C. elegans food choice exhibits effort discounting-like behavior

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ABSTRACT

Cost-benefit decisions are ubiquitous in both human and animal behavior. Economists have developed formal models of cost-benefit decision-making by focusing on discounting behavior, the devaluation of a reward based on the costs associated with it. The phylogenetic limits of discounting behavior remain unknown. Here, we provide evidence that the nematode *C. elegans* exhibits behavior closely resembling effort discounting. Given a choice between food options that are easy or difficult to consume, worms devalue the latter in a manner predicted by economic models. We identified a plausible mechanism for this behavior based on differential rates of leaving food patches and demonstrated that this mechanism is disrupted by deficits in dopamine signaling, as in rodents. Together, these results establish *C. elegans* as a potential invertebrate model for discounting behavior and set new phylogenetic bounds on this type of cost-benefit decision-making.

INTRODUCTION

Cost-benefit decisions are ubiquitous in animal behavior, driven by universal evolutionary pressures to maximize survival and reproductive success. Foraging, mate selection, parental investment, and territoriality each demand a balance between costs, such as energy expenditure and risk, and benefits such as resources, mates, and offspring. A better understanding of cost-benefit decision-making, including its biological basis, would advance a variety of fields, including economics, psychology, neuroscience, and behavioral ecology.

Economic theorists have developed a variety of formal models of cost-benefit decision-making by focusing on *discounting* behavior (Samuelson, 1937; Mazur and Coe, 1987). In this context, discounting refers to the tendency of individuals to *devalue* a reward based on the costs associated with it. Costs commonly investigated include delay, risk, and effort. In general, as cost increases, perceived or subjective value decreases. For example, in the case of delay discounting, receiving a guaranteed payment of $35 after 30 days is worth less than receiving $25 today if a person accepts the smaller immediate reward. In risk discounting, participants might choose to receive $50 for certain rather than a 50% chance of receiving $100. In effort discounting, participants might choose to receive $1 with no effort rather than $5 after repeatedly squeezing a stiff handgrip (Stanek and Richter, 2021).

Studies of discounting are not limited to humans. The literature on discounting in rodents is particularly rich, with multiple paradigms for all three forms of discounting (Bailey, Simpson and Balsam, 2016a; Salamone *et al.*, 2018; Gray *et al.*, 2019; Castrellon *et al.*, 2021). In models of delay discounting, for example, mice choose between low-reward and high-reward nose pokes, where the higher reward comes with a delay (Isles *et al.*, 2004). Risk discounting can be modeled by giving subjects a choice between a low-probability lever yielding a large reward (4 food pellets) and a high-probability lever yielding a small reward (1 food pellet) 100% of the time (St. Onge, Chiu and Floresco, 2010). Effort discounting can be modeled by giving rodents a choice between low-reward (2 food pellets) and high-reward (4 food pellets) arms of a T-maze

where a physical barrier must be climbed to obtain the higher reward (Salamone, Cousins and Bucher, 1994). However, behaviorists have also observed discounting in several species of nonhuman apes, monkeys, lemurs, jays, chickens, pigeons, honeybees, and other animals (Isles *et al.*, 2004; Green, Myerson and Calvert, 2010; Hayden, 2016).

The phylogenetic limits of discounting behavior are unknown. We therefore asked whether foraging decisions in the nematode worm *C. elegans* exhibit discounting-like behavior. *C. elegans* is an omnivorous bacterivore. Worms swallow bacteria through rhythmic contractions of the pharynx, a tube-shaped muscular organ that delivers bacterial particles to the gut; each pharyngeal contraction is called a pump. *C. elegans* primarily inhabits rotting plant material such as decaying fruits and stems (Frézal and Félix, 2015). Its natural habitat contains thousands of different bacterial species (Samuel *et al.*, 2016). Each species has a characteristic nutritional quality, defined in terms of the growth rate of individual worms cultured on that species (Avery and Shtonda, 2003; Samuel *et al.*, 2016). *C. elegans* food preferences are sensitive not only to nutritional quality but also to food density in a manner that satisfies quantitative economic criteria for choices based on subjective value (Katzen *et al.*, 2023). *C. elegans* foraging behavior has been used to investigate the neuronal and genetic basis of exploration-exploitation trade-offs (Bendesky *et al.*, 2011; Milward *et al.*, 2011), a classical example of cost-benefit decision-making. In the laboratory, worms feed on patches of bacteria grown on agarose plates. They leave and re-enter a patch many times. Leaving rates are low on high-quality lawns but increase if the bacterial food is depleted or is difficult to ingest (Shtonda and Avery, 2006; Bendesky *et al.*, 2011; Milward *et al.*, 2011; Olofsson, 2014; Scheer and Bargmann, 2023). This response pattern suggests that leaving promotes exploration to find a better food source.

The fact that *C. elegans* exhibits subjective-value based choice and cost-benefit feeding decisions suggested to us that it might also exhibit discounting behavior. Of the various forms of discounting, we focused on effort discounting as it uniquely does not require learned instrumental responses, like lever pressing for rewards, which are not feasible in *C. elegans*. We modeled our experimental procedure on rodent T-maze studies, where one arm requires more

effort to reach the food reward (Salamone *et al.*, 2018). We baited the arms of a miniature T-maze with selected densities of a single species of bacteria. One arm contained normal bacteria, while the other arm contained a bacterial preparation in which the cells had been elongated, which we predicted would make them more effortful to ingest.

We found that worms exhibit choice behavior that closely resembles effort discounting. Given a choice between equal densities of normal and elongated bacteria, worms prefer normal bacteria. This preference can be reversed by presenting elongated bacteria at a higher density than normal bacteria, indicating that high food density compensates for greater effort, as in rodent models. Furthermore, worms can be made indifferent to the two food options by adjusting relative food densities. It is therefore possible to measure the extent of devaluation and accurately predict novel indifference points. Preference for normal bacteria can be attributed to an increased patch-leaving rate on elongated food. Additionally, typical levels of discounting require intact dopamine signaling. Our results expand the repertoire of cost-benefit behaviors in *C. elegans* and demonstrate that lower invertebrates are capable of effort discounting-like behavior.

RESULTS

Elongated bacteria require more effort to eat

We conceptualized feeding effort in *C. elegans* as the amount of pharyngeal muscle activity required to ingest a given quantity of food. The starting point of our experiments was to make bacteria more effortful to consume. Computer simulations of pharyngeal mechanics predict that large bacterial cells are swallowed less efficiently than small cells (Avery and Shtonda, 2003). Consistent with this result, worms develop more slowly when cultured on large bacteria, possibly because they are acquiring nutrients less efficiently. These findings led to the hypothesis that larger bacteria require more effort to eat, necessitating more pharyngeal pumps per unit of food consumed. To test this hypothesis, we artificially increased the size of an easily ingested bacterium, *Comamonas* spp., using the antibiotic cephalexin. In Gram-negative bacteria such as *Comamonas*, cephalexin interferes with bacterial cell septation during mitosis, resulting in elongated, spaghetti-like bacterial filaments that nevertheless remain edible (Millet *et al.*, 2022).

Pharyngeal pumping rates in normal and elongated bacteria were measured in the reference strain N2 by placing individual worms in a microfluidic channel filled with a bacterial suspension and fitted with electrodes that record the electrical signals associated with each pharyngeal contraction (Lockery *et al.*, 2012). Bacteria were washed with cephalexin-free buffer before being fed to worms. Pumping frequencies in normal and elongated bacteria were equivalent (Fig. 1A; Table 1, row 1) indicating that worms expend approximately the same amount of effort per unit time feeding on normal and elongated food. To test the hypothesis that elongation interferes with food absorption, we measured nutrient uptake in worms cultured on normal or elongated bacteria, using fat stores as a proxy for carbohydrate and lipid consumption. This was done by staining fat stores with the specific label Oil-Red-O (Soukas *et al.*, 2009) and quantifying the density of staining after a two-day culture period extending from the L1 to the L4 stage. We found that fat levels were significantly lower in worms cultured on elongated food (Fig. 1B2; Table 1, row 2). Given that pumping rate was unchanged in the presence of normal versus elongated bacteria (Fig. 1A), the lower fat stores observed in worms fed elongated bacteria

suggest that the amount of nutrition incorporated per pump is lower in worms feeding on elongated versus normal bacteria. This supports our hypothesis that elongation impairs food transport, requiring longer feeding bouts and thus increased muscular activity to consume an equivalent amount of food.

