culture provides further evidence that hybridomas are eliminated from cell fusion cultures exposed to HAT medium as a result of X chromosome segregation.

The loss of spleen cell chromosomes is an important cause of unstable antibody production by hybridomas (*1–3, 5–8*). Previous studies have demonstrated that the heavy chain synthesis is preferentially lost before light chain synthesis and that these losses correlate with segregation of the spleen cell chromosomes encoding the genes for the respective Ig chains (*3, 11, 12*). The hybridoma selection method described here, however, promotes the growth of those hybridomas which retain the spleen cell–donated heavy chain Ig gene and eliminates the loss of large numbers of hybridomas caused by X chromosome segregation during culture in HAT medium. This phenomenon may have contributed to the limited success in producing human lymphocyte–human myeloma hybridomas (*23*).

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References and Notes

1. B. A. Diamond, D. E. Yelton, M. D. Scharff, *N. Engl. J. Med.* **304**, 1344 (1981).
2. D. E. Yelton, D. H. Margulies, B. A. Diamond, M. D. Scharff, in *Monoclonal Antibodies: Hybridomas: A New Direction in Biological Analyses*, R. H. Kennett, T. J. McKearn, K. B. Bechtol, Eds. (Plenum, New York, 1980).
3. G. Kohler, H. Hengartner, C. Milstein, in *Protides of the Biological Fluids*, H. Peeters, Ed. (Pergamon, New York, 1978).
4. G. Kohler and C. Milstein, *Eur. J. Immunol.* **6**, 511 (1976).
5. V. T. Oi and L. A. Herzenberg, *Sel. Methods Cell. Immunol.* **17**, 351 (1980).
6. G. Kohler, in *Immunological Methods*, I. Lefkovits and B. Pernis, Eds. (Academic Press, New York, 1979 and 1981), vols. 1 and 2.
7. G. Kohler and C. Milstein, *Nature (London)* **256**, 495 (1975).
8. G. Kohler, S. C. Howe, C. Milstein, *Eur. J. Immunol.* **6**, 292 (1976).
9. J. W. Littlefield, *Science* **145**, 709 (1964).
10. M. F. Lyon, *Nature (London)* **232**, 229 (1971); B. Migeon, *ibid.* **239**, 87 (1972).
11. H. Hengartner, T. Meo, E. Muller, in *Monoclonal Antibodies: Hybridomas: A New Direction in Biological Analyses*, R. H. Kennett, T. J. McKearn, K. B. Bechtol, Eds. (Plenum, New York, 1980).
12. ———, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4494 (1978); M. T. Davison and T. H. Roderick, *Cytogenet. Cell Genet.* **22**, 552 (1978); P. D'Eustachio, A. L. M. Bothwell, T. K. Takaro, D. Baltimore, F. H. Ruddle, *J. Exp. Med.* **153**, 793 (1981); P. D'Eustachio, D. Pravtcheva, K. Marcu, F. H. Ruddle, *ibid.* **151**, 1545 (1980).
13. R. T. Taggart, *Am. J. Hum. Genet.* **33**, 149A (1981).
14. E. Capana, A. Group, H. Winking, G. Noack, M. V. Civitelli, *Chromosoma* **58**, 341 (1976). The Rb(8.12) mice (with 8.12 translocation chromosomes) were obtained from M. Davisson at Jackson Laboratory.
15. C. Kozak, E. Nichols, F. H. Ruddle, *Somatic Cell Genet.* **1**, 371 (1975).
16. R. P. Creagan and F. H. Ruddle, in *Chromosomes in Biology and Medicine*, J. J. Yunis, Ed. (Academic Press, New York, 1977).
17. Cellular extracts of myeloma or hybridoma cell lines (10^7 cells) were prepared as described (*18*), subjected to polyacrylamide gel electrophoresis (*19*) and stained specifically for APRT or HPRT enzyme activity by including either ^{14}C-labeled adenine or ^{14}C-labeled hypoxanthine in the reaction mixture. The FOX-NY myeloma line contained no detectable HPRT or APRT enzyme activity.
18. U. Francke and R. T. Taggart, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5230 (1979); *ibid.* **77**, 3595 (1980).
19. R. T. Taggart, R. B. Miller, R. C. Karn, J. A. Tribble, M. Craft, J. Ripberger, A. D. Merritt, in *Proceedings of Electrophoresis '78, International Conference of Electrophoresis*, N. Catsimpoalas, Ed. (Elsevier/North-Holland, New York, 1978), pp. 231–242.
20. R. T. Taggart, R. C. Karn, A. D. Merritt, P. L. Yu, P. M. Conneally, *Hum. Genet.* **52**, 227 (1979).
21. A female Rb(8.12) mouse was immunized with a purified preparation of human pepsinogen I as follows: one intraperitoneal injection of 80 μg in complete Freunds adjuvant and another of 50 μg in incomplete Freunds adjuvant 3 weeks later, then an intravenous (via tail vein) injection of 20 μg in saline was given 4 weeks later.
22. Microwell cultures were assayed by incubating 100 μl of ^{125}I-labeled human pepsinogen I (2×10^4 count/min) with 100 μl of the respective culture supernatant in 96-well polyvinyl chloride plates coated with human pepsinogen I for 2 to 3 days at 4°C. Positive cultures immobilized between 3 and 51 percent of ^{125}I-labeled antigen.
23. G. Kohler, *Hybridoma* **1**, 1 (1981).
24. This investigation was supported by a research grant from the Veterans Administration and by seed grants from the Center for Ulcer Research and Education Foundation, the UCLA Cancer Center (USPHS 1 P01 CA 16042) and the California Institute for Cancer Research. We thank R. Andersen, I. Anderson, D. Artner, C. Cardaci, S. Carpenter, K. Savage, P. Shamblin, L. Wilkins, and I. Williams for assistance. M. Fox contributed essential support for this report.
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An Excitatory Amino Acid Antagonist Blocks Cone Input to Sign-Conserving Second-Order Retinal Neurons

