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Chemotaxis mediated by non-adaptive dynamics

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Abstract

Chemotaxis is the ability of bacteria to locate high attractant sources in the environment. The extensive knowledge gained from the pathway in the model organism Escherichia coli shows that adaptation to stimuli is a hallmark underlying chemotaxis. Studies on certain mutant strains and other bacterial species, however, indicate that some form of chemotaxis could also be achieved without adaptation. It is not clear how efficient such chemotaxis is, how it could be mediated, and how widespread it is among different bacterial species.

In order to explore alternative pathway structures and dynamics that can underlie chemotaxis, we employ an evolutionary approach. This approach starts with a population of bacteria that move in a virtual environment based on the dynamics of simple pathways they harbour. As mutations lead to changes in pathway structure and dynamics, bacteria with better ability to localize with high attractant sources gain a selective advantage. We find that chemotaxis via non-adaptive dynamics evolves consistently under different model assumptions and environments. These dynamics directly couple tumbling probability of the cell to increasing attractant levels. Further analyses of evolved pathway structures show that this alternative behaviour can be mediated with as few as two components.

The non-adaptive mechanisms mediating chemotaxis provide an explanation for experimental observations made in mutant strains of E. coli and in wild type Rhodobacter sphaeroides and that could not be explained with existing knowledge. These mechanisms could allow a straightforward link between cell metabolism and chemotaxis. Furthermore, they could have acted as the origin of the conventional chemotaxis involving adaptation.

INTRODUCTION

Bacterial chemotaxis and the pathways mediating it are a model system for studying the molecular basis of behaviour. More than 30 years after the first studies of this behaviour in *Escherichia coli* [1,2], we now have extensive knowledge of the underlying pathway in this species [3,4]. Briefly, *E. coli* swims in a forward direction (undergoing some degree of rotational diffusion) when one or more of the reversible motor proteins on its outer membrane rotate counter-clockwise (CCW) and the attached flagella intertwine to form an effective propeller. When the motors reverse and rotate clockwise (CW), the flagella disassociate and cause the bacterium to tumble, resulting in a new swimming direction. The switching frequency of the motor is coupled to receptor activity by a series of proteins constituting a signalling pathway. With increasing attractant levels, the excitatory branch of the pathway causes direct suppression of CW rotation and tumbling, while the adaptation branch causes the cell to resume its original tumbling levels at constant attractant concentrations independently of this concentration level. The former branch involves the response regulator CheY, which in its phosphorylated form binds the motor and increases the probability of CW rotation. The adaptation is achieved via control of receptor methylation, and hence receptor sensitivity, through the proteins CheR and CheB. The combination of these two branches results in the tumbling frequency approximately following a time-derivative of the attractant concentration [5]. Adaptation is the hallmark of this response, allowing bacteria to perform temporal comparisons of attractant with high sensitivity and achieve proper chemotaxis [5-9]. In fact, perfect adaptation is the most robust feature of the system in face of fluctuations in protein concentrations [10].

While the importance of adaptation in proper chemotaxis is well established, there are several indications that some form of chemotaxis could be possible without it. The earliest of these came from a mutant strain of *E. coli* that lacks CheR and CheB but is still capable of chemotaxis [11]. This ‘anomalous’ chemotaxis was suggested to result from random diffusion coupled with partial adaptation [12]. While there are possible mechanisms that could allow such receptor-independent adaptation [13,14], their relation to the chemotaxis seen in CheR-CheB mutant was not explored. Another observation involves the ‘gutted’ strain of *E. coli*, which lacks all proteins of the chemotaxis pathway except CheY [15]. While the dynamics mediating chemotaxis in this mutant is unknown, involvement of adaptation is highly unlikely. The most convincing case for non-adaptive chemotaxis comes from studies on *Rhodobacter sphaeroides*. In this species, adaptation to persisting stimuli seems to work much slower or not at all [16]. Interestingly, there are other observations from *R. sphaeroides* that cannot be explained by the knowledge gained from the *E. coli* system; cells grown under aerobic conditions give an ‘inverted’ response with limited adaptation [17] and chemotaxis to certain attractants requires transporters [18]. Currently, we lack detailed understanding of the molecular systems mediating chemotaxis in this species, however, it is known that it has a large number of proteins involved in this chemotaxic behaviour and that these are arranged into several distinct pathways [19].

