ferns with high chromosome numbers may be diploid (*11*). The results also call into question evidence supporting a selective value of polyploidy in maintaining genetic variability in *Pteridium* and other homosporous pteridophytes (*5*, *9*).

REFERENCES AND NOTES

1. F. R. Ganders, *Bot. J. Linn. Soc.* **65**, 211 (1972).
2. C. H. Haufler and D. E. Soltis, *Am. J. Bot.* 71, 878 (1984).
3. G. J. Gastony and L. D. Gottlieb, *ibid.* **69**, 634 (1982).
4. D. E. Soltis and P. S. Soltis, *Am. Natur.*, in press.
5. E. J. Klekowski, Jr., and H. G. Baker, *Science* **153**, 305 (1966).
6. E. J. Klekowski, Jr., *Am. J. Bot.* **60**, 535 (1973).
7. D. W. Bierhorst, *ibid.* **62**, 448 (1975).
8. E. J. Klekowski, Jr., and L. G. Hickok, *ibid.* **61**, 422 (1974); L. G. Hickok, *ibid.* **65**, 516 (1978).
9. R. H. Chapman *et al.*, *Science* **204**, 1207 (1979).
10. G. J. Gastony and D. C. Darrow, *Am. J. Bot.* **70**, 1409 (1983).
11. C. H. Haufler and D. E. Soltis, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4389 (1986).
12. D. E. Soltis *et al.*, *Syst. Bot.* **5**, 30 (1980).
13. H. Harris and D. A. Hopkinson, *Handbook of Enzyme Electrophoresis in Human Genetics* (North-Holland, Amsterdam, 1976), chap. 6.
14. L. D. Gottlieb, *Science* **216**, 373 (1982).
15. N. F. Weeden, *J. Mol. Evol.* **17**, 133 (1981).
16. D. E. Soltis, C. H. Haufler, D. C. Darrow, G. J. Gastony, *Am. Fern J.* **73**, 9 (1983). Modifications listed in C. H. Haufler, *Syst. Bot.* **10**, 92 (1985).
17. Details of collection data, techniques, and segregation ratios are available from the first author, or consult P. G. Wolf, thesis, University of Kansas, Lawrence (1986).
18. E. J. Klekowski, Jr., *Bot. J. Linn. Soc.* **62**, 361 (1969).
19. C. H. Haufler and W. W. Adams III, *Am. J. Bot.* **69**, 1560 (1982).
20. Techniques as described in G. J. Gastony and D. C. Darrow [*ibid.* **70**, 1409 (1983)], modified such that chloroplasts were centrifuged through a Percoll gradient with layers of 50, 25, and 20% Percoll. Intact chloroplasts separated from other material and remained between the 20 and 25% layers.
21. R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1981), chap. 17.
22. L. G. Hickok and E. J. Klekowski, Jr., *Am. J. Bot.* **60**, 1010 (1973); C. H. Haufler, M. D. Windham, D. M. Britton, S. J. Robinson, *Can. J. Bot.* **63**, 1855 (1985); G. J. Gastony, *Am. J. Bot.* **73**, 1563 (1986).
23. Cytological techniques were modified from G. J. Gastony and C. H. Haufler, *Biotropica* **8**, 1 (1976). Gametophytes were treated for 5 hours in $0.002M$ 8-hydroxyquinoline at room temperature, fixed in 3:1 absolute alcohol:glacial acetic acid for 24 hours, then hydrolyzed in full-strength Glusulase for 2 hours. The material was then rinsed and returned to fixative for storage at −20°C. Meristematic notches and antheridia were stained with iron acetocarmine for detecting mitotic cells.
24. E. Pichersky and L. D. Gottlieb, *Genetics* **105**, 421 (1983).
25. C. H. Haufler, *Am. J. Bot.*, in press.
26. D. E. Dykhuizen, C. Mudd, A. Honeycutt, D. L. Hartl, *Evolution* **39**, 1 (1985).
27. Actual quantification of the mating system in *P. aquilinum* requires populational analysis of genotype frequencies in order to establish outcrossing rates. Preliminary data indicate that random mating of gametes occurs in natural populations of bracken (P. G. Wolf, C. H. Haufler, E. Sheffield, unpublished data).
28. We thank T. A. Ranker and M. D. Windham for extensive advice and assistance during the study and P. S. Soltis and D. E. Soltis for comments on the manuscript. This study was supported by grants from University of Kansas General Research Fund and the North Atlantic Treaty Organization.