Abstract. *cis-2,3-Piperidinedicarboxylic acid (PDA), an excitatory amino acid antagonist, reversibly blocked cone input to OFF bipolars and horizontal cells, whereas ON bipolars were relatively unaffected. Kainic acid effects were also blocked, indicating a postsynaptic mechanism of action. The use of PDA helps to characterize one of two classes of excitatory amino acid synaptic receptors that mediate cone influence in the outer retina.*

The photoreceptors of the vertebrate retina form chemical synapses on three classes of second-order neurons: horizontal cells, ON bipolars, and OFF bipolars (*1*). A single photoreceptor may synapse on two or possibly all three types (*2*). For this reason, it is widely accepted that a single transmitter activates all three second-order neurons. Aspartate and glutamate have been the prime photoreceptor transmitter candidates since they mimic the endogenous transmitter when applied to all three cell types in several vertebrate species (*3*). In the mud puppy retina, one of these second-order neurons, the ON bipolar, contains a distinct synaptic receptor that binds to 2-amino-4-phosphonobutyrate, a glutamate analog (*4*). This selectivity has been confirmed in several vertebrate species (*5, 6*). The one-transmitter hypothesis requires that the other two second-order neurons also have excitatory amino acid synaptic receptors, although differing from that of the ON bipolar. We now report on the effects of an excitatory amino acid antagonist, (±)-*cis*-2,3-piperidinedicarboxylic acid (PDA), which acts on the horizontal cell and the OFF bipolar and thus helps to characterize this other receptor type.

Experiments were performed in the light-adapted, superfused retina-eyecup preparation of the mud puppy *Necturus maculosus*. Intracellular recordings were obtained from retinal neurons while an amphibian Ringer superfusate containing a control solution was interchanged with one or more solutions containing pharmacological agents. Rapid exchange in this system permits detectable drug effects within 10 to 25 seconds. The light stimulus consisted of small spot (200 μm), annulus (inner diameter, 400 μm; outer diameter > retinal diameter), or full-field illumination. Changes in input resistance were frequently monitored with a ± 0.1-nA current pulse applied through the electrode and a bridge-balancing device [WP707; see (*7*) for details of methodology].

When PDA, in concentrations up to 10 m*M*, was applied to the retina (Fig. 1a), there was no apparent effect on cone photoreceptors (*8*). In addition, PDA did not block synaptic transmitter release from photoreceptors because ON bipolars (Fig. 1b) functioned normally in the presence of these high levels of PDA.