Our effort-discounting paradigm assumes that the normal and elongated bacteria are equivalent except for the amount of effort required to consume them. For example, elongation could affect nutrient content or olfactory attractiveness of bacteria. *C. elegans* food preferences are sensitive to the nutrient content of bacteria (Feng *et al.*, 2025). We therefore asked whether cephalexin treatment affects nutrient content. We focused on key macronutrients: carbohydrates, lipids, and proteins. For protein and carbohydrates, we used colorimetric assays (Materials and Methods; Fig. 1C,D). For lipids, we used phase separation to harvest and measure lipid content (Materials and Methods; Fig. 1E). In all three tests, we detected no differences between control and cephalexin-treated bacteria (Table 1, rows 3-5). We conclude that cephalexin treatment does not alter bacterial nutritional composition.

Worms are attracted to particular species of bacteria by the characteristic mixture of volatile organic compounds each species emits (Worthy *et al.*, 2018). Thus, another concern is that cephalexin treatment might alter the odors produced by bacteria thereby changing their attractiveness. To control for this possibility, we performed a T-maze assay (Materials and Methods) in which patches of normal and elongated bacteria were placed at the ends of each respective arm. The patches contained equal volumes of bacteria at an optical density (OD) of 1.0. Patches were spiked with sodium azide, a paralytic agent, to trap worms once they reached a patch. Worms were placed at the base of the T-maze and allowed to explore it for 60 minutes after which the number of worms in each patch was counted. A food preference index ($-1 \leq I \leq 1$) was calculated, where positive and negative values indicate preference for normal and elongated bacteria respectively, and $I \cong 0$ denotes indifference between the two options (Materials and Methods). We found that worms under these conditions were indifferent (Fig.

1F2; Table 1, row 6). This result indicates that cephalexin treatment does not alter the relative attractiveness of normal or elongated bacteria.

Figure 1

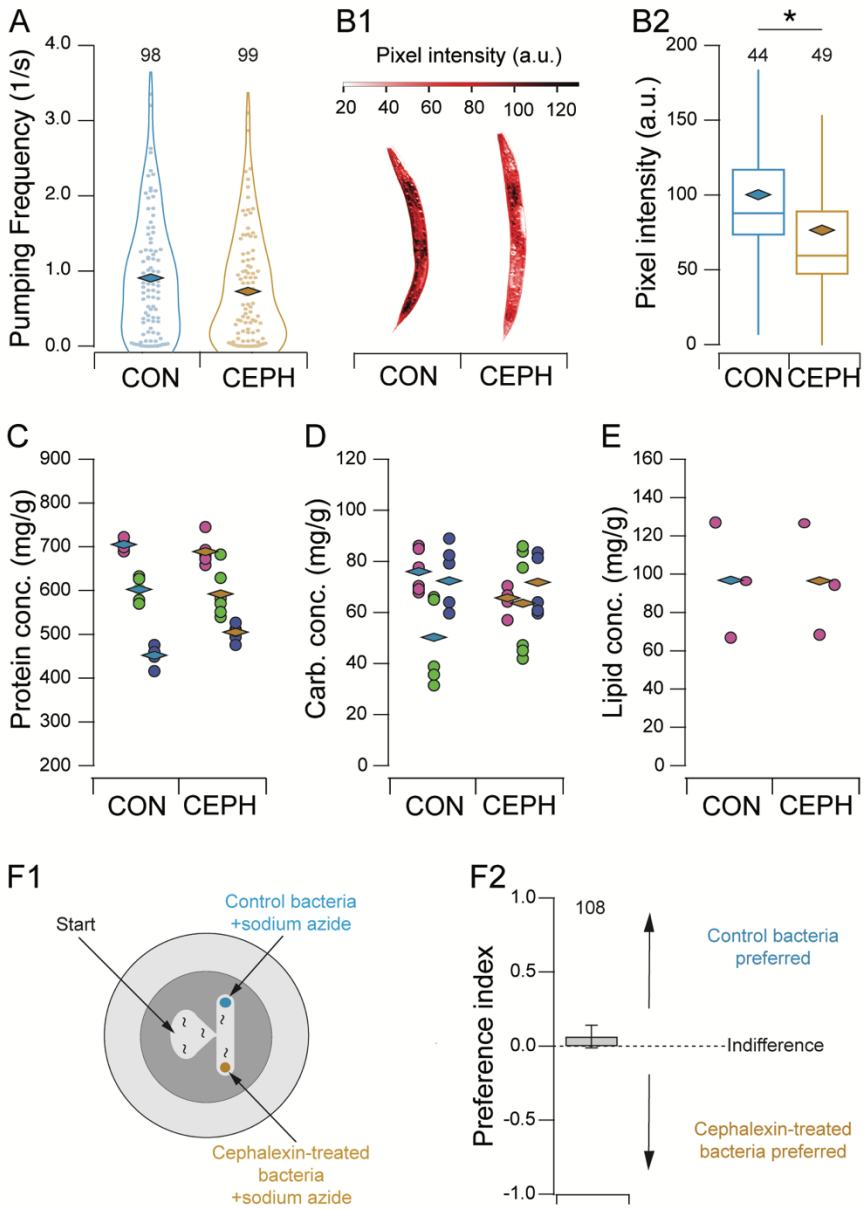


Figure 1. Establishment of an effort discounting paradigm in C. *elegans*.
A. Pharyngeal pumping frequency in suspensions of normal (CON) and elongated (CEPH) bacteria. Symbols (box and whisker plot): *filled circles*, single-worm data points; *diamonds*, means; *numbers*, sample size.
B1. Quantification of Oil-Red-O staining of lipid in worms cultured on normal (CON) or elongated (CEPH) bacteria. Median worms from each condition are shown.

170 B2. Mean pixel intensity of Oil-Red-O staining of lipid in worms cultured on normal (CON) or
171 elongated (CEPH) bacteria. *Diamonds*, means; *numbers*, sample size.
172 C-D. Protein and carbohydrate in worms cultured on normal (CON) and elongated bacteria
173 (CEPH). Columns of data points of the same color are biological replicates run in parallel.
174 Symbols: *filled circles*, technical replicates (n = 6); *diamonds*, means of technical replicates.
175 E. Lipid concentration in worms cultured on normal (CON) and elongated bacteria (CEPH).
176 Symbols: *filled circles*, biological replicates (n = 3); *diamonds*, means of biological replicates.
177 F1. T-maze assay for relative attractiveness of normal and elongated bacteria. Food patches
178 contained sodium azide as a paralytic agent.
179 F2. Mean preference index in the T-maze assay. Error bars, ± 95% CI.

Worms devalue food that requires more effort to eat

Having demonstrated that elongated bacteria require more effort to eat are but equivalent to normal bacteria in terms of nutrition and attractiveness, we next asked whether *C. elegans* exhibits a preference for the normal, easier-to-eat bacteria – evidence of effort discounting-like behavior. We tested this by measuring food preference in the T-maze assay using the same methodology as in Fig. 1F but without the paralytic agent, allowing individual worms to sample both patches. One arm of the T-maze was baited with normal bacteria at an OD of 1.0, while the other arm contained elongated bacteria at ODs of 1.0, 1.5, or 2.0. Worms explored the maze for 60 minutes and the food preference index was computed based on worm counts at the end of this period. There was a significant overall effect of optical density of elongated bacteria on preference (Fig. 2B; Table 1, row 7). When normal and elongated bacteria were at the same concentration (OD 1), worms preferred normal bacteria (I = 0.10; Fig. 2B; Table 1, row 8). This demonstrates that worms do, in fact, devalue elongated food. When normal and elongated bacteria were at OD 1.0 and 2.0, respectively, worms preferred elongated bacteria (I = -0.17; Fig. 2B; Table 1, row 9). This suggests that, in the worm's valuation system, higher food density compensates for greater effort. This outcome was not a foregone conclusion. For instance, it was possible that no amount of elongated food would be sufficient to make worms prefer it. Our results are consistent with findings in rodents, where high-effort options are chosen when the food reward density is sufficiently high (Floresco, Tse and Ghods-Sharifi, 2008). When the optical densities of normal and elongated food were 1.0 and 1.5, respectively, worms were indifferent between the two options (I = 0.03; Fig. 2B; Table 1, row 10). Indifference points are also observed in rodent studies of effort discounting (Salamone, Cousins and Bucher, 1994; van den Bos *et al.*, 2006; Ostrander *et al.*, 2011). Overall, we conclude that in feeding decisions, worms take into account both relative effort and relative food density.

An economic model predicts novel indifference points

Indifference points are significant because, by definition, the presented options have equal perceived value, allowing for the quantification of devaluation. This quantification is only possible at an indifference point. For example, consider a hypothetical human scenario in which

a participant is asked to choose between receiving $20 immediately or $20 with the added cost of climbing 20 flights of stairs. Most participants would choose the immediate $20. Given a choice between $20 immediately and $100 at the cost of climbing 20 flights of stairs, some participants might choose the $100 option. However, suppose that when given a choice between $20 immediately and $80 at the cost of climbing 20 flights of stairs, a particular participant is indifferent between the two options. For this person, under these circumstances, we can conclude that the $20 option is perceived as equal in value to the $80 option. In other words, the $80 option has been discounted (devalued) to $20.

