larger, flying pollinators, and also from those adapted to small, generalist, short-tongued pollinators that differ from ants primarily in their ability to fly. Its ultimate value can be determined only through broader application and refinement.

The wet or seasonally rainy tropics, where studies of other ant-plant mutualisms have concentrated, do not support many plant species exhibiting the characters of the ant-pollination syndrome. Hagerup has suggested (*10*) that the best habitats for ant pollination are extreme deserts, and these results offer some support for that hypothesis. Three of the four demonstrated and likely ant-pollinated species which grow consistently in hot dry areas are annuals. This suggests that more ant-pollinated species are to be found among the inconspicuous-flowered desert and mediterranean-climate annuals of the world.

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12. *Helianthus annuus* L.; *Mimulus guttatus* DC.
13. Range depends primarily on whether individual flowers or many-flowered compact umbels are considered the visual and functional pollination units, and secondarily on variable traits.
14. I thank S. A. Cook, P. R. Ehrlich, L. Gass, D. E. Gill, C. S. Hickman, R. W. Holm, D. H. Janzen, P. H. Raven, J. Roughgarden, and C. D. White for helpful comments on the manuscript; C. S. Hickman for assistance in the field; A. Francoeur for identification of the ants; and G. Savage for translations. The work was supported in part by a Sloan Foundation grant to Swarthmore College.

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"Decision"-Making in Bacteria: Chemotactic Response of Escherichia coli to Conflicting Stimuli

Abstract. *Motile bacteria presented simultaneously with both attractant and repellent respond to whichever one is present in the more effective concentration. Apparently bacteria have a processing mechanism that compares opposing signals from the chemoreceptors for positive and negative taxis, sums these signals up, and then communicates the sum to the flagella.*

What will a motile bacterium do if confronted simultaneously with a gradient of attractant (let us say increasing concentrations of attractant to the right) and a gradient of repellent (let us say increasing concentrations of repellent to the right)? In this "conflict" situation a bacterium must "decide" whether to pursue the attractant, ordinarily a nutritious chemical (*1, 2*), or flee from the repellent, ordinarily a harmful chemical (*3*).

Already in 1888 Pfeffer (*4*) reported that the relative strength of the two gradients determines whether attraction or repulsion will occur. He determined this microscopically by observing the entrance of bacteria into a capillary containing both attractant (KCl, peptone, or meat extract) and repellent (acid, base, or ethanol) at various concentrations. Work of Tsang *et al.* (*5*) (see below) supports this study.

Using an objective assay (*6*) based on Pfeffer's, we have confirmed and extended his report. *Escherichia coli* were exposed to a capillary tube containing both attractant and repellent, and then after an hour the number of bacteria that had entered the capillary tube was determined. For attractant we used L-aspartate, a chemical that is beneficial because it can be readily metabolized (*2*), and for repellent we used L-valine, a chemical that is harmful because it inhibits the growth of *E. coli* (*7*).

At a relatively low concentration ($10^{-6}M$) of aspartate, the bacteria fail to be attracted at relatively high concentrations ($10^{-1}M$) of valine (Fig. 1A). The repelling effect of $10^{-1}M$ valine can be largely overcome by increasing the concentration of aspartate to 10^{-4} to $10^{-3}M$ (Fig. 1B, left). However, at yet higher concentrations of aspartate (Fig. 1B, right) the repelling effect of valine reappears; this can be explained by saturation of the aspartate chemoreceptor, the sensing mechanism, making aspartate poorly detectable at these high concentrations (*8, 9*).

Similar results have been obtained with DL-α-methylaspartate, an attractant that is a nonoxidizable analog of aspartate (*2*), and with L-leucine, a repellent found to be harmless (*3*); hence the phenomena under study are not mechanistically related to benefit or harm. We have obtained such results also with other attractants, L-serine and D-galactose (*10*), and another repellent, acetate.

With valine present in both the bacterial suspension and the capillary tube at $10^{-2}M$ [the concentration found just inside the mouth of the capillary when the capillary is filled with $10^{-1}M$ valine and the pond contains none (*6*)], there was no inhibition of aspartate taxis; hence a gradient of repellent is required for inhibition of attraction, and the mere presence of repellent does not inhibit. A mutant that is not repelled by valine (*3*) failed to show the inhibition of aspartate taxis that is illustrated in Fig. 1A, even when the capillary contained $10^{-1}M$ valine; hence the inhibition cannot be ascribed to some nonspecific effect, but rather it is specific for repellents.

