Identification of Animal Behavioral Strategies by Inverse Reinforcement Learning

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Abstract

Animals are able to flexibly adapt to new environments by controlling different behavioral patterns. Identification of the behavioral strategy used for this control is important for understanding animals' decision-making, but methods available for quantifying such behavioral strategies have not been fully established. In this study, we developed an inverse reinforcement-learning (IRL) framework to identify an animal's behavioral strategy from behavioral time-series data. As a particular target, we applied this framework to thermotactic behavior in *C. elegans*. We found that the identified strategy comprised two different modes. The strategy also clarified how the worms control thermosensory states throughout migration, in terms of control theory. Furthermore, we applied our method to thermosensory neuron-deficient worms, to identify the neural basis underlying these strategies. Thus, this study validates and presents a novel approach that should propel the development of new, more effective experiments to identify behavioral strategies and decision-making in animals.

Introduction

Animals develop behavioral strategies, a set of sequential decisions necessary for organizing appropriate actions in response to environmental stimuli, to ensure their survival and reproduction. Such strategies lead the animal to its preferred states. For example, foraging animals are known to optimize their strategy to most efficiently exploit food sources [1]. Although the behavioral sequence can reflect the overall behavioral strategy, a method to quantitatively identify a behavioral strategy from behavioral time-series data has not been well established. Therefore, we here propose a new computational framework based on the concept of reinforcement learning (RL).

RL is a mathematical paradigm to represent how animals adaptively learn behavioral strategies to maximize cumulative rewards via trial and error [2] (upper panel in **Figure 1A**). Over the last two decades, neuroscience studies have clarified that dopaminergic neural activity in the ventral tegmental area (VTA) encodes the prediction error of reward [3], similar to temporal difference (TD) learning in RL [4]. It is therefore widely believed that RL is localized within the basal ganglia, a group of brain nuclei heavily innervated by VTA dopaminergic neurons [5–8]. An animal's behavioral strategy should thus be associated with reward-based representations in its neural system.

Here, our aim was to identify the reward-based representation for the animal's behavioral strategy. In particular, we utilized inverse reinforcement learning (IRL), a recently-developed machine learning-related framework that solves an inverse problem of RL (lower panel in **Figure 1A**) [9,10]. IRL estimates state-dependent rewards from the history of an agent's actions and states, working under the assumption that the agent has already acquired the optimal strategy to maximize the cumulative rewards. One application is apprenticeship learning. For example, the seminal studies on IRL employed a radio-controlled helicopter, for which the state-dependent rewards of an expert were estimated by using the observed time-series of both the human expert's manipulation and the helicopter's state. Consequently, autonomous control of the helicopter was achieved by (forward) RL that utilized the estimated rewards [11,12]. This engineering application prompted studies in which IRL was used to identify the behavioral strategies of animals and humans. Recently, application studies of IRL have emerged, mostly regarding human behavior, with a particular interest in constructing artificially intelligent systems that mimic human behavior [13–15]. In these studies, the experimenters designed the behavioral tasks with specific objectives, and the observed behavioral strategies are therefore usually expected.

In contrast to these advanced applications, IRL applications to animal behavior in a natural environment are far from established. In the helicopter control example above, the measurable variables of the helicopter (e.g., location, velocity, and acceleration) were fully monitored for IRL. In human behavior experiments, the dimensions of the state and reward spaces, which represent behavioral strategies, are restricted by the artificially-designed task, and can thus easily be set for RL and IRL. However, the natural environment in which animals live is quite different; animals always face a degree of uncertainty, and can only partially observe a current state due to sensory constraints. Moreover, animals exhibit stochastic behaviors even under the same condition. This makes it very difficult to set the state and reward space, as well as the dimension to be used. This difficulty has limited the applicability of IRL to the field of basic bioscience. In this study, hence, we tried to identify the hidden strategies of freely-moving animals.

To this end, we chose freely migrating *Caenorhabditis elegans*, because this nematode is a well-studied model animal whose whole-body movements are tractable. In addition, a tracking system for freely migrating *C. elegans* has been established, and provides behavioral time-series data that are required for IRL [16]. *C. elegans* have a behavioral strategy by which the worms sense external environments and migrate to preferred places; we especially focused on thermotaxis, in which worms cultivated at a certain temperature tend to migrate toward that temperature when placed within a thermal gradient [17,18]. Although the worms are not aware of either the spatial temperature profile or the location of the place with the target temperature, they nevertheless often reach their preferred (target) place.

In this study, we propose a new IRL framework for animal behavior analyses. Applying this framework to *C. elegans* behavioral data measured by the established worm tracking system, we successfully identified the reward-based representations associated with the worms' thermotactic behavioral strategy.

Results

Heart of the IRL framework

To identify an animal's behavioral strategy based on IRL, we initially make the assumption that the animal's behaviors are the result of a balance between two factors: passive dynamics (blue worm in **Figure 1B**) and reward-maximizing dynamics (red worm in **Figure 1B**). These factors correspond to inertia-based and purpose-driven body movements, respectively. Even if a worm moving in a straight line wants to make a purpose-driven turn towards a reward, it cannot turn suddenly, due to the inertia of its already moving body. Thus, it is reasonable to consider that an animal's behaviors are optimized by taking both factors into account, namely by minimizing resistance to the passive dynamics and maximizing approach to the destination (reward). Such a behavioral strategy has recently been modeled as a linearly-solvable Markov decision process (LMDP) [19], in which the agent requires not only a state-dependent cost (i.e., a negative reward), but also a control cost for quantifying resistance to the passive dynamics (**Figure 1C**) (see **Materials and Methods**). Importantly, the optimal strategy in the LMDP is analytically obtained as a probability of controlled state transition:

$$\pi\left(s_{t+1} \mid s_t\right) \propto P(s_{t+1} \mid s_t)\exp\left\{-v(s_{t+1})\right\}, \tag{1}$$

where s_t and $v(s)$ indicate the animal's state at time step t and a value function defined as the expected sum of state-dependent costs, $q(s)$, and control cost, $KL\left[\pi(\cdot \mid s)\middle\|p(\cdot \mid s)\right]$, from state s toward the future, respectively; $P(s_{t+1}|s_t)$ represents a probability of uncontrolled state transition, which represents the passive dynamics from s_t to s_{t+1}. In this equation, the entire set of $v(s)$ represents the behavioral strategy. Thus, the identification of the animal's behavioral strategy is equivalent to an estimation of the value function $v(s)$ based on observed behavioral data (s_1, s_2,…s_t,…s_T) (lower panel in **Figure 1A**). This estimation was performed by maximum likelihood estimation (MLE) [20], and is an instance of IRL. Note that in this estimation, we introduced a constraint to make the value function smooth, because animals generate similar actions in similar states. This constraint is essential to stably estimate the behavioral strategy of animals. In summary, identification of an animal's behavioral strategy from behavioral data requires only the appropriate design of state representation and passive dynamics.

Guidelines for the application of the IRL framework

Please see the flowchart in Figure 2 for a visualization of this guideline. Suppose one is interested in a certain animal's behavioral strategy. The first step would be to perform a behavioral experiment (**phase 1 in Figure 1D**), which can be either a freely-moving task or a conditional task. The second step would then be to design the states, s, and the passive dynamics, $P(s_{t+1}|s_t)$, based on which the animal develops its strategy (**phase 2 in Figure 1D**). At this time, prior knowledge about the kinds of sensory information the animal processes provides useful information for the appropriate selection of the states and the passive dynamics. The third step is the quantification of the time-series of selected states (**phase 3 in Figure 1D**) and the fourth step the implementation of the LMDP-based IRL to estimate the VF (**phase 4 in Figure 1D**). At this point, the behavioral strategy can be identified. Following this guideline, we applied the IRL framework to the behavioral strategy of freely-migrating *C. elegans* under a thermal gradient.