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Effect of Lizards on Spider Populations: Manipulative Reconstruction of a Natural Experiment

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Which species affect one another, how intensely, and the mechanisms of those effects are crucial data for understanding how ecological communities work. Tropical islands without lizards, the major top predators, have about ten times as dense web spider populations as those with lizards; processes responsible for this effect were experimentally simulated by removing lizards from randomly selected mainland plots. Spider densities in removal plots averaged 2.5 times as high as controls. Spider survival, prey abundance, and prey consumption were all negatively affected by lizards. Contrary to most studies, predator removal caused an increase in the number of spider species.

INSECTIVOROUS VERTEBRATES ARE potentially both predators upon and competitors of insectivorous arthropods. Persistence of such arthropods in places having vertebrates may therefore be quite tenuous. In many tropical areas, lizards are the dominant vertebrate insectivore; these feed upon spiders and eat many of the same prey types as spiders eat (*1*, *2*). In 1983 we reported (*3*) that orb spider densities are about ten times high on islands without lizards than on those with lizards. Also, numbers of spider species are about twice as high on islands without lizards. These data, from about 100 islands, strongly suggest that lizards reduce spider abundance and species diversity; however, rather than directly demonstrating the causal factor, they are correlative. Furthermore, such data in themselves shed limited light on the mechanism of the effect. Is it predation, competition, or both? We report now a manipulation of lizard populations that was designed to test directly the causal agent and elucidate the mechanisms responsible for drastically smaller spider populations on lizard-inhabited islands. Our experiment is similar to a less extensive one performed by Pacala and Roughgarden (*4*) in a different tropical system; ours both confirms and extends their results.

Our study site was Staniel Cay, Bahamas, a very large island (hence a "mainland") in the midst of the smaller islands used in the 1983 study. Initially, we staked out nine 83.6-m^2 plots whose mean vegetation height ranged from 0.14 to 0.53 m and whose vegetation consisted primarily of sea grape (*Coccoloba*). Plots were divided into three groups, with high, medium and low vegetation, called blocks (*5*); each block had three plots. This experimental design corrects for the effect of plot heterogeneity, in this case in vegetation height, which may otherwise mask the effect of the manipulation. Enclosures were built on two plots in each block, leaving the third plot unenclosed. Then one enclosure in each block was randomly chosen for lizard removal. Thus, there were three replicates of three treatments: lizard-removal enclosures, control enclosures where lizards were not removed, and unenclosed plots where lizards were not removed. Comparisons between lizard and control enclosures were used to determine the effect of lizards. Comparisons between control enclosures and unenclosed plots were used to determine the effect of enclosures. Enclosures were constructed between 29 April and 5 May 1985 and generally followed the design of Pacala *et al.* (*6*); wood-framed fences with one-eighth inch hardware cloth were sunk 0.31 m into the ground and stood 0.93 m high, with 0.41-m sheets of plastic mounted horizontally on top to prevent passage by lizards. Most lizards on the study site were *Anolis sagrei*, with some *Anolis carolinensis* and *Ameiva festiva*. Because lizard and spider densities were reduced during enclosure construction, the control enclosures and open plots were restocked with lizards to about their natural densities (0.06 to 0.10/m^2) before the first census (11 May), and all plots were restocked immediately after the first census with three female and two male *Metepeira datona* (the commonest spider species). Plots were censused at about 4-week intervals for slightly more than a year; numbers and species identities of web spiders and lizards (*7*) were recorded, and four (22 cm by 14 cm tanglefoot) sticky traps were placed for 24 hours in each plot to estimate abundance of other arthropods, the potential prey of both lizards and spiders.