Formally, at an indifference point,

$$V(E) = d(E)V_0$$

where E represents effort, V_0 represents the original value of the reward before devaluation and $V(E)$ is the discounted value of V_0. The quantity $d(E)$ is the discount factor, which, in economic models of discounting, is a function of E. From the above equation,

$$d(E) = \frac{V(E)}{V_0}$$

with $0 \leq d(E) \leq 1$; in the above example $d(E)$ = 20/80 or 1/4. In economic models, $d(E)$ is inversely related to E but the precise form of $d(E)$ varies between models (Figure 2—figure supplement 1).

In our experiments, E is determined by the degree of bacterial elongation. Although this quantity is unknown, it remained unchanged because we used the same cephalexin exposure protocol across experiments (see Materials and Methods). It is analogous to the fixed effort of climbing 20 flights of stairs in the hypothetical example. Assuming a linear relationship between optical density and perceived value, we can determine $d(E)$ from the indifference point shown in Fig. 2B, where V_0 = OD 1.5 and $V(E)$ = OD 1.0, yielding $d(E)$ = 2/3. In the most basic models

of discounting, $d(E)$ is independent of V_0. Assuming independence, other values of V_0 can be inserted into equation 1 to predict novel indifference points. For instance, setting V_0 = OD 3 predicts indifference at $V(E)$ = OD 2, and setting V_0 = OD 0.75 predicts indifference at $V(E)$ = OD 0.5. That is, any pair of optical densities (normal versus elongated) in a 2:3 ratio should be an indifference point. We tested these predictions by measuring preference in T-mazes baited with normal and elongated food at OD ratios (normal:elongated) of 2:3 and 0.5:0.75, finding indifference as predicted (Fig. 2C, Table 1, rows 11-14). The fact that standard discounting models correctly predict indifference points reinforces the conclusion that *C. elegans* exhibits discounting-like behavior.

Figure 2

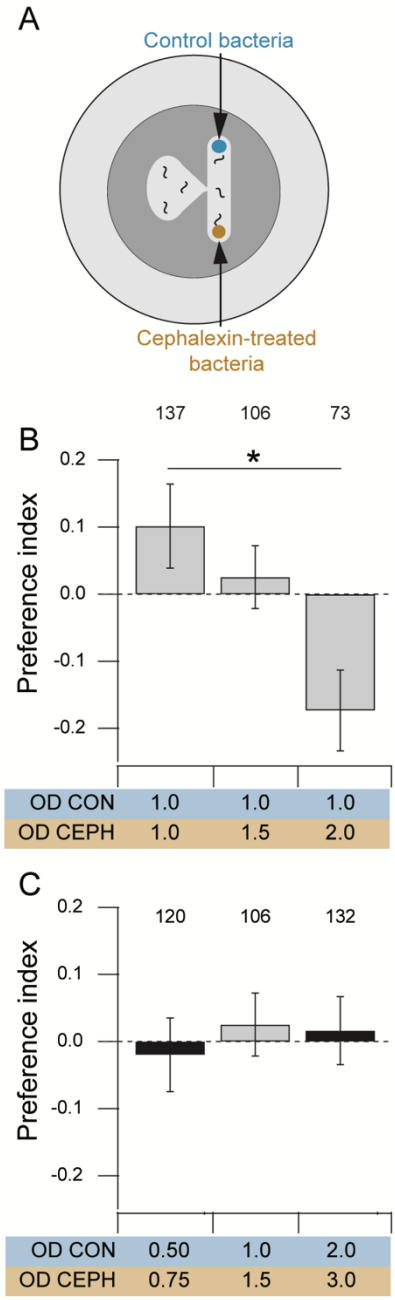


Figure 2. Food preference in T-maze assays.
A. T-maze assay for preference for normal (Control) versus elongated bacteria (cephalexin).
B. Effect on preference of raising the optical density of elongated bacteria (CEPH). Mean preference index is shown for assays in which three different optical densities (OD) of elongated bacteria (CEPH) were paired with normal bacteria (CON) at OD 1.0. Worms are indifferent when elongated bacteria are at OD 1.5. *Numbers*, sample size. Error bars ± 95% CI

C. Tests of predicted indifference points. Mean preference index is shown for three different pairs of optical densities of normal (CON) and elongated (CEPH) bacteria. The grey bar is the indifference point found in panel B. The black bars are novel indifference points found by scaling the optical density of elongated bacteria by the discount factor of 2/3 obtained from the grey bar. *Numbers*, sample size. Error bars ± 95% CI.

Figure 2—figure supplement 1

Model	Equation	Discount factor
Hyperbolic	$V(E) = \frac{V_0}{1 + \alpha E}$	$\frac{1}{1 + \alpha E}$
Generalized hyperbolic	$V(E) = \frac{V_0}{(1 + \alpha E)^s}$	$\frac{1}{(1 + \alpha E)^s}$
Exponential	$V(E) = \frac{V_0}{e^{\alpha E}}$	$\frac{1}{e^{\alpha E}}$

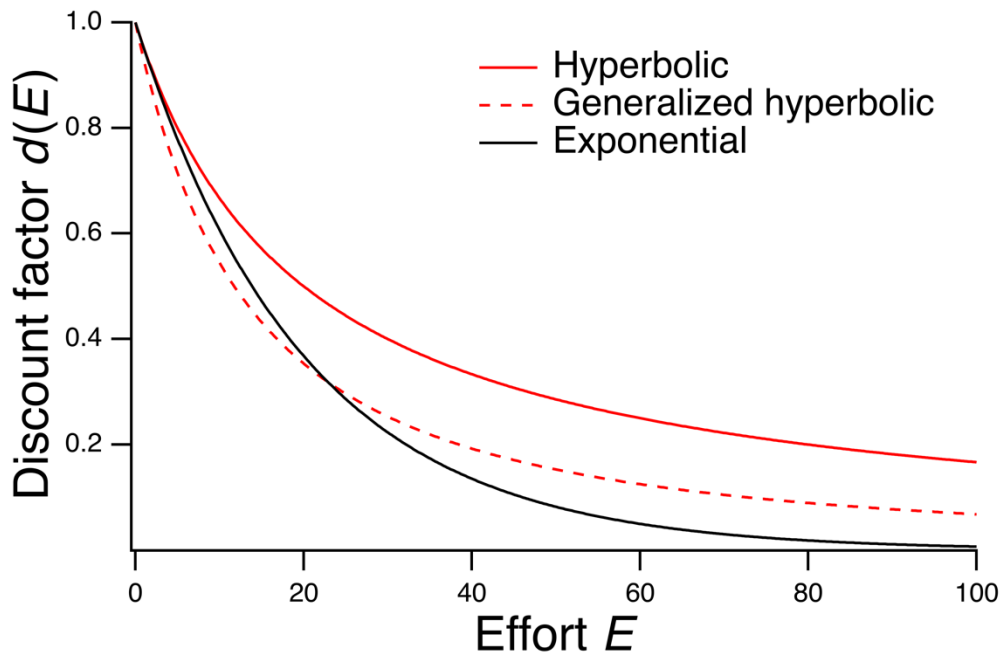


Figure 2—figure supplement 1. Standard models of effort discounting.
There are three main models of effort discounting, each with a unique equation relating effort and reward. V_0 is the value of the reward if it were given effort-free. E is the level of effort required to obtain the effortful reward. $V(E)$ is the discounted value of V_0 under conditions of indifference, given level of effort E. The quantity α is a positive constant representing the chooser's sensitivity to effort. The quantity s in the generalized hyperbolic model is the shape parameter. Discount factors are constant at fixed E. In the graph, V_0 = 100, α = 0.05, and s = 1.5.

Effort discounting-like behavior is based on assessment of the local food environment

We next investigated the behavioral mechanism through which effort influences food preference, examining how worms interact with patches of normal versus elongated food. We studied these interactions at the microscopic level of individual worms to explain behavior at the macroscopic level of T-maze assays. This was done by performing food-patch leaving assays (Scheer and Bargmann, 2023) using either normal or elongated bacteria. Individual worms were transferred to a 1 cm diameter, nematode growth medium-filled arena containing a small patch (3–4 mm diameter) of normal or elongated bacteria (OD 1.0) at its center. The patch size was comparable to those in the T-maze assay. Each worm was video recorded for 15 minutes while it entered and left the patch multiple times (Fig. 3A).