Phase 1: Experiment on C. elegans *thermotaxis*

To identify the behavioral strategy underlying the thermotactic behavior of *C. elegans*, we performed population thermotaxis assays, in which 80–150 worms that had been cultivated at 20 °C were placed on the surface of an agar plate with controlled thermal gradients (see **Figure 2A**). Behavioral crosstalk is negligible, because the rate of physical contact is low at this worm density. We prepared three different thermal gradients centered at 17 °C, 20 °C, and 23 °C, to collect behavioral data; these gradients would encourage ascent up the gradient, movement around the center, and descent down the gradient, respectively. We confirmed that the fed worms aggregated around the cultivation temperature in all gradients (**Figure 2B**).

Phase 2: Design of C. elegans' *states and passive dynamics*

We defined two elements of LMDP: state representation and passive dynamics, which are signified by s and $P(s_{t+1}|s_t)$ in equation (1), respectively.

First, we characterized the state, which represents the sensory information that the worms process during thermotaxis. Because previous studies have shown that the nematode's AFD thermosensory neuron encodes the temporal derivative of temperature [21,22], we assumed that the worm made decisions in order to select appropriate actions (i.e., migration direction and speed) based not only on temperature, but also on its temporal derivative. We then represented a state by a two-dimensional (2D) sensory space, $s=(T, dT)$, where T and dT denote temperature and its temporal derivative, respectively. This means that the value function in equation (1) is given as a function of T and dT, $v(T, dT)$.

Second, we characterized the passive dynamics, which are given by state transitions as a consequence of random behavior. We considered that *C. elegans* likely migrated in a persistent direction, but in a sometimes fluctuating manner. Thus, it is reasonable to define the passive transition from a state $s_t=(T_t, dT_t)$ to the next state $s_{t+1}=(T_{t+1}, dT_{t+1})$, where dT_{t+1} maintains dT_t with white noise and T_{t+1} is updated as T_t+dT_t with white noise. Thus, $P(s_{t+1}|s_t)$ is to be simply modeled as a normal distribution (see **Materials and Methods**). Please note the distinction between T and t throughout this manuscript.

Phase 3: Quantification of time-series of thermosensory states

To quantify thermosensory states selected in phase 2, we tracked the trajectories of individual worms over 60 min within each gradient, using multi-worm tracking software [16] (**Figure 2C**). We then obtained time-series of the temperature that each individual worm experienced (upper panel in **Figure 2D**). We also calculated the temporal derivative of the temperature using a Savitzky-Golay filter [23] (lower panel in **Figure 2D**).

Phase 4: Thermotactic strategy identified by IRL

Using the collected time-series of state, $s=(T, dT)$, and passive dynamics, $P(s_{t+1}|s_t)$, we performed IRL (the estimation of the value function $v(s)$) based on behavioral data. We modified an existing estimation method called OptV [20] by introducing a smoothness constraint (see **Materials and Methods**), and confirmed that this smoothness constraint was indeed effective in accurately estimating the value function when applied to artificial data simulated by equation (1) (**Supplementary figure 1**). Since the method was able to powerfully estimate a behavioral strategy based on artificial data, we next applied it to the behavioral data of fed *C. elegans*.

Our method successfully estimated the value function of T and dT, $v(T, dT)$ (**Figure 3A**), and visualized $\exp(-v(T, dT))$, called the desirability function (**Figure 3B**). Because both the value and desirability functions essentially represent the same thermotactic strategy, we discuss only the desirability function below. We found that the identified desirability function peaked at T=20 (°C) and dT=0 (°C/s), encouraging the worms to reach and stay close to the cultivation temperature; moreover, we identified both diagonal and horizontal components. The diagonal component represents directed migration (DM), a strategy that enables the worms to efficiently reach the cultivation temperature. For example, at lower temperatures than the cultivation temperature a more positive dT is favored, whereas at higher temperature a more negative dT is favored. This DM strategy is consistent with the observation that the worms migrate toward the cultivation temperature, and also clarifies how the worms control their thermosensory state throughout migration, which has not been known until now (see **Figure 6** and Discussion). The horizontal component represents isothermal migration (IM), which explains a well-known characteristic called isothermal tracking; the worms typically exhibit circular migration under a concentric thermal gradient. Note that although we used a linear, not a concentric gradient in our thermotaxis assay, our IRL algorithm successfully extracted the isothermal tracking-related IM strategy. We further revealed that the IM strategy worked not only at the cultivation temperature, but also at other temperatures. It must be stressed that the identified desirability function (**Figure 3B**) is not equivalent to the state distribution of T and dT (**Supplementary figure 2**), but rather represents the desirability of the next state.

Moreover, the reward function, which is equivalent to the negative state cost function, could be calculated from the identified desirability function using equation (4) (**Figure 3C**). The reward function primarily represents the worms' preference; the desirability function represents the behavioral strategy, and is thus a result of optimizing cumulative reward and control cost. Taken together, our method quantitatively visualized the behavior strategy of cultivated *C. elegans.*

Reliability of the identified strategy

We examined the reliability of the identified DM and IM strategies by means of surrogate method-based statistical testing. Specifically, we tested whether the DM and IM strategies were obtained by chance, under the null hypothesis that the worms randomly migrated under a thermal gradient with no behavioral strategy. We first constructed a set of artificial temperature time-series that could be observed under the null hypothesis. By using the iterated amplitude adjusted Fourier transform (IAAFT) method (24), we prepared 1000 surrogate datasets by shuffling observed temperature time-series (**Figure 4A**), while preserving the autocorrelation of the original time-series (**Figure 4B**). We then applied our IRL algorithm to this surrogate dataset to estimate the desirability functions (**Figure 4C**). To assess the significance of the DM and IM strategies, we calculated sums of the estimated desirability functions within the previously described horizontal and diagonal regions, respectively (**Figure 4D**). Empirical distributions of these test statistics for the surrogate datasets could then serve as null distributions (**Figure 4E**). For both DM and IM, the test statistic derived using the original desirability function was located above the empirical null distribution (p=0 for the DM strategy; p=0 for the IM strategy), indicating that both strategies were not obtained by chance but reflected an actual strategy of thermotaxis.

In addition, we estimated the behavioral strategy based on a one-dimensional (1D) state representation, i.e., $s=(T)$. Comparing 1D and 2D cases, we used cross-validation (see **Materials and Methods**) to confirm

that the prediction ability for a future state transition in the 2D behavioral strategy was significantly higher than that in the 1D behavioral strategy (p=0.0002; Mann-Whitney U test) (**Supplementary figure 3**).

Strategies of starved worms and thermosensory neuron-deficient worms

We also estimated the desirability and value functions in a starved condition, in which worms dispersed under a thermal gradient (**Figure 5A and Supplementary figure 4**). We found that the DM strategy was abandoned, while the IM strategy could still be observed (**Figure 5Ab**), compared with the desirability function of the fed worms (**Figure 3B**). This suggests that the starved worms were not using directed migration. Interestingly, the desirability function at the cultivation temperature was lower than at surrounding temperatures, showing how the worms avoided the cultivation temperature (see **Figure 6** and Discussion). These data indicate that our method could distinguish between strategies of normally fed and starved *C. elegans*.

Next, we performed IRL on behavioral data from two *C. elegans* strains in which one of the two thermosensory neurons, AWC or AFD, were deficient [17,18,24]. AWC had been genetically ablated via cell-specific expression of caspases (see **Materials and Methods**), and we used *ttx-1* mutants as AFD-deficient animals [25]. The AWC-deficient worms appeared to show normal thermotaxis (**Figure 5Ba**). We also found the desirability function to be similar to that of wild type (WT) animals (**Figure 5Bb**), suggesting that the AWC did not play an essential role during thermotaxis.

The AFD-deficient worms in contrast demonstrated cryophilic thermotaxis (**Figure 5Ca**). The desirability function consistently increased with a decrease in temperature (**Figure 5Cb**). We further found that the desirability function lacked the dT-dependent structure, indicating that the DM strategy observed in the WT worms had disappeared. This suggests that the AFD-deficient worms inefficiently aimed for lower temperatures by a strategy primarily depending on the absolute temperature T, but not on the temporal derivative of the temperature, dT (**Figure 5Cb**). Moreover, the fact that the AFD encodes temporal derivatives of temperature [21] further corroborates the loss of the dT-dependent structure. Taken together, our results show that the AFD, but not the AWC neuron, is essential for efficiently navigating to the cultivation temperature.