Lizard removal dramatically increased web spider density (Fig. 1). The first mar-

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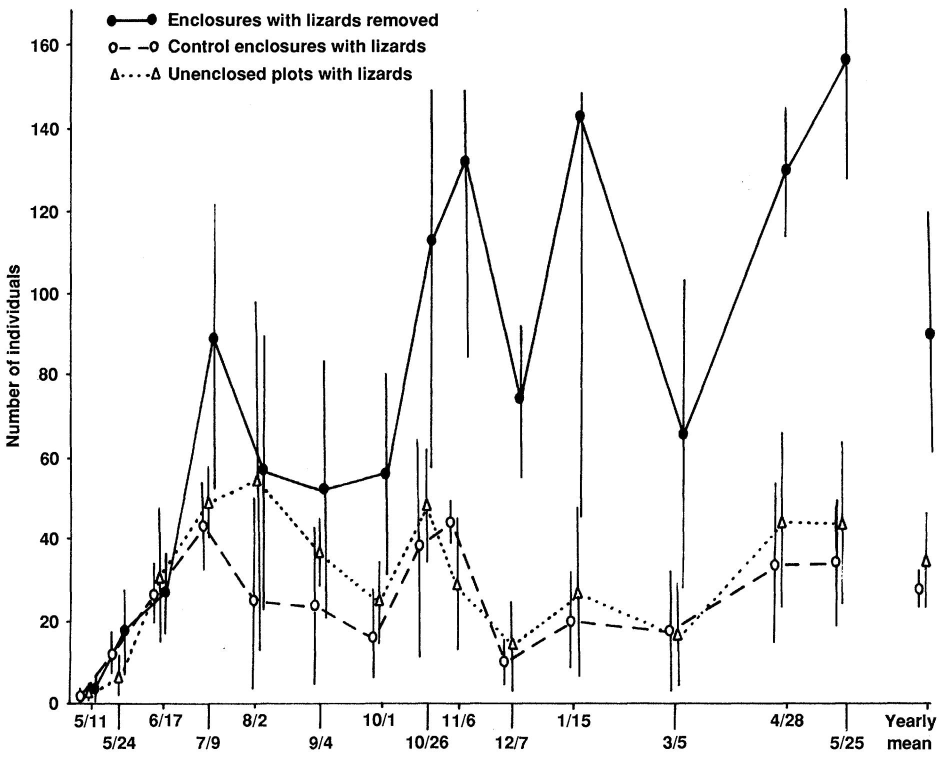

Fig. 1. Number of web spider individuals during the course of the lizard-removal experiment. Error bars are the standard deviations of the three plots within each treatment. "Yearly mean" is reported as in Table 1.

ginally significant difference between removal and control enclosures was recorded 9 July, about 2 months after the experiment began (Table 1). Differences between removal and control enclosures increased, irregularly but persistently; after 7 December, three out of four dates showed a statistically significant effect. Significant differences tended to occur at the end of periods when both removal and control enclosures increased in spider numbers; five of the six significant differences were of this sort (Fig. 1). Maximum difference between removal and control enclosures was about sevenfold; the removal mean averaged over a full year (beginning 17 June) was about 2.5 times the control mean. This compares with an average factor of about ten for no-lizard over lizard islands under natural, unmanipulated conditions. Enclosures with lizards (controls) and unenclosed plots (necessarily having lizards) showed no significant differences at any of the 15 monitoring dates and tracked one another closely (Fig. 1). A block (vegetation height) effect was significant at only one census, although *F* values ran rather high, averaging 3.20 over all dates.

We also found significant differences in survival rates, measured over an 8-day period, of adult female spiders (*M. datona*) established in webs; survival was higher in removal than either control or unenclosed plots (*2*). Although evidence for predation on established spiders thus exists, predation on unestablished spiders, which are mostly juveniles, may be substantially more important in this system. First, direction of the effect of lizard removal was set after only 12 days, when many spiders were still unestablished (Fig. 1). Second, significant differences in spider abundance between removal and other types of plots occurred mainly during overall increases in spider abundance (see above); this suggests that reproductive pulses of juveniles are rapidly consumed in lizard plots but not in removal enclosures. Third, separate experiments in which spiders were introduced onto islands with and without lizards showed immediate and marked effects of lizards in reducing colonizing spiders (*8*). In addition, dissection of lizard individuals that leaked into the no-lizard enclosures showed that they do consume our principal spider species, *M. datona*.