For analysis, we took a kinetic approach to investigate the rates at which worms stochastically changed their position relative to the food patch. This approach is both simple, as it does not require assigning specific locomotory states at particular times to individual worms, and powerful, as it forms a bridge between the microscopic and macroscopic behavior of a stochastic system, thereby elucidating underlying dynamics. Accordingly, we defined three kinetic states based on occupancy of specific zones in the arena (Fig. 3B):

1. State F, *On Food*: both head and tail of the worm were in contact with the food patch.
2. State B, *At Border*: either the head or tail was in contact with the food patch, but not both.
3. State O, *Off Food*: neither head nor tail was in contact with the food patch.

This system contains four state transitions: F→B, B→O, O→B, and B→F. Behavior was analyzed in terms of state probabilities ($P_{\mathrm{F}}, P_{\mathrm{B}}, P_{\mathrm{O}}$) and rate constants ($k_{\mathrm{FB}}, k_{\mathrm{BO}}, k_{\mathrm{OB}}, k_{\mathrm{BF}}$). State probability was defined as the fraction of total observation time the worm was in each state. Rate constants were defined as the number of transitions divided by the total time spent in the state of origin. Rate constants determine the probability per unit time of transitioning from one state to another and thus represent the underlying physical causes of state transitions.

We observed a variety of changes in kinetic parameters on elongated versus normal food. P_{B} and P_{O} increased at the expense of P_{F}, consistent with decreased preference for elongated food in T-maze assays (Fig. 3C; Table 1, rows 15,16). The rate constants exhibited two key features. First, in control animals, between any pair of states, the mean rate constants leading to patch entry (O→B→F transitions) were greater than the mean rate constants leading to patch leaving (F→B→O transitions). In particular, $k_{\mathrm{BF}} > k_{\mathrm{FB}}$ and $k_{\mathrm{OB}} > k_{\mathrm{BO}}$ (Fig. D3 vs. D1 and Fig. D4 vs. D2; Table 1, rows 17-23). This indicates a greater propensity to enter food patches than to leave them, which is consistent with efficient foraging. Second, changes in rate constants brought about by elongated food exhibited a distinctive pattern: the rates for patch leaving ($k_{\mathrm{FB}}, k_{\mathrm{BO}}$) were increased whereas the rates for patch entry (k_{OB}, k_{BF}) were unchanged (Fig. 3D; Table 1, rows 24-28). Thus, worms exhibited a heightened propensity to leave elongated food, consistent with previous observations (Scheer and Bargmann, 2023). The fact that rates for patch entry were unaffected by elongated bacteria rules out a mechanism in which worms, exiting patches of elongated food (which is relatively undesirable), become reluctant to re-enter them. Finally, we found that speed on elongated food increased, whereas speed off food remained unchanged (Fig. 3E; Table 1, rows 29-33). Applying the kinetic data of individual worms to the population level in T-maze assays suggests that effort discounting-like behavior could result from feeding decisions based on the local food environment rather than a direct comparison of patch contents across the arms of the T-maze.

Figure 3

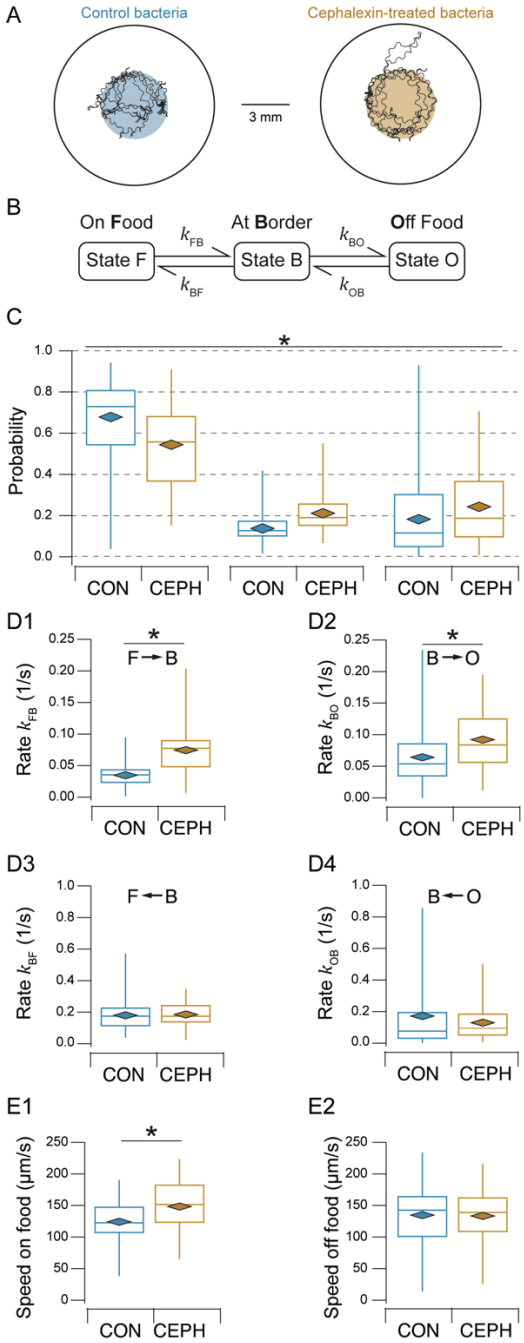


Figure 3. Kinetic analysis of food-patch leaving assays.
A. Food-patch leaving assays for normal (control) and elongated (cephalexin-treated) bacteria. *Open circles*, arenas; *colored circles*, food. A representative track of a single worm is superimposed on each diagram.
B. Three-state kinetic model of behavior in the assay. Each state represents the indicated zone in the arena.

C. Probability of states F, B, and O for normal (CON) and elongated (CEPH) bacteria. Significance was assessed using compositional statistics as described in Fig. 3—figure supplement 1.
D. Rate constants for normal (CON) and elongated (CEPH) bacteria.
E. Locomotion speed for normal (CON) and elongated (CEPH) bacteria on or off food.

Figure 3—figure supplement 1

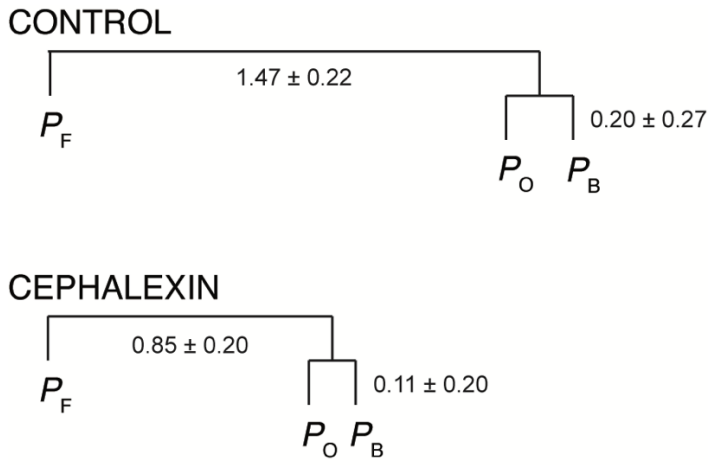


Figure 3—figure supplement 1. Statistical test of the effect of cephalexin on state probabilities in Figure 3C.
State probabilities are an instance of *compositional data*, meaning data that sum to a constant (1 in this case). Such data require special treatment because a decrease in one variable means an increase in at least one other variable. Accordingly, data were first subjected to an isometric log ratio transform (see Materials and Methods). The transformation quantifies the change in *balance* between subsets of the data. Balances are indicated by the numbers in the figure. Here, the control balance between P_{F} and the reciprocal probabilities P_{B} and P_{O} (1.47) is decreased by cephalexin treatment (0.85). A *t*-test on individual worm balances revealed that P_{F} significantly decreased while the joint P_{B} and P_{O} data significantly increased. In contrast, a similar approach showed that the balance between P_{B} and P_{O} was not significantly affected. This indicates that P_{B} and P_{O} increased by similar amounts.

339

340 **Intact dopamine signaling is required for effort discounting-like behavior**

341 A salient aspect of effort discounting in rodents is its sensitivity to changes in midbrain
342 dopamine signaling (Salamone *et al.*, 2018). Increased signaling promotes preference for high-
343 value, high-effort rewards, whereas decreased signaling promotes preference for low-value,
344 low-effort rewards (Bailey, Simpson and Balsam, 2016b; Salamone *et al.*, 2018; Hart and
345 Izquierdo, 2019); in other words, increased dopamine signaling results in greater effort
346 tolerance. Dopamine signaling is a candidate mechanism for the regulation of effort
347 discounting-like behavior in *C. elegans* because it affects how worms respond to the presence
348 and absence of food as they enter or leave food patches. Worms slow down when they
349 encounter a food patch, a response that requires intact dopamine signaling (Sawin,
350 Ranganathan and Horvitz, 2000). When worms exhaust their food supply or are removed from a
351 food patch, they engage in a bout of frequent, high-angled turns called area-restricted search
352 (Hills, Brockie and Maricq, 2004). This response also requires dopamine signaling.