Discussion

We proposed an IRL framework to identify animals' behavioral strategies based on collected behavioral time-series data. We validated the framework using artificial data, and then applied it to *C. elegans* behavioral data collected during thermotaxis experiments in wild type worms. We quantitatively identified the worms' thermotactic strategy, which was represented by the desirability function of a 2D sensory space (but not a 1D space), that is, based on absolute temperature and its temporal derivative. We then visualized the properties of the thermotactic strategy in terms of the desirability function, which successfully identified what states are pleasant and unpleasant for *C. elegans*. Finally, we demonstrated the ability of this technique to discriminate alterations in components within a strategy by comparing the desirability functions of two strains of the worm with impaired thermosensory neuron function; we found that the AFD neuron (but not the AWC) is fundamental to efficiently guided navigation to the cultivation temperature.

Validity of LMDP

It is worth comparing the LMDP and the animals' behaviors to determine if the original assumption of an LMDP is suitable. First, animals' movements are usually restricted by external constraints such as inertia and gravity, and by internal (musculoskeletal) constraints, including the body's own passivity. These constraints were reflected by the passive dynamics in the LMDP. Second, animals resist entering unlikely states in which these restrictions are more powerful; that is, they prefer natural, unrestricted movements. This feature was reflected by the cost of resistance to the passive dynamics, and represented by the KL divergence (see equation (2)). Because it can deal with these issues satisfactorily, we believe the LMDP is suited for modeling the animals' behavioral strategy.

Validity of the identified strategies

We applied our IRL approach to several cases of worms (WT and two deficient strains), and confirmed that the identified behavioral strategies, that is, the desirability functions, showed no discrepancy in thermotactic behaviors. Fed WT worms aggregated at the cultivation temperature (**Figure 2B**), which can be explained by highest amplitudes of the desirability function at the cultivation temperature (**Figure 3B**). Starved WT worms dispersed around the cultivation temperature (**Figure 5Aa**), accompanied by lowered amplitudes of the desirability function at the cultivation temperature (**Figure 5Ab**). The AWC-deficient worms showed normal thermotaxis (**Figure 5Ba**), and their desirability function was similar to that of WT animals (**Figure 5Bb**). The AFD-deficient worms demonstrated cryophilic thermotaxis (**Figure 5Ca**), consistent with higher amplitudes of the desirability function at lower temperatures (**Figure 5Cb**). In summary, these results demonstrate the validity of our approach as well as the potential of the method to determine changes in behavioral strategy.

Findings beyond what is already known

Although the identified strategies seem to just reproduce the actual behavior of C. elegans as discussed above, we offered a novel interpretation of the thermotactic strategy in terms of control theory[26]. First, regarding the DM strategy (**Figure 6A**), we could provide several examples of alternative strategies that also enable the worm to navigate to the goal (cultivation) temperature. **Figure 6B** shows the desirability function for the

worms switching their preference from a positive to a negative gradient in temperatures lower and higher than the goal temperature, namely "bang-bang control". **Figure 6C** shows the resulting desirability function if the worms simply prefer the goal temperature, regardless of the temporal derivative of the temperature. This might seem like "proportional (P) control". However, the identified DM strategy is based on both the absolute temperature and its temporal derivative as shown in **Figure 6A**, so that the worms in fact exercise "proportional-derivative (PD) control". Second, regarding the strategy of the starved worms (see **Figure 6D**), as discussed above, there are several possible alternative strategies. The worms could escape the cultivation temperature by exercising "bang-bang control" or "PD control", as shown in **Figure 6E and F**. The identified starved strategy is however closer to "P control", which only uses the absolute temperature. Our IRL-based approach is therefore able to clarify how the worms control their thermosensory state throughout migration, which has not been understood until now.

Functional significance of DM and IM strategies

We found that the WT worms used a thermotactic strategy consisting of two components, DM and IM strategies (**Figure 3B**). What is the functional meaning of these two strategies? We propose that the existence of these two strategies could be interpreted in terms of balancing exploration and exploitation. Exploitation is the use of pre-acquired knowledge in an effort to obtain rewards, and exploration is the effort of searching for possibly greater rewards. For example, the worm knows that food is associated with the cultivation temperature, and it can exploit that association. On the other hand, the worm could explore different temperatures to search for a larger amount of food than what is available at the cultivation temperature. In an uncertain environment, animals usually face an "exploration-exploitation dilemma" [27]; exploitative behaviors reduce the chance to explore for greater rewards, whereas exploratory behaviors disrupt the collection of the already-available reward. Therefore, an appropriate balance between exploration and exploitation is important for controlling behavioral strategies. We propose the hypothesis that the DM strategy generates exploitative behaviors, whereas the IM strategy generates explorative ones: the worms, through the DM strategy, exploit the cultivation temperature, and at the same time explore the reward (food) through the IM strategy, with each change in temperature.

How do the worms acquire these two strategies? We found that in the starved condition, temperature and feeding were dissociated, and as a result the DM strategy disappeared, whereas the IM strategy was still applied (**Figure 5Ab**). According to these findings, we hypothesize that the DM strategy emerges as a consequence of associative learning between the cultivation temperature and food access; the IM strategy, however, could be innate. Further investigation of these hypotheses should be expected in the future.

Comparison between strains and WT animals

In addition to WT worms, we identified the desirability functions of the AWC- and AFD-deficient worms (**Figure 5B and C**). The AWC and AFD neurons are both known to sense the temporal derivative of temperature, dT [17,21]. However, the AWC-deficient worms showed a desirability function profile similar to that of WT worms (**Figure 5Bb**), whereas the AFD-deficient worms had a different profile (**Figure 5Cb**); the profile lacked the DM's diagonal component and showed no bias along the dT axis. It can be assumed that an impaired AFD neuron prevents the worm from deciding whether an increase or decrease in

temperature is favorable, which could lead to inefficient thermotactic migration. Thus, the AFD, but not the AWC neuron, is involved in oriented migration behavior based on temporal changes in temperature.

Advantages of our IRL framework

Our IRL framework has several advantages. First, it is generally applicable to behavioral data not only of *C. elegans*, but to that of any animal, by following our guideline (**Figure 1D**). Thus, our approach has the potential for use in other biological fields like ecology and ethology. Second, this approach can be applied independently of experimental conditions. Our approach is especially suitable for analyzing behavior in natural conditions where target animals are behaving freely. To the best of our knowledge, this is the first study to identify the behavioral strategy of a freely-behaving animal by IRL. Third, our approach is able to identify behavioral strategies in terms of desirability functions, of which the neural substrates are expected to comprise many different functionally networked cortical (prefrontal cortex) and subcortical (basal ganglia) areas [5,6]. The approach presented here thus allows analyses of neural correlates, such as comparing regional neural activities of freely-behaving animals with strategy-related variables calculated by IRL. In an era when high-throughput experiments and "big data" analyses produce massive amounts of the behavioral data that is required for our IRL approach, it has the potential to become a fundamental tool with broad applicability in neuroscience, especially for the study of the neural mechanisms underlying behavior and behavior strategies.

Materials and Methods

Reinforcement learning

Reinforcement learning (RL) is a machine learning model that describes how agents learn to obtain an optimal policy, that is, a behavioral strategy, in a given environment. RL consists of several components: an agent, an environment, and a reward function. The agent learns and makes decisions, and the environment is defined by everything else. The agent continuously interacts with the environment, in which the state of the agent transits based on its action (behavior), and the agent gets a reward at the new state according to the reward function. The aim of the agent is to identify an optimal strategy (policy) that maximizes cumulative rewards in the long term.

