REPORT

EVOLUTIONARY BIOLOGY

Cognition-mediated evolution of low-quality floral nectars

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Plants pollinated by hummingbirds or bats produce dilute nectars even though these animals prefer more concentrated sugar solutions. This mismatch is an unsolved evolutionary paradox. Here we show that lower quality, or more dilute, nectars evolve when the strength of preferring larger quantities or higher qualities of nectar diminishes as magnitudes of the physical stimuli increase. In a virtual evolution experiment conducted in the tropical rainforest, bats visited computer-automated flowers with simulated genomes that evolved relatively dilute nectars. Simulations replicated this evolution only when value functions, which relate the physical stimuli to subjective sensations, were nonlinear. Selection also depended on the supply/demand ratio; bats selected for more dilute nectar when competition for food was higher. We predict such a pattern to generally occur when decision-makers consider multiple value dimensions simultaneously, and increases of psychological value are not fully proportional to increases in physical magnitude.

When presented with a choice, hummingbirds and nectar-feeding bats prefer concentrated solutions of up to 60% sugar (1–3). Plants that are specialized for vertebrate pollination, however, generally offer relatively dilute nectars of 18 to 23% sugar (4, 5). Nectar value depends on both quality and quantity. Quality is primarily determined by sugar concentration, which is genetically controlled and relatively consistent for individual flowers (6–9). However, multiple foragers normally visit the same flowers, which causes nectar quantity to be highly variable and dependent on the elapsed time since the previous visit. Consumer behavior thus determines availability, introducing a complex dynamic into the decision-making process. To study the factors contributing to the evolution of dilute nectars, we incorporated consumer behavior into a virtual evolution experiment by having free-range bats visit artificial flowers.
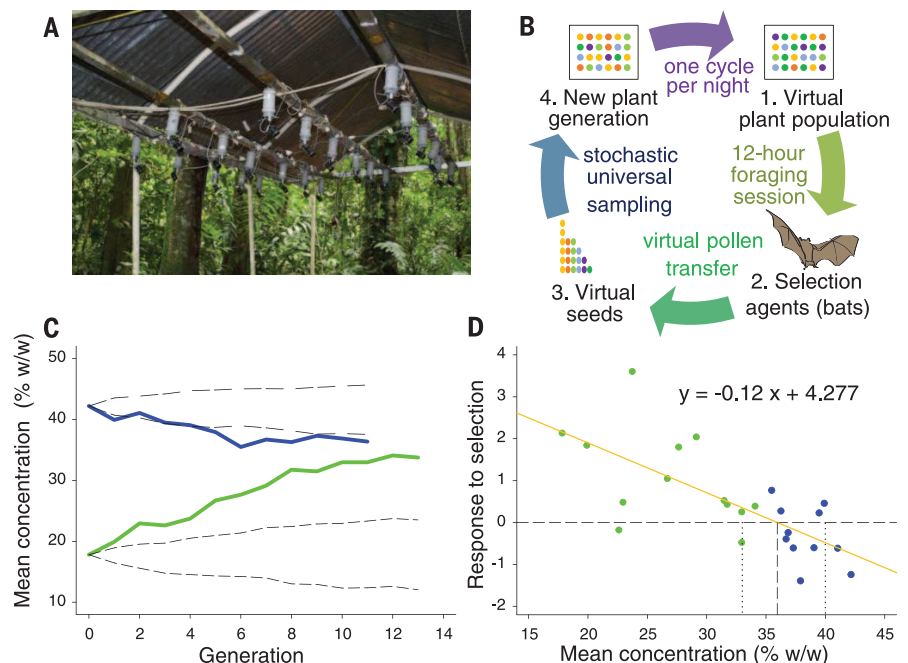
Field experiments were conducted with free-range adult *Glossophaga commissarisi* bats that had been outfitted with radio-frequency ID tags in the rainforest at La Selva Biological Station, Costa Rica. We presented bats with a computer-controlled array of 23 artificial flowers (Fig. 1A). Each flower was equipped with an ID sensor and a photogate (a device that recorded the duration of a bat visit) and connected to a nectar-pump system capable of delivering nectar of defined sugar concentration and volume (10). The density of the array approximated the distribution of a local bat-pollinated bromeliad (*Werauhia gladioliflora*) that provides nectar with sugar concentrations between 14 and 16% (11). Visiting bats were rewarded with nectar, and the amount of nectar was determined by the secretion rate and the elapsed time since the previous bat visit.