353

354 To test whether dopamine signaling is involved in effort discounting-like behavior in *C. elegans*,
355 we examined food preference in a variety of mutants with altered dopamine signaling. The gene
356 *cat-2* encodes a tyrosine hydroxylase essential to the main synthesis pathway for dopamine; *cat-*
357 *2* mutants are commonly used to reduce dopamine signaling in *C. elegans*. The gene *dat-1*
358 encodes a dopamine transporter that mediates the reuptake of dopamine into presynaptic
359 neurons; *dat-1* mutants are used to increase dopamine signaling. The effects of reduced
360 dopamine signaling can also be investigated in dopamine receptor mutants. *C. elegans* has four
361 genes encoding dopamine receptors with homology to dopamine receptors in mammals. Of
362 these, *dop-1*, *dop-2*, and *dop-3* are the most thoroughly characterized to date. *dop-1* is a DRD1
363 homolog, whereas *dop-2* and *dop-3* are DRD2 homologs. The genes *dop-1* and *dop-3* are
364 required for normal responses to the presence of food (Chase, Pepper and Koelle, 2004); *dop-2*
365 is required for increased levels of activity in the absence of food (Bastien *et al.*, 2024). We found
366 a significant overall effect of strain on food preference measured in the T-maze assay (Fig. 4;
367 Table 1, row 34). Post hoc testing showed that *cat-2*, *dat-1*, *dop-1*, and *dop-2* were

368 indistinguishable from N2, whereas *dop-3* mutants were significantly different from N2 (Table 1,
369 row 35). In particular, they were indifferent between the two food options, indicative of
370 decreased effort discounting-like behavior (Table 1, row 36). We conclude that fully intact
371 dopamine signaling is required for normal effort discounting-like behavior in *C. elegans*, as in
372 rodents. However, the fact that impaired dopamine signaling rendered worms indifferent
373 between the food options suggests that decreased dopamine signaling increases tolerance for
374 high-effort food, in contrast to its effect in rodents. The absence of an effect of the *cat-2* and
375 *dat-1* mutations is puzzling in view of the requirement for *dop-3*. One possibility is that residual
376 dopamine in the *cat-2* mutant (Sanyal *et al.*, 2004) is sufficient for normal effort discounting-like
377 behavior and that normal levels of dopamine signaling are saturating, such that *dat-1* mutations
378 have no effect.

Figure 4

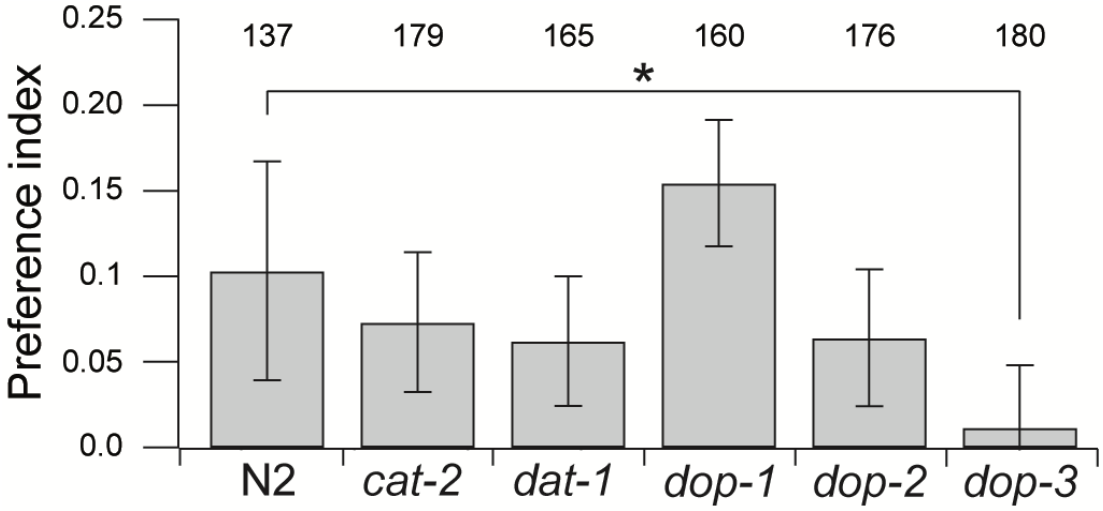
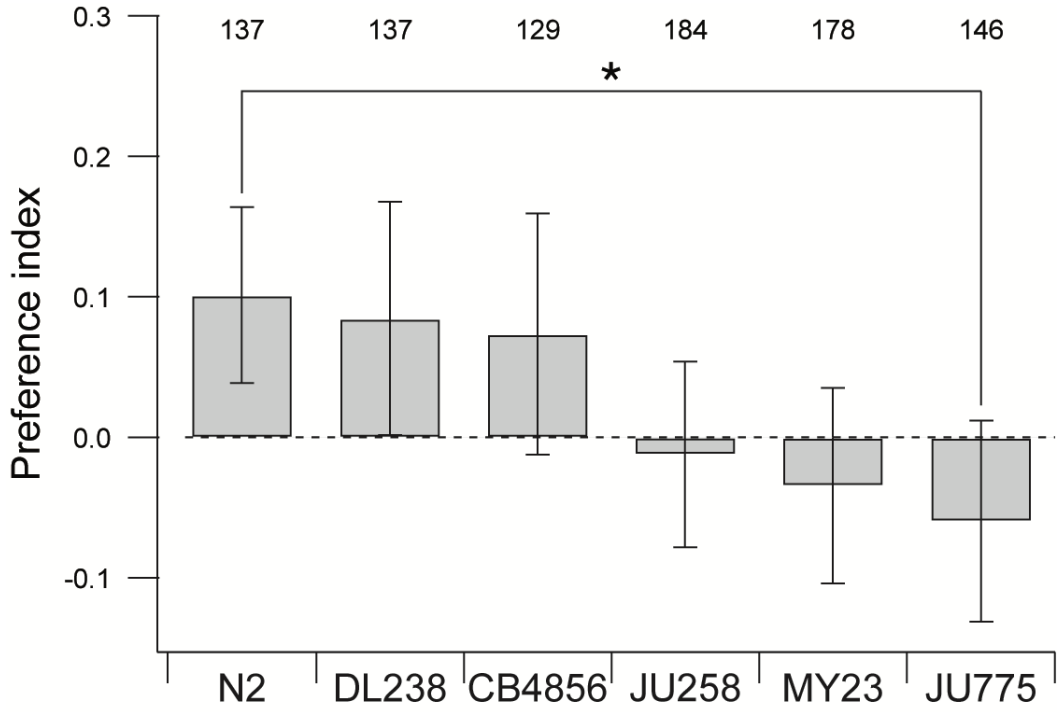


Figure 4. Effect of dopamine signaling mutations on preference for normal versus elongated bacteria. Data are mean preferences measured in T-maze assays. Dopamine signaling was reduced by a nonsense mutation in *cat-2* or by deletions of three dopamine receptor genes (*dop*). Dopamine signaling was increased by a deletion in *dat-1*. *Numbers,* sample size. Error bars ± 95% CI.

Effort discounting-like behavior is not an adaptation to the laboratory environment

Finally, we considered the question whether effort discounting-like behavior may reflect adaptation to the laboratory environment. The reference strain used in this study, N2, has been cultured for over 50 years in the laboratory setting, where it is typically fed small, easily consumed bacteria, such as *E. coli* strain OP50. Consequently, it may have lost the ability to consume larger bacteria efficiently. To address this question, we tested whether any natural isolate strains would prefer normal over elongated bacteria. Accordingly, we performed T-maze assays on five such strains with normal and elongated food at OD 1.0. Natural isolate strains proved to be challenging to work with as they frequently escaped the maze or burrowed in the substrate. These traits reduced the number of countable worms per assay, leading to considerable variance in the data. Nevertheless, there was a significant overall effect of strain on mean preference index (Fig. 5; Table 1, row 37). Post hoc testing revealed that only the strain JU775 had a preference index that was significantly different from N2 (Table 1, row 38) and that JU775's preference was not distinguishable from zero (Table 1, row 39). Thus, JU775 was indifferent between normal and elongated food. On the other hand, mean preference of DL238 was both different from zero (Table 1, row 40) and indistinguishable from N2 (Table 1, row 38). That is, DL238 behaved like N2. We conclude that N2's preference is not an adaptation to the laboratory environment. Between DL238 and JU775, there was a range of different responses, suggesting the possibility of genetic variation in preference for normal versus elongated food. This could reflect differences in effort tolerance or ease of consumption of elongated bacteria.