In this study, the environment and the agent's behavioral strategy were modeled as LMDP, one of the settings of RL. An LMDP is characterized by the passive dynamics of the environment in the absence of control, and by controlled dynamics that reflect the behavioral strategy. Passive and controlled dynamics were defined by transition probabilities from state s to s', $p(s'|s)$ and $\pi(s'|s)$, respectively. At each state, the agent not only acquires a cost (negative reward), but also receives resistance to the passive dynamics (**Figure 1C**). Thus, the net cost is described as

$$l\left(s,\pi(\cdot\mid s)\right)=q(s)+KL\left[\pi(\cdot\mid s)\middle\|p(\cdot\mid s)\right], \tag{2}$$

where $q(s)$ denotes a state cost and $KL[\pi(.|s)||p(.|s)]$ indicates the Kullback–Leibler (KL) divergence between $\pi(.|s)$ and $p(.|s)$; this represents the resistance to the passive dynamics.

The optimal policy that minimizes the cumulative net cost has been analytically obtained as

$$\pi^{*}\left(s'\mid s\right)=\frac{P(s'\mid s)\exp\left\{-v(s')\right\}}{\sum_{s'}P(s'\mid s)\exp\left\{-v(s')\right\}}, \tag{3}$$

where $v(s)$ is a value function, that is, the expected cumulative net costs from state s toward the future, which satisfies Bellman's self-consistency:

$$\exp(-v(s))=\exp(-q(s))\sum_{s'}P(s'\mid s)\exp\left\{-v(s')\right\}. \tag{4}$$

Inverse reinforcement learning (*estimation of the value function*)

To estimate the value function $v(s)$, we assumed that the observed sequential state transitions $\{s_t, s_{t+1}\}_{t=1:T}$ were generated by the optimal policy π^*. We then maximized the likelihood of the sequential state transition:

$$L=\prod_{t}\pi^{*}\left(s_{t+1}\mid s_{t}\right), \tag{5}$$

where $\pi^*(s_{t+1}|s_t)$ corresponds to equation (3). This maximum likelihood estimation (MLE) was called OptV [20]. Based on the estimated value function, the primary cost function, $q(s)$, can be calculated by using equation (4).

It is reasonable to assume that animals have value functions that are smooth in their state space in order to compensate for noisy sensory systems. To obtain smooth value functions, we regularized MLE as

$$\hat{v}(s)=\underset{v(s)}{\arg\min}\left[-\log L(v(s))+\lambda\sum_{s}\sum_{s'\in\chi(s)}\left|v(s)-v(s')\right|^{2}\right], \tag{6}$$

where the first term represents negative log-likelihood, and the second term represents a smoothness constraint introduced to the value function; a positive constant λ indicates the strength of the constraint, and $\chi(s)$ indicates a set of neighboring states of s in the state space. Notice that the cost function, the regularized negative log-likelihood, is convex with respect to $v(s)$, which means there are no local minima in its optimization procedure.

Passive dynamics of thermotaxis in C. elegans

To apply IRL to thermotactic behaviors of *C. elegans*, state s and passive dynamics $p(s'|s)$ must be defined. We previously found that the thermosensory AFD neuron encodes the temporal derivative of the environmental temperature [21], and thus assumed that the worm can sense not only absolute temperature T, but also the temporal derivative of temperature dT/dt. We therefore set a 2D state representation as (T, dT). Note that dT/dt is simply denoted as dT.

The passive dynamics were described by the transition probability of a state (T, dT) as

$$P\left((T',dT')\middle|(T,dT)\right)=\mathcal{N}\left(T'\middle|T+dT\Delta t,\sigma_T\right)\mathcal{N}\left(dT'\middle|dT,\sigma_{dT}\right), \tag{7}$$

where $\mathcal{N}(x|\mu, \sigma)$ indicates a Gaussian distribution of variable x with mean μ and variance σ, and Δt indicates the time interval of monitoring in behavioral experiments. This passive dynamics aspect can be loosely interpreted as the worms inertially migrating in a short time interval under a thermal gradient, but it may also be perturbed by white noise.

Artificial data

We confirmed that our regularized version of OptV (equation (6)) provided a good estimation of the value function using simulation data. First, we designed the value function of T and dT as the ground truth (**Supplementary figure 2A**), and passive dynamics through equation (7). Thus, the optimal policy was defined by equation (3). Second, we generated a time-series of state transitions according to the optimal policy, and separated these time series into training and test datasets. After that, we estimated the value function from the training dataset, varying the regularization parameter λ in equation (6) (**Supplementary figure 2B**). We then evaluated the squared error between the behavioral strategy based on the ground truth and the estimated value function, using the test dataset. Since the squared error on the test data was substantially reduced (by 88.1%) due to regularization, we deemed it effective for avoiding overfitting (**Supplementary figure 2C**).

Cross-validation

In estimation of the value function, we performed cross-validation to determine λ in equation (6), and σ_T and σ_{dT} in equation (7), with which the prediction ability is maximized. We divided the behavioral time-series data equally into nine parts. We then independently performed estimation of the value function nine times; for each estimation, eight of the nine parts of the data were used for estimation, while the remaining part was used to evaluate the prediction ability of the estimated value function by the likelihood (equation (5)). We then optimized those parameters at which we obtained the lowest negative log-likelihood as averaged from the nine estimations.

C. elegans *preparation*

All worms were hermaphrodites and cultivated on OP50 as bacterial food using standard techniques [28]. The following strains were used: N2 wild-type Bristol strain, IK0615 *ttx-1(p767)*, IK2808 *njIs79*[*ceh-36p::cz::caspase-3(p17)*, *ceh-36p::caspase-3(p12)::nz*, *ges-1p::NLS::GFP*]. The AWC-ablated strain (IK2808) was generated by the expression of reconstituted caspases [29]. Plasmids carrying the reconstituted caspases were injected at 25 ng/µl with the injection marker pKDK66 (*ges-1p::NLS::GFP*) (50 ng/µl). Extrachromosomal arrays were integrated into the genome by gamma irradiation, and the resulting strains were outcrossed four times before analysis. To assess the efficiency of cell killing by the caspase transgenes, the integrated transgenes were crossed into integrated reporters that expressed GFPs in several neurons, including the neuron of interest, as follows: IK2811 *njIs82*[*ceh-36p::GFP*, *glr-3p::GFP*] for AWC. Neuronal loss was confirmed by the disappearance of fluorescence; 100% of *njIs80* animals displayed the loss of AFD, and 98.4% of *njIs79* animals displayed the loss of AWC.

Thermotaxis assay

Thermotaxis (TTX) assays were performed as previously described [30]. Animals cultivated at 20 °C were placed on the center of an assay plate (14 cm × 10 cm, 1.45 cm height) containing 18 ml of TTX medium with 2% agar, and were allowed to freely move for 60 min. The center of the plate was adjusted to 17 °C, 20 °C, or 23 °C, to create three different gradient conditions, and the plates were then maintained at a linear thermal gradient of approximately 0.45 °C/cm.

Behavioral recording

Worm behaviors were recorded using a multi-worm Tracker [16] with a CMOS sensor camera-link camera (8 bits, 4,096 × 3,072 pixels; CSC12M25BMP19-01B; Toshiba-Teli), a Line-Scan Lens (35 mm, f/2.8; YF3528; PENTAX), and a camera-link frame grabber (PCIe-1433; National Instruments). The camera was mounted at a distance above the assay plate that consistently produced an image with 33.2 µm per pixel. The frame rate of recordings was approximately 13.5 Hz. Images were captured and processed by custom software written in LabView (National Instruments), and a custom image analysis library written in C++, to detect worm bodies and measure behavioral parameters such as the position of the centroid.

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Author contributions

H.N. and S.I. conceived the project. S.Y. performed the computational analysis. M.I., Y.T, and S.N. performed the experiments. H.N. and S.Y. wrote the draft, and H.N., S.Y., M.I., S.N., I.M., and S.I. prepared the final version of the manuscript.

Competing interests

The authors declare that there are no competing interests.

