The cellular anatomy of the BAc is that of a typical tympanal chordotonal organ (*6, 15*). Its sensory units are multicellular scolopidia, each consisting of a bipolar sensory neuron and several accessory cells, including the diagnostic scolopale cell. A defining feature of scolopale cells is a hexagonal arrangement of scolopale rods, which contribute to the cell's fusiform shape and which fuse and terminate distally to meet a bullet-shaped scolopale cap. We found that each BAc of *O. ochracea* contains between 60 and 70 scolopidia. The scolopale cells in the ear of the tachinid fly, like those of orthopteroid insect ears, show the classical scolopale anatomy, including hexagons of scolopale rods (Fig. 2C) and unmistakable scolopale caps (Fig. 2D). The BAc is innervated by an anterior branch of thoracic nerve II (frontal nerve).

Because the PTM apparently functions as an "eardrum" in the auditory apparatus of ormiine flies, it is of interest that it is sexually dimorphic and enlarged in females relative to males. Our conclusion that the PTM is an ormiine adaptation for hearing is supported by the results of an examination of a nonormiine (presumably nonhearing) tachinid fly, *Myiopharus doryphorae*, in which the prosternum and the prosternal membranes in both sexes are much smaller than in *O. ochracea*.

Earlier workers reported that larviparous female, but not male, tachinids are attracted to singing crickets or to loudspeakers playing tape-recorded songs (*1*). Our field experiments with captive flies indicate that intact tympanal membranes are crucial for hearing cricket songs. When 17 intact and sham-operated flies were released in a flight cage (2 by 2 by 2 m), 11 were attracted to a loudspeaker broadcasting a cricket song. None of ten flies with punctured tympanal membranes and broken auditory apodemes were attracted to the loudspeaker, although they could fly. The behavior of the two groups is significantly different (Fisher's exact test, $P < 0.001$) (*16*). Moreover, we have physiological evidence for the auditory function of the prosternal membranes; puncturing both PTMs and associated auditory apodemes eliminated the neural response described above.

From evolutionary as well as mechanistic points of view, both female crickets and female parasitoid flies are under similar selective pressures on the design characteristics of their ears. Their sense of hearing must serve the same ends: completion of reproduction depends on hearing and locating a singing male cricket over great distances. In insects, long-distance, high-frequency hearing is subserved by scolopophorous tympanal organs, as in moths, praying mantises, cicadas, locusts, katydids, and crickets. To this list we now add these tachinid flies (*17*), and we propose that this dipteran tympanal organ evolved through convergent evolution due to similar selective pressures: for a fly to act like a cricket, it must hear like one.