Figure 5



408

409 **Figure 5. Preference for normal versus elongated bacteria in N2 and five wild-isolate strains.**
410 Data are mean preferences measured in T-maze assays. N2 and DL238 are significantly different
411 from zero. *Numbers*, sample size. Error bars ± 95% CI.
412

DISCUSSION

Effort discounting is a form of cost-benefit decision-making with implications for economics, psychology, neuroscience, behavioral ecology, and other fields. To investigate effort discounting in *C. elegans*, worms were given a choice between two patches of the same species of bacteria. One patch contained normal bacteria, whereas the other patch contained elongated bacteria, which we showed to be more effortful to consume yet similar to normal bacteria in nutrition and attractiveness. When the two types of bacteria were presented at equal density, worms preferred normal bacteria. Three lines of evidence suggest that this preference is analogous to the devaluation of a reward based on increased effort to obtain it: (1) Preference can be reversed by increasing the density of the effortful option. (2) The relative density of normal and effortful food can be titrated to an indifference point. (3) Widely used economic models of discounting correctly predict novel indifference points. Points (1) (Floresco, Tse and Ghods-Sharifi, 2008) and (2) (Salamone, Cousins and Bucher, 1994; van den Bos *et al.*, 2006; Ostrander *et al.*, 2011), together with the requirement for intact dopamine (Salamone *et al.*, 2018) signaling, establish similarities to effort discounting in other animal models. The demonstration of effort discounting-like behavior in a lower invertebrate sets a new phylogenetic boundary on discounting behavior.

Relationship to previous work

The decision of whether to stay or leave a patch of food has been well studied in *C. elegans* (Shtonda and Avery, 2006; Bendesky *et al.*, 2011; Milward *et al.*, 2011; Olofsson, 2014; Scheer and Bargmann, 2023). Leaving is rare on rich patches of normal food but increases on depleted patches or when the mechanics of feeding have been genetically impaired. Both conditions would be expected to decrease feeding efficiency and thereby increase the amount of muscular effort required to obtain food. It is likely that patch-leaving rate in these studies and ours is linked to an internal signal of feeding efficiency. The nature of this signal remains to be determined, but it likely integrates pharyngeal pumping rate with the rate of food accumulation in the gut.

An increase in patch-leaving frequency in response to elongated bacteria has been reported previously (Scheer and Bargmann, 2023). There are some significant differences between that study and ours: (1) *E. coli* was used instead of *Comamonas*; (2) bacteria were elongated with the antibiotic aztreonam which renders food inedible to *C. elegans* (Gruninger, Gualberto and Garcia, 2008; Ben Arous, Laffont and Chatenay, 2009), whereas the cephalexin treatment we used resulted in elongated but edible food (Millet *et al.*, 2022); (3) worms were video-recorded for 40 minutes instead of 15 minutes; (4) food-patch leaving was defined using different behavioral criteria; (5) patch-entry rates were not considered. Nevertheless, our results confirm and extend the previous findings by showing that the effect of elongated food on patch-leaving rate is robust across bacterial species, varied degrees of edibility of elongated food, and differences in details of behavioral analysis.

Utility of economic modeling

Although *C. elegans* patch-leaving behavior has been described in detail before, placing it in an explicit economic context is new. This context provided a quantitative framework that enriched our study in two respects. First, it gave us a metric – the discount factor – for quantifying the extent to which effortful food was devalued by the worm. We observed that worms were indifferent when normal and elongated bacteria were at ODs 1.0 and 1.5, respectively, yielding a discount factor of 2/3. Second, this framework made testable predictions. At the level of effort determined by our cephalexin exposure protocol, any combination of optical densities of normal and elongated food in a 2:3 ratio should be an indifference point. This prediction was verified by measuring preference under conditions in which the optical density of elongated food was both higher and lower than the original value of 1.5. This result shows that standard models of effort discounting may apply to *C. elegans*. It will now be interesting to test whether the discount factor is sensitive to effort, as assumed in many discounting models (Figure 1—figure supplement 1). This could be done by identifying indifference points when effort is manipulated by changing cephalexin exposure conditions to increase or decrease the extent of elongation.

A model of effort-discounting like behavior

We propose the following simple model of effort discounting-like behavior in T-maze assays based on our kinetic analysis of patch entry and exit. In the T-maze assay, worms preferentially accumulate in the patch containing normal food. In theory, this could be because worms leave patches of elongated food (F →B→O transitions) at a higher rate, enter patches of elongated food (O →B→F transitions) at a lower rate, or both. Our kinetic analysis favors the former. We found that the rates governing patch entry, k_{OB} and k_{BF}, were unchanged by the presence of elongated food, whereas the rates governing patch leaving, k_{FB} and k_{BO}, were increased. The increase in these two rate constants is likely the result of increased roaming, a state of arousal characterized by increased speed and a greater tendency to leave food patches (Fujiwara, Sengupta and McIntire, 2002; Ben Arous, Laffont and Chatenay, 2009; Flavell *et al.*, 2013; Scheer and Bargmann, 2023). Our observation that speed increased on elongated food (Fig. 3E) is consistent with increased roaming on these patches in the T-maze.

Role of dopamine signaling in effort discounting-like behavior

Fully intact dopamine signaling is required for normal effort discounting-like behavior in *C. elegans*. This finding is broadly consistent with the known requirements of dopamine signaling for foraging behavior in *C. elegans*, as our T-maze assay is essentially a foraging assay. *C. elegans* dopamine receptors DOP-1, DOP-2, and DOP-3 are required for normal responses to the presence or absence of food (Chase and Koelle, 2007; Suo, Culotti and Van Tol, 2009; Bastien *et al.*, 2024). Dopamine itself is required for area-restricted search in which worms, suddenly finding themselves outside a food patch, engage in a period of frequent locomotory reorientations in an attempt to relocate the food (Hills, Brockie and Maricq, 2004). However, the relationship between dopamine signaling and effort discounting-like behavior in *C. elegans* remains an area for future research. The fact that decreased dopamine signaling in the *dop-3* mutant rendered worms indifferent between the food options suggests that decreased dopamine signaling *increases* tolerance for high-effort food, in contrast to its effects in rodents. This discrepancy may reflect a true difference in the function of dopamine in effort discounting

in *C. elegans* and rodents, or methodological differences. Manipulations of dopamine signaling in rodent studies mainly involve acute experiments using pharmacological agents, whereas we studied worms with chronic, lifelong alterations in dopamine signaling.

In summary, our study provides strong evidence that *C. elegans* exhibits effort discounting-like behavior, reinforcing the idea that decision-making strategies based on effort-cost trade-offs are evolutionarily conserved. By demonstrating that worms devalue effortful food like higher organisms, we extend the phylogenetic boundary of this behavior. The application of an economic framework allowed us to quantify this effect and make testable predictions, confirming its robustness. Furthermore, our findings highlight the role of dopamine signaling in modulating effort sensitivity, suggesting potential parallels with vertebrate systems. Future research can now exploit the utility of *C. elegans* as a genetically tractable system to explore how environmental factors and genetic variations influence effort-based decision-making in *C. elegans*, providing deeper insights into the fundamental principles and molecular basis of cost-benefit decisions.

