lation sizes precluded sampling both embryo and fledgling sex ratios at the same place during a season.
11. The angular transformation is required for parametric analysis of proportional data [R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1969), p. 386; F. J. Rohlf and R. R. Sokal, *Statistical Tables* (Freeman, San Francisco, 1969), p. 129]. Correlations are also significant for 1976 and for combined analyses without the extreme clutch of five females in 1976 ($r = -.42, P < .05$ and $r = -.40, P < .01$, respectively).
12. For samples including clutches in which some eggs failed to develop, negative correlations are significant for 1976 ($r = -.34$, $P = .05$, $N = 33$), for 1977 ($r = -.41, P < .03, N = 29$), and for pooled data ($r = -.33, P < .01$). It is not known whether embryonic mortality favors either sex, as data from domestic fowl are inconsistent [W. Landauer, *The Hatchability of Chicken Eggs as Influenced by Environment and Heredity* (Univ. of Connecticut Agricultural Experimental Station, Storrs, 1961), p. 170].
13. Parental care decreases after the young fledge, but it is not known how long or variable this period is in this or comparable species. Trends set during maximal parental effort may be accentuated after fledging but are unlikely to be reversed.
14. E. Novitski, *Genetics* **32**, 526 (1947); *ibid.*, **36**, 267 (1951); ——, W. J. Peacock, J. Engel, *Science* **148**, 516 (1965); E. Novitski and I. Sandler, *Proc. Natl. Acad. Sci. U.S.A.* **43**, 318 (1957); W. J. Peacock and J. Erickson, *Genetics* **51**, 313 (1965); R. Lewontin, *Annu. Rev. Ecol. Syst.* **1**, 1 (1970).
15. G. C. Williams, *Adaptation and Natural Selection* (Princeton Univ. Press, Princeton, N.J., 1966).
16. R. L. Trivers, in *Sexual Selection and the Descent of Man, 1871-1971*, B. Campbell, Ed. (Aldine-Atherton, Chicago, 1972), pp. 136-179; *Am. Zool.* **14**, 249 (1974); R. D. Alexander, *Annu. Rev. Ecol. Syst.* **5**, 325 (1974).
17. H. F. Howe, thesis, Univ. of Michigan (1977).
18. Trivers and Willard (*5*) hypothesized correlations between female condition and investment in offspring and between this investment and the future reproductive success of potentially polygynous male offspring. This grackle is sometimes polygynous, but early breeders (likely to be the healthiest in a bird population) are most likely to produce female young. In this species, a correlation between differential investment in the sexes and future reproductive success of offspring is likely to be an artifact of parental need to ensure that males attain a fast enough rate of growth and a high enough fledging weight to make survival to reproductive age possible. This is a separate hypothesis. It does not require that female condition and parental investment covary with the reproductive success of that small subset of males that does survive to reproductive age. Nor does it confuse the origin of sexual dimorphism—which may well have evolved in response to sexual selection (*1*)—with selection on sex ratio. Reproductive consequences of parental condition have been discussed by D. Lack [*Population Studies of Birds* (Oxford Univ. Press, London, 1966)], C. M. Perrins [*Ibis* **112**, 242 (1970)], and R. E. Ricklefs [in *Avian Energetics*, R. Paynter, Jr., Ed. (Publ. No. 15, Nuttall Ornithological Club, Cambridge, Mass., 1974), pp. 152-297]. Polygyny in this species has been reported by R. H. Wiley [*Z. Tierpsychol.* **40**, 59 (1976)], and Howe (*7*, *17*).
19. I thank Dr. and Mrs. W. Burns of Dexter, Michigan, for the use of their property and the staff of the University of Michigan Botanical Gardens for their cooperation. This research was accomplished in partial completion of the Ph.D. requirements at the University of Michigan. I am grateful for useful criticisms of the manuscript from R. D. Alexander, D. De Steven, K. Fiala, D. H. Janzen, L. Masters, J. Maynard Smith, R. B. Payne, and D. W. Tinkle. This work was supported by a Walker Scholarship of the Museum of Zoology and the Graduate School, University of Michigan.
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A Mutant of *Paramecium* Defective in Chemotaxis