REFERENCES AND NOTES

1. W. Cade, *Science* **190**, 1312 (1975); T. J. Walker, *Fla. Entomol.* **69**, 678 (1986); J. R. Mangold, *ibid.* **61**, 57 (1978).
2. L. M. Roth, *Am. Midl. Nat.* **40**, 265 (1948).
3. H. C. Bennet-Clark and A. W. Ewing, *J. Exp. Biol.* **49**, 117 (1968); J. C. Hall, in *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*, G. Kerkut and L. Gilbert, Eds. (Pergamon, New York, 1985), vol. 9, pp. 287–373.
4. A. Michelsen and O. N. Larsen, in *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*, G. Kerkut and L. Gilbert, Eds. (Pergamon, New York, 1985), vol. 6, pp. 495–556.
5. A. W. Ewing, *Arthropod Bioacoustics* (Cornell Univ. Press, Ithaca, NY, 1989).
6. M. Moulins, in *Structure and Function of Proprioceptors in Invertebrates*, P. J. Mill, Ed. (Chapman & Hall, London, 1976), pp. 387–426.
7. Flies were shipped to Ithaca, NY, for physiological and anatomical studies.
8. We recorded from the connectives that run between the fused thoracic ganglion and the subesophageal-cerebral complex of *O. ochracea* with 35-μm stainless steel hook electrodes.
9. A. Moiseff and R. R. Hoy, *J. Comp. Physiol. A* **152**, 155 (1983); D. D. Yager and R. R. Hoy, *Science* **231**, 727 (1986).
10. T. J. Walker, *Evolution* **16**, 407 (1962).
11. The neurophysiological recordings were made at 22° to 25°C. The recordings of the cricket song were made in the field at 25°C. Frequency mismatch due to a temperature effect is unlikely because the carrier frequency of the cricket song is not related to the ambient temperature (*10*).
12. Sound pressure levels (SPL), expressed in decibels (reference: 20 μPa), were measured at the location of the fly with a B&K type 2209 sound level meter (calibrated with a model 4220 pistonphone) and B&K type 4135 and type 4138 condenser microphones. Frequency calibration and spectral purity were controlled by means of fast Fourier transform analysis on a Nicolet 444A mini-ubiquitous spectrum analyzer. The sound stimulus consisted in trapezoidal pulses (rise/fall time: 5 ms) of 20 ms. Sound amplitude (first calibrated at 80 dB SPL) was controlled by a HP-350D step attenuator. The loudspeaker was positioned 90° in azimuth and 30° in elevation to the longitudinal axis of the animal.
13. P. M. Narins and R. R. Capranica, *Science* **192**, 378 (1976); B. Cardone and J. H. Fullard, *Physiol. Entomol.* **13**, 19 (1988); D. D. Yager, *J. Zool.* **221**, 517 (1990); W. J. Bailey and H. Römer, *J. Comp. Physiol. A* **169**, 349 (1991).
14. Morphological work was done by P. M. Choate (University of Florida, Gainesville), using dissection of fresh material, and scanning electron microscopy was the basis for further investigation of the ormiine sense of hearing.
15. E. G. Gray, *Philos. Trans. R. Soc. London Sect. B* **243**, 75 (1960).
16. R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, ed. 2, 1981).
17. It has come to our attention that R. Lakes-Harlan and K.-G. Heller [*Naturwissenschaften* **79**, 224 (1992)] have independently, and at the same time, discovered a similar hearing organ in another ormiine fly, *Therobia leonidei*.

18. We thank T. J. Walker, S. Wineriter, and P. M. Choate for assistance in Gainesville. We also thank M. Read for the histology, M. Nelson for preparing figures, R. Roush for tachinid specimens, and T. J. Walker for tape recordings of *G. rubens* from Florida. R. Capranica, T. Seeley, G. Eickwort, T. Eisner, T. J. Walker, and investigators in the laboratory of R.R.H. read the manuscript. D.R. and R.R.H. are funded by NINDCD-DC00103 and Hatch NYC-191403.

12 June 1992; accepted 9 September 1992

The Complete Skull and Skeleton of an Early Dinosaur

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The unearthing of a complete skull and skeleton of the early dinosaur *Herrerasaurus ischigualastensis* sheds light on the early evolution of dinosaurs. Discovered in the Upper Triassic Ischigualasto Formation of Argentina, the fossils show that *Herrerasaurus*, a primitive theropod, was an agile, bipedal predator with a short forelimb specialized for grasping and raking. The fossils clarify anatomical features of the common ancestor of all dinosaurs. *Herrerasaurus* and younger dinosaurs from Upper Triassic beds in Argentina suggest that the dinosaurian radiation was well under way before dinosaurs dominated terrestrial vertebrate communities in taxonomic diversity and abundance.

Fragmentary skeletons of the earliest and most primitive dinosaurs were discovered more than 30 years ago in Upper Triassic rocks in South America and include *Staurikosaurus pricei* from the Santa María Formation of southern Brazil (*1*) and *Pisanosaurus mertii* and *Herrerasaurus ischigualastensis* from the Ischigualasto Formation of northwestern Argentina (*2–5*). These formations, deposited approximately 225 million years ago, record the initial phase of dinosaur evolution before their domination of terrestrial vertebrate communities (*6*). The remains of these early dinosaurs, however, are too fragmentary to provide a coherent view of the origin and early radi-

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ation of dinosaurs. Recent work in the Ischigualasto Formation resulted in the discovery of several skeletons of *Herrerasaurus* (7) that provide the first complete picture of an early dinosaur and clarify the sequence of anatomical changes that occurred during early dinosaur evolution.