In contrast to the photoreceptors and ON bipolars, the horizontal cells and OFF bipolars were very sensitive to PDA. Horizontal cells and OFF bipolars are hyperpolarized in the light as a result of a decrease in transmitter release from photoreceptors (*9*). Horizontal cells were also hyperpolarized by PDA (Fig. 1c), and there was a corresponding decrease

in the size of the response to light. Between light flashes, 0.1-nA biphasic current pulses (negative, then positive) were applied through the electrode and the bridge device. A slight increase in resistance occurred during PDA application. This indicates that the action of PDA is not the result of an increase in conductance to an ion or ions whose equilibrium potential is near the maximum light-evoked membrane potential (approximately −70 mV). The effect of PDA on the OFF bipolar (Fig. 1d) was similar to its effect on the horizontal cell; 6 m*M* PDA caused a reversible hyperpolarization and a decrease in the light response.

These responses were graded with PDA concentration, a partial block being seen at 1 to 2 m*M* PDA and a maximal effect at 6 to 10 m*M* PDA. Even at these high concentrations, a small light response (approximately 5 to 10 percent of control levels) persisted.

The center-depolarizing response of the ON bipolar was not blocked by PDA, indicating that synaptic transmission from photoreceptors is unaffected by this agent. However, the hyperpolarizing antagonistic surround response was eliminated by PDA (Fig. 1b). This observation was anticipated since the antagonistic surround is attributed to horizontal cell activity.

Both horizontal cells and OFF bipolars show anomalous rectification (*10, 11*). Thus, the conductance decrease associated with a decrease in transmitter-receptor ionophore activation is counteracted by a resistance decrease of the nonsynaptic membrane resulting from a voltage-dependent increase in conductance (probably to potassium ions) with membrane hyperpolarization. In the horizontal cell, these two factors are closely balanced so that usually no difference in membrane resistance is detected in light versus dark (*12*). Since we were interested in determining whether the PDA-evoked hyperpolarization was due to an action on the synaptic receptors of the cell, we compared the effect of PDA with that of cobalt, an agent that diminishes synaptic transmission by blocking the calcium-dependent presynaptic release of neurotransmitter (*13*) (Fig. 2a). When 4 m*M* PDA was applied to this cell, the dark membrane potential hyperpolarized, the light response decreased, and there was a slight increase in the cell's input resistance as detected by the negative deflection of the current pulse. Shortly after application of PDA was stopped, the light response, membrane potential, and input resistance recovered their original levels. Then the cell was exposed to 5 m*M* cobalt to block transmitter release. Again, this caused a hyperpolarization with a loss of the light response, but there was no change in the input resistance. Thus cobalt and PDA have similar actions. If PDA was acting nonsynaptically to block the light response by shunting the cell, the resulting decrease in resistance would add to the decrease in resistance caused by anomalous rectification, and a decline in the input resistance of the horizontal cell should have been seen. Our results suggest that PDA is acting postsynaptically to block the photoreceptor transmitter. The results cannot be explained as the result of gap junction uncoupling between horizontal cells, since this would result in an increase in the response to a small spot (*14*), which does not occur (Fig. 2b).