TABLES

Row	Figure	Test	Effect or comparison tested	Units of replication or sampling	Number of replicates	Statistic	Statistic value	DF 1 or combined DF	p	Effect size metric	Effect size
1	1A	Mann-Whitney	Cephalexin	Worms	99, 98	*U*	4178	–	9.23E-02	Rank-biserial correlation (r)	0.13 (small)
2	1B2	Mann-Whitney	Cephalexin	Worms	44, 49	*U*	618	–	**3.20E-04**	Rank-biserial correlation (r)	0.43 (moderate)
3	1C	*t*-Test	Cephalexin	Biol. Replicate	3, 3	*t*	0.10	2	9.28E-01	Cohen's d	0.08 (small)
4	1D	*t*-Test	Cephalexin	Biol. Replicate	3, 3	*t*	0.10	2	9.31E-01	Cohen's d	0.08 (small)
5	1E	*t*-Test	Cephalexin	Tech. Replicate	3, 3	*t*	0.12	2	9.91E-01	Cohen's d	0.01 (small)
6	1F2	*t*-Test	Mean not = 0	Assay plates	108	*t*	1.65	104	3.56E-01	Cohen's d	0.16 (small)
7	2B	1-way ANOVA	OD ratios	Assay plates	137, 106, 73	*F*	19.07	2	**1.52E-08**	Eta squared	0.11 (moderate)
8	2B	*t*-Test	Mean not = 0	Assay plates	137	*t*	3.20	136	**1.69E-03**	Cohen's d	0.27 (small)
9	2B	*t*-Test	Mean not = 0	Assay plates	73	*t*	1.99	72	**2.06E-07**	Cohen's d	0.67 (large)
10	2B	*t*-Test	Mean not = 0	Assay plates	106	*t*	1.08	105	2.82E-01	Cohen's d	0.10 (small)
11	2C	1-way ANOVA	OD ratios	Assay plates	120, 106, 132	*F*	0.83	2	4.37E-01	Eta squared	0.00
12	2C	*t*-Test	Mean not = 0	Assay plates	120	*t*	0.71	119	4.80E-01	Cohen's d	0.06 (small)
13	2C	*t*-Test	Mean not = 0	Assay plates	106	*t*	1.08	105	2.82E-01	Cohen's d	0.10 (small)
14	2C	*t*-Test	Mean not = 0	Assay plates	132	*t*	0.64	131	5.22E-01	Cohen's d	0.06 (small)
15	3C	*t*-Test	P_F vs (P_O, P_B)	Single worm	66, 60	*t*	4.22	123.57	**4.62E-05**	Cohen's d	0.75 (medium)
16	3C	*t*-Test	P_O vs P_B	Single worm	66, 60	*t*	0.57	116.99	5.73E-01	Cohen's d	0.10 (small)
17	3D	1-way ANOVA	Transition type	Single worm	66, 60	*F*	24.71	3	**4.22E-14**	Eta squared	0.22 (large)
18	3D	Tukey	k_{FB}, k_{BF}	Single worm	66, 60	*q*	3.68	122	**<1.00E-07**	Cohen's d	2.199391 (large)
19	3D	Tukey	k_{BO}, k_{OB}	Single worm	66, 60	*q*	3.68	122	**4.30E-06**	Cohen's d	0.68 (medium)
20	3D	Tukey	k_{BO}, k_{BF}	Single worm	66, 60	*q*	3.68	122	**6.00E-07**	Cohen's d	1.60 (large)
21	3D	Tukey	k_{OB}, k_{BF}	Single worm	66, 60	*q*	3.68	122	9.77E-01	Cohen's d	0.051 (negligible)
22	3D	Tukey	k_{FB}, k_{BO}	Single worm	66, 60	*q*	3.68	122	4.86E-01	Cohen's d	0.91 (large)
23	3D	Tukey	k_{OB}, k_{FB}	Single worm	66, 60	*q*	3.68	122	**0**	Cohen's d	0.88 (large)
24	3D	MANOVA	Cephalexin	Single worm	66, 60	*F*	16.44	4121	**8.77E-11**	Eta squared (partial)	0.35
25	3D1	*t*-Test	Cephalexin	Single worm	66, 60	*t*	7.38	79.39	**1.36E-10**	Cohen's d	1.36 (large)
26	3D2	*t*-Test	Cephalexin	Single worm	66, 60	*t*	3.32	118.69	**1.18E-03**	Cohen's d	0.60 (medium)
27	3D3	*t*-Test	Cephalexin	Single worm	66, 60	*t*	0.28	123.25	7.83E-01	Cohen's d	0.05 (negligible)
28	3D4	*t*-Test	Cephalexin	Single worm	66, 60	*t*	1.38	95.7	1.70E-01	Cohen's d	0.24 (small)
29	3E	2-way ANOVA	Cephalexin	Single worm	66, 60	*F*	5.24	1	**2.29E-02**	Eta squared	0.02 (small)
30	3E	2-way ANOVA	Food	Single worm	66, 60	*F*	0.70	1	4.00E-01	Eta squared	0.00 (negligible)
31	3E	2-way ANOVA	Cephalexin × Food	Single worm	66, 60	*F*	6.66	1	**1.10E-02**	Eta squared	0.03 (small)
32	3E	*t*-Test	Cephalexin	Single worm	66, 60	*t*	3.68	115.91	**4.59E-04**	Cohen's d	0.66 (medium)
33	3E	*t*-Test	Cephalexin	Single worm	66, 60	*t*	0.19	123.94	8.47E-01	Cohen's d	0.034 (negligible)
34	4	1-way ANOVA	Strain	Assay plates	137, 179, 165, 160, 175, 180	*F*	7.69	3	**4.71E-05**	Eta squared	0.03 (small)
35	4	Dunnett test	N2 vs other strains	Assay plates	See above	*q*	2.78	649	**1.92E-02**	Cohen's d	0.50 (medium)
36	4	*t*-Test	Mean not = 0	Assay plates	180	*t*	0.65	159	5.14E-01	Cohen's d	0.05 (small)
37	5	1-way ANOVA	Strain	Assay plates	137, 137, 129, 184, 178, 146	*F*	3.30	5	**5.83E-03**	Eta squared	0.018 (small)
38	5	Dunnett test	N2 vs other strains	Assay plates	See above	*q*	3.03	905	**1.10E-02**	Cohen's d	0.68 (medium)
39	5	*t*-Test	Mean not = 0	Assay plates	146	*t*	0.36	145	1.03E-01	Cohen's d	0.13 (small)
40	5	*t*-Test	Mean not = 0	Assay plates	137	*t*	2.02	136	**4.56E-02**	Cohen's d	0.17 (small)

Table 1. Statistical information. Bold p values denote significant effects.

MATERIALS AND METHODS

Key resources table

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Strain (*E. coli*)	OP50	CGC[1]	RRID:WB-STRAIN:WBStrain00041969	Worm maintenance
Strain, strain background (*Comamonas* spp.)	DA1877	CGC	RRID:WB- STRAIN:WBStrain00040995	Behavioral testing
Strain (C. elegans)	N2	CGC	RRID:WB- STRAIN:WBStrain00000001	All WT assays
Strain (C. elegans)	*cat-2(e1112)*	CB	CB1112	Behavioral testing
Strain (C. elegans)	*dat-1(ok157)*	CGC	RM2702	Behavioral testing

Strain (C. elegans)	*dop-1(vs101)*	CGC	LX636	Behavioral testing
Strain (C. elegans)	*dop-2(vs105)*	CGC	LX702	Behavioral testing
Strain (C. elegans)	*dop-3(vs106)*	CGC	LX703	Behavioral testing
Drug	cephalexin hydrate	Thermo Fisher	J6317206	Bacteria elongation
Dye	Oil-Red-O	Sigma-Aldrich	00625	Lipid storage analysis
Software algorithm	Igor Pro	Wavemetrics	Version 9.05	Behavioral testing, image analysis
Software	R	R Core Team (2024)	Version 4.4.2 (2024-10-31)	Statistical analysis
Software	WormLab	MBF Bioscience	Version 2024	Video analysis

[1]*Caenorhabditis* Genetics Center.

Bacteria culture

Two bacterial species were used: *E. coli* (OP50) used for worm culture, and *Comamonas* spp. (DA1877) for assays. Bacterial cultures were grown in liquid lysogeny broth (LB) under sterile conditions at 37°C with agitation. For all assays, DA1877 was grown for one day, then "Control" cultures were solubilized with M9 buffer (3g KH_2PO_4, 6g Na_2HPO_4, 5g NaCl, 1mL 1 mol/L $MgSO_4$, H_2O to 1L); "cephalexin" cultures were supplemented with a solution of M9 and cephalexin hydrate 97% to a concentration of 450 µmol/L and incubated for 2 hours. Cultures then underwent three rounds of concentration by centrifugation, followed by rinsing with 10 mL of M9 buffer before being resuspended to their final concentration. Concentration was defined as optical density at 600 nm (OD), as measured with a cell density meter (Laxco, DSM, Bothell, WA, USA). All measurements were performed on samples diluted into the linear range of the instrument (OD 0.1–1.0).

Worm maintenance & synchronization

All strains were maintained at 20°C on 5 cm plates containing nematode growth medium (NGM) seeded with *Escherichia coli* OP50 bacteria as a food source (Brenner, 1974). For synchronization, 20 young adult worms were transferred to fresh 5 cm NGM plates. Adults were