Abstract. *In an effort to study the sensory-motor pathway of chemotaxis in* Paramecium tetraurelia, *I have generated mutants defective in their responses to chemicals. One mutant in particular, d4-530, is repelled by sodium acetate, which attracts normal paramecia by klinokinesis. The mutant is repelled by the mechanism of orthokinesis. To my knowledge, this is the first report of orthokinesis in chemotaxis of paramecia.*

Swimming and avoiding reaction are the two main components of behavior in *Paramecium*. Jennings (*1*) first described the avoiding reaction as a transient backing away from a stimulus, turning, and renewed forward swimming in a randomly chosen direction. This corresponds to normal ciliary beating, transient reversal of the ciliary beat, and a return to normal beating, respectively. We now understand a great deal about the membrane potential control of this behavior in the wild type (*2*) and in behavioral mutants defective in membrane electrogenesis (*3*).

Paramecia combine these two behaviors, swimming and avoiding reaction, in the more complex behavior of chemotaxis, or more accurately, chemokinesis (*4*). In chemokinesis, paramecia accumulate near or escape from the vicinity of certain chemicals. They accomplish this by modulating either the frequency of avoiding reaction (klinokinesis) or the velocity of forward swimming (orthokinesis) (*4*): an increase in frequency of avoiding reaction or increased velocity in a solution will cause repulsion from that solution and a decreased frequency of avoiding reaction or decreased velocity will cause attraction.

A genetic approach was used to dissect the chemosensory pathway. I describe here d4-530, a mutant of *Paramecium tetraurelia* that is repelled by sodium acetate, which attracts normal paramecia (*5*, *6*). Previously, the mechanism of chemokinesis in *Paramecium* was believed to be solely klinokinesis (*1*, *7*). However, d4-530 is repelled from sodium acetate by orthokinesis, and, to my knowledge, this is the first report of orthokinesis in *Paramecium* chemokinesis.

The response of paramecia to chemicals was measured by a T-maze assay, designed to present a test and control solution to a population of animals (*5*). The number of animals swimming into the arm with the test solution divided by the number of animals swimming into both test and control solution arms of the T gives an index of chemokinesis (I_{che}). An $I_{che} > 0.5$ indicates attraction to the test solution relative to the control; $I_{che} < 0.5$ indicates repulsion from the test solution into the control solution. Mutant d4-530 is defective in chemokinesis, and by this assay, it was similar to the wild type in response to all chem-

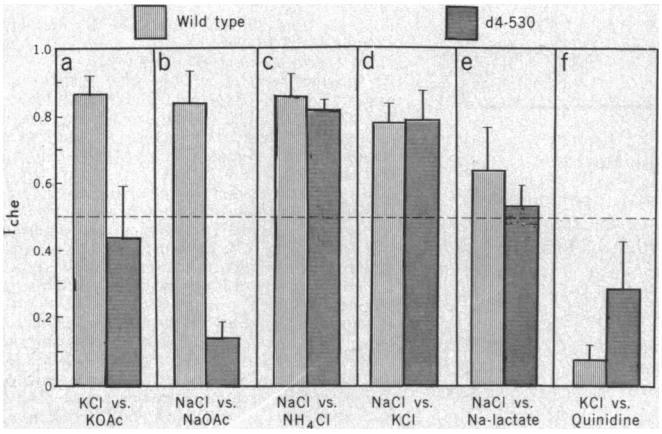

Fig. 1. Chemokinesis assayed by means of a T-maze (*5*). The index of chemokinesis (I_{che}) is defined as the number of animals in the test arm divided by the number of animals in both the test and control arms of the T. An $I_{che} > 0.5$ denotes attraction; $I_{che} < 0.5$ denotes repulsion from the test solution. All solutions included salts indicated in the chemokinesis buffer described in Table 1. The first solution under the histogram fills the control arm and all of the maze except the test arm; the second solution fills the test arm. (a) to (e) Concentrations are 5 m*M* of salt indicated; (f) concentrations are 0.1 m*M* quinidine hydrochloride or KCl. *KOAc*, potassium acetate; *NaOAc*, sodium acetate.

