References and Notes

1. F. Loewus, *Federation Proc.* **24**, 855 (1965); R. M. Roberts and F. Loewus, *Plant Physiol.* **41**, 1489 (1966); R. M. Roberts, R. H. Shah, F. Loewus, *Arch. Biochem. Biophys.* **119**, 590 (1967); ———, *Plant Physiol.* **42**, 659 (1967).
2. R. G. Stanley and F. Loewus, in *Pollen Physiology and Fertilization*, H. F. Linskens, Ed. (North-Holland, Amsterdam, 1964), p. 128; L. C. T. Young, R. G. Stanley, F. Loewus, *Nature* **209**, 530 (1966).
3. D. B. Dickinson, *Physiol. Plantarum* **20**, 118 (1967).
4. ———, *Plant Physiol.* **43**, 1 (1968).
5. F. Loewus, *Intern. J. Appl. Radiation Isotopes* **12**, 6 (1961).
6. Y. Mäkinen and J. L. Brewbaker, *Physiol. Plantarum* **20**, 477 (1967).
7. D. B. Dickinson, *Science* **150**, 1818 (1965).
8. M. Kroh, *Rev. Palaeobotan. Palynol.* **3**, 197 (1967).
9. W. G. Rosen and S. R. Gawlik, in *Electron Microscopy*, R. Uyeda, Ed. (Proc. Sixth Intern. Congr. Electron Microscopy, Maruzen, Tokyo, 1966), vol. 2, p. 313; P. D. Ascher and S. J. Peloquin, *Am. J. Botany* **53**, 99 (1966).

10. Supported by grant GM-12422 from USPHS. We thank Dr. W. G. Rosen for providing the *L. Longiflorum* pollen used in this study and for his helpful comments. We also thank Miss Charlene Snow for her assistance.

* National Science Foundation Senior Foreign Scientist. Permanent address: Department of Botany, University of Nijmegen, the Netherlands.

19 March 1968

Homing Orientation by Olfaction in Newts (Taricha rivularis)

Abstract. *Newts displaced after perfusion of the olfactory epithelium with formaldehyde failed to orient toward home, whereas control animals subjected to oral perfusion with formaldehyde oriented as readily as did untreated controls. Newts with surgically extirpated olfactory nerves failed to home unless their nerves had regenerated. These results strongly suggest a critical role for olfaction in homing behavior.*

The newt *Taricha rivularis* is able to return to its home area after being displaced for distances as great as 8.0 km. This feat is accomplished by direct, oriented migration toward the home area, regardless of its compass direction or the ruggedness of the intervening mountain terrain (*1*). Moreover, newts take correct initial homeward headings upon leaving the release site, even when the displacement distance is as great as 12.8 km (*2*). The maximum distance at which *T. rivularis* can orient its initial migratory movements has yet to be established, though we have found that the farther a newt is displaced, the more likely it is to leave the release site in a random, nonoriented direction. Since blinded animals can home successfully (*3*), it seems unlikely that vision is essential for orientation, although the possible existence of alternate photosensory systems has not been tested.

The experiments we report here provide evidence that in these animals olfaction plays a major role in initial orientation and in the eventual return to the home site.

In the first experiments designed to assess the importance of olfaction in homing, we effected anosmia surgically by extirpating a section of the olfactory nerves. Of 617 such operated newts displaced from Pepperwood Creek to Jim Creek (about 3.2 km displacement distance), only 15 are known to have returned home. These were killed and dissected, and each proved to have regenerated olfactory nerves. The control group for this homing study consisted of 692 normal animals that experienced the same displacement. Of these, 564 were later recaptured at their home sites along Pepperwood Creek. Daily patrols of the stream and frequent collections in the land traps adjacent to Pepperwood Creek preclude the possibility that large numbers of animals could have returned undetected. Although the total return of operated newts was too small to permit any broad conclusions, it is at least clear that not one newt with unregenerated nerves was recaptured at the home location.

