylogeny (Fig. 2). In accordance, ripe fruit scent does not show phylogenetic signal ($K_{\text{mult}} = 0.45, 1000$ permutations, $P = 0.25$). The implication is that fruit scent in this system is not phylogenetically conserved and cannot explain the results reported here.

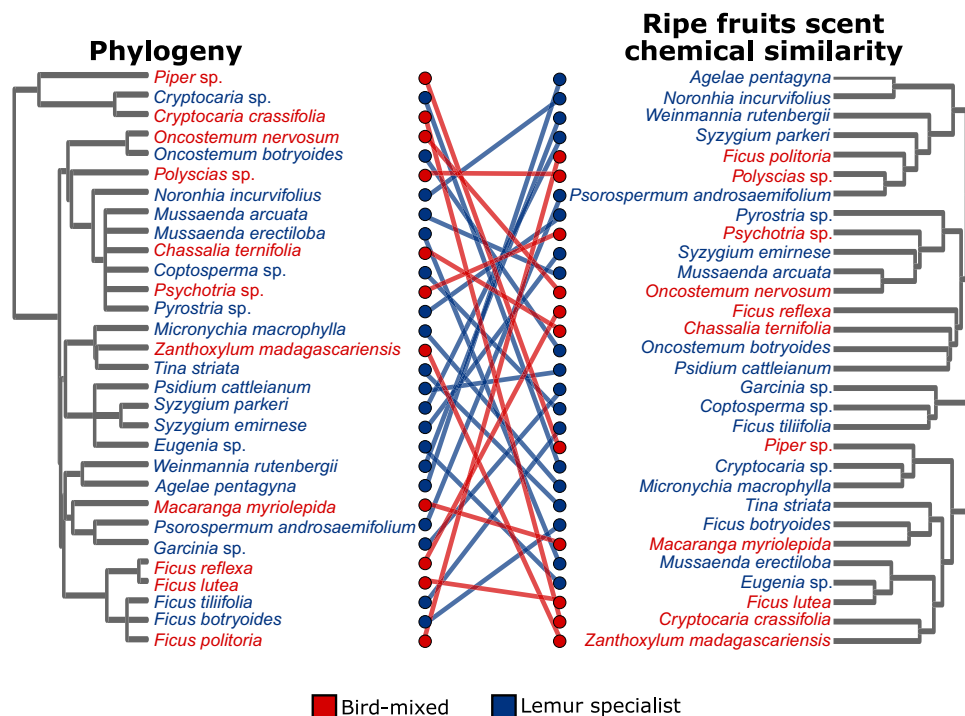


Fig. 2. A tangram of the model plants: Phylogeny versus ripe fruit chemical similarity. The strong dissimilarities between closely related taxa such as the congeneric *Mussaenda*, *Oncostemum*, and *Ficus* indicate a relatively low phylogenetic conservatism in VOC emission. (Left) Phylogeny of model plant species based on Zanne *et al.* (42). (Right) Cluster analysis of ripe fruits based on the Bray-Curtis dissimilarity matrix, calculated on the relative amounts of 389 VOCs.