We assumed individual plants in our population to have equal rates of photosynthesis and invest equal amounts of photosynthate, as sugar, into nectar (9). However, the sugar concentration of the nectar was determined by a flower's virtual genome, which consisted of a diploid set of four diallelic genes. The effect of the eight codominant alleles determined the water component of the nectar and thus its final sugar concentration. Some alleles coded for smaller water components and some alleles coded for larger water components, making the sugar concentration of nectar a multilocus trait (12).

Upon leaving a flower, a bat was assumed to transfer virtual pollen to the next flower and generate a virtual offspring seed there. Twenty-three such seeds, selected from a night's production by stochastic universal sampling, formed the next generation of virtual plants (Fig. 1B).

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Fig. 1. Wild bats exert stabilizing selection on nectar concentration. (**A**) Artificial flower array. (**B**) Formation of plant generations. Starting population of plants (1); each plant has its own genome coding for water production rate in flower nectar. Bats visit flowers for nectar (2) and generate virtual seeds (3). The most common genotypes are most likely represented in the new generation of plants (4), which is then presented to bats. (**C**) Whether the initial mean sugar concentration [% weight of sugar/total weight of the solution (% w/w)] was low or high, bats selected for intermediate concentrations; solid lines indicate low (green) and high (blue) initial mean sugar concentrations. Phenotypic trajectories lie outside those expected under genetic drift; dashed lines indicate 95% prediction intervals. (**D**) Each dot represents a difference in mean sugar concentration between the offspring generation and the generation from the previous night, where green indicates low and blue indicates high initial mean sugar concentrations. Dashed lines show equilibrium concentration, and dotted lines show 95% confidence intervals. The orange line shows regression.