Arthropods caught in sticky traps, which we are using as an indication of the abundance of the prey of both lizards and spiders (*2*), showed significant differences in yearly mean in both number and biomass between removal and control enclosures. Enclosures without lizards had 30 to 40% more numbers and biomass than those with lizards. Significant differences were also found at several dates during the study, especially toward the end (*2*). In addition, prey consumption rates by spiders were higher in removal than control or unenclosed plots (*2*). This suggests competition for food. However, because unenclosed plots had more arthropods than either type of enclosed plot (probably due to the fences), other mechanisms are possible (*2*). A measure of reproductive rate (mean egg sacs per adult female) was increased by a third in the lizard-removal plots over the control plots, although this is not statistically significant (*2*).

Changes in number of species of web spiders were similar to those in the number of individuals but were less dramatic (Fig. 2). Removal and control enclosures significantly differed at only one date and for the yearly mean (Table 2). Enclosures with lizards and unenclosed plots again were quite similar, especially in yearly mean; no statistically significant differences were seen. The maximum mean number of species for any

Table 1. Statistical analysis of number of web spider individuals in the lizard-removal enclosures (R), the control enclosures with lizards (C), and the unenclosed plots with lizards (U).

Date	*F* value†	Pairwise comparisons‡		
		R versus C	R versus U	U versus C
11 May 1985	1.37 (ns)	ns	ns	ns
24 May 1985	4.92 (+)	ns	+	ns
17 June 1985	0.06 (ns)	ns	ns	ns
9 July 1985	4.90 (+)	+	ns	ns
2 August 1985	2.01 (ns)	ns	ns	ns
4 September 1985	2.45 (ns)	ns	ns	ns
1 October 1985	9.51 (*)	*	ns	ns
26 October 1985	2.15 (ns)	ns	ns	ns
6 November 1985	9.69 (*)	+	*	ns
7 December 1985	5.14 (+)	ns	+	ns
15 January 1986	10.87 (*)	*	*	ns
5 March 1986	4.38 (+)	ns	ns	ns
28 April 1986	12.28 (*)	*	*	ns
25 May 1986	24.28 (**)	**	*	ns
Yearly mean§	16.67 (*)	*	*	ns

†Treatment effect, two-way analysis of variance (ANOVA), df = 2, 4; data were $\log_{10}(x + 1)$ transformed; ns = $P > 0.10$; + $P < 0.10$; *$P < 0.05$; **$P < 0.01$. ‡Analysis of treatment means, Tukey method (*4*) which is two-tailed; *P* values are family significance levels. §Each variate in the yearly mean analysis was the mean number observed in a particular plot from 17 June 1985 to 25 May 1986.

date is four, and only eight different species were found at the site throughout the study, so that variation in species number, unlike that in number of individuals, is severely constrained by availability. Maximum difference between removal and control enclosures was about 1.75-fold, and the yearly removal mean was about 1.3 times the control mean. This compares with a factor of about 2 for numbers of species between lizard and no-lizard islands under natural, unmanipulated conditions. A significant block effect was found on two dates; *F* ratios at other times were often quite low.