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ical attractants tested, except to acetate relative to chloride (see Fig. 1). Potassium acetate was not an attractant to d4-530, but was a strong attractant to the wild type (Fig. 1a); sodium acetate acted as a repellent to d4-530, but as an attractant to the wild type (Fig. 1b). The mutant may also be defective in its response to lactate. The response to sodium lactate relative to NaCl (Fig. 1e) was weaker attraction than for the wild type, but it was within the wild type range.

Mutant d4-530 was more weakly repelled than the wild type by quinidine hydrochloride (Fig. 1f); however, it can be normally repelled. The repulsion from quinine hydrochloride, the optical isomer of quinidine hydrochloride, in Dryl's solution (*8*) was equivalent to repulsion of the wild type (I_{che} = 0.01 ± 0.01 and 0.06 ± 0.04, respectively). It was normally attracted to KCl and to NH_4Cl relative to NaCl (Fig. 1, c and d) and was not repelled by sodium lactate relative to NaCl (Fig. 1e). Therefore, sodium and acetate must have a synergistic effect on d4-530 to cause such a drastic change in behavior from the wild type.

This mutant (d4-530) has an associated behavioral phenotype, that is, it is a partial "paranoiac" (*9*). It shows the same forward swimming as the wild type, but the avoiding reaction is sometimes prolonged and the cells swim backward for 1 to 15 seconds. However, it does not show this behavior frequently. After a 5-minute incubation in 5 m*M* NaCl chemokinesis solution, a solution that stimulated paranoiac behavior (*5*, *9*), none of d4-530 and none of the wild type cells showed long backward swimming, while 87 percent of full paranoiac d4-90 cells were swimming backward.

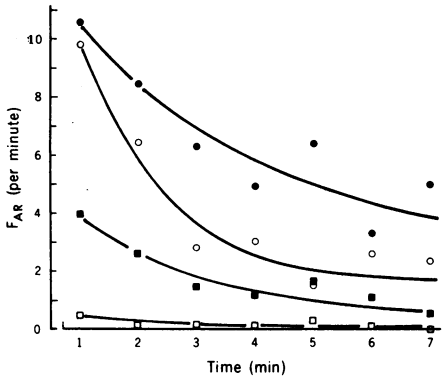

Fig. 2. Frequency of avoiding reactions in NaCl and sodium acetate solutions. The number of avoiding reactions in 1-minute intervals (F_{AR}) is plotted versus the minute ending the interval. Animals were incubated in 5 m*M* NaCl chemokinesis solution for 30 minutes and transferred to depressions of 5 m*M* NaCl or 5 m*M* sodium acetate solution. Data are averages from 20 cells. Closed circles and closed squares, 5 m*M* NaCl test solution; open circles and open squares, 5 m*M* sodium acetate; circles, wild type; and squares, d4-530.

The paranoiac phenotype is not sufficient by itself to change chemokinesis behavior; d4-90 showed normal responses to attractants and repellents (Table 1). The response to sodium lactate, a weak attractant to the wild type, showed wide variation that cannot presently be explained. More importantly, d4-90 was not repelled by, but was normally attracted to, sodium acetate after transfer from NaCl.

The two components of behavior, avoiding reaction and velocity of forward swimming, are both under membrane potential control. Hyperpolarization decreases the frequency of action potentials and increases ciliary beat frequency; depolarization increases frequency of action potentials and decreases ciliary beat frequency (*10*). The action potential causes a transient ciliary reversal, the avoiding reaction. Therefore, a solution that hyperpolarizes decreases the frequency of avoiding reactions and tends to cause attraction by klinokinesis. The hyperpolarization in this solution will also increase velocity and tend to cause repulsion by orthokinesis. The opposite effects occur with depolarization. Therefore, klinokinesis and orthokinesis are at odds in *Paramecium*.