557 removed from the plates after laying eggs for 3 hours. The plates were maintained at 20°C until
558 worms reached the young adult stage.
559
560 **Pharyngeal pumping assay**
561 Pharyngeal pumping was measured electrophysiologically using the ScreenChip microfluidic
562 system (InVivo Biosystems, Eugene, OR, USA). Synchronized young adults were preincubated
563 with control or cephalexin-treated bacteria at OD 1 for 20 minutes before being transferred into
564 the reservoir of a microfluidic device fitted with electrodes. Individual worms were moved one
565 at a time from the reservoir into the recording channel and given one to two minutes to
566 acclimate. Voltage transients associated with pharyngeal pumping were recorded for five
567 minutes. All recordings were performed within 90 minutes of the worms being loaded into the
568 microfluidic reservoir. Mean pumping frequency was extracted using custom code written in
569 Igor Pro (WaveMetrics, Lake Oswego, OR, USA).
570
571 **Food-preference assay**
572 T-mazes (Levichev *et al.*, 2023) were constructed by cutting masks from sheets of 2 mm thick
573 ethylene-vinyl acetate foam (Darice Craft Foam, Strongsville, OH, USA) using a fabric-cutting
574 machine (Cricut, South Jordan, UT, USA). Masks were placed on blank 5 cm NGM plates. Maze
575 arms were baited with 4.5 µL of various bacterial suspensions as described in the text.
576 Synchronized young adults were washed by five rounds of centrifugation (30 sec, 300 g)
577 followed by aspiration of the supernatant, then deposited by liquid transfer at the starting point
578 of the maze. A transparent plastic disc was placed over the maze to eliminate air currents.
579 Twelve plates were placed on a flatbed scanner (EPSON, model J221B, Los Alamitos, CA, USA)
580 and imaged after an elapsed time of 60 minutes. Imaged worms were counted manually. The
581 food preference index was computed as $I = (n_{\mathrm{N}} - n_{\mathrm{L}})/(n_{\mathrm{N}} + n_{\mathrm{L}})$, where n_{N} and n_{L} are the
582 number of worms in the normal and elongated patches, respectively. In some experiments, a
583 paralytic agent (sodium azide, NaN_3, 3 µL at 20 mM) was added to each food patch to prevent
584 animals from leaving the patch of food after reaching it. Sodium azide diffuses through the agar
585 over time, and its action is not instantaneous. To account for these effects, all worms within 5

586 mm of the end of the maze arm, rather than only those on food, were counted when calculating
587 the preference index.
588
589 **Patch-leaving assay**
590 Custom 8-well plates (25 mm × 45 mm) were fabricated from acrylic plastic. Wells (diameter: 10
591 mm; depth: 6 mm) were filled with NGM agar. Bacterial suspension (2 µL, OD 1) was placed at
592 the center of each well, forming a patch 3-4 mm in diameter. Synchronized one-day-old adult
593 worms were cleaned by transferring them to an unseeded NGM plate for several minutes. One
594 worm was transferred to each well using a pick and placed at the center of the patch. The well
595 plate was immediately placed on a stage 8 cm above a diffuse white-light source and imaged (2
596 or 5 frames per second, 23.4 µm/pixel) for 15 minutes using a video camera (AmScope, 1080P,
597 Irvine, CA, USA) fitted with a telephoto lens (Nikon USA, AF Micro Nikkor 60 mm, Melville, NY,
598 USA). The distance between the lens and the stage was 50 cm. Worm tracks and locomotion
599 parameters were extracted using WormLab software (MBF Bioscience, Williston, VT, USA) and
600 analyzed using custom routines in Igor Pro (WaveMetrics, Lake Oswego, OR, USA). To define the
601 worm's position with respect to the patch, its margin was traced manually.
602
603 **Bacterial composition analysis**
604 Bacteria were grown under sterile conditions at 37°C with agitation in 500 mL of LB overnight,
605 pelleted (5,000 rpm, 3 minutes), washed with water, and re-pelleted. Pellets were stored at -
606 80°C prior to analysis. Bacterial stocks were weighed and then lysed with CelLytic B Plus kit
607 (Sigma-Aldrich, CB0050, Saint Louis, MO, USA) on the day of the assay.
608
609 Protein. Protein was assayed with the Pierce BCA Protein Assay kit (Thermo Fisher Scientific,
610 A55864, Waltham, MA, USA). Readings were taken with a spectrophotometer (NanoDrop,
611 Thermo Fisher Scientific, ND-2000, Waltham, MA, USA) using the bicinchoninic acid assay
612 setting.
613

614 Carbohydrate. Carbohydrate was measured with the Abbexa Total Carbohydrate Assay Kit
615 (Abbexa, abx298986, Cambridge, UK). Spectrophotometer readings were made at OD 540 nm.
616
617 Lipid. Lipid was quantified using the method of Aggeler *et al*. (Aggeler, Then and Ghosh, 1987).
618 We prepared 100 µL of cell lysate and added: 500 µL methanol, 250 µL dichloromethane, and
619 100 µL distilled H_2O. The mixture was vortexed before adding an additional 375 µL of distilled
620 water. The solution was vortexed again to form an emulsion and then centrifugated at 4800 g
621 for 10 minutes. The bottom layer of solution was transferred to a pre-weighed tube and then
622 weighed again after the lipid layer dried. The difference in weight was used to measure the
623 quantity of lipid per 100 µL of cell lysate.
624
625 **Fat storage analysis**
626 Fat storage was assessed using the procedures of Soukas *et al*. (Soukas *et al.*, 2009) and Stuhr
627 et al. (Stuhr and Curran, 2020). A 0.5% isopropanol solution of Oil-Red-O (Sigma-Aldrich, Oil-
628 Red-O powder 00625, Saint Louis, MO, USA) was prepared several days before the assay.
629 Bacterial cultures (Control and cephalexin) were prepared as described above and adjusted to
630 OD 3 before being loaded onto fresh NGM plates. Seventeen young adult worms were placed
631 on these plates and allowed to lay eggs for 5 hours before being removed. The plates were
632 incubated at 20°C for two days. L4 larvae were harvested and washed in PBS + 0.01% Triton X-
633 100. Worms were fixed with a 60% isopropanol solution and incubated with the Oil-Red-O
634 solution for two hours on a rotating plate. Worms were washed twice with PBS + Triton 0.01%
635 solution, first for 30 minutes, then for one hour, on a rotating plate. Stained worms were
636 imaged on a microscope slide at 32×, and staining intensity was quantified using custom
637 routines in Igor Pro (WaveMetrics, Lake Oswega, OR, USA).
638
639 **Statistical analysis**
640 General. Statistical details for each test (figure panel, statistical test, effect or comparison
641 tested, definition of units of replication, number of replicates, statistic value, degrees of
642 freedom, *p*-value, and effect size) are compiled in Table 1. These details are cited in the text

using the notation "Table 1, row *n*," where *n* is the row number in the table. For all comparisons, the centers of distributions were their arithmetic mean or median. For dispersion measures, we used 95% confidence intervals. The following statistical tests were employed according to the experimental design: We used *t*-tests to determine whether the mean differed from zero. To assess differences in pairs of means, we used *t*-tests and, when the data were not normally distributed (according to the Shapiro-Wilk test), the Mann-Whitney U test. For comparisons of three or more means we used 1-way or 2-way ANOVA with *post hoc* tests.

State probabilities. State probabilities are an instance of *compositional data*, meaning data that sum to 1 (or 100%), which requires special treatment. For analysis, we subjected these data to the isometric log ratio transform (Aitchison, 1986; Egozcue *et al.*, 2003) using the "compositions" package in R (Team, 2020; van den Boogaart, Tolosana-Delgado and Bren, 2024). Following transformation, *t*-tests were used to infer statistical significance (Figure 3—figure supplement 1).

Box plots. The box represents the first and third quartiles of the data, with a horizontal line representing the median. The whiskers extend to the maximum and minimum values following the removal of extreme outliers. These were defined as points with values below $Q1 - 3 \times IQR$ or above $Q1 + 3 \times IQR$, where $Q1$ is the first quartile, $Q3$ the third quartile and IQR is the interquartile range $Q3 - Q1$. A diamond symbol represents the mean.

Number of replicates. In the case of *assay plates*, a replicate is unique cohort of worms on an assay plate, such as a T-maze. In the case of *worms*, a replicate is a unique animal tested once. Nutrient replicates (Fig. 1C–E) were biological or technical, as indicated in Table 1. The minimum sample size for the T-maze assays was based on pilot experiments that demonstrated the ability to detect moderate to small effects with 10–30 replicates per experimental condition. Previously published EPG data (Katzen *et al.*, 2021) showed that mutants or treatments could be distinguished with approximately 20 replicates; however, to ensure the detection of small effect sizes across experimental conditions, larger sample sizes were used.

Effect size. For *t*-tests we used Cohen's *d*. For Mann-Whitney U tests, we used the rank-biserial correlation with $r = 2\,U/(n_1 n_2) - 1$, where U is the Mann-Whitney statistic and n_1 and n_2 are group sizes. For 1-way ANOVA, we used eta squared with $\eta^2 = SS_{\text{between}}/SS_{\text{total}}$ where SS_{between} is the sum of squares between groups and SS_{total} is the total sum of squares. For 2-way ANOVA we used $\eta^2 = SS_{\text{effect}}/SS_{\text{total}}$ where SS_{effect} is the sum of squares for a factor or interaction. For MANOVA we used partial eta squared with $\eta_p^2 = SS_{\text{effect}}/(SS_{\text{effect}} + SS_{\text{error}})$ where SS_{error} is sum of squares for error.

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COMPETING INTERESTS

S.R.L. has a competing financial interest in InVivo Biosystems, Inc., which produces the ScreenChip system utilized in the pharyngeal pumping assay. The other authors have no competing interests to declare.

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