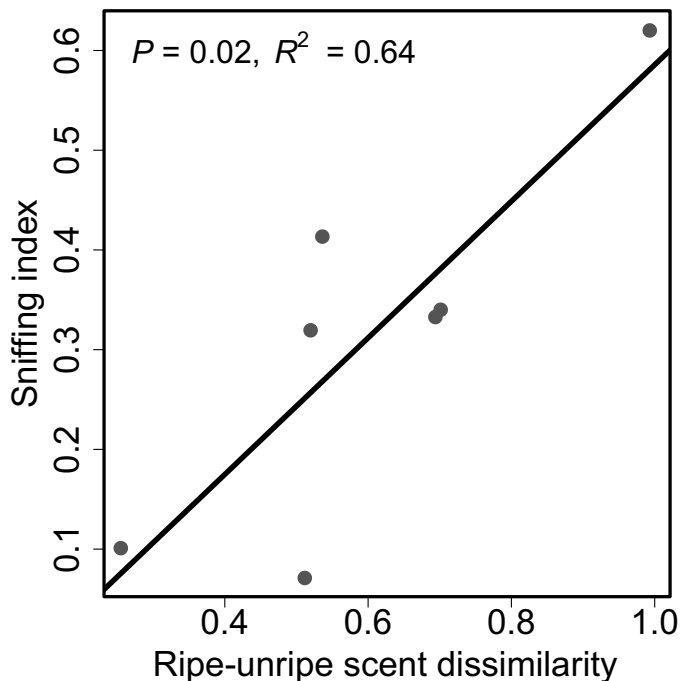


Fig. 3. The relationship between ripe-unripe scent dissimilarity and sniffing index in red-bellied lemurs (*E. rubriventer*). When choosing fruits, lemurs tend to rely on their sense of smell more when foraging on species in which the chemical distance between ripe and unripe fruits is larger. P value and adjusted R^2 are from a linear model.

We then tested the prediction that lemurs rely on fruit scent to identify ripe fruits and that the changes in fruit scent would drive an increase in reliance on olfaction. We followed three groups of red-bellied lemurs (*E. rubriventer*) in Ranomafana National Park for 6 weeks and used focal animal sampling technique to record the food-selection behavior of nine adults and subadults. Each time an individual interacted with an individual fruit, we recorded whether it was sniffed (see Materials and Methods and movie S1). The rationale was that sniffing is an active sampling of scent that is associated with olfactory examination (23). We obtained 534 observations of feeding on seven of the species for which we also sampled fruit scent. For each of the seven species, we calculated a “sniffing index”—the mean probability of the nine lemurs sniffing the fruits of the seven plants. The sniffing index was positively correlated with both scent increase ratio (Spearman correlation: $r = 0.65$) and chemical dissimilarity ($r = 0.86$), although possibly because of the low power of the statistical test, only the latter was statistically significant (linear models: scent increase ratio, $P = 0.12$; scent dissimilarity, $P = 0.02$) (Fig. 3 and fig. S2). Although these results should be interpreted with caution owing to the small number of species, they imply that the greater the difference in the scent of ripe and unripe fruits, the more lemurs rely on olfaction to decide whether or not to eat a fruit.

Our results show that in plant species that specialize on seed dispersal by the olfactorily oriented lemurs, fruits tend to substantially increase the amount of scent emitted and change their chemical composition when ripe, such that lemurs can distinguish them more easily from unripe fruits. This pattern is not simply an inevitable by-product of fruit maturation because these tendencies are

significantly weaker in plant species that do not specialize on olfactorily oriented lemurs for seed dispersal. Nor can these patterns be attributed to common ancestry. Lemurs, in turn, increase their use of olfaction when feeding on those species that change their scent upon ripeness. This indicates that they use fruit scent for fruit selection. Since plants benefit from the lemur’s ability to identify ripe fruits, over time, this behavior is expected to exert selective pressure for increased olfactory conspicuity of ripe fruits and drive the evolution of the patterns reported here. Together, these results strongly support the hypothesis that fruit scent in lemur-dispersed species is an evolved trait, the function of which is to signal ripeness and thus facilitate the mutualistic interaction between primates and plants. This provides strong support for the dispersal syndrome hypothesis, according to which fruit traits are shaped by their respective animal mutualists.

Madagascar is relatively species-poor in bats and birds that interact with plants, and lemurs and plants show a long, tight, and unique history of interaction (31). The evolution of fruit scent as a signal for lemurs is yet another example of the coevolution of lemurs and plants in Madagascar. While it is likely that the lemurs’ unique set of sensory adaptations and long history of isolation from other frugivore guilds make this an extreme case, similar processes may have occurred in other tropical systems (19). It is therefore reasonable to assume that chemical interaction promoting the identification of ripe fruits is pertinent to frugivory and the sensory evolution of all primates, including humans.

MATERIALS AND METHODS

Model system

Samples were collected in the montane rainforest of the Talatakey region of Ranomafana National Park, eastern Madagascar. The frugivore community of the park is composed of four large lemurs, two small nocturnal lemurs, seven birds, and one bat (32). Samples were collected opportunistically. We included all plants from which we could obtain both ripe and unripe fruits from the same individuals, and because of their relative rarity, we tried to include species that are either exclusively or at least, to a large extent, dispersed by birds. We divided the model system into two classes: lemur specialists and bird-mixed. We classified species as lemur specialists if both published records (32–38) and local knowledge concurred that only lemurs eat the fruits of these species. We classified species as bird-mixed when they are either exclusively, primarily, or partially bird-dispersed (table S2 and S3). This broader category includes taxa that are eaten exclusively or primarily by birds and generalist species that are eaten by both birds and lemurs. The assumption is that partial or full reliance on bird seed dispersal would substantially weaken the selection pressure to emit scent signals.