$$y = -0.12\,x + 4.277$$

Fig. 2. Psychophysical and population effects on decision making. (**A** and **B**) Probabilities of choosing an option compared to a reference (black circles) for nectar sugar concentration (A) or volume (B). The different line types (solid, dashed, or dotted) represent curves calculated from psychometric functions of intensity perception based on different references (black circles). Probabilities change more for decreased than increased values (*2*), and this asymmetry is stronger at low magnitude. Symbols with error bars labeled "3 bats" or "9 bats" are medians (± interquartile ranges) of concentration (% w/w) or volume experienced during laboratory experiments over the complete runs; distributions differ primarily in volume. (A, inset) A psychometric function of intensity perception. Such functions were used to calculate choice probabilities in (A) and (B) for specific reference values (black circles). (**C**) An analytical example of reward value maximization when sugar energy is fixed but water content is variable, assuming logarithmic preference for alternatives. Consider a flower with a fixed rate of sugar production and an independent rate of water added to this sugar. When harvested by a single forager at regular time intervals (20, 60, and 120 min), the amount of sugar collected per visit decreases when collection intervals decrease. Iso-caloric lines connect combinations of volume and concentration with identical quantities of sugar in a portion resulting from the fixed revisit interval and constant secretion rate. Black dots show the nectar concentration at which perceived value is maximal. As the amount of sugar in a portion decreases, the optimal concentration also decreases (arrow).
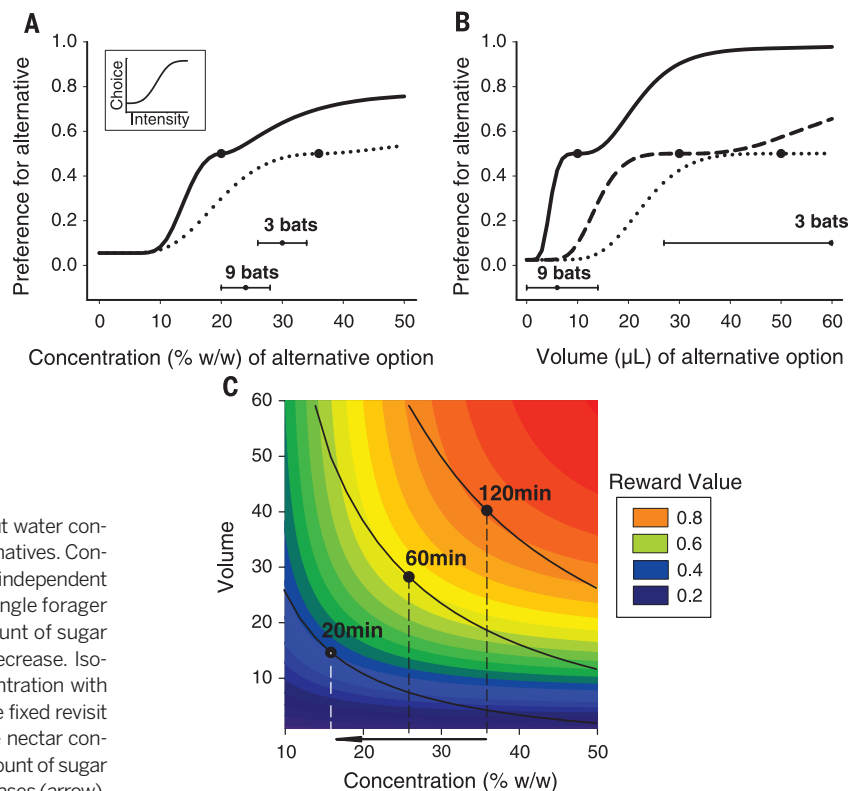
Flowers that were visited more frequently were thus more likely to have their alleles represented in the next generation.

We tracked the evolution of two lineages of artificial flowers over multiple generations. The evolutionary outcome differed significantly from that expected under genetic drift (Fig. 1C). Bats made fewer visits to flowers with either very dilute or very concentrated nectar (fig. S1). As a result, sugar concentration of nectar in both lineages, which started from 17.8% for dilute nectar or 42.2% for concentrated nectar, evolved to about 36% [95% confidence interval (CI) 33 to 40%] within 10 to 12 generations, where one generation was produced per night (Fig. 1, C and D). This result was consistent with stabilizing selection converging on an equilibrium [Fig. 1D; linear regression: $P < 0.001$, adjusted coefficient of determination (R^2) = 0.48].

An optimal forager should choose among available alternatives based on the highest energy return. However, studies of the ability of bats to discriminate nectar volume (*13*) and concentration (*2, 10*) in binary choice designs have yielded psychometric curves consistent with Weber's law (Fig. 2, A and B). This law states that the ability to perceive a stimulus as different from another requires a minimum difference in intensity that is proportional to the intensity of the initial stimulus (*2, 14*). The proportional processing of volume and concentration may result from underlying subjective value (psychophysical) functions, in which value progressively increases with stimulus magnitude, but with a decreasing slope (*10, 13–16*).

With such concave-down value functions (e.g., logarithmic or power functions), if nectar quality changes by a specific amount, then a reduction in magnitude changes choice probabilities more strongly than a corresponding increase in magnitude. As seen in the psychometric function, the slope decreases with higher magnitudes (Fig. 2, A and B). This is relevant here, where nectar rewards are evaluated along two dimensions (volume and sugar concentration) and where there is a trade-off between a decrease in value along one dimension and an increase along the other dimension.

To explore potential effects of proportional processing on natural selection of nectar concentrations, we modeled the experimental flower array and tested the evolutionary consequences of virtual nectar-foraging bats. The virtual bats made choices by integrating information about nectar volume and concentration into a single representation of value (supplementary materials and methods). Our simulations contrasted logarithmic value functions (Fig. 2) with functions with a linear relationship between choice probability and caloric value. We also examined how the supply/demand ratio influences selection dynamics. At low supply, bats encountered smaller volumes of nectar, which made discriminating nectar quantity easier because smaller outcomes are represented internally more sharply than larger ones (*14*) (Fig. 2B).