The skull, previously undiscovered, lacks nearly all of the specializations of later dinosaurs. Narrow from side to side and low in profile (Fig. 1), the skull has small external nares and a narrow antorbital fossa without diverticulae (8). The most significant cranial specialization is a well-developed sliding joint within each lower jaw. As in theropods, this intramandibular joint allowed the lower jaws to flex, with the anterior toothed half capable of rotating approximately 15° against the posterior half in *Herrerasaurus*. Extant anguimorphine lizards have evolved an analogous intramandibular joint and a flexible, grasping bite, which may be adaptations to subdue live prey (9).

The neck, pectoral girdle, and forelimb are also represented among the new fossils and show that *Herrerasaurus* was an obligatory biped with a slender, flexible neck (Fig. 2) (8, 10). The forelimb, which is less than half the length of the hind limb, has short proximal segments (humerus and radius-ulna) and an elongate manus (Figs. 3 and 4A). Manual digits IV and V are reduced and would not have extended as free digits beyond the palm of the hand (phalangeal formula 2-3-4-1-0). The elongate penultimate phalanges and large recurved unguals on digits I through III indicate that the manus is specialized for grasping (8).

This material of *Herrerasaurus* clarifies anatomical features that arose in the common dinosaurian ancestor and revises our understanding of the structure and timing of the early dinosaurian radiation. In addition, the depositional setting in which these fossils were found and the associated fauna and flora provide insight into the paleoecology of an important Late Triassic community.

The monophyly of dinosaurs (that is, their descent from a single common ancestor) has gained wide acceptance after it was first explicitly proposed (*11*). The monophyly of a particular group is based on novel anatomical traits (synapomorphies), which are inferred to have been inherited from a common ancestor. Numerous dinosaurian synapomorphies have been listed in discussions of early dinosaur phylogeny (4, *11*–*14*). The material of *Herrerasaurus* presents an opportunity to test these characters, with appropriate outgroup comparisons (*Lagosuchus*, *Lagerpeton*, Pterosauria, Crurotarsi) (*15*).

Of the nine cranial characters earlier proposed as dinosaurian synapomorphies (4, *13*), only the absence of the postfrontal bone appears to be valid (8). The postfrontal is absent in *Herrerasaurus* and other dinosaurs and is present in dinosaurian outgroups (Pterosauria and Crurotarsi) (*15*). Additional dinosaurian cranial synapomorphies that are present in the skull of *Herrerasaurus* include the extension of adductor musculature onto the frontal, lateral exposure of the quadrate head (Fig. 1) and a reduction of the posttemporal opening to a foramen (8).

Of the approximately 50 postcranial synapomorphies listed earlier in support of Dinosauria (4, *13*), only seven are supported by the new material of *Herrerasaurus*. These include the addition of a dorsal vertebra to the sacrum, an elongate deltopectoral crest on the humerus, loss of the ungual on the fourth digit of the hand, a brevis fossa on the ilium, a subrectangular femoral head, a cnemial crest on the tibia, and an ascending process on the astragalus (*16*). The new fossils provide evidence of additional dinosaurian synapomorphies, including an antitrochanter within the acetabulum that is divided by a notch, reduction of the medial process of the calcaneum, and reduction of the heel on distal tarsal 4.

The early dinosaurs *Herrerasaurus* and *Staurikosaurus* were originally described and classified as saurischians (*1*–*5*, *11*), although their precise phylogenetic relations were not specified (*17*, *18*). A more recent view, stimulated by the application of cladistic methodology, is that *Herrerasaurus* and *Staurikosaurus* are basal dinosaurs that lie outside true ornithischians and saurischians (*12*–*14*). Indeed, several features in the original partial skeletons of *Herrerasaurus* and *Staurikosaurus* supported this view, such as the absence of a third, fully incorporated sacral vertebra and the narrow distal end of the tibia.