In order to determine the mechanism of attraction and repulsion, I measured frequency of avoiding reaction and velocity of forward swimming in test and control solutions (*11*). Cells were incubated 30 minutes in control solution and were transferred singly to depressions of test or control solution. The frequency of avoiding reaction was measured by observation of individual cells in depressions while recording each avoiding reaction on an event recorder. Velocity was also measured after a 30-minute incubation in control solution. Long-exposure, dark-field photomacrographs (*12*) of the cells after transfer to test or control solution show lines in the paths of the swimming animals. Measurements of the path lengths were used to calculate the average velocity (*13*).

Wild type animals are attracted to sodium acetate by klinokinesis. Figure 2 shows the frequency of avoiding reaction of the wild type after transfer from 5 m*M* NaCl solution to either 5 m*M* sodium acetate or 5 m*M* NaCl. Frequency of avoiding reaction decreased in acetate solution relative to the chloride control, which is consistent with attraction by klinokinesis. However, wild type animals in the same solutions still swam slightly faster 15 minutes after transfer to acetate than to chloride (0.96 ± 0.22 mm/sec, N = 14; 0.77 ± 0.21 mm/sec, N = 15, respectively). Nakatani found similar increases in velocity in attractants of *P. caudatum* (*14*). This is consistent only with repulsion from acetate by orthokinesis. Therefore, the wild type must respond to the attractant sodium acetate by klinokinesis.

Mutant animals are repelled from sodium acetate by orthokinesis. Figure 2 shows the frequency of avoiding reaction in sodium acetate and NaCl solutions after transfer from NaCl. One expects an increased frequency of avoiding reaction in sodium acetate relative to NaCl for the mutant to be repelled by classical klinokinesis. Instead, the frequency of avoiding reaction dropped to almost zero in sodium acetate. The animals swam significantly faster in sodium acetate solutions (0.96 ± 0.16 mm/sec, N = 16; 0.64 ± 0.14 mm/sec, N = 8) even after 15 minutes. They are repelled by swimming faster in sodium acetate, that is, by orthokinesis.

Mutant d4-530 gave fewer avoiding reactions than the wild type in both NaCl and sodium acetate solutions (Fig. 2). However, the differences between the number of avoiding reactions in sodium acetate and in NaCl at the initial time points were greater for d4-530 than for the wild type. It is not yet clear whether it is the difference between the frequency of avoiding reaction in test and control solutions or the magnitude of frequency of avoiding reaction in test solution that is important in determining whether

Table 1. Chemokinesis of paranoiac d4-90 in the T-maze assay. Data are averages of the number of experiments (N) ± 1 standard deviation. Abbreviations: KOAc, potassium acetate; NaOAc, sodium acetate; Dryl, Dryl's solution.

Control solution* (5 m*M*)	Test solution†	I_{che}	N
KCl	KOAc	0.72 ± 0.05	4
NaCl	NaOAc	0.71 ± 0.12	8
NaCl	NH_4Cl	0.90 ± 0.07	4
NaCl	KCl	0.80 ± 0.16	8
NaCl	Na-lactate	0.54 ± 0.26	16
Dryl‡	0.15m*M* quinine-HCl in Dryl	0.08 ± 0.10	7

*Solutions contain the salts indicated and 1 m*M* tris, 1 m*M* citric acid, and 1 m*M* $Ca(OH)_2$, *p*H 7.05. †The concentration of all test solutions was 5 m*M* unless otherwise indicated. ‡Dryl solution contains 1 m*M* Na_2HPO_4, 1 m*M* NaH_2PO_4, 1.5 m*M* $CaCl_2$, and 2 m*M* sodium citrate, *p*H 7 (*8*).

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klinokinesis or orthokinesis will prevail.