Both psychophysics and pollinator density may thus affect nectar evolution. To elucidate their influences, we performed four simulations comparing linear versus nonlinear value functions in conditions of either high or low supply/

demand ratios. Only the simulations that incorporated nonlinear value functions (Fig. 3, A, B, E, and F) were congruent with the main result of our field experiments in that evolved concentrations converged to intermediate equilibrium values. In contrast, simulations incorporating linear value functions resulted in either directional selection to sugar concentrations greater than 43% or no selection (Fig. 3, C, D, G, and H). Because all simulations incorporated the designed dynamics of our flower array and the frequency and consistency of flower visits by bats in similar foraging situations, concave-down value functions appear essential to understanding our experimental results. Furthermore, we found that the simulations with a low supply/demand ratio (nine bats) resulted in lower equilibrium sugar concentration [Fig. 3F; 18.7 ± 0.1% (mean ± 95% CI); linear regression: $P < 0.001$, adjusted R^2 = 0.34] than simulations with a high supply/demand ratio (three bats) [Fig. 3E; 26.8 ± 0.2% (mean ± 95% CI); linear regression: $P < 0.001$, adjusted R^2 = 0.12]. This suggests that the evolutionary trajectory of the sugar concentration of nectar is influenced by the availability of nectar. We therefore tested our cognitive evolutionary model in the laboratory, which enabled independent control of the amount of sugar available and the number of bats.