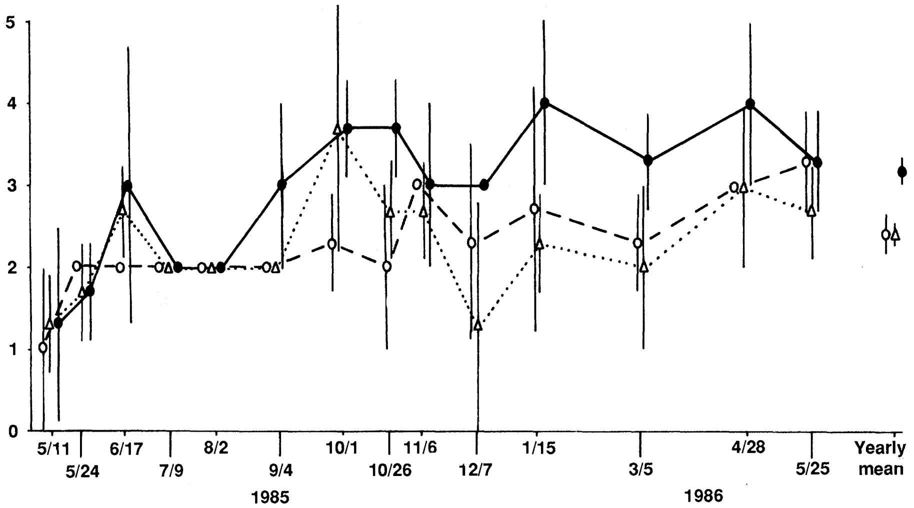

Fig. 2. Number of web spider species during the course of the lizard-removal experiment. Error bars as described in Fig. 1. "Yearly mean" is reported as in Table 1.

An increase in the number of prey species upon removal of a predator is not typical (*9*). In 11 of 16 studies, predator removal decreased prey species number; only five studies showed results such as ours. Most investigations, however, were performed in aquatic systems; only three were terrestrial, all of which have plants as "prey" and arthropods as "predators," and none of which showed the effect we obtained. Thus ours appears to be the first study having terrestrial arthropods as prey, and it goes against the prevailing trend.

The explanation for the more typical result is that predation reduces the abundance of dominant competitors, rendering competitive extinction among prey species less likely and thus allowing more to coexist (*10*). One or more of several considerations may explain our contrary result. First, the interaction between lizards and spiders appears not to be purely predation; evidence for food competition is present, and this would lower species number. Second, predation may be so severe as to lower spider populations precariously near threshold sizes for rapid extinction (*11*); the state "having lizards" may simply imply too much predation to increase species number by reducing competition. Third, even in the absence of predation, spider species in this system may rarely reach densities at which they compete. Supporting this possibility is the fact that maximum spider densities in our plots (1.5/m^2) are similar to those in studies failing to find interspecific competition (*12*) and are substantially less than densities (10 to 15/m^2) in the only experimental field study of spiders (*13*) finding such competition (*14, 15*). Moreover, the two precipitous drops in spider abundance during the second half of the experiment (Fig. 1) occurred after a hurricane and severe winter weather, respectively, suggesting that physical factors are important and may keep population densities too low for competition between spider species in this system.

This study illustrates the complementarity between "manipulative experiments," that is, those in which areas to receive different treatments are randomly selected by the experimenter, and "natural experiments," those in which the "selection" has already been made by natural forces (*15, 16*). The natural experiment, reported in 1983, showed a strongly negative relation between lizard presence and spider abundance, but the process producing this pattern could not be observed. The present manipulative experiment showed that the relation between lizard presence and spider abundance is causal and that underlying mechanisms include predation and, probably to a lesser extent, competition for food. While the manipulative experiment was thus invaluable, the natural experiment both suggested the manipulation and guaranteed its relevance to natural conditions. Moreover, the natural experiment showed about four times the effect on abundance as did the manipulative experiment. Probably this is because our plots were located on a very large island and therefore were surrounded by huge amounts of similar habitat. Immigration into plots of both spider colonists and their food resources would thus be substantially greater than on natural islands, obscuring somewhat a treatment effect. In general, when manipulative experiments are relatively unisolated, natural experiments may show the greater effect.

In conclusion, our results, combined with the even stronger effects found by Pacala and Roughgarden (*4*) in a completely different system, compose a clear case for a major reduction by lizards of spiders in tropical systems.

Table 2. Statistical analysis of number of web spider species in the lizard-removal enclosures (R), the control enclosures with lizards (C), and the unenclosed plots with lizards (U).