In similar experiments designed to test initial orientation rather than complete homing performance, we compared surgically treated anosmic newts with a control group in which an identical opening was made in the skull but the olfactory nerves were left intact. Members of both groups showed random orientation at the release site. Since in the salamander the cerebral hemispheres are predominantly olfactory in function (*4*), the sham operation may have produced anosmia through traumatization of this region. Thus, though the surgical experiments gave unclear results, they did suggest that interventions involving olfactory regions of the central nervous system produced deficits in the ability to take correct initial headings and to complete a homing performance.

We were thus encouraged to attempt more controlled and rigorous lesions in the olfactory system, this time testing initial orientation. We rendered newts anosmic by perfusing the nasal cavities with a 10 percent solution of formaldehyde. A blunted hypodermic syringe was inserted into each external naris, and the solution was injected until it flowed freely from the internal nares into the oral cavity. Excess formaldehyde in the oral cavity was immediately flushed away with fresh water. Histological examination of animals treated in this way showed that the olfactory epithelium had become necrotic.

In another large group of newts only the oral cavity was perfused with 10 percent formaldehyde, which was immediately flushed out with fresh water. A third group was used as a standard with which to compare initial orientation.

All were collected from the same segment of stream and released on land about 1.2 km upstream (Fig. 1). Displacements were made early in the breeding season and each release consisted of approximately equal numbers of anosmic animals and treated controls selected randomly from the total collected each day. The normal control group had experienced the same displacement and recapture routine 1 year earlier. All three groups were marked distinctively by coded toe clipping: 502 anosmic animals, 451 treated controls, and 617 normal controls were released.

Recapture fences were constructed of ¼-inch mesh hardware cloth, arranged so as to funnel migrating newts into escape-proof traps. They were arranged predominantly on the steep, wooded hillside that forms the south bank of Pepperwood Creek in Sonoma County, California. Three fences (30.48 m long) were spaced 41.1, 91.4, and 219.4 m from—and perpendicular to—the stream (Fig. 1). We built a fourth fence on the north side of Pepperwood Creek on flat, open terrain where little migration of newts occurs. Complete four-fence networks of this type were located 228.5 m downstream and 228.5 m upstream from the release point. Captures in two additional fences, one in a densely wooded area farther upstream on the north bank and a comparable one farther downstream augmented the number of recaptures in the main fence networks. In the present tests we made all of our displacements from a downstream collecting site (about 1.2 km) (Fig. 1).

The newt's initial orientation movements upon its departure from the release site were measured by comparing the number of captures in the traps upstream from the release site with the number of captures in the downstream traps. The newts were allowed as much as 3 months to choose their migratory direction when leaving the release site.

The reason for conducting these experiments over long periods and in a

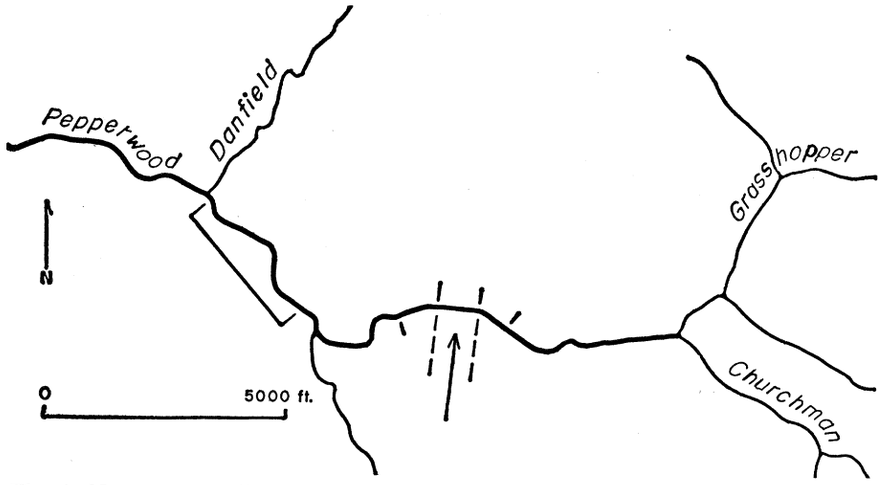

Fig. 1. Newts were collected from the portion of Pepperwood Creek indicated by the bracket and displaced upstream to the release site (arrow) midway between land traps perpendicular to the stream. The displacement distance is approximately 1.2 km.