It appears that half-inhibition occurs when the concentrations of repellent and attractant both exceed their respective thresholds by the same factor. In Fig. 1A, half-inhibition occurs at $10^{-2}M$ valine and $10^{-6}M$ aspartate, both roughly 30-fold above threshold. Thresholds determined by this method are $2.5 \times 10^{-4}M$ and $6 \times 10^{-8}M$, respectively. In Fig. 1B (left), half-inhibition occurs at $10^{-1}M$ valine and $2 \times 10^{-5}M$ aspartate, roughly 350-fold

above threshold. For α-methylaspartate, an attractant with a much higher threshold ($2.5 \times 10^{-6}M$), half-inhibition by $10^{-1}M$ valine occurred at $10^{-3}M$ α-methylaspartate, both about 400-fold above threshold. Thus repellent and attractant appear to be "pitted" against each other, and whichever is higher above threshold wins out.

Does each bacterium process the positive and negative sensory data, or is the result just a statistical one with bacteria following the attractant part of the time and the repellent part of the time? Observations of individual bacteria in a conflict situation can help to answer this.

During the past few years, the movement of individual enteric bacteria in a gradient of attractant (*11–12*) or a gradient of repellent (*5*) has been studied. Increasing attractant concentrations cause cells to tumble (or "twiddle") less frequently, while increasing repellent concentrations cause more tumbling; on the other hand, decreasing concentrations cause the opposite effect (more tumbling for attractants and less tumbling for repellents).

Tsang, Macnab, and Koshland (*5*) stated that a response of one type (either less or more frequent tumbling) could be progressively reduced by a simultaneous opposing stimulus of increasing strength, and eventually could be replaced by a response of the opposite type. For example, it was stated that the smooth (less frequent tumbling) response due to a decrease in concentration of the repellent phenol could be reduced and overcome by superimposing a decrease in concentration of the attractant L-serine. These workers concluded that this is consistent with the idea of algebraically additive stimuli leading into a common mechanism. We have reached the same conclusion by a different experiment, as follows.

We have shown (*13*) that attractants cause flagella to rotate counterclockwise and repellents cause them to rotate clockwise, and that counterclockwise rotation of flagella leads to "runs" (absence of tumbles), while clockwise rotation leads to tumbles. Actually, it is the rotation of the cells whose flagella are tethered to a glass slide that is observed, but this indicates the direction of rotation of the flagella, were they free to rotate (see *13, 14*).

Essentially all the rotating cells go counterclockwise after the addition of $5 \times 10^{-8}M$ aspartate, but the simultaneous addition of $5 \times 10^{-2}M$ valine overcomes this, making essentially all the cells rotate clockwise (Fig. 2A). Thus the cells that rotate in one direction in the presence of attractant will rotate in the opposite direction if sufficient repellent is also present. At lower concentrations of valine (Fig. 2A), the attractant and repellent are balanced off to give intermediate responses. The converse situation is shown in Fig. 2B. All the cells rotate clockwise in the presence of $5 \times 10^{-2}M$ valine, but this can be fully overcome by the simultaneous presence of sufficient aspartate ($3 \times 10^{-7}M$).