All these observations indicate existence of alternative ways of mediating chemotaxis, some of which might involve non-adaptive dynamics. The exact nature of these alternative mechanism(s), their relation to the conventional chemotaxis pathway, and the extent of their presence in diverse bacterial species all remain unclear. These issues become more relevant as we start to realize the diversity of chemotaxis pathways in other bacterial species and discover significant differences from *E. coli* both in structure and dynamics [20-22].

Here, we use a computational approach to address these questions and study the evolutionary history of chemotaxis using simulations of bacterial movement and pathway dynamics. These simulations use a population of virtual bacteria existing in a virtual world complete with an attractant source. Bacteria start with a set of initially non-interacting proteins, as well as a receptor and a reversible motor. Interactions between the proteins evolve through random mutations, with bacteria selected based on their ability to localise with the attractant. These evolutionary simulations consistently result in bacteria with a strong ability to chemotax. Interestingly, the underlying pathways in these bacteria show non-adaptive dynamics where tumbling probability is directly coupled to attractant levels. We find that such non-adaptive dynamics can be mediated by as few as two signalling components, allowing for the possibility of metabolites acting as effectors. Combined with experimental observations, these results suggest that non-adaptive dynamics underlie the chemotaxis observed in gutted *E. coli* strains and have a role in the complex chemotaxis behaviour of *R. sphaeroides*. Furthermore, they allow us to speculate that mechanisms leading to such dynamics exist in current-day bacteria as alternative pathways or remnants of evolution and provide a way to fine-tune chemotaxis in different conditions, or link it to metabolism.

RESULTS

In order to study the evolution of bacterial chemotaxis, we use virtual bacteria that move in a computer-based two-dimensional environment containing a fixed and pre-determined attractant source. The movement of these bacteria is coupled to the dynamics of a signalling pathway consisting of several proteins that catalyze each other's activation and deactivation, corresponding to kinases and phosphatases in real cells (see *Methods*). These proteins include a receptor that can interact with the local attractant concentration as well as an effector that, when activated, can bind to a reversible motor, reversing its direction and causing the bacteria to tumble. Evolutionary simulations start with a population of bacteria, each of which contains a certain number of proteins that are initially not interacting. These bacteria are allowed to explore the environment for a 'generation-time' consisting of a certain number of time steps. At each time step, the bacteria either can continue to swim forward or can tumble to orient to a new random direction. Additionally, the concentrations of activated proteins in the pathways of each bacterium are updated, and the probability of tumbling during the next time step is computed based on the concentration of activated effector. After this generation-time, bacteria are selected and replicated based on the amount of attractant they have encountered. During replication, there is a probability for mutations to occur, which alters the structure and parameters of the biochemical pathway. To summarize, these evolutionary simulations couple mutational events occurring at molecular level (i.e. pathway level) with selection at behavioural level (i.e. chemotaxis).

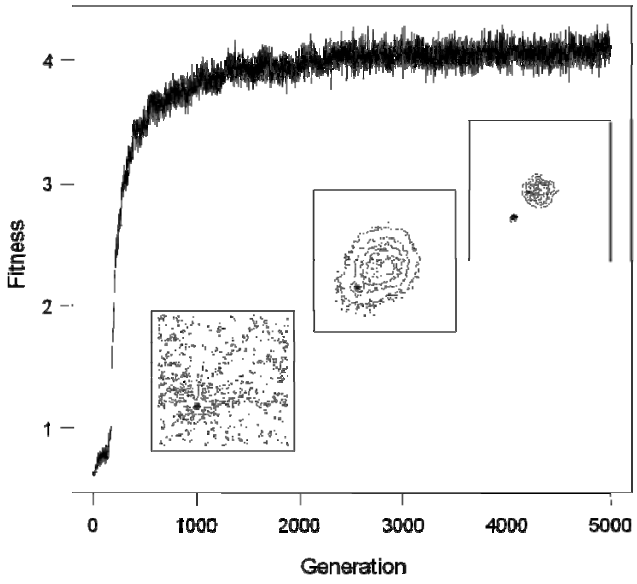

Figure 1: Evolution of chemotaxic bacteria in silico. The average fitness in an evolving population of virtual bacteria. The inset shows the time-averaged distribution of positions of the population at generation 0, 200 (corresponding to a fitness of approximately 2.0), and 5000 (final generation) as a contour plot. Areas enclosed by darker lines indicate more time spent there. Note that in these simulations the entire population starts at grid location (30,30) while attractant source is fixed at (50,50).