Sample collection

Ripe and unripe fruits were collected from the tree and brought to the laboratory within 3 hours. To eliminate intraindividual variation and to achieve a representative sample of the scent emitted by fruits of a single individual, two to eight fruits obtained from an individual plant were pooled together as a single sample. Scent was sampled using a semistatic headspace procedure similar to that of Nevo *et al.* (19). Fruits were placed in a sampling bag (40 cm; Toppits oven bags, Toppits). One end of the bag was tightly closed with a zip tie, and the other was tightened around a Teflon tube on which a

chromatoprobe scent trap (39) was mounted. The chromatoprobes contained 1.5 mg of Tenax, 1.5 mg of Carbotrap, and 1.5 mg of Carbosieve III (all from Sigma-Aldrich) trapped between layers of glass wool. Samples were left in the chamber for 30 min, after which the air in the bag was pumped for 1 min onto the trap using a membrane pump at 200 ml/min. Scent was then left to build up for another 1.5 hours, after which the air in the bag was pumped onto the same probe for 10 min. The probe was then stored in a 2-ml glass vial sealed with a Teflon cap and stored at -20°C . Control samples were collected by applying the same procedure with empty bags.

Chemical analysis

Samples were analyzed using an Agilent gas chromatograph 7890B equipped with an Agilent DB-5 unpolar capillary column (DB-5, 30 m, 0.25 mm diameter, Agilent Technologies) and a cold injection system (CIS 4C, Gerstel), coupled with an Agilent mass spectrometer 5977A. Samples were introduced to the thermal desorption unit (TDU) at 10°C . After 1 min, the TDU started heating up at 15°C until it reached 300°C , a temperature at which it rested for 15 min. The liner was cooled to -100°C . After the transfer to the liner, it was heated up at $12^{\circ}\text{C}/\text{min}$ until the temperature reached 290°C , which was maintained for 6 min. Initial oven temperature was 50°C . This temperature was maintained for 1 min and then increased by $10^{\circ}\text{C}/\text{min}$ to 325°C , which was held for 20 min. The MS transfer line temperature was set to 280°C , the MS source temperature was set to 230°C , and the MS quad temperature was set to 150°C . The MS operated at electron ionization mode and scanned between 35 and 450 Da.

Samples were analyzed using Amdis 2.71. VOCs were identified on the basis of their mass spectra using the NIST11 mass spectra library and their retention indices, which were calculated on the basis of an *n*-alkane reference mixture. We excluded compounds that are known contaminants (for example, siloxanes and phthalates). Since the control samples included small amounts of genuine plant VOCs, we calculated the mean amount in the controls and subtracted this amount from all samples. Thus, this mean amount was considered a baseline above which the presence of a compound was considered genuine. We quasi-quantified the absolute amounts by comparing the output to multiple runs of an external standard [1 μl of *n*-heptadecane and hexane stock solution (20 ng/ml)], which were run separately and regularly while running the scent samples.

Statistical analysis of chemical data

For analysis of the scent increase ratio, we summed the estimated amount of all VOCs in a sample and divided this amount by the number of fruits in the sample. We then divided the amount per fruit in the ripe fruits by that in the unripe fruits obtained from the same individual plant. Since large ripe fruits develop from large unripe fruits and vice versa, this approach removes the effect of fruit size on total scent production and allows direct comparison of the mean change in scent production across taxa. For ripe-unripe dissimilarity indices, we used the relative amounts of VOCs by dividing the amount of each VOC by the overall amount of VOCs in the sample, thus removing any effect of overall amount of scent. We then calculated the Bray-Curtis dissimilarity index between ripe and unripe fruits obtained from the same individual plant. Bray-Curtis dissimilarities are the most appropriate for these kinds of data because they ignore zeros and therefore do not misclassify two samples as similar when they do not contain the same compounds (40). In both, to verify the robustness of our analysis, we used two sepa-