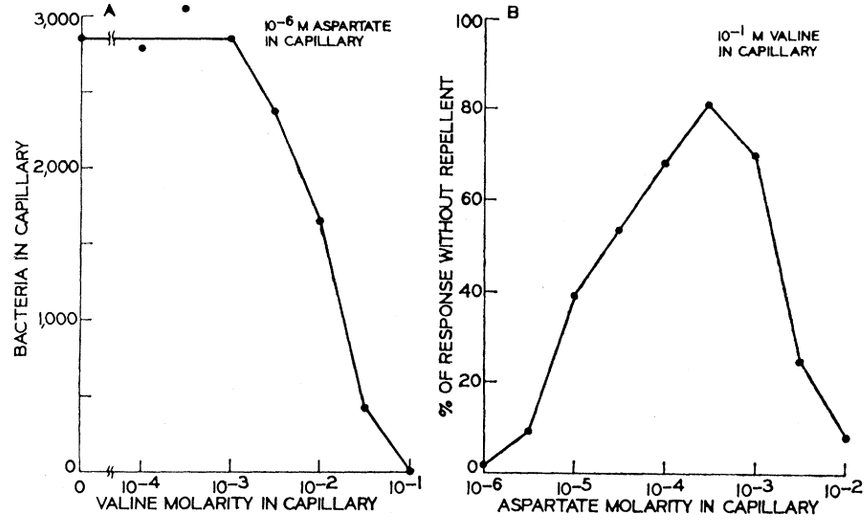

Fig. 1. (A) Effect of the repellent L-valine on the accumulation of *E. coli* strain AW607, wild-type for chemotaxis (*3*), in a capillary tube containing also an attractant, $10^{-6}M$ L-aspartate. A blank value (no attractant or repellent in capillary) of 240 has been subtracted from all values. Average of two experiments. (B) Effect of the attractant L-aspartate on the accumulation of *E. coli* strain AW607 in a capillary tube also containing a repellent, $10^{-1}M$ L-valine. In the absence of repellent, the accumulations in the capillary were 2.5, 5, 18, 34, 47, 66, 80, 76, and 66×10^3 bacteria for each concentration of aspartate shown on the abscissa from left to right. A blank value (no attractant or repellent in capillary) of 190 has been substracted from all values. Average of two experiments.

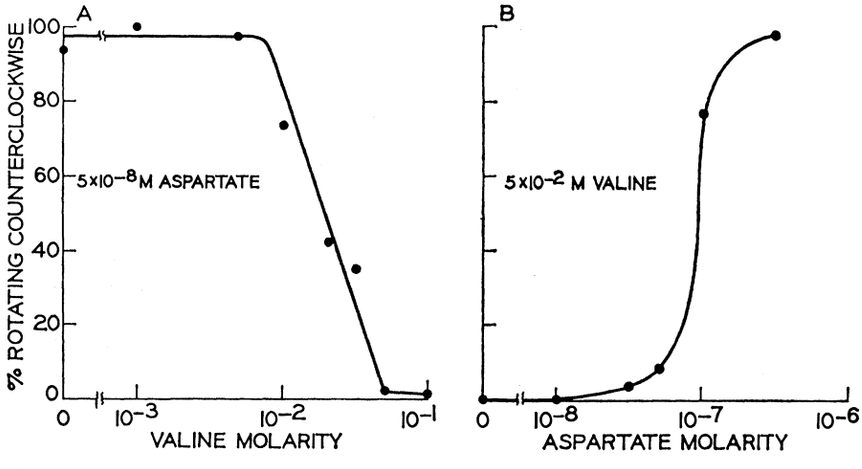

Fig. 2. (A) The effect of adding a mixture of the attractant L-aspartate (always $5 \times 10^{-8}M$) and L-valine (at various concentrations) on the direction of rotation of AW607 cells grown in glucose whose flagella are tethered to glass by means of antiserum to flagella. (B) The effect of adding a mixture of L-valine (always $5 \times 10^{-2}M$) and L-aspartate (at various concentrations) to AW607 tethered cells. The experimental procedure is described in (*13*) and legend to Fig 1. Untethered cells were rinsed off with 0.5 ml of chemotaxis medium, as described in (*13*). Cells were scored for clockwise or counterclockwise rotation for 30 seconds after the addition of the attractant-repellent mixture. Each rotating cell was observed just long enough to determine its direction of rotation. Typically, about 20 cells were scored during this period for each datum. Data were collected in duplicate or triplicate, and averages are presented. In the absence of attractant or repellent, 40 to 80 percent of the rotating cells were scored as counterclockwise.

By this assay the thresholds for aspartate and valine are $5 \times 10^{-9}M$ and $5 \times 10^{-3}M$, respectively. As in the capillary assay, a 50 percent effect is again observed when attractant and repellent are equally above threshold: roughly tenfold for Fig. 2, A and B.