There is a simple explanation for the aberrant behavior of mutant d4-530 based on a model of membrane potential control of chemokinesis (*15*). A hyperpolarization of the wild type in sodium acetate relative to NaCl would account for a decreased frequency of avoiding reaction and an increased velocity in the attractant. The decreased frequency of avoiding reaction (klinokinesis) dominates and the animals are attracted. A larger hyperpolarization of mutant d4-530 in sodium acetate relative to NaCl would account for the even greater decrease in frequency of avoiding reaction and increase in velocity in sodium acetate. However, the increase in velocity (due to both the increased ciliary beat and the decrease in time spent backing in the avoiding reaction) dominates and the cells are repelled by orthokinesis. Preliminary experiments show that the wild type does hyperpolarize in sodium acetate and that the mutant hyperpolarizes to a greater extent (*16*).

The discovery of orthokinesis of mutant d4-530 has led to other examples of repulsion by orthokinesis and to the development of a model for membrane potential control of chemokinesis (*15, 16*). A mathematical model is needed to determine the contribution from velocity and frequency of avoiding reaction, and hence, the dominance of klinokinesis or orthokinesis.

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References and Notes

1. H. S. Jennings, *Behavior of the Lower Organisms* (Indiana Univ. Press, Bloomington, 1967), pp. 48 and 61.
2. R. Eckert, *Science* **176**, 473 (1972).
3. C. Kung, S.-Y. Chang, Y. Satow, J. Van Houten, H. Hansma, *ibid.* **188**, 898 (1975).
4. Paramecia show a kinesis rather than a taxis in response to chemicals. They do not orient and swim directly toward or away from a stimulus as in a taxis. Instead, they reach or avoid a stimulus by a modulation of their motor behavior in either velocity or rate of change of direction. G. S. Fraenkel and D. L. Gunn, *Orientation of Animals* (Dover, New York, 1961), pp. 10–23, 43–57.
5. J. Van Houten, H. Hansma, C. Kung, *J. Comp. Physiol.* **104**, 211 (1975).
6. Strains used were or were derived from wild type 51-S of *Paramecium tetraurelia* (*P. aurelia*, species 4). Cells were cultured in Cerophyl medium with 3.5 m*M* Na_2HPO_4; *Aerobacter aerogenes* was added 24 hours before use. T. M. Sonneborn, *J. Exp. Zool.* **113**, 57 (1950); in *Handbook of Genetics* (Plenum, New York, 1974), pp. 469–594.
7. S. Dryl, in *Behavior of Microorganisms*, A. Perez-Miravete, Ed. (Plenum, New York, 1973).
8. _____, *J. Protozool.* **6** (Suppl.), 15 (1959).
9. C. Kung, *Z. Vgl. Physiol.* **71**, 142 (1971).
10. Y. Satow and C. Kung, *Nature (London)* **247**, 69 (1973); C. Kung and R. Eckert, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 93 (1972); H. Machemer, *J. Comp. Physiol.* **92**, 293 (1974); _____ and R. Eckert, *ibid.* **104**, 247 (1975).
11. Measurement of frequency of avoiding reaction and velocity involve temporal rather than the spatial and temporal gradients that the animals experience in the T-maze. Instead of swimming in a changing concentration of attractant, the animals are suddenly transferred from a solution without attractant to one with attractant. It is not known to what extent the measured velocities and frequencies differ from those in the T-maze. The measurements are at least qualitatively useful because animals do show strong chemokinesis in response to step gradients in a modified assay (*16*), which simulates the rapid change of attractant concentration experienced in the temporal gradient (I_{che} = 0.88 for sodium acetate versus NaCl). Animals were incubated 30 minutes in control solution before transfer to control or test solution. C. Kung and E. Gee have observed the frequency of avoiding reactions decrease with time to a basal level (personal communication). Therefore, the measured frequency of avoiding reaction in control solution may be an underestimate, making the real difference in frequency of avoiding reaction in NaCl and sodium acetate even greater. The incubation procedure was used for the following reasons: (i) cells had to be washed from culture fluid, which is undefined and more variable than control buffer; (ii) any solution transferred with cells would maintain the concentrations of all chemicals except chloride and acetate in transfer to test solution; (iii) cells show geotaxis in buffer and are easily collected with a minimum of solution at the top of tubes with buffer; and (iv) variable incubations up to 30 minutes prior to chemokinesis assays showed no significant change in I_{che}.
12. S.-Y. Chang and C. Kung, *Science* **180**, 1197 (1973).
13. Only tracks that stayed in focus were measured. Exposure times were chosen to minimize the number of avoiding reactions in the tracks. Avoiding reactions decrease the average velocity and tend to turn the animals into another plane. Therefore, tracks with avoiding reactions often go out of focus and are seldom used.
14. I. Nakatani, *J. Fac. Sci. Hokkaido Univ. Ser. 6* **17**, 401 (1970).
15. J. Van Houten, in preparation.
16. _____, thesis, University of California (1976).
17. I thank P. Foletta and D. Kusher for their technical assistance, and C. Kung and E. Orias for discussion of this work.
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Amacrine Cells in *Necturus* Retina: Evidence for Independent γ-Aminobutyric Acid- and Glycine-Releasing Neurons