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Figures

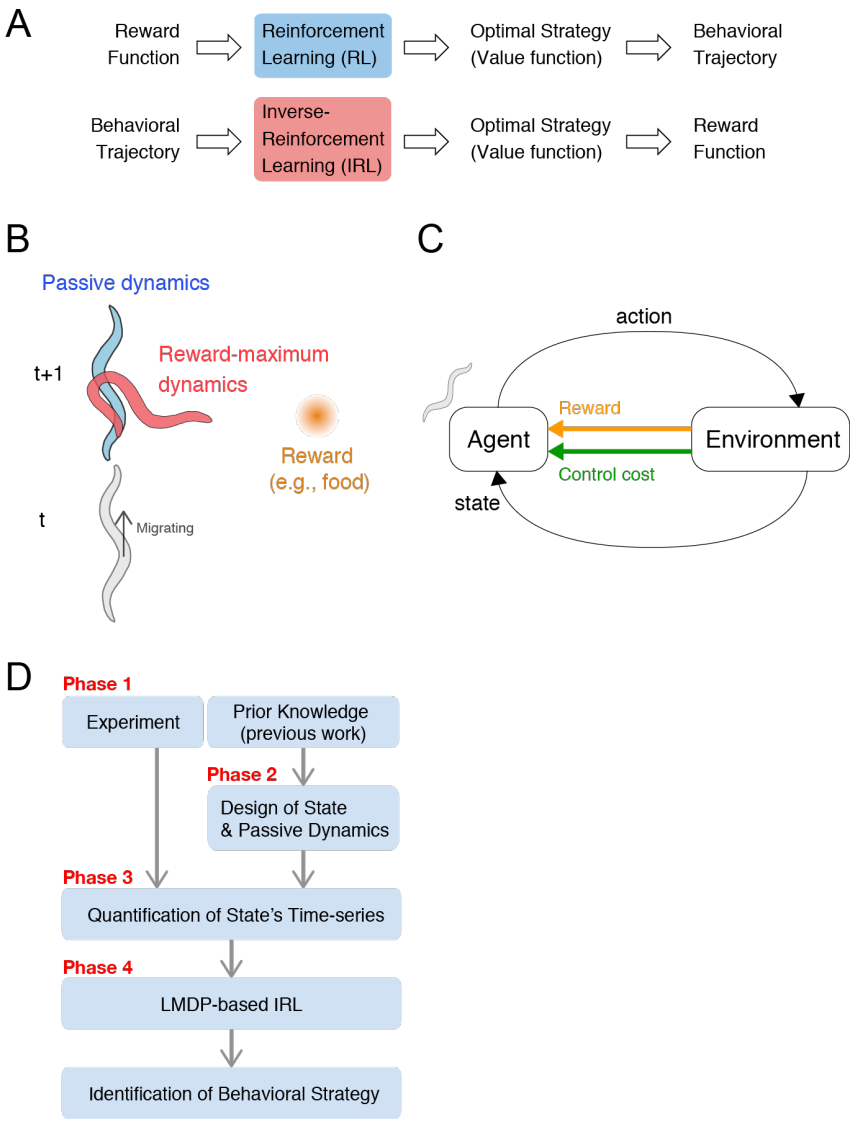

Figure 1: Concept and procedure of the inverse reinforcement learning (IRL)-based approach

(A) RL represents a forward problem, in which a behavioral strategy is determined to maximize the cumulative reward given as a series of state-dependent rewards. IRL represents an inverse problem, in which a behavioral strategy, or its underlying value and reward functions, is estimated in order to reproduce an observed series of behaviors. The behavioral strategy is evaluated by the profiles of the identified functions.

(B) Examples of passive dynamics and controlled dynamics. Here, an animal migrates upwards whereas the food (reward) is placed to its right. In this situation, if the animal continues to migrate upwards, the distance to the food increases. If the animal exercises harder body control, that is, changes its migrating direction toward the food, the distance to the food decreases. The animal should therefore make decisions based on a tradeoff between these two dynamics.

(C) The agent-environment interaction. The agent autonomously acts in the environment, observes the resultant state-transition through its sensory system, and receives not only the state reward but also the body control cost. The behavioral strategy is optimized in order to maximize the accumulation of the net reward, which is given as state reward minus body control cost.

(D) Guideline for the IRL framework. This guideline outlines the general procedures of the IRL framework for the identification of animal behavioral strategies. Details are explained in the main text.

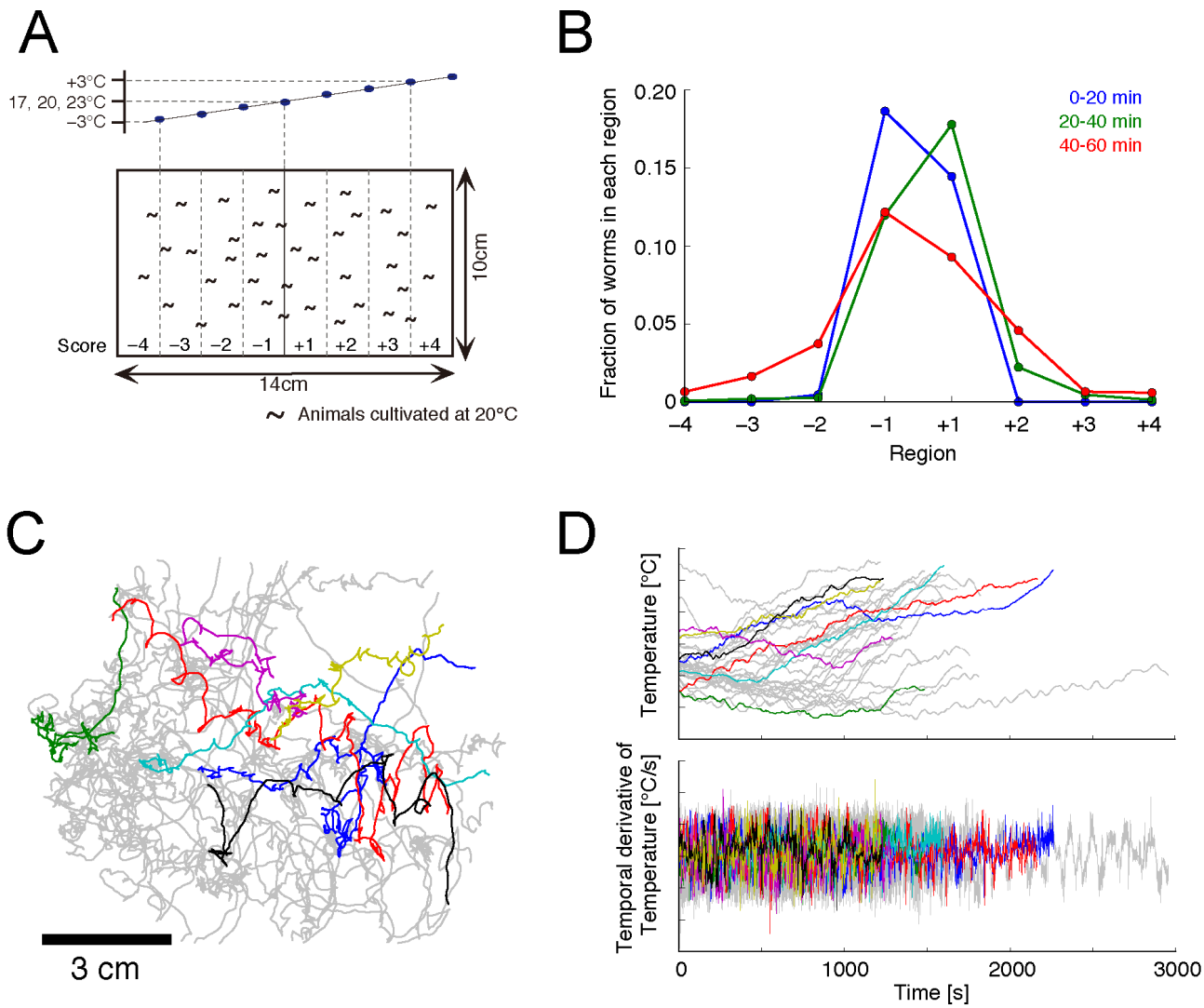

Figure 2: Data acquisition of *C. elegans* behavior

(A) Thermotaxis assays with a thermal gradient. In each assay, a linear temperature gradient was set along the agar surface, whose center was set at either 17, 20, or 23 °C. At the onset of the assays, fed or starved worms were uniformly placed on the agar surface.