Figure 1 shows the population average of fitness (encountered attractant) during one such evolutionary simulation for a signalling pathway consisting of four proteins. As shown, the fitness value rapidly improves over a few generations and reaches a plateau. Clearly, the pathway structure and dynamics in virtual bacteria are evolving in such a way to mediate a form of chemotaxis. This behaviour can be seen from the average time spent of the population at different parts of the environment (see insets of Figure 1). While un-evolved bacterial populations are distributed irrespective of attractant source, final populations are able to quickly localize with the attractant source and spend most time there. Such chemotaxis is mediated by a particular pathway structure

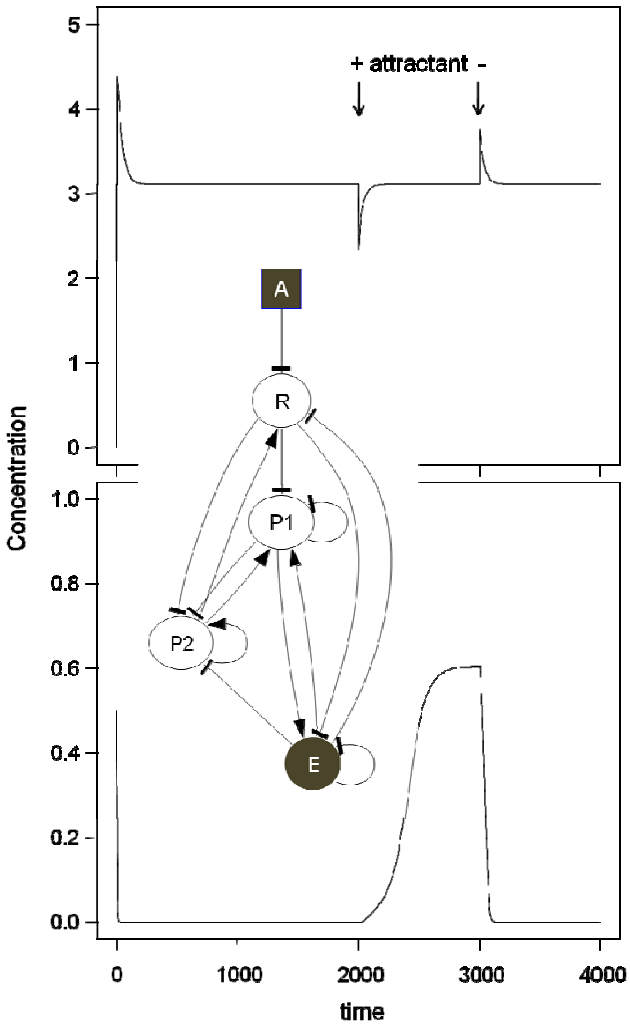

Figure 2: Adaptive vs. non-adaptive pathway dynamics. Time course of phosphorylated CheY concentration (top), as simulated by the model presented in [22] and the time course of active effector concentration for the most frequent pathway in the evolutionary simulation described in Figure 1 (bottom). The inset shows the cartoon representation of this pathway. In both simulations, an attractant of arbitrary concentration is added at time 2000 and removed at time 3000.

with specific response dynamics (kinetic parameters are given in *Supplementary Material*). At steady state, in absence of any signal, the concentration of activated effector is at a low level and the bacterium mostly swims without tumbling (see Figure 2). When the bacterium encounters attractant, the effector is rapidly activated and stays activated as long as this is present, resulting in increased bacterial tumbling. Such pathway dynamics allow the bacteria to spend more time in regions of high attractant and swim straight when the attractant decreases. In other words, the pathway dynamics lead to an alternative chemotaxis strategy, whereby bacteria can maximally exploit an attractant source when it encounters one, but does not put too much effort in searching for attractant. In evolutionary simulations repeated five times for pathways of 2 to 5 proteins, this mechanism always evolved as the dominant one.