Abstract. *About one-half of on-off ganglion cells have inhibitory postsynaptic potentials (IPSP's) which are blocked by strychnine, while the remainder have IPSP's which are blocked by picrotoxin or bicuculline. These antagonists do not abolish light activity of the presynaptic inhibitory neuron, the amacrine cell. The existence of separate γ-aminobutyric acid– and glycine-releasing amacrine cells is implied by these results.*

The amacrine cells of the vertebrate retina constitute a class of nerve cells which lack axons and have dendrites that are both post- and presynaptic. As interneurons of the inner retina, these cells receive input from bipolar cells and, in turn, form a feedback synapse onto bipolar cell terminals; "feed forward" synapses are formed onto ganglion cells and other amacrine cells (*1*). Electrophysiological studies of the mud puppy retina have demonstrated that amacrine cells are "on-off" in that they respond to the onset and termination of a light stimulus with transient depolarizations (*2*). In addition, both the dendrites and the soma of these cells generate tetrodotoxin-sensitive impulse activity (*3*).

Miller and Dacheux (*4*) have demonstrated that the amacrine cells are inhibitory to on-off ganglion cells, through a chloride-dependent, hyperpolarizing inhibitory postsynaptic potential (IPSP). Since the amino acids glycine and γ-aminobutyric acid (GABA) have been implicated as possible transmitters of amacrine cells (*5*), we studied the effects of these agents as well as their antagonists on ganglion cells and amacrine cell responses. Our findings suggest that there are two populations of amacrine cells, one of which releases GABA and the other glycine. These cells appear to be about equal in number, but in most cases a single on-off ganglion cell receives input from only one type of amacrine cell. A very few cells apparently are influenced by both types.

The studies reported here were carried out in a perfused mud puppy eyecup preparation, which has been previously described (*4*). Intracellular recordings were first obtained while perfusing the eyecup with a normal Ringer solution. After impalement and stabilization of intracellular recordings, the perfusate was changed to a test solution.

Figure 1 shows recordings obtained from three different on-off ganglion cells. In most recordings only the excitatory postsynaptic potentials (EPSP's) and IPSP's are evident, since impulse activity was usually abolished by depolarization caused by injury from electrode penetration (*4*). This recording condition usually obscured the EPSP's but enhanced the IPSP's, which are the prominent responses of the recordings. The cell illustrated in Fig. 1a was exposed to strychnine ($10^{-5}M$) for 90 seconds, which enhanced the IPSP's and also made the EPSP's more apparent. After the exposure to strychnine, a solution containing picrotoxin ($10^{-5}M$) perfused the eyecup and completely abolished the IPSP's within 2 minutes, leaving on and

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