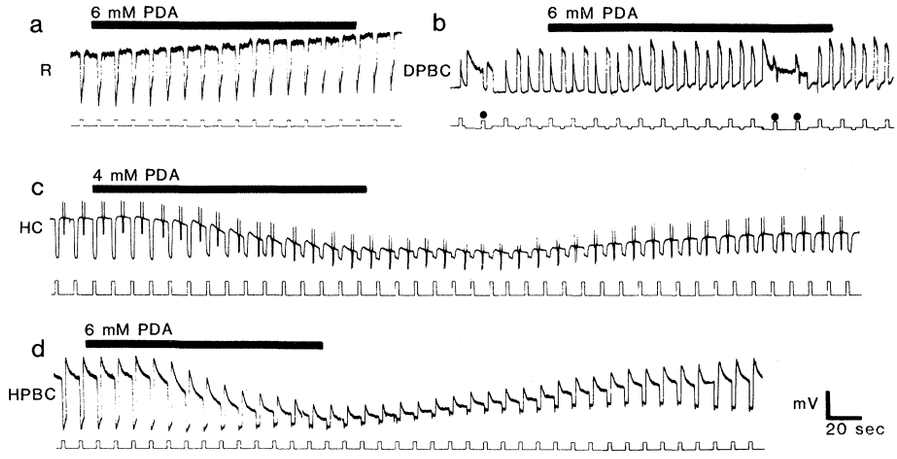

Fig. 1. Intracellular recordings from distal retinal neurons during bath application of PDA (bar above each trace). The square wave below each trace indicates the occurrence of a 2-second light stimulus (irradiance, 5×10^{-9} W/cm^2), which was a diffuse light except in (b) where a 200-μm small spot (downward step) was alternated with an annulus (inner diameter, 400 μm; outer diameter > retinal diameter). (a) PDA did not alter the response of the cone photoreceptor (*R*) but it caused a hyperpolarization and attenuation of the light response of (c) the horizontal cell (*HC*) and (d) the OFF bipolar (*HPBC*). (b) In the ON bipolar (*DPBC*), PDA did not block the response of focal illumination but did block the hyperpolarizing surround response (dark circles). Calibration: (a) and (d) 10 mV, (b) 20 mV, and (c) 30 mV.

We tested the effectiveness of PDA, which had been developed and described as an excitatory amino acid antagonist (*15*), against excitatory amino acids agonists in the retina. In the outer retina, aspartate or glutamate application often results in "desensitization," so that repeated application of these agonists can produce progressively diminishing pharmacological effects. Since it would be difficult to distinguish desensitization from PDA antagonism, we used agonists that were not subject to this effect. Two common and potent excitatory amino acid agonists are kainic acid and *N*-methyl aspartate, but we have found that only the former is effective in the outer retina. In the mud puppy, kainic acid mimics the effect of the photoreceptor transmitter on all three types of second-order neurons (*16*), and in dogfish it acts synaptically at the ON bipolar synapse (*16*). When 25 μ*M* kainic acid was applied to a horizontal cell (Fig. 2b), it caused a depolarization and a large decrease in the cell's light response, as might be expected of a photoreceptor transmitter agonist. The kainic acid application was suspended, and after recovery, 3 m*M* PDA was applied. This caused a hyperpolarization and a decrease in the light response. In the continued presence of PDA, the reapplication of 25 μ*M* kainic acid resulted in only a slight depolarization. Figure 2c provides a comparison of the effect of kainic acid on a horizontal cell when neurotransmission is blocked presynaptically with cobalt. Thus PDA seems to be an effective antagonist of excitatory amino acid agonists as well as the endogenous photoreceptor transmitter.

Few suspected neurotransmitters, except excitatory amino acid analogs, affect outer retinal neurons, but high concentrations of γ-aminobutyric acid (GABA) can depolarize mud puppy horizontal cells (*17*). Furthermore, GABA is a monocarboxylic acid that has the same chain length as glutamate and therefore should be useful in determining the specificity of PDA's action. In the horizontal cell illustrated in Fig. 2d, 5 m*M* GABA caused a depolarization and greatly reduced the light response, an effect similar to that caused by kainic acid. After the neuron recovered from the effects of the brief GABA application, 3 m*M* PDA was applied. When the cell had hyperpolarized and the light

response diminished, another brief application of 5 m*M* GABA caused a large depolarization with no suggestion of PDA antagonism. A comparison of (b) and (d) in Fig. 2 suggests that PDA is a specific and effective excitatory amino acid antagonist.