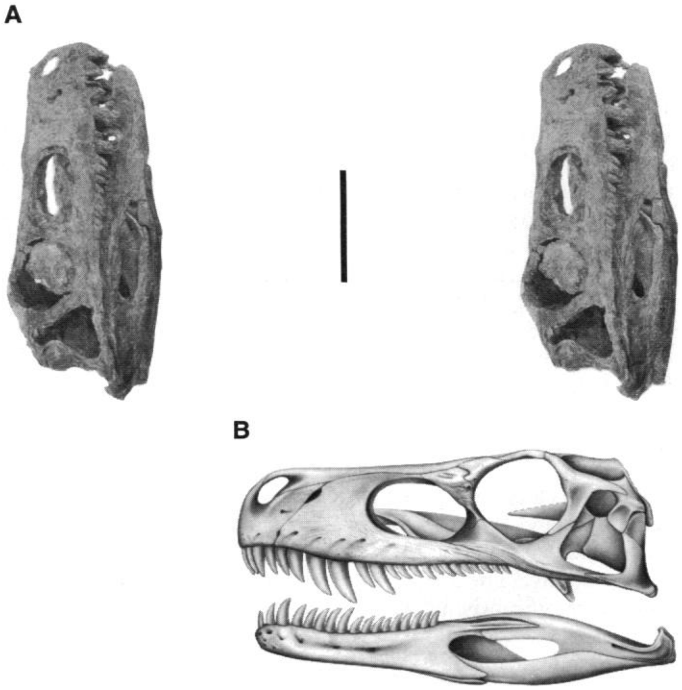

Fig. 1. Skull of *Herrerasaurus ischigualastensis*. (**A**) Stereopair of skull (PVSJ 407) in right lateral view. Scale bar = 10 cm. (**B**) Reconstruction of skull in left lateral view. PVSJ, Museo de Ciencias Naturales, Universidad Nacional de San Juan.

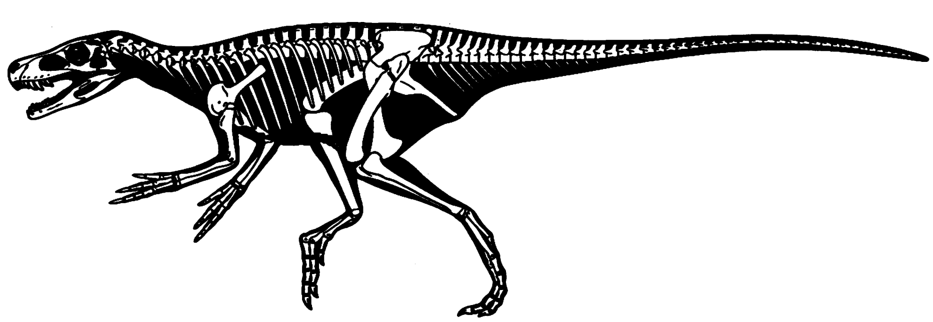

Fig. 2. Skeletal reconstruction of *Herrerasaurus ischigualastensis* based on fossils MCZ 7064, PVL 2566, and PVSJ 53, 373, and 407. Adult skeletal length range is 3 to 6 m. MCZ, Museum of Comparative Zoology, Harvard University; PVL, Fundación Miguel Lillo, Universidad Nacional de Tucumán, San Miguel de Tucumán; PVSJ, as in Fig. 1.