Date	*F* value†	Pairwise comparisons‡		
		R versus C	R versus U	U versus C
11 May 1985	0.40 (ns)	ns	ns	ns
24 May 1985	1.00 (ns)	ns	ns	ns
17 June 1985	0.83 (ns)	ns	ns	ns
9 July 1985	0 (ns)	ns	ns	ns
2 August 1985	0 (ns)	ns	ns	ns
4 September 1985	3.00 (ns)	ns	ns	ns
1 October 1985	1.39 (ns)	ns	ns	ns
26 October 1985	19.00 (**)	*	*	ns
6 November 1985	0.40 (ns)	ns	ns	ns
7 December 1985	1.19 (ns)	ns	ns	ns
15 January 1986	1.40 (ns)	ns	ns	ns
5 March 1986	2.36 (ns)	ns	ns	ns
28 April 1986	1.20 (ns)	ns	ns	ns
25 May 1986	1.00 (ns)	ns	ns	ns
Yearly mean§	11.05 (*)	*	*	ns

†Treatment effect, two-way ANOVA, df = 2, 4; ns = $P > 0.10$; *$P < 0.05$; **$P < 0.01$. ‡Analysis of treatment means, Tukey method (*4*) which is two-tailed; *P* values are family significance levels. §Each variate in the yearly mean analysis was the mean number observed in a particular plot from 17 June 1985 to 25 May 1986.

REFERENCES AND NOTES

1. T. W. Schoener, *Science* **155**, 474 (1967); *Ecology* **49**, 704 (1968); ______ and G. C. Gorman, *ibid.*, p. 819; S. W. Pacala and J. Roughgarden, *Science* **217**,

444 (1982); *Ecology* **66**, 129 (1985).
2. Reported in detail in D. A. Spiller and T. W. Schoener, in preparation.
3. T. W. Schoener and C. A. Toft, *Science* **219**, 1353 (1983); see also C. A. Toft and T. W. Schoener, *Oikos* **41**, 411 (1983).
4. S. Pacala and J. Roughgarden, *Oecologia* **64**, 160 (1984). A 6-month removal resulted in a 20- to 30-fold increase in three web spider's abundance, a two- to threefold increase in arthropods on the forest floor, and a small decrease in arboreal insects coincident with the spider increase.
5. J. Neter and W. Wasserman, *Applied Linear Statistical Models* (Irwin, Homewood, IL, 1974).
6. S. Pacala, J. Rummel, J. Roughgarden, *J. Herpetol.* **17**, 94 (1983).
7. Lizard removal was not entirely successful, especially at first when juveniles were apparently hatching from unremoved eggs. However, densities were always well below those in control enclosures, averaging 15% of the latter, and were reduced to zero at various points (*2*).
8. T. W. Schoener, in *Community Ecology*, J. M. Diamond and T. Case, Eds. (Harper & Row, New York, 1986), pp. 556–586.
9. A. Sih, P. Crowley, M. McPeek, J. Petranka, K. Strohmeier, *Annu. Rev. Ecol. Syst.* **16**, 209 (1985). They reviewed all studies in seven major journals between 1965 and 1984.
10. See, for example, R. T. Paine, *Am. Nat.* **100**, 65 (1966) and references in (*8*).
11. N. S. Goel and N. Richter-Dyn, *Stochastic Models in Biology* (Academic Press, New York, 1974).
12. D. H. Wise, *Oecologia* **48**, 252 (1981); C. C. Horton and D. H. Wise, *Ecology* **64**, 929 (1983).
13. D. A. Spiller, *Ecology* **69**, 909 (1984); *Oecologia* **64**, 322 (1984).
14. D. H. Wise, in *Ecological Communities*, D. R. Strong, D. Simberloff, L. G. Abele, Eds. (Princeton Univ. Press, Princeton, NJ, 1984), pp. 42–53.
15. T. W. Schoener, *Am. Nat.* **122**, 240 (1983).
16. J. M. Diamond, *Nature (London)* **304**, 586 (1983); in *Community Ecology*, J. M. Diamond and T. Case, Eds. (Harper & Row, New York, 1986), pp. 3–22.

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Genetic Analysis of Halothane Sensitivity in *Caenorhabditis elegans*

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The nematode *Caenorhabditis elegans* appears to be a useful model for studying the action of volatile anesthetics. A mutant strain that is hypersensitive to the widely used anesthetic halothane was described earlier. The mutation is now shown to be an allele of *unc-79*. Other alleles of *unc-79* are also associated with hypersensitivity to halothane. A strain with a mutation in a second gene, *unc-80*, is also hypersensitive to halothane. Nematodes bearing mutations in both *unc-79* and *unc-80* are slightly more sensitive to halothane than those bearing only one of these mutations. Mutations in a third gene, *unc-9*, suppress both *unc-79* and *unc-80*. Nematodes bearing the suppressor mutations alone have normal sensitivity to halothane. These results show that sensitivity to halothane can be altered by mutations in several different genes.