Our results indicate that the photoreceptor transmitter is an excitatory amino acid. Whether mud puppy cones use more than one excitatory amino acid could not be determined because of the lack of specificity of PDA. In goldfish, red and green sensitive cones take up both aspartate and glutamate (*18*), whereas rods take up only glutamate, suggesting rod-cone differences in neurotransmitters. However, results with the glutamate analog 2-amino-4-phosphonobutyrate and the aspartate analog 2-amino-3-phosphonopropionate in the cone-dominated mud puppy (*4*) and the rod-dominated dogfish (*6*) indicate that rods and cones use the same neurotransmitter. Furthermore, fish horizontal cells appear to be much more sensitive to glutamate than to aspartate (*19*). As judged from our experiments on the effects of glutamate and aspartate agonists on second-order neurons, and in view of the ineffectiveness of aspartate antagonists (α-aminoadipate and α-aminosuberate) on these neurons, glutamate is the more likely transmitter candidate. It is clear, however, that there are two types of synaptic excitatory amino acid receptors. The ON bipolar contains one type of receptor, and the OFF bipolar and the horizontal cell have similar, if not identical, receptors of the second type. This second type of receptor is also important in excitatory transmission in the inner retina (*20*).

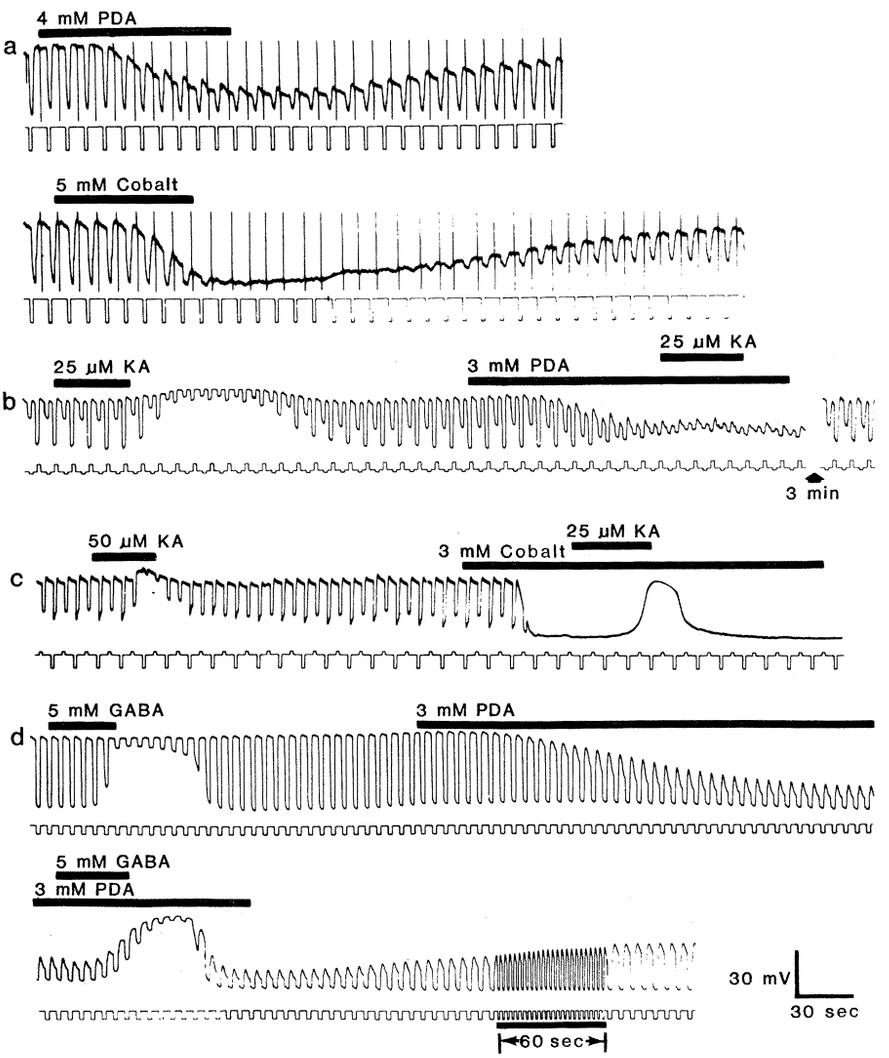

Fig. 2. Intracellular recordings from horizontal cells. The period of drug application is shown in the bar above each trace; the square wave below each trace indicates the occurrence of a 2-second light stimulus (irradiance, 5×10^{-9} W/cm^2). (a) Recordings from one horizontal cell in which diffuse light stimulation was alternated with a −0.1-nA current pulse. Application of 4 m*M* PDA caused a hyperpolarization, a decrease in the light response, and a slight increase in the cell's input resistance. Cobalt at 5 m*M* also caused a hyperpolarization with a loss of the light response, but no change in the input resistance. (b) Kainic acid (*KA*) at 25 μ*M* caused a depolarization and a decrease in the light response, but this effect of kainic acid was blocked by PDA. (c) When the input to the horizontal cell was blocked presynaptically with cobalt, kainic acid still caused a large depolarization. (d) Continuous recording from the horizontal cell demonstrated that PDA does not block the GABA-induced depolarization of horizontal cells. The light stimulus in (a) and (d) was a diffuse light, and in (b) and (d) a 200-μm small spot, with a downward deflection in (b) and an upward deflection in (c), alternating with an annulus (inner diameter, 400 μm; outer diameter > retinal diameter).