Laboratory experiments were conducted using captive bats from our laboratory colony. We analyzed the evolution of an array of computer-controlled flowers in situations of high demand (nine bats foraging) versus situations of low demand (three bats foraging). There were between
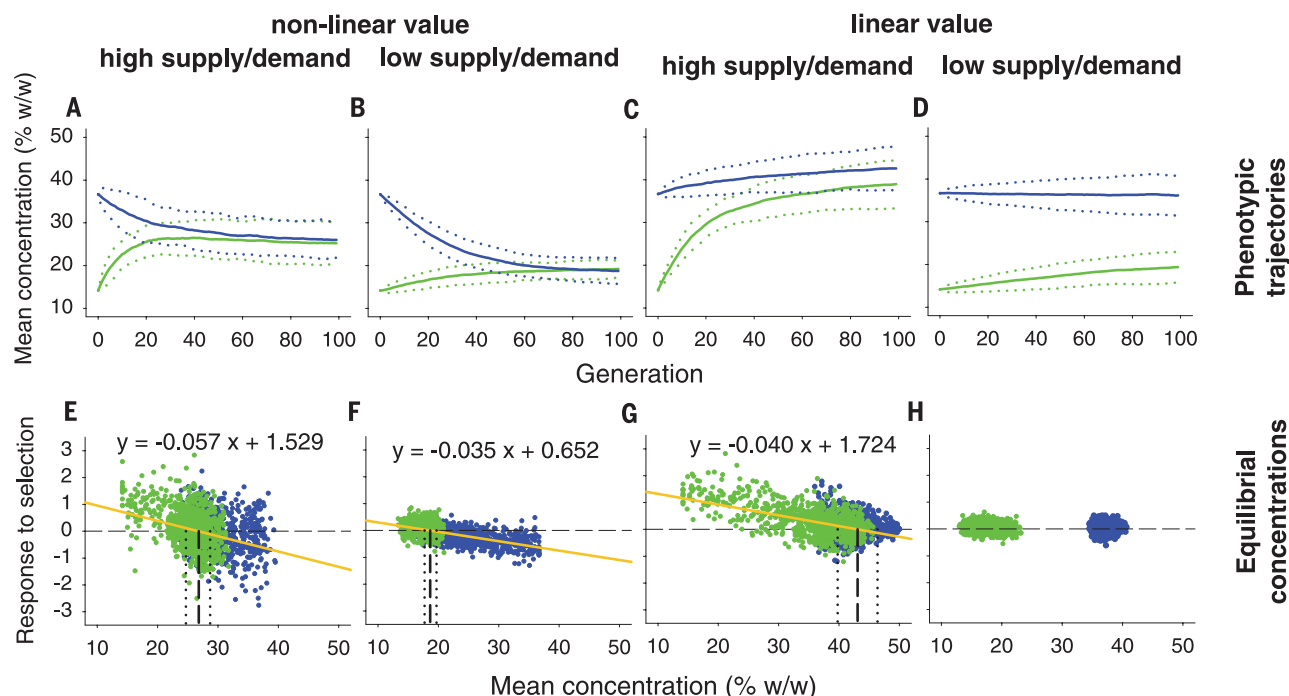
Fig. 3. Selection for dilute nectar can be driven by nonlinear reward evaluation. Selection in a low-demand situation, where only three bats share available nectar (**A**, **C**, **E**, and **G**), and in a high-demand situation, where nine bats share available nectar (**B**, **D**, **F**, and **H**). Virtual bats encoded volume and sugar concentration either logarithmically (A, B, E, and F) or linearly (C, D, G, and H) with respect to caloric contents. Simulations had 100 generations, and *n* equals 100 replications. (A to D) Phenotypic trajectories; notation as in Fig. 1C, except here 95% prediction intervals indicated by dotted lines. (E to H) Responses to selection; notation as in Fig. 1D. Only a random selection of 10% of data points are shown.
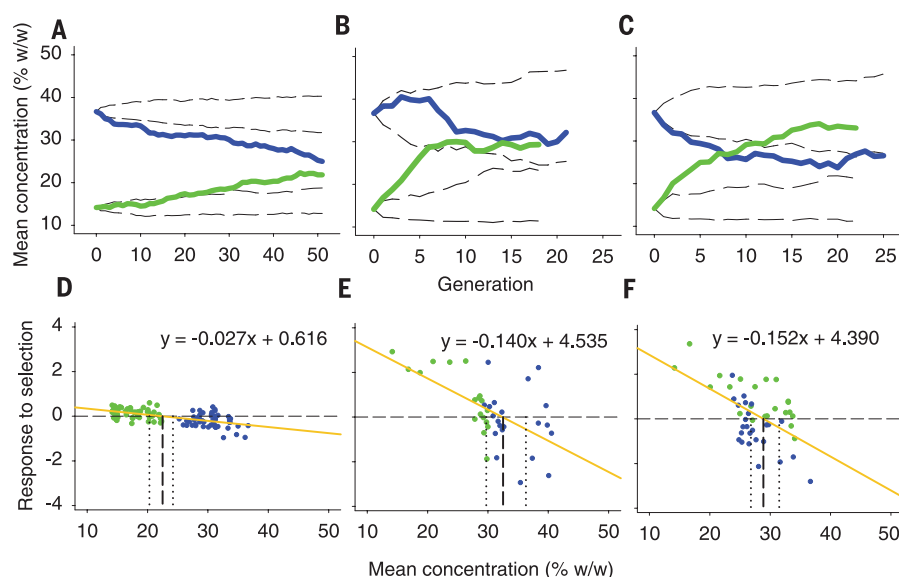
Fig. 4. The mean sugar concentration at equilibrium depends on nectar demand. Selection exerted by nine bats (high demand) (**A** and **D**) caused slower convergence and a shift to a lower equilibrium sugar concentration than selection exerted by three bats (low demand) (**B**, **C**, **E**, and **F**). (A to C) Phenotypic trajectories; notation as in Fig. 1C. (D to F) Responses to selection; notation as in Fig. 1D. The supply was kept at the same constant rate for all experiments.

15 and 50 successive generations in each selective lineage. The flowers of the initial parental generations produced nectar with either high or low mean levels of sugar concentration. Data were collected automatically, and the results were analyzed using algorithms that were essentially identical to those used in the field study (supplementary materials).