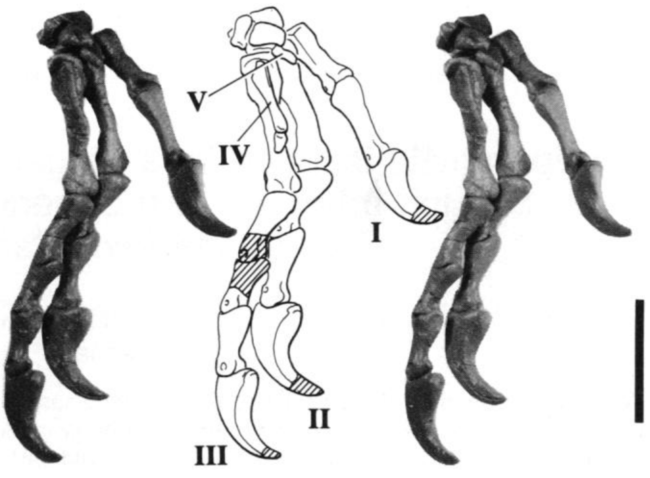

Fig. 3. Stereopair of the left carpus and manus of *Herrerasaurus ischigualastensis* (PVSJ 373) in ventrolateral view. Cross-hatching indicates missing fragments; scale bar = 5 cm. I through V indicate manual digits I through V; PVSJ, as in Fig. 1.

The material of *Herrerasaurus*, however, casts doubt on this initial cladistic assessment of early dinosaur phylogeny. The skull, neck, and forelimb, in particular, show several characters that occur only in saurischian dinosaurs, such as a subnarial foramen between the premaxilla and maxilla (Fig. 1), hyposphene-hypantrum articulations in the dorsal vertebrae (*11, 14*), epipophyses on mid- and posterior cervical vertebrae, and an elongate manus that approaches, or exceeds, half of the length of the more proximal part of the forelimb (Fig. 4A) (*14*). Furthermore, other features of *Herrerasaurus* provide evidence of theropod affinity, such as the pubic foot and elongate prezygapophyses in the distal caudal vertebrae. In light of this evidence, the presence of only two fully incorporated sacral vertebrae in *Herrerasaurus* and *Staurikosaurus* now suggests that full incorporation into the sacrum of at least one additional dorsal vertebra must have occurred independently in ornithischians and saurischians. This is not surprising, given that dorsal and caudal vertebrae have been incorporated into the sacrum many times in subsequent dinosaur phylogeny (Fig. 4B). Likewise, the narrow distal end of the tibia in *Herrerasaurus*, *Staurikosaurus*, and the basal ornithischian *Pisanosaurus* also suggests independent broadening of the tibia and astragalus in ornithischians and saurischians (Fig. 4C).

If *Herrerasaurus* and *Staurikosaurus* represent early theropods, then several branch points at the base of the phylogenetic tree of Dinosauria must have occurred earlier than previous phylogenies have implied. The split between saurischians and ornithischians must have occurred before the middle Carnian, when the Ischigualasto Formation was deposited (6). The presence of herrerasaurid theropods in the Ischigualasto Formation and more specialized saurischians [prosauropods and tetanurian theropods (*19*)] in the overlying Los Colorados Formation suggests that Saurischia had already split into at least four morphologically disparate lineages by the Norian. Three of these lineages (prosauropods and ceratosaurian and tetanurian theropods) are documented by fossils. A sauropod lineage must also have been present in the Late Triassic even though the earliest sauropods are known from Jurassic strata (*20*).

Despite the early appearance of several major clades of dinosaurs, dinosaur species do not dominate the vertebrate faunas in either the Ischigualasto or Los Colorados formations but rather co-exist with a variety of crurotarsal archosaurs and synapsids (*6, 21*). In the Ischigualasto Formation, for example, dinosaurs (*Herrerasaurus* and the herbivore *Pisanosaurus*) constitute a minority of the recorded vertebrate species and are much less abundant than rhynchosaurian (*Scaphonyx*) and traversodontid (*Exaeretodon*) herbivores (*22*). If these terrestrial vertebrate faunas typify Late Triassic communities, the early dinosaurian radiation may be characterized by a high degree of morphologic innovation with low species diversity and abundance, an evolutionary pattern that has been linked with open ecologic space (*23*).

Herrerasaurus ranges through approximately 40 m of section in the lower third of the Ischigualasto Formation, which is characterized by coarse-grained, lenticular sandstones deposited in narrow channels and overbank deposits represented by variegated silty mudstones with evidence of soil development (*22*). These facies, along with locally abundant fossil plants [sphenopsids, ferns, seed ferns, and conifers (*21*)], suggest that the early dinosaur *Herrerasaurus* inhabited an upland, riparian forest with a seasonal climate (*24*).