rate statistical approaches. We first conducted a GLMM using $n = 90$ individuals in which either the log-transformed scent increase ratio or the Bray-Curtis dissimilarity index was used as a response variable, dispersal syndrome was a single fixed factor, and species was a random factor. We verified the assumptions of the models (independence and normal distribution of the residuals) using quantile-quantile plots and histograms and by plotting the residuals versus the model fitted values. We further verified that the results were not strongly affected by individual samples (leverage) by systematically running the models while excluding a single sample in each iteration and calculating the estimated slopes. To verify the robustness of the results, we also calculated the mean ripe-unripe scent increase ratio and Bray-Curtis dissimilarity index in a species and ran two-tailed Welch two-sample *t* tests. The relationship between the two indices of ripe fruit olfactory conspicuousness (fig. S1) was estimated using a linear model. To estimate the phylogenetic signal, we calculated Pagel's lambda (41) using a phylogeny by Zanne *et al.* (42). We used Pagel's lambda because it is more compatible with phylogenies that are not fully resolved (43). To calculate the phylogenetic signal in the full scent profile of ripe fruits, we used K_{mult} (44)—a method originally developed for morphometric analysis that calculates a statistic parallel to Blomberg's *K* (45) in multidimensional data. We used VOC relative amounts averaged in each species and calculated Bray-Curtis dissimilarity indices between all samples. We then conducted a principal coordinate analysis and moved on to calculate K_{mult} on the scores of each species. Thus, our analysis measures the tendency of closely related taxa to score similarly and thus be chemically similar in dissimilarity space.

Behavior: Sampling and analysis

Sampling of behavior took place between 31 October and 10 December 2016 and was done in parallel to scent sampling, thus preventing us from knowing anything about patterns of scent production and chemistry in these species before behavioral data collection. We followed three groups of red-bellied lemurs (*E. rubriventer*), from which nine adults and subadults were identified individually. We followed each group for a week and then switched to the next one. We used focal animal sampling to obtain data on their behavior. We focused on a single individual for 15 min or until we lost visual contact with that individual and then switched to another individual, preferably one that did not precede the current one. During focal animal observations, we recorded every interaction with an individual fruit as a single data point. For each individual fruit, we recorded the plant species and whether the animal sniffed the fruit before either ingesting or rejecting it. We defined "sniffing" as bringing the fruit to immediate proximity to the nostrils without biting it (see also movie S1). We obtained 534 such data points for seven of the plant species for which we also conducted chemical analysis. For each species, we calculated the sniffing index by first dividing the number of fruits sniffed by the total number of fruits interacted with by each individual lemur separately and then averaged this figure across all individuals. We analyzed the data using Spearman correlations between the sniffing index and either the scent increase ratio or ripe-unripe scent dissimilarity. Statistical significance was tested using a linear regression model in which sniffing index was the response variable and scent increase ratio or dissimilarity was a single predictor. All analyses were conducted on R 3.4.3 (46) and the following packages: vegan (47), ape (48), phytools (49), lme4 (50), lmerTest (51), car (52), plotrix (53), and geomorph (54).

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at <http://advances.sciencemag.org/cgi/content/full/4/10/eaat4871/DC1>

Fig. S1. The relationship between scent increase ratio and ripe-unripe dissimilarity.

Fig. S2. The relationship between scent increase ratio and sniffing behavior.

Table S1. VOCs identified in 90 fruit samples of 30 species.

Table S2. Raw data, field season 2016.

Table S3. Raw data, field season 2017.

Table S4. Raw data, behavior.

Movie S1. Female red-bellied lemur (*E. rubriventer*) feeding on Vondavenina fruits at Ranomafana National Park, Madagascar.