large test area is related to our observation, over many years of experimenting with displaced newts in test enclosures, that *T. rivularis* is subject to great changes in behavior through handling. In early displacement tests, the enclosures were small (18.3 to 30.48 m across), and the displacement distances were short, requiring a minimum of handling. The results in these short-time, short-distance tests were satisfactory. But with greater displacement distances, longer periods of confinement during processing, and added handling for operating and coding, we found that results in small test enclosures were unsatisfactory. Many animals refused to leave the release site; those that did leave appeared to be seeking escape or shelter regardless of the home direction. We eliminated these difficulties by using larger test areas and allowing more time. The newts may remain immobile for long periods, but then have an opportunity to probe the release area seeking homing cues without committing themselves to capture in a trap. Once the directional choice is made, the newt must travel in the chosen direction for at least 228.5 m before encountering a trap.

The first trap captures in each group occurred about 2 weeks after release, when a few test animals encountered the fences. Captures mounted steadily through the next month, then fell off with the onset of hot, dry weather in late spring when newts cease migrating and seek underground hiding places to avoid summer desiccation.

Although the treated newts did not move completely at random, accuracy in orienting toward the home area was greatly reduced (Fig. 2). The treated controls oriented with the same accuracy as the normal controls.

Studies of amphibian orientation and homing have produced several hypotheses concerning the sensory basis of this phenomenon. Landreth and Ferguson have shown that newts and frogs use a sun-compass mechanism in maintaining a compass course (*y*-axis) that bisects the home shore at right angles (*5*). Frogs tested in an aquatic arena failed to orient under cloud cover or when the sky was of uniform light intensity, indicating a visual mechanism. Shoop, on the other hand, found that the salamander *Ambystoma maculatum* does most of its migrating during cloudy and rainy nights (as does *T. rivularis*) when no celestial cues are available. He doubts that visual cues could guide these migrations (*6*). When the frog *Pseudacris triseriata* was tested in a T-maze, 71 percent chose odors from a breeding habitat rather than upland forest odors (*7*). Our own earlier results (*3*) give no indication that the homing performance of blinded newts is impaired.

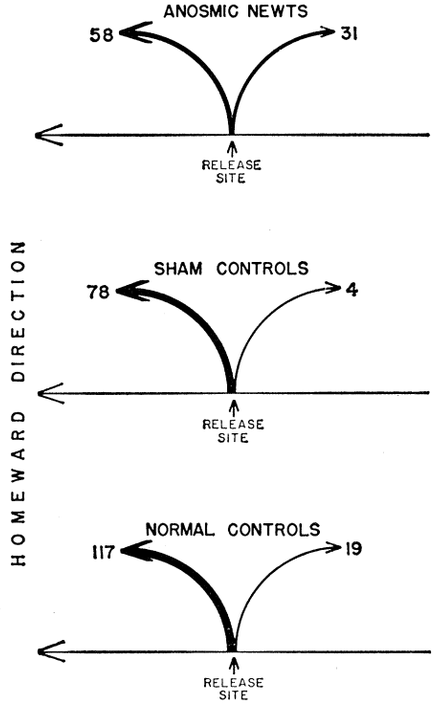

Fig. 2. The distribution of displaced anosmic newts released between the two trap networks is significantly different ($P = .01$) from the distributions of the two control groups. The distribution of the treated control group does not differ significantly from that of the normal controls (test based upon normal approximation to the binomial distribution). Actual release and recapture figures: 502 anosmic released, 89 recaptured; 451 treated controls released, 82 recaptured; 617 normal controls released, and 136 recaptured.

It appears from the results with *T. rivularis* treated with formaldehyde, in which only olfactory tissue is destroyed, that odor plays an important, though not necessarily exclusive, role in initial orientation. The fact that anosmic newts did not distribute themselves completely randomly suggests that other mechanisms may partially take over when olfaction fails. This is consistent with Ferguson's proposal that orientation is probably not dependent on one sense exclusively.