(B) Temporal changes in the worms' spatial distribution under the 20 °C-centered thermal gradient in the fed condition.

(C) Trajectories of a number of worms extracted by the multi-worm tracking system. Different colors indicate different individual worms.

(D) Time series of the temperature experienced by the migrating worms shown in C (colors correspond to those in C) and its derivative.

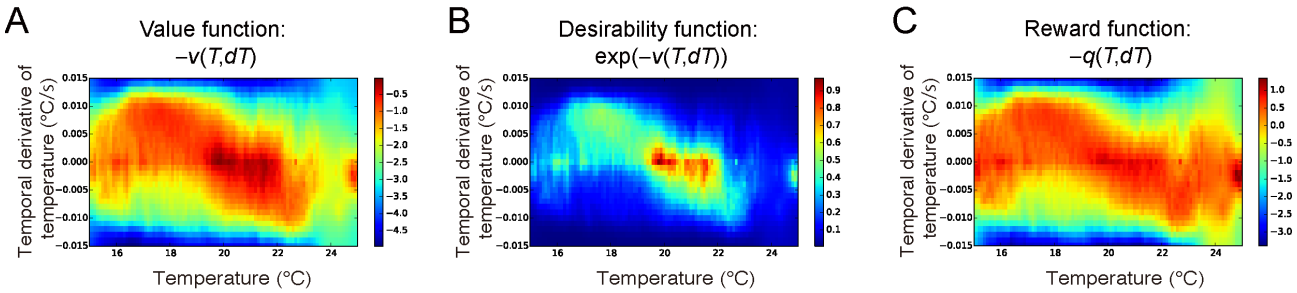

Figure 3: Behavioral strategy identified for fed wild type (WT) worms

The behavioral strategies of fed WT worms represented by the value (A), desirability (B), and reward (C) functions. The worms prefer and avoid the red- and blue-colored states, respectively.

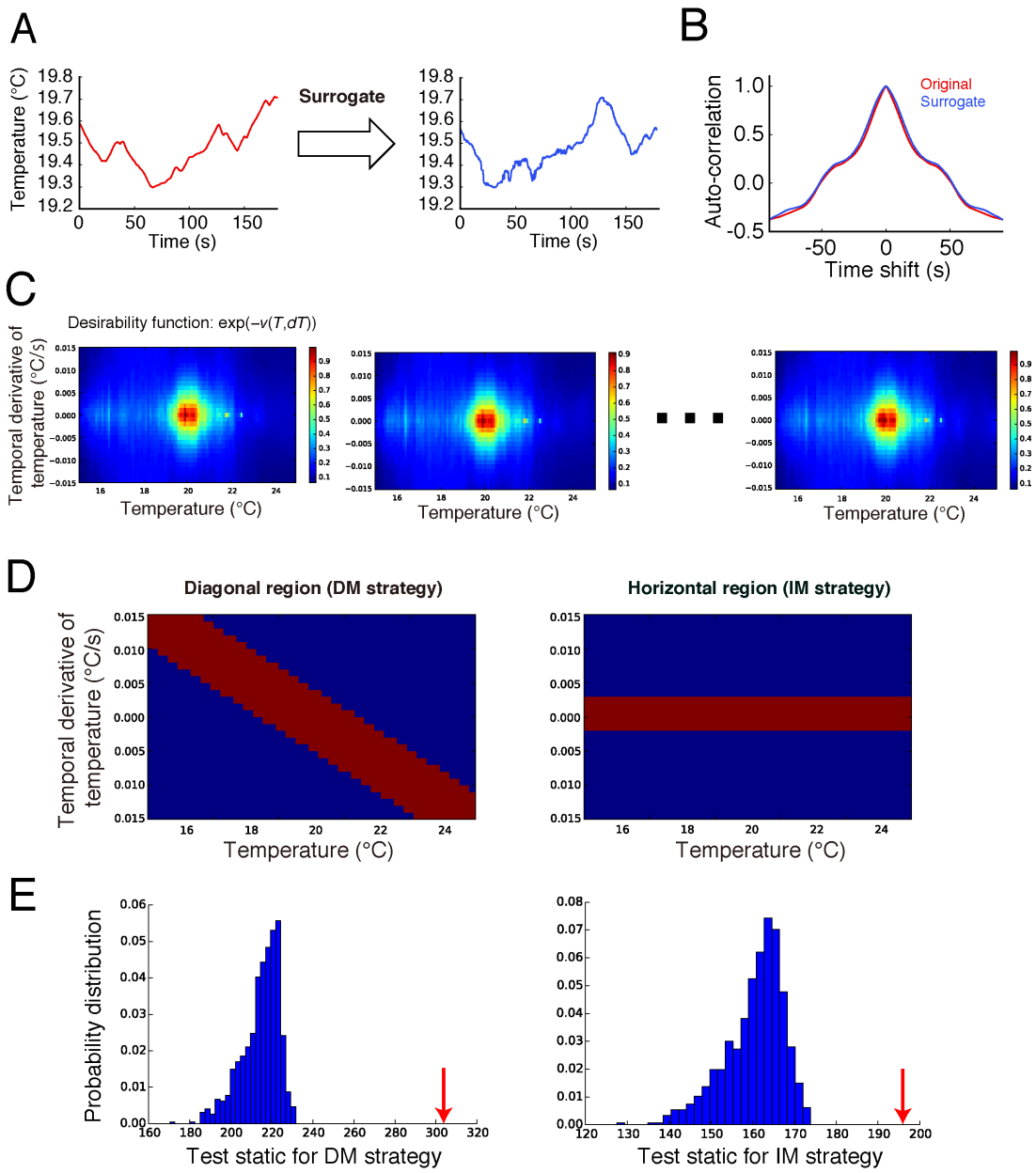

Figure 4: Statistical test for reliability of behavioral strategies with the surrogate method

The reliability of the directed migration (DM) and isothermal migration (IM) strategies (see Figure 3) was assessed by means of statistical testing with the null hypothesis that the worms randomly migrate with no behavioral strategy. (A) To generate time-series data under the null hypothesis, original time-series data of temperature (left panel) was surrogated by the IAAFT method (right panel). (B) Before and after the surrogation, the autocorrelations were almost preserved. (C) The desirability functions estimated from the surrogate datasets. (D) The DM and IM strategies correspond to the red-highlighted diagonal and horizontal regions of the desirability function, respectively. Within these regions, sums of the estimated desirability functions were calculated as test statistics. (E) Histograms of the empirical null distributions of the test statistics for the DM and IM strategies. The test statistics derived by the original desirability function (red arrows) are located above the empirical null distributions (p=0 for the PT strategy; p=0 for the IT strategy).