REFERENCES AND NOTES

- H. F. Howe, L. C. Westley, *Ecological Relationships of Plants and Animals* (Oxford Univ. Press, 1988).
- K. Bolmgren, O. Eriksson, Fleshy fruits—Origins, niche shifts, and diversification. *Oikos* **109**, 255–272 (2005).
- M. G. M. van Roosmalen, *Fruits of the Guiana Flora* (Institute of Systematic Botany, 1985).
- C. H. Janson, Adaptation of fruit morphology to dispersal agents in a Neotropical forest. *Science* **219**, 187–189 (1983).
- S. B. Lomáscolo, D. J. Levey, R. T. Kimball, B. M. Bolker, H. T. Alborn, Dispersers shape fruit diversity in *Ficus* (Moraceae). *Proc. Natl. Acad. Sci. U.S.A.* **107**, 14668–14672 (2010).
- L. van der Pijl, *Principles of Dispersal in Higher Plants* (Springer, 1982).
- P. Jordano, Angiosperm fleshy fruits and seed dispersers: A comparative analysis of adaptation and constraints in plant-animal interactions. *Am. Nat.* **145**, 163–191 (1995).
- A. Bollen, G. Donati, J. Fietz, D. Schwab, J. B. Ramanamanjato, L. Randrihasipara, L. Van Elsacker, J. U. Ganzhorn, An intersite comparison of fruit characteristics in Madagascar: Evidence for selection pressure through abiotic constraints rather than through co-evolution, in *Tropical Fruits and Frugivores*, J. L. Dew, J. P. Boubli, Eds. (Springer, 2005), pp. 93–119.
- S. B. Lomáscolo, H. M. Schaefer, Signal convergence in fruits: A result of selection by frugivores? *J. Evol. Biol.* **23**, 614–624 (2010).
- J. F. Brodie, Evolutionary cascades induced by large frugivores. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 11998–12002 (2017).
- H. M. Schaefer, A. Valido, P. Jordano, Birds see the true colours of fruits to live off the fat of the land. *Proc. Biol. Sci.* **281**, 20132516 (2014).
- K. Valenta, O. Nevo, C. Martel, C. A. Chapman, Plant attractants: Integrating insights from pollination and seed dispersal ecology. *Evol. Ecol.* **31**, 249–267 (2017).
- H. M. Schaefer, G. D. Ruxton, *Animal-Plant Communication* (Oxford Univ. Press, 2011).
- M. Galetti, R. Guevara, M. C. Cortés, R. Fadini, S. von Matter, A. B. Leite, F. Labacca, T. Ribiero, C. S. Carvahlo, R. G. Collevatti, M. M. Pires, P. R. Guimarães Jr., P. H. Brancalion, M. C. Ribiero, P. Jordano, Functional extinction of birds drives rapid evolutionary changes in seed size. *Science* **340**, 1086–1090 (2013).
- E. K. V. Kalko, M. A. Condon, Echolocation, olfaction and fruit display: How bats find fruit of *flagellichorus cucurbitis*. *Funct. Ecol.* **12**, 364–372 (1998).
- M. Ordano, P. G. Blendinger, S. B. Lomáscolo, N. P. Chacoff, M. S. Sánchez, M. G. Núñez Montellano, J. Jiménez, R. A. Ruggera, M. Valoy, The role of trait combination in the conspicuousness of fruit display among bird-dispersed plants. *Funct. Ecol.* **31**, 1718–1727 (2017).
- R. Hodgkison, M. Ayasse, C. Häberlein, S. Schulz, A. Zubaid, W. A. Mustapha, T. H. Kunz, E. K. V. Kalko, Fruit bats and bat fruits: The evolution of fruit scent in relation to the foraging behaviour of bats in the New and Old World tropics. *Funct. Ecol.* **27**, 1075–1084 (2013).
- R. M. Borges, J.-M. Bessière, M. Hossaert-McKey, The chemical ecology of seed dispersal in monoecious and dioecious figs. *Funct. Ecol.* **22**, 484–493 (2008).
- O. Nevo, E. W. Heymann, S. Schulz, M. Ayasse, Fruit odor as a ripeness signal for seed-dispersing primates? A case study on four Neotropical plant species. *J. Chem. Ecol.* **42**, 323–328 (2016).
- O. Nevo, R. O. Garrí, L. T. Hernandez Salazar, S. Schulz, E. W. Heymann, M. Ayasse, M. Laska, Chemical recognition of fruit ripeness in spider monkeys (*Ateles geoffroyi*). *Sci. Rep.* **5**, 14895 (2015).
- P. C. Wright, V. R. Razafindratsita, S. T. Pochron, J. Jernvall, The key to Madagascar frugivores, in *Tropical Fruits and Frugivores: The Search for Strong Interactors*, J. L. Dew, J. P. Boubli, Eds. (Springer, 2005), pp. 121–138.
- K. Valenta, M. Edwards, R. R. Rafaliarison, S. E. Johnson, S. M. Holmes, K. A. Brown, N. J. Dominy, S. M. Lehman, E. J. Parra, A. D. Melin, Visual ecology of true lemurs suggests a cathemeral origin for the primate cone opsin polymorphism. *Funct. Ecol.* **30**, 932–942 (2016).
- O. Nevo, E. W. Heymann, Led by the nose: Olfaction in primate feeding ecology. *Evol. Anthropol.* **24**, 137–148 (2015).
- M. J. E. Charpentier, J. C. Crawford, M. Boulet, C. M. Drea, Message 'scent': Lemurs detect the genetic relatedness and quality of conspecifics via olfactory cues. *Anim. Behav.* **80**, 101–108 (2010).
- K. Valenta, R. J. Burke, S. A. Styler, D. A. Jackson, A. D. Melin, S. M. Lehman, Colour and odour drive fruit selection and seed dispersal by mouse lemurs. *Sci. Rep.* **3**, 2424 (2013).