An olfactory basis for homing presupposes a stable source of odorant capable of remaining recognizable year after year. Because normal newts orient toward home before and after, as well as during, their aquatic breeding season, and because their migration is terrestrial, it seems unlikely that anything in the water attracts them to the home territory. Terrestrial odors from trees, smaller plants, and decaying plant material could provide such long-term odors. Even a microsmatic biologist can easily differentiate between that portion of Pepperwood Creek bounded by fir trees and another stretch where California laurel predominates. The odor complex which identifies an area must be a composite of numerous individual odors, which can maintain its collective identity over long periods.

DAVID GRANT
OSCAR ANDERSON
VICTOR TWITTY*
Department of Biological Sciences, Stanford University, Stanford, California 94305

References and Notes

1. V. C. Twitty, D. Grant, O. Anderson, *Proc. Nat. Acad. Sci. U.S.* **56**, 864 (1966); *ibid.* **51**, 51 (1964).
2. ———, *ibid.* **57**, **342** (1967).
3. V. C. Twitty, *Of Scientists and Salamanders*

(Freeman, San Francisco, 1966),, pp. 134–38.
4. C. J. Herrick, *The Brain of the Tiger Salamander* (Univ. of Chicago Press, Chicago, 1948).
5. H. Landreth and D. Ferguson, *Nature* **215**, 516 (1967); *Herpetologica* **23**, 81 (1967); *Science* **158**, 1459 (1967).
6. C. R. Shoop, *Science* **149**, 558 (1965).
7. B. S. Martof, *Physiol. Zool.* **35**, 270 (1962).
8. Supported by NSF grant GB-4164. We thank D. Kennedy (Stanford) for his editorial advice and especially for his efforts in arranging the continuation of this research following the death of Victor Twitty. We also thank J. Ware for help with the statistical analysis.
* Died 22 March 1967 before the completion of these experiments.

10 May 1968

Malate Dehydrogenase: Evidence for Tetrameric Structure in Mus musculus

Abstract. *Two electrophoretically distinct variants of supernatant nicotinamide-adenine dinucleotide phosphate–dependent malate dehydrogenase exist in mice* (Mus musculus). *They are controlled by codominant alleles segregating at an autosomal locus. The two forms exist in a polymorphic condition in wild populations of* Mus musculus *and are fixed in a homozygous condition in inbred lines. These genetic electrophoretic variants are used here to study the subunit structure of this enzyme. Evidence indicating a tetrameric structure for mouse nicotinamide-adenine dinucleotide phosphate–dependent malate dehydrogenase is presented. This interpretation is based on the occurrence in heterozygote tissue extracts of five electrophoretically distinct enzymes. This is the predicted phenotype for tetramers composed of two types of subunits which associate randomly in heterozygotes forming three hybrid enzymes having mobilities intermediate between the parental forms.*

Malate dehydrogenase (decarboxylating; E.C. 1.1.1.40) reversibly catalyzes the oxidative decarboxylation of malate in the presence of NADP (*1*) and manganous ions to pyruvate and carbon dioxide. The enzyme also decarboxylates oxalacetate to pyruvate in the presence of Mn^{++} (*2*, *3*). Henderson has shown that at least two distinct and unrelated forms of NADP-malate dehydrogenase (NADP-MDH) (*1*) exist in *Mus musculus* (*4*). She demonstrated that there were two enzymes which had markedly different electrophoretic mobilities, restricted to either the mitochondrial or supernatant fractions, with allelic variants being in the supernatant, but not in the mitochondrial fraction. These observations provided evidence for two distinct enzymes with similar substrate specificities determined by independent loci. Henderson suggested that the different electrophoretic forms of the supernatant enzyme were determined by two alleles which segregated in a simple Mendelian fashion; the locus specifying the enzyme was possibly situated on linkage unit II; and these alleles coded for subunits which associated randomly, forming a single, though diffuse, hybrid enzyme. Her observations suggested a dimeric association of subunits.

We now report that the supernatant form of mouse NADP-MDH is a tetramer composed of four freely associating subunits, confirm and extend Henderson's genetic interpretation, and tabulate the expression of the two allelic forms of the supernatant enzyme in 30 inbred lines of mice.