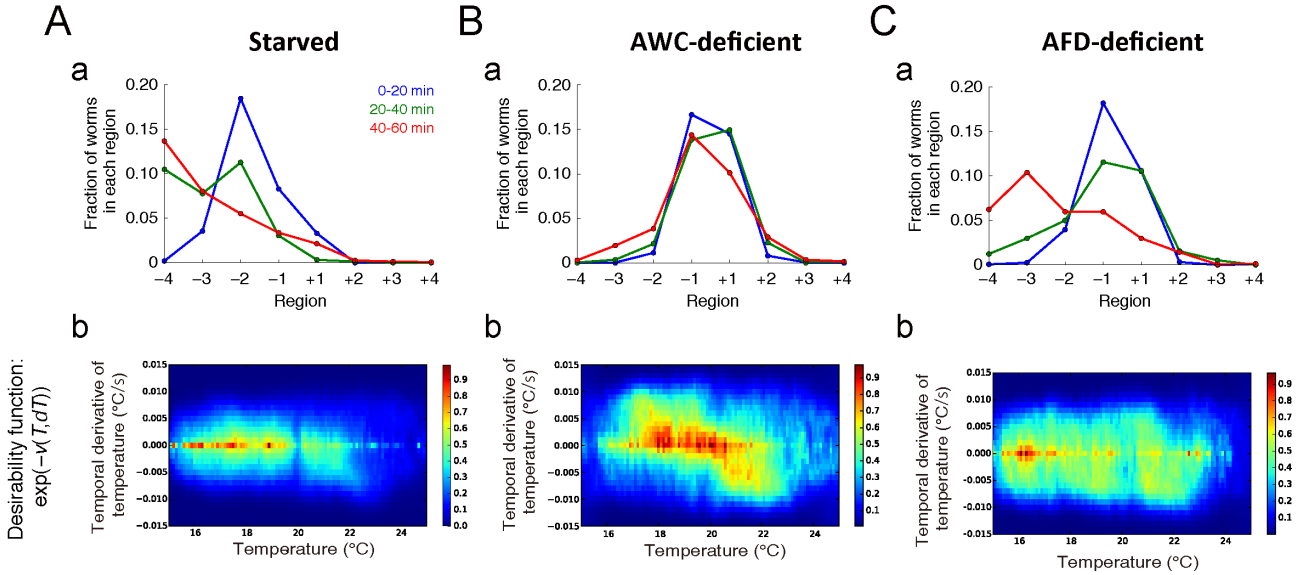

Figure 5: Inverse reinforcement learning (IRL) analyses of starved worms, AWC-, and AFD-deficient worms

Temporal changes in distributions of starved worms, AWC-deficient worms, and AFD-deficient worms in the 20 °C-centered thermal gradient after the behavior onset are presented in row a of A, B, and C, respectively. Starved worms disperse under a thermal gradient; AWC-deficient worms migrate to the cultivation temperature, similarly to fed wild type worms, and AFD-deficient worms show cryophilic thermotaxis. Corresponding desirability functions are shown in row b of A, B, and C.

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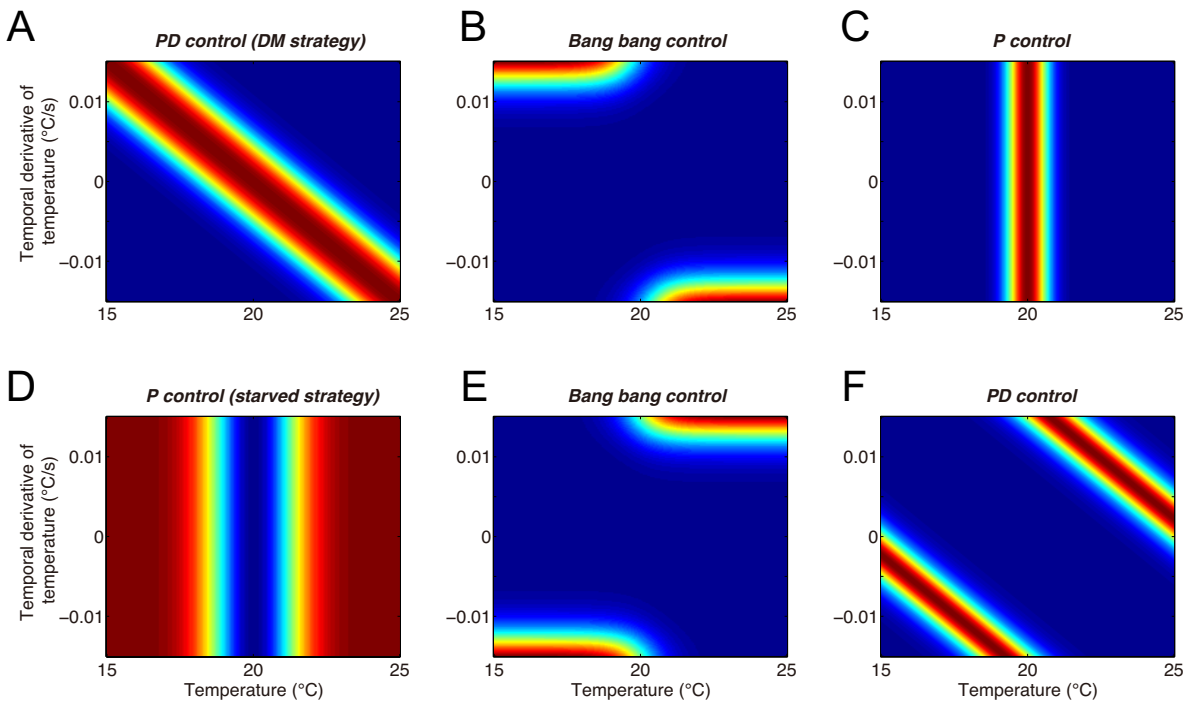

Figure 6: Possible strategies of preferring and avoiding the cultivation temperature

Each panel represents the desirability function of a possible strategy. The prior knowledge that fed worms navigate to the cultivation temperature and starved worms escape the cultivation temperature allowed to propose several possible strategies, but not to identify the worms' actual strategy (fed worms: A-C, starved worms: E-G). Our approach identified that the fed worms used the proportional-derivative (PD) control-like DM strategy shown in (A), while the starved worms used the proportional (P) control-like strategy shown in (D).

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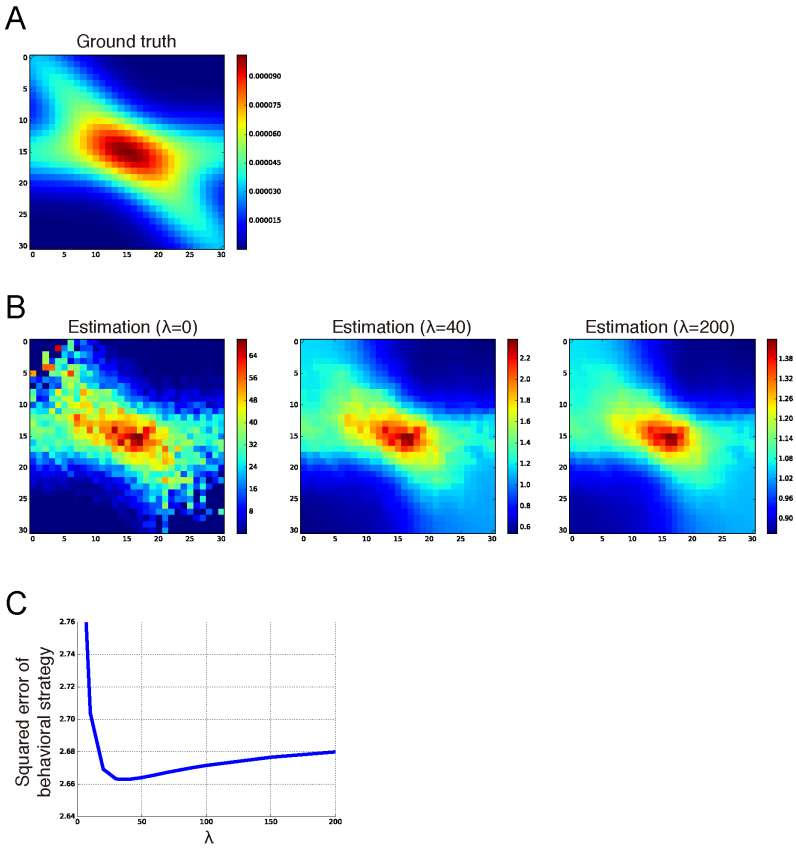
Supplementary figures