- O. Nevo, K. Valenta, The ecology and evolution of fruit odor: Implications for primate seed dispersal. *Int. J. Primatol.* **39**, 338–355 (2018).
- S. S. Steiger, A. E. Fidler, M. Valcu, B. Kempenaers, Avian olfactory receptor gene repertoires: Evidence for a well-developed sense of smell in birds? *Proc. Biol. Sci.* **275**, 2309–2317 (2008).
- Y. Niimura, A. Matsui, K. Touhara, Acceleration of olfactory receptor gene loss in primate evolution: Possible link to anatomical change in sensory systems and dietary transition. *Mol. Biol. Evol.* **35**, 1437–1450 (2018).
- A. T. D. Bennett, M. Théry, Avian color vision and coloration: Multidisciplinary evolutionary biology. *Am. Nat.* **169**, S1–S6 (2007).
- H. E. M. Dobson, Relationship between floral fragrance composition and type of pollinator, in *Biology of Floral Scent*, N. Dudareva, E. Pichersky, Eds. (CRC Press, 2006), pp. 147–198.
- R. W. Sussman, P. H. Raven, Pollination by lemurs and marsupials: An archaic coevolutionary system. *Science* **200**, 731–736 (1978).
- O. H. Razafindratsima, A. E. Dunham, Co-fruiting plant species share similar fruit and seed traits while phylogenetic patterns vary through time. *J. Ecol.* **104**, 1789–1798 (2016).
- S. Atsalis, Diet of the brown mouse lemur (*Microcebus rufus*) in Ranomafana National Park, Madagascar. *Int. J. Primatol.* **20**, 193–229 (1999).
- O. H. Razafindratsima, thesis, Rice University (2015).
- F. Dowsett-Lemaire, Fruit choice and seed dissemination by birds and mammals in the evergreen forests of Malawi. *Rev. Ecol.* **43**, 251–285 (1988).
- B. H. Daru, K. Yessoufou, C. Nuttman, J. Abalaka, A preliminary study of bird use of fig *Ficus* species in Amurum Forest Reserve, Nigeria. *Malimbus* **37**, 1–15 (2015).
- E. A. Martin, L. Ratsimisetra, F. Laloë, S. M. Carrière, Conservation value for birds of traditionally managed isolated trees in an agricultural landscape of Madagascar. *Biodivers. Conserv.* **18**, 2719–2742 (2009).
- J. L. Dew, P. C. Wright, Frugivory and seed dispersal by four species of primates in Madagascar's eastern rain forest. *Biotropica* **30**, 425–437 (1998).
- S. Dötterl, A. Jürgens, Spatial fragrance patterns in flowers of *Silene latifolia*: Lilac compounds as olfactory nectar guides? *Plant Syst. Evol.* **255**, 99–109 (2005).
- R. R. Junker, A biosynthetically informed distance measure to compare secondary metabolite profiles. *Chemoecology* **28**, 29–37 (2017).
- M. Pagel, Inferring the historical patterns of biological evolution. *Nature* **401**, 877–884 (1999).
- A. E. Zanne, D. C. Tank, W. K. Cornwell, J. M. Eastman, S. Smith, R. G. FitzJohn, D. J. McGlinn, B. C. O'Meara, A. T. Moles, P. B. Reich, D. L. Royer, D. E. Soltis, P. F. Stevens, M. Westoby, I. J. Wright, L. Aarssen, R. I. Bertin, A. Calaminus, R. Govaerts, F. Hemmings, M. R. Leishman, J. Oleskyn, P. S. Soltis, N. G. Swenson, L. Warman, J. M. Beaulieu, Three keys to the radiation of angiosperms into freezing environments. *Nature* **506**, 89–92 (2014).
- R. Molina-Venegas, M. Á. Rodríguez, Revisiting phylogenetic signal: strong or negligible impacts of polytomies and branch length information? *BMC Evol. Biol.* **17**, 53 (2017).
- D. C. Adams, A generalized *K* statistic for estimating phylogenetic signal from shape and other high-dimensional multivariate data. *Syst. Biol.* **63**, 685–697 (2014).
- S. P. Blomberg, T. Garland Jr., A. R. Ives, Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution* **57**, 717–745 (2003).
- R Core Team, R: A language and environment for statistical computing (R Foundation for Statistical Computing, 2017); www.R-project.org/.
- J. Oksanen, F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, H. Wagner, vegan: Community Ecology Package (R package version 2.5-2, 2018); <https://CRAN.R-project.org/package=vegan>.
- E. Paradis, J. Claude, K. Strimmer, APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290 (2004).
- L. J. Revell, phytools: An R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**, 217–223 (2012).
- D. Bates, M. Mächler, B. Bolker, S. Walker, Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48 (2015).
- A. Kuznetsova, P. B. Brockhoff, R. H. B. Christensen, lmerTest package: Tests in linear mixed effects models. *J. Stat. Softw.* **82**, 1–26 (2017).
- J. Fox, S. Weisberg, *An R Companion to Applied Regression* (Sage, ed. 2, 2011).
- J. Lemon, Plotrix: A package in the red light district of R. *R News* **6**, 8–12 (2006).
- D. C. Adams, M. L. Collyer, A. Kaliontzopoulou, E. Sherratt, Geomorph: Software for geometric morphometric analyses (R package version 3.0.5, 2017); <https://cran.r-project.org/package=geomorph>.