NO ONE KNOWS HOW THE VOLATILE anesthetics routinely used in operating rooms produce unconsciousness. Study of the mechanism of anesthetic action requires an appropriate yet tractable model system. One method currently used is to measure various biochemical and biophysical properties of anesthetics in vitro (*1*), but the relation of these measurements to each other and to consciousness is not clear. We have chosen instead to use the nematode *Caenorhabditis elegans* as a model for studying anesthesia. The anatomy of *C. elegans* can be described at the level of individual cells; the cell lineages are completely known and are largely invariant among individuals (*2*). There are 302 neurons in the adult hermaphrodite, organized in dorsal and ventral nerve cords, a nerve ring, and several ganglia. A complete wiring diagram of the synaptic connections has been compiled by reconstructions from serial electron micrographs (*3*). Furthermore, *C. elegans* has been extensively analyzed genetically, and a number of mutations are known that derange the structure and the function of the nervous system (*4*). Most important for our work, the response of *C. elegans* to volatile anesthetics is similar to that of mammals (*5*).

As the dose of an anesthetic gas increases, wild-type worms first become hyperactive, then become progressively more uncoordinated until they stop moving altogether and assume a straight posture. At this point, they no longer withdraw when tapped on the snout. All effects are reversed within minutes after removal from anesthetic. The potency of various anesthetics in producing this response in *C. elegans* parallels their potency in mammals. In addition, a mutant with an abnormal response to anesthetics has been isolated; this mutant is resistant to some anesthetics but is hypersensitive to the most lipid-soluble anesthetics, including halothane. The mutation associated with hypersensitivity to halothane previously was called HS1 (*5*); we have renamed this mutation *ec1* in accordance with standard nomenclature (*6*).

Worms were grown and handled by standard procedures, and the anesthetic response was assayed as described in detail (*5*). Briefly, worms are grown on petri plates filled with an agar-based growth medium. Plates of worms to be tested were placed in a glass dish, and a flat glass cover was clamped to the dish to create an airtight chamber. A volume of halothane, estimated to give an appropriate concentration based on the volume of the chamber, was injected into the sealed chamber by means of a glass syringe and a three-way stopcock. The worms were observed through the lid of the chamber with a dissecting microscope; they were judged to be anesthetized when they stopped moving and assumed a straight posture, as described (*5*). After 2 hours, a sample of the gas in the chamber was removed and its exact concentration was determined with a gas chromatograph.

Dose-response curves for the wild-type strain N2 and the strain bearing the mutation *ec1* are shown in Fig. 1. Our results are similar to those of Morgan and Cascorbi (*5*) for both N2 and the strain bearing the *ec1* mutation. We show below that *ec1* is an allele of the gene *unc-79*, and it is labeled as such in the figures. The effective dose at which 50% of the worms are immobile (the ED$_{50}$) is 1.1 ± 0.05% (SEM) for *ec1* and 3.2 ± 0.06% for N2. Virtually all animals with the *ec1* mutation are immobile in 2% halothane, but no wild-type animals are. The 2% dose could be used when scoring both halothane-hypersensitive and normal populations. Using halothane hypersensitivity as a marker phenotype, we mapped *ec1* to a region on linkage group III (*7*) and tested for allelism with several previously identified genes in that region (*8*); *ec1* did not complement *unc-79 (e1068)* for either halothane hypersensitivity or uncoordinated movement. In order to be sure that the altered anesthetic response was due to the defect in *unc-79*, we tested two other *unc-79* alleles, *e1068* and *e1291*, for hypersensitivity to halothane. All three *unc-79* alleles are associated with hypersensitivity to halothane, and animals with these genes do not differ among themselves to a statistically significant extent in their response to halothane. This result indicates that halothane hypersensitivity is a characteristic of *unc-79* mutants and not some special property of *ec1*.

Wild-type worms move almost constantly in the absence of anesthetic (*5*). Worms with

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