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References and Notes

1. J. E. Dowling and F. Werblin, *J. Neurophysiol.* **32**, 315 (1969); F. Werblin and J. E. Dowling, *ibid.*, p. 339; A. Kaneko, *J. Physiol. (London)* **207**, 622 (1970).
2. B. B. Boycott and H. Kolb, *J. Comp. Neurol.* **148**, 91 (1973); H. Kolb, *Phil. Trans Roy. Soc. London Ser. B* **258**, 261 (1970); W. K. Stell, *Invest. Ophthalmol.* **15**, 895 (1976).
3. M. Murakami, K. Ohtsu, T. Ohtsuka, *J. Physiol. (London)* **227**, 889 (1972); M. Murakami, T. Ohtsuka, H. Shimazaki, *Vision Res.* **15**, 456 (1975); L. Cervetto and E. F. MacNichol, Jr., *Science* **178**, 767 (1972); S. M. Wu and J. E. Dowling, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5205 (1978).
4. M. M. Slaughter and R. F. Miller, *Science* **211**, 182 (1981).
5. S. C. Massey, M. L. J. Crawford, D. A. Redburn, *Invest. Ophthalmol.* **20**, 44 (1981); P. H. Schiller, *ibid.* **22**, 11 (1982).
6. R. A. Shiells, G. Falk, S. Naghshineh, *Nature (London)* **294**, 592 (1981).
7. R. F. Miller and R. F. Dacheux, *J. Gen. Physiol.* **67**, 639 (1976).
8. In the retina eyecup of the light-adapted mud puppy, the rods play little or no role in synaptic transmission [G. L. Fain, *J. Physiol. (London)* **252**, 735 (1975)].
9. Y. A. Trifonov and A. L. Byzov, *Biofizika* **10**, 673 (1965); R. F. Dacheux and R. F. Miller, *Science* **191**, 963 (1976); J. E. Dowling and H. Ripps, *Nature (London)* **242**, 101 (1973); A. Kaneko and H. Shimazaki, *Cold Spring Harbor Symp. Quant. Biol.* **40**, 537 (1975).
10. A. L. Byzov and Y. A. Trifonov, *Vision Res.* **21**, 1573 (1981).
11. F. S. Werblin, in *The Neurosciences Fourth Study Program,* F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, Mass., 1979), p. 193.
12. J. Toyoda, *Vision Res.* **13**, 283 (1973); R. Nelson, *J. Neurophysiol.* **36**, 519 (1973).
13. J. N. Weakley, *J. Physiol. (London)* **234**, 597 (1973).
14. R. F. Miller, *Brain Res.* **139**, 178 (1978).
15. J. Davies, R. H. Evans, A. A. Francis, A. W. Jones, J. C. Watkins, *J. Neurochem.* **36**, 1305 (1981).
16. M. M. Slaughter and R. F. Miller, *Invest. Ophthalmol.* **18** (Suppl.), 131 (1980).
17. R. F. Miller, T. E. Frumkes, M. Slaughter, R. F. Dacheux, *J. Neurophysiol.* **45**, 743 (1981).
18. R. E. Marc and D. M. K. Lam, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7185 (1981); J. Kleinschmidt, *Invest. Ophthalmol.* **20** (Suppl.), 184 (1982).
19. A. T. Ishida and G. L. Fain, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5890 (1981); E. M. Lasater and J. E. Dowling, *ibid.* **79**, 936 (1982).
20. M. M. Slaughter and R. F. Miller, unpublished data.

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