Acknowledgments: We thank P. Wright, J. C. Razafimahimodison, P. Rabeson, and the entire staff of Centre ValBio for their help and support during field work. We thank P. Thomas for sharing the video available as online supplementary material, J. Kuppler for helping with chemical analysis of the samples, and K. Valenta for help in writing this manuscript. We also

thank D. Adams for his advice on the K_{mult} analysis. We thank the editor and two anonymous reviewers for their helpful comments on a previous version of this manuscript. Samples were collected under research permit number 203/16/MEEF/SG/DGF/DSAP/SCB.Re and exported under permit number 322N-EV11/MG16. We thank Madagascar Institute pour la Conservation des Ecosystèmes Tropicaux (MICET) for help in obtaining research and export permits.

Funding: The project was funded by the Deutsche Forschungsgemeinschaft (grant no. NE 2156/1-1). **Author contributions:** O.N. obtained funding, designed the project, collected and analyzed the samples, conducted statistical analysis, and wrote the manuscript. D.R. helped collect and process the samples and collected all behavioral data. J.A.J.J. helped in laboratory work. S.S. participated in chemical analysis. M.A. helped in project development and funding acquisition, helped in writing the manuscript, and provided access to laboratory facilities. All authors have read and approved the final version of this manuscript. **Competing interests:**

The authors declare that they have no competing interests. **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

Submitted 3 March 2018

Accepted 28 August 2018

Published 3 October 2018

10.1126/sciadv.aat4871

Citation: O. Nevo, D. Razafimandimby, J. A. J. Jeffrey, S. Schulz, M. Ayasse, Fruit scent as an evolved signal to primate seed dispersal. *Sci. Adv.* **4**, eaat4871 (2018).

Fruit scent as an evolved signal to primate seed dispersal

Omer Nevo, Diary Razafimandimby, Juan Antonio James Jeffrey, Stefan Schulz and Manfred Ayasse

Sci Adv 4 (10), eaat4871.
DOI: 10.1126/sciadv.aat4871

ARTICLE TOOLS	http://advances.sciencemag.org/content/4/10/eaat4871
SUPPLEMENTARY MATERIALS	http://advances.sciencemag.org/content/suppl/2018/10/01/4.10.eaat4871.DC1
REFERENCES	This article cites 42 articles, 7 of which you can access for free http://advances.sciencemag.org/content/4/10/eaat4871#BIBL
PERMISSIONS	http://www.sciencemag.org/help/reprints-and-permissions

Use of this article is subject to the [Terms of Service](#)