Using a simple model, we can capture the movement of bacteria as mediated by such pathway dynamics (see *Methods*). This model shows that in simple environments the presented dynamics should lead to bacteria accumulating proportionally with the local concentration of the attractant. In other words, an efficient form of chemotaxis can be achieved with the presented dynamics that does not display any adaptation to attractant and results in increases in tumbling probability with increasing attractant. Both features are in striking contrast to *E. coli*, where the pathway ensures decreasing tumbling probability with increasing attractant followed by rapid adaptation [5] (see Figure 2).

There are a number of different explanations for why the chemotaxis pathways evolved in these simulations are underlined by an 'inverted' response and non-adaptive dynamics. Firstly, it might be that the modelled environmental situations are particularly well suited for chemotaxis mediated via non-adaptive dynamics. In particular, the consistency of the attractant source might reduce the need for adaptation. Secondly, the evolutionary processes as modelled here might make non-adaptive pathways more evolutionarily accessible. Thirdly, there could be several other factors, such as metabolism of attractant source, intra-cellular communication, and multi-state receptors that are not included in the model and that could be important for the evolution of chemotaxis mediated by other dynamics.

To explore the effectiveness of non-adaptive mechanisms in more realistic fluctuating environments, we ran simulations where the attractant source, background attractant distribution, and initial starting location of bacteria were chosen at random for each generation (see *Methods*). As adaptation has been suggested as a method to preserve robustness of the response to fluctuations in the external environment or internal parameters [23], it could be possible that such simulations lead only to evolution of chemotaxis pathways with adaptive dynamics. In five separate simulations, we observe three simulations evolving pathways with strictly non-adaptive dynamics and inverted response. One simulation evolved pathways that showed limited adaptation, also with an inverted response. The fifth simulation resulted in pathways with a 'normal' response at lower levels of attractant, where increasing attractant caused a decrease in tumbling probability. The dynamics of these pathways, however, were dependent on the level of attractant, and higher attractants resulted in inverted responses. The structure and dynamics of representative pathways from these simulations are shown in Figure 3 (kinetic parameters are given in *Supplementary Material*).

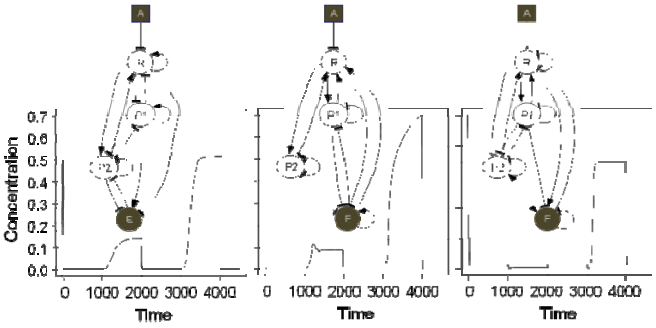

Figure 3: Diverse mechanisms mediating chemotaxis. Time course of active effector concentration for the most frequent and unique pathways obtained from five different evolutionary simulations with fluctuating attractant dynamics (see *Methods*). Each row displays dynamics for a specific pathway structure shown in the inset. An attractant of low (high) concentration is added at time 1000 (3000) and removed at time 2000 (4000). The first pathway structure was found in three simulations, while others were found in one each.

These results indicate that inverted responses and non-adaptive dynamics result in efficient chemotaxis even in fluctuating environments. To further explore and contrast the efficiency of chemotaxis mediated by such dynamics to that mediated by conventional dynamics, we performed additional simulations. These started with an initial bacterial population containing biochemical pathways with dynamics similar to that found E. coli [24]. In five separate simulations, the bacteria always evolved pathways with non-adaptive dynamics and inverted response. In other words, under the conditions of these simulations, there always existed a pathway with non-adaptive dynamics and that could mediate more efficient chemotaxis than the original adaptive pathway. This indicates that the results we obtain are not due to lack of an evolutionary route to the conventional dynamics observed in E. coli. It does not indicate, however, that chemotaxis mediated by non-adaptive dynamics is superior as it was not possible to reproduce all environmental conditions and other possibly important features as mentioned above.