These results replicated the fundamental finding from the field experiment: An intermediate equilibrium sugar concentration of nectar evolved (Fig. 4). The equilibrium concentration

was also shown to be affected by the supply/demand ratio; the concentration was lower under high competition [Fig. 4, A and D; 22 ± 2% (mean ± 95% CI); linear regression: $P < 0.001$, adjusted $R^2 = 0.35$] than under low competition [Fig. 4, B and E; 33 ± 3% (mean ± 95% CI); linear regression: $P < 0.001$, adjusted $R^2 = 0.33$; and Fig. 4, C and F; 29 ± 2%; linear regression: $P < 0.001$, adjusted $R^2 = 0.29$]. The equilibrium from the high-competition treatment was also closer to the range of sugar concentrations found naturally in bat-pollinated flowers (Fig. 4, A and D). There was no significant difference in equilibrium sugar concentrations due to treatment order in the groups with three bats [Fig. 4, B, C, E, and F; lm in R (linear model function in R language), $t = 0.11$, $P = 0.92$; overall model $F_{3,82} = 13.28$, $P < 0.001$, adjusted $R^2 = 0.30$].

Our studies of the dynamic interactions of bats and flowers show that the evolution of nectars with lower sugar concentration can be driven by pollinators acting as economic decision-makers subject to the principles of psychophysics and reinforcement. If pollinators simply maximized energetic gains through linear-value encoding, they would not exert stabilizing selection pressure on the flowers, and an intermediate concentration would not be selected for (Fig. 3).

Our approach of combining field experiments, simulations, and confirmatory laboratory studies allowed us to elucidate an intricate evolutionary narrative. The dynamic interaction between nectar volume, sugar concentration, and psychophysics would have made predicting the direction

of selection from analyses based on only a single reward dimension impossible. The competition among bats that determines the supply/demand ratio provided an additional layer of causal complexity (figs. S2 and S3). These results demonstrate the power of iterating between simulation and experimentation and suggest a plausible account of the transition to producing nectars with low sugar concentrations in bat-pollinated plants as well as in other species that undergo evolutionary shifts between different pollinators (12, 17–20).

We replicated the natural situation most closely when we reduced the supply/demand ratio by increasing the number of consumers exploiting limited resources. The increase in demand reduced median food portions available at flowers from 60 to 6 μL (Fig. 2B), but weakly affected sugar concentration (Fig. 2A, 30% versus 24%). When overall value is the product of multiple reward dimensions, proportional magnitudes attain importance. Since perceived differences are stronger at smaller physical magnitudes, discrimination along the volume dimension could take priority over the concentration dimension. Therefore, as predicted by our model (Fig. 2C), bats favored increases in volume instead of concentration, shifting the balance toward more dilute nectars. Similar processes are likely to affect the behavior of invertebrate pollinators, where the different shape of the psychometric function for concentration (2) and the preference for nectars with higher sugar concentrations even at the cost of profitability, (21) presumably cause selection for more concentrated nectars. For vertebrate and invertebrate pollinators,

body size correlates positively with nectar production rates and negatively with sugar concentration (5).

In contrast to some psychological models of economic choice that assume nonlinearity in utility (15), our approach is based directly on physiological processes underlying proportional (Fechnerian) reward evaluation. When multiple perceptual dimensions determine value, a trade-off (22) situation may arise as does between nectar volume (13) and sugar concentration (2, 10). These effects of proportional psychophysics on reward evaluation are of a general nature and should be applicable to other choice situations.

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Editor's Summary

Sweet, but not too sweet

Nectar-feeding pollinators, when given a choice, tend to prefer nectar with high concentrations of sugar. Nectar-producing plants, however, tend to produce more dilute nectar. This mismatch between selective force and trait value has long been seen as an evolutionary paradox. Nachev *et al*. used a dynamic flower array that evolved in real time in a Costa Rican rainforest to show that the "paradox" is, in fact, driven by pollinator choices (see the Perspective by Farris). Bat pollinators based their choices on small, nonlinear differences in nectar sweetness, which led to selection for less sweet nectar overall.

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