The gene for the supernatant enzyme is designated *Mdh-1*, and the alleles *Mdh-1*^{a} and *Mdh-1*^{b} control, respectively, the slow and fast cathodally migrating bands (*4*). These alleles code for the *a* and *b* subunits (slow and fast, respectively), which assort randomly and give rise to the three possible F_2 patterns. These phenotypes are designated A (Fig. 1, channels 2, 6), B (Fig. 1, channel 4) for the two homozygote types, and AB for the heterozygote (Fig. 1, channels 1, 3, 5).

Henderson's data was based on hybrid animals from crosses between inbred strains of mice (C3H/HeJ × AKR/J and C57BL/6J × DBA/2J) homozygous for different *Mdh-1* alleles, and a few F_2 mice showing segregation of the two alleles. We made crosses (F_1, F_2, and BC) between inbred strains SJL/J and C57BL/6J in order to validate the genetic nature of the *Mdh-1* polymorphism. The results of the crosses (Table 1) demonstrate (i) the expression of a hybrid enzyme pattern in all F_1 animals; (ii) an F_2 distribution in agreement with a 1 : 2 : 1 ratio for A, AB, and B phenotypes respectively; (iii) agreement with a 1 : 1 ratio in the backcrosses; and (iv) no sex association of the *Mdh-1* gene. These results indicate that *Mdh-1*^{a} and *Mdh-1*^{b} are codominant alleles controlled by an autosomal locus.

The NADP-MDH phenotypes in 30 lines of inbred mice are given in Table 2. A number of wild populations of mice from diverse sources have been screened, and all three NADP-MDH phenotypes (A, AB, B) have been observed. No new electrophoretic variants

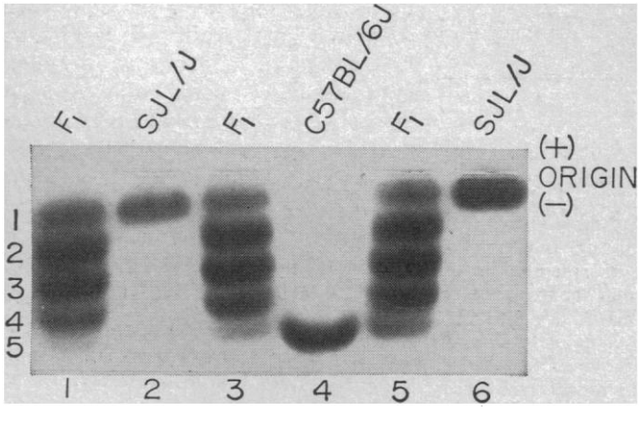

Fig. 1. Kidney NADP-MDH phenotypes after starch-gel electrophoresis, demonstrating the genetic variation and tetrameric nature of the supernatant isozyme. Vertical column of numbers represent enzyme bands, and the horizontal row represents gel channels. (Channels 1, 3, and 5) Hybrid phenotypes showing the five bands characteristic of a protein composed of four subunits; (channels 2 and 6) slow-migrating form, A; and (channel 4) fast-migrating form, B. The same electrophoretic patterns are observed in liver homogenates. Fresh homogenates were prepared for electrophoresis (*5*) from kidneys and livers of adult mice. Starch-gel electrophoresis was carried out vertically at 3°C for 20 hours at 280 volts across the system, the gel buffer consisted of 0.008*M* tris and 0.003*M* citric acid adjusted to *p*H 6.7 with NaOH; the bridge buffer consisted of 0.223*M* tris and 0.086*M* citric acid adjusted to *p*H 6.3 with NaOH. After electrophoresis, the gel was incubated at 37°C for 2 hours in a staining solution consisting of NADP (20 mg), nitro blue tetrazolium (10 mg), and phenozine methosulfate (4 mg) dissolved in a mixture of 90 ml of 0.2*M* tris-HCl, *p*H 8.0; 10 ml of 0.5*M* of L-malic acid (adjusted to *p*H 7.0), and 0.5 ml of 0.25*M* $MnCl_2$.