Both non-adaptive dynamics and inverted responses are observed in wild type *R. sphaeroides* [16,17]. Inverted responses are also observed in gutted strains of *E. coli* [15]. In both cases, efficient chemotaxis was observed, although the exact nature of the underlying molecular mechanisms could not be determined. It is highly likely that these mechanisms form systems similar to the pathways presented here. An analysis of results from simulations with two proteins reveals the minimum signalling systems to achieve chemotaxis mediated by non-adaptive dynamics (see Figure 4). They involve coupling of the signal to an effector via a receptor, with self-regulation of both proteins (through allosteric interactions or processes such as auto-phosphorylation). The striking simplicity of these minimal systems lead to the speculation that non-adaptive chemotaxis could even be achieved without any signalling proteins; a small

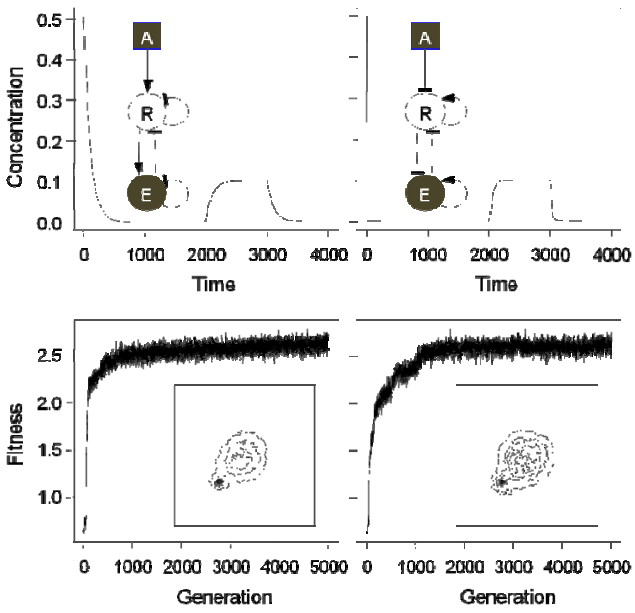

Figure 4: Minimal non-adaptive pathways mediating chemotaxis. Cartoon representation and time course of active effector concentration for the most frequent and unique pathways with two proteins obtained from five different evolutionary simulations. The pathway on the left and right were found in one and four simulations respectively. Bottom panels show fitness curves for the corresponding simulations and the time-averaged distribution of positions of the final population.

molecule, that is a by-product of metabolism or is taken into the cell via a transporter, could directly regulate tumbling probability of the cell. We hypothesize that exactly such a scenario is responsible for chemotaxis observed in gutted *E. coli*. Attractant related metabolism causes increases in fumarate levels inside the cell, which binds the motor and increases tumbling probability. That fumarate can involve in chemotaxis [25] and can control motor switching [26] have been demonstrated experimentally but the exact dynamics of how it could lead to chemotaxis was unknown. Further, the finding that transporters involve in chemotaxis in R. sphaeroides [18] suggest that non-adaptive dynamics could provide a straightforward way to link metabolism and chemotaxis.

DISCUSSION

Adaptation to stimuli is the hallmark of proper chemotaxis. Several experimental observations from other bacterial species and mutant strains of *E. coli*, however, indicate that other, possibly non-adaptive, dynamics could also lead to a form of chemotaxis. Here we provide direct evidence for one such dynamics that leads to efficient chemotaxis. The main features of this dynamics is an inverted response, leading to increasing tumbling frequency with increasing attractant levels and an absence of adaptation.

Using computational models of bacteria movement and biochemical pathways, we show that such non-adaptive dynamics readily evolve under different

environmental conditions and model assumptions and allow bacteria to achieve chemotaxis. Further, we find the efficiency of such chemotaxis to be equal to a case where bacteria would accumulate in space proportional to local attractant concentration. This type of chemotaxis favours exploitation of attractant sources over searching and could be especially efficient in conditions of abundant attractant. The minimal system for achieving non-adaptive dynamics mediating chemotaxis requires only two signalling components.

These findings provide a possible explanation for the chemotaxic ability of gutted *E. coli* cells and the complex chemotaxis behaviour of wild-type *R. sphaeroides*. In both cases and possibly in other current-day bacteria, chemotaxis pathways with non-adaptive and inverted responses could work in conjunction with the conventional chemotaxis pathway. These pathways could be remnants of evolution or functional pathways. They could involve in the fine-tuning of chemotaxis under certain environmental conditions or in providing a link between cell metabolism and chemotaxis.

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