Apparently bacteria have a "data-processing" system that receives opposing signals from the chemoreceptors for positive and negative chemotaxis, sums these signals, and sends the result to the flagella for action. The chemoreceptors for attractants might move the bacterial membrane potential in one direction while the chemoreceptors for repellents might move it in the opposite direction; or there may be a chemical whose level, influenced by the chemoreceptors, determines flagellar action (*12*).

The present and prior (*4*, *5*) results eliminate the following possibilities: (i) That the attractant might be dominant. Thus no matter how high the concentration of repellent, the bacterium ignores the repellent and is attracted. (ii) That the repellent might be dominant. Thus no matter how high the concentration of attractant, the bacterium will not be attracted so long as any repellent can be detected. (iii) Indecision. The bacterium might be neither repelled nor attracted, but vacillates ineffectively no matter what the concentration of attractant or repellent so long as they are detectable. Instead, the results show that bacteria presented simultaneously with both attractant and repellent respond to whichever is present in the more effective concentration.

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10. In the case of D-galactose, the effect shown in Fig. 1A can be readily demonstrated, but the inhibition by $10^{-1}M$ valine can be overcome only slightly by increasing the concentration of galactose (as in Fig. 1B, left). This can be explained by the observation that the galactose chemoreceptor is relatively unresponsive; see (*8*).
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Mobility and the Restriction of Mobility of Plasma Membrane Lectin-Binding Components

Abstract. *Labeling by ferritin-conjugated agglutinins from* Ricinus communis *was used to demonstrate the relative mobilities of the agglutinin receptors located in specific regions on plasma membranes of rabbit spermatozoa. The relative mobility of lectin receptors was higher on postacrosomal regions of sperm than on acrosomal and tail regions. Lectin-induced clustering could not be demonstrated in the acrosomal and tail regions, an indication of the existence of localized restraints on the mobilities of lectin receptors. A system of transmembrane restraints may maintain the segregation of plasma membrane components into membrane domains on certain highly differentiated cells.*

Evidence has accumulated that the plasma membranes of animal cells are dynamic, fluid structures (*1*). For example, membrane lipids have been shown to undergo rapid lateral movements during electron paramagnetic and nuclear magnetic resonance experiments (*2*).

Other membrane components such as glycoprotein antigens and lectin-binding constituents are also capable of lateral motion. In experiments on the intermixing of surface antigens on heterokaryons after virus-induced cell fusion, Frye and Edidin were able by immunofluorescent methods to demonstrate the movement of membrane (glycoprotein) components (*3*). Also, fluorescent antibodies applied by micropipetting bind in small patches to cultured muscle fibers, but the patches quickly spread—an indication of rapid planar diffusion (*4*). Utilizing fluorescent antibodies (*5*, *6*) and fluorescent lectins (*7–9*), several investigators have described the movement of membrane components into "clusters" or "caps."

Electron microscopy has been used to determine small lateral movements (of the order of hundreds of nanometers) on cell plasma membrane surfaces. Ferritin-antibody (*5*, *10*), ferritin-lectin (*11*), and lectin-peroxidase (*12*) labeling of membrane components has demonstrated a change in the distribution of these receptors from uniform or random to aggregated or clustered distributions. Freeze-etch electron microscopy was used to study *p*H-dependent reversible changes in the distribution of human erythrocyte membrane-intercalated particles (*13*) that are associated with outer surface anionic groups such as sialic acid (*14*). Surface labeling with ferritin conjugates or hemocyanin markers have been used to show dynamic changes in the distribution of lymphocyte, fibroblast, and erythrocyte membrane antigens and saccharides (*10*, *11*, *15*). The movement of particles or lectins attached to cultured cells growing on substrate has suggested a flow process of mobile membrane components (*16*).

The lateral mobility of certain membrane components has been described (*1–16*), but there are indications that particular components may have specific rates of lateral motion. Frye and Edidin noticed that fluorescent antibodies against mouse and human antigens intermixed at different temperature-dependent rates on fused heterokaryons (*3*) and certain cell surface antigenic components form antibody-induced or lectin-induced temperature-dependent caps more readily than others (*5*, *9*). Edidin and Weiss have extended these findings to include the intermixing of mouse H-2 antigens and human membrane antigens on several normal and transformed fibroblasts and found that the antigenic sites on normal cell surfaces could not be easily clustered or capped by antibodies, while they were quickly capped on