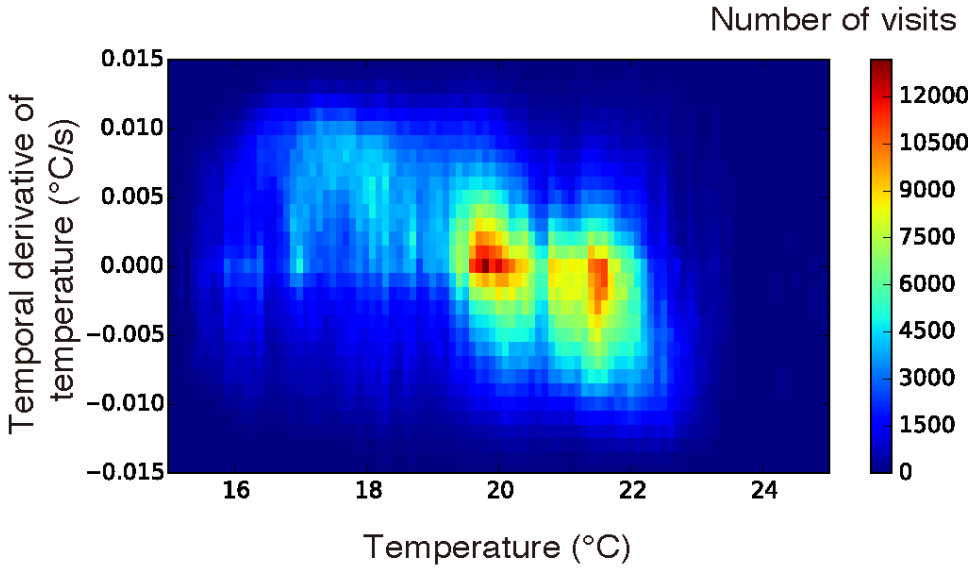


Supplementary figure 1: Validation of the regularized (OptV) estimation method in artificial data

(A) The desirability function corresponding to the ground truth value function used for generation of artificial data. Time-series data were artificially generated as training and test data sets by sampling equation (1), given the ground truth of the value function.

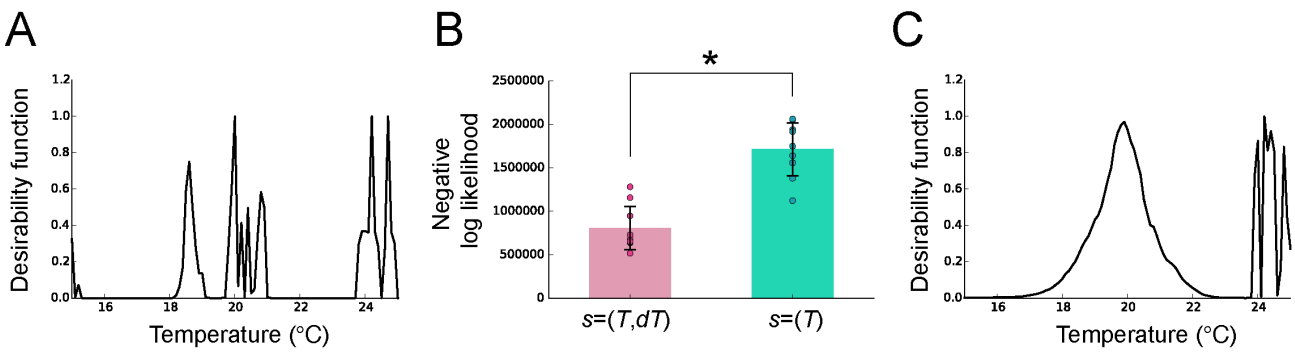
(B) The desirability functions described by equation (6) under three different regularization parameters (λ) were visualized from the estimated value functions.

(C) Squared error between the behavioral strategies based on the ground truth and estimated value functions using the test data set. The presence of an optimal λ, at which the minimal squared error is obtained, indicates that the regularization was effective for accurately estimating the value function.



Supplementary figure 2: State distributions in fed wild type (WT) worms

Observed distributions of T and dT in fed WT worms are shown as a heat map. Notice that the distribution is substantially different from the desirability function (see Figure 3B).



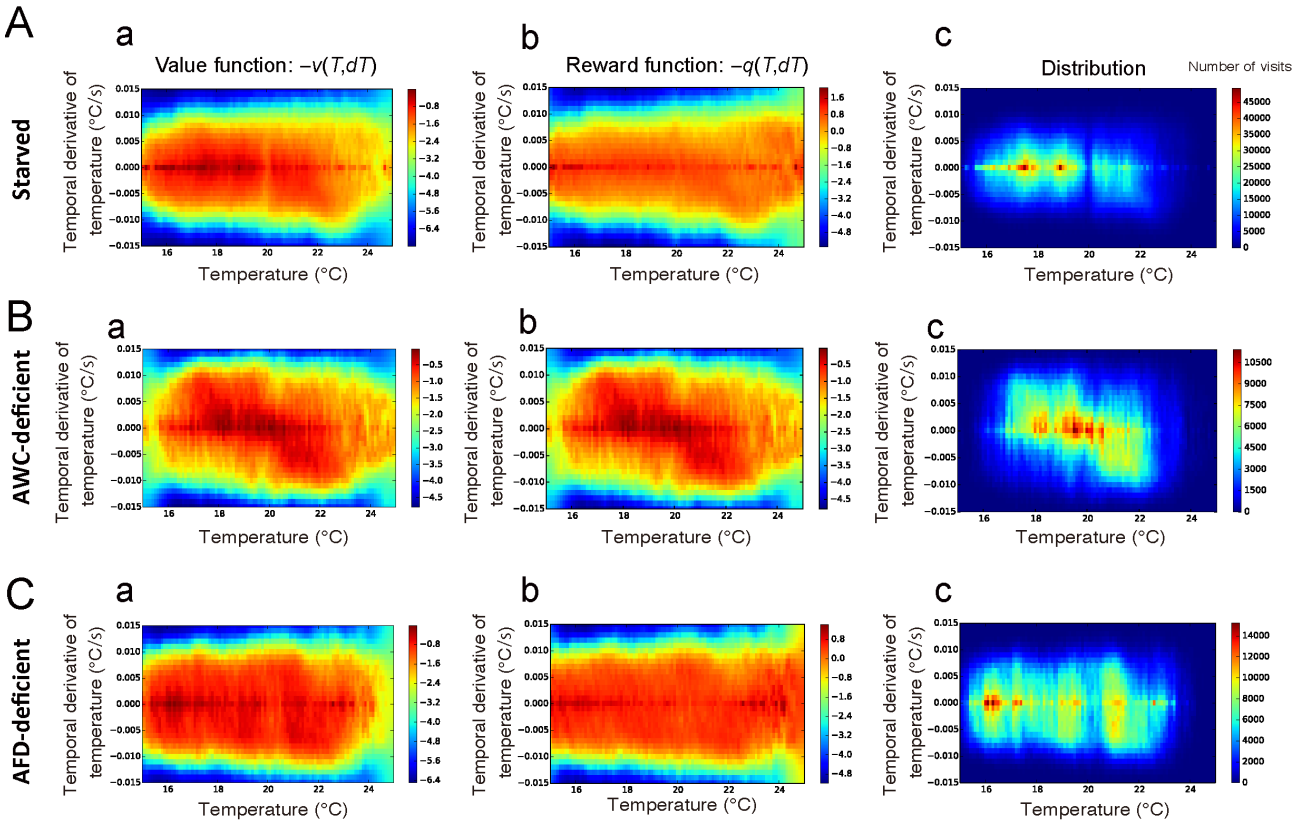
Supplementary figure 3: Inverse reinforcement learning (IRL) analysis with one-dimensional state representation

IRL was analyzed with one-dimensional state representation (s=(T)).

(A) The desirability function was calculated using the estimated value function. In the estimation, the regularization parameter, λ, in equation (6) was optimized by cross-validation.

(B) Prediction ability was compared between IRLs with s=(T, dT) and s=(T) using a cross-validation dataset. The negative log-likelihood of the behavioral strategies (equation (1)) with the estimated value function of both T and dT (see Figure 3B) was significantly smaller than that with the estimated value function of T alone (Supplementary figure 3A) (p=0.0002; Mann-Whitney U test). Thus, the behavioral strategy with s=(T, dT) was more appropriate than that with s=(T).

(C) The desirability function became smoother as λ increased. This desirability function peaks around the cultivation temperature (20 °C).



Supplementary figure 4: Estimated value/reward functions and state distributions

The estimated value functions (a), reward functions (b), and state distributions (c) are depicted for the starved wild type worms (A), the AWC-deficient worms (B) and the AFD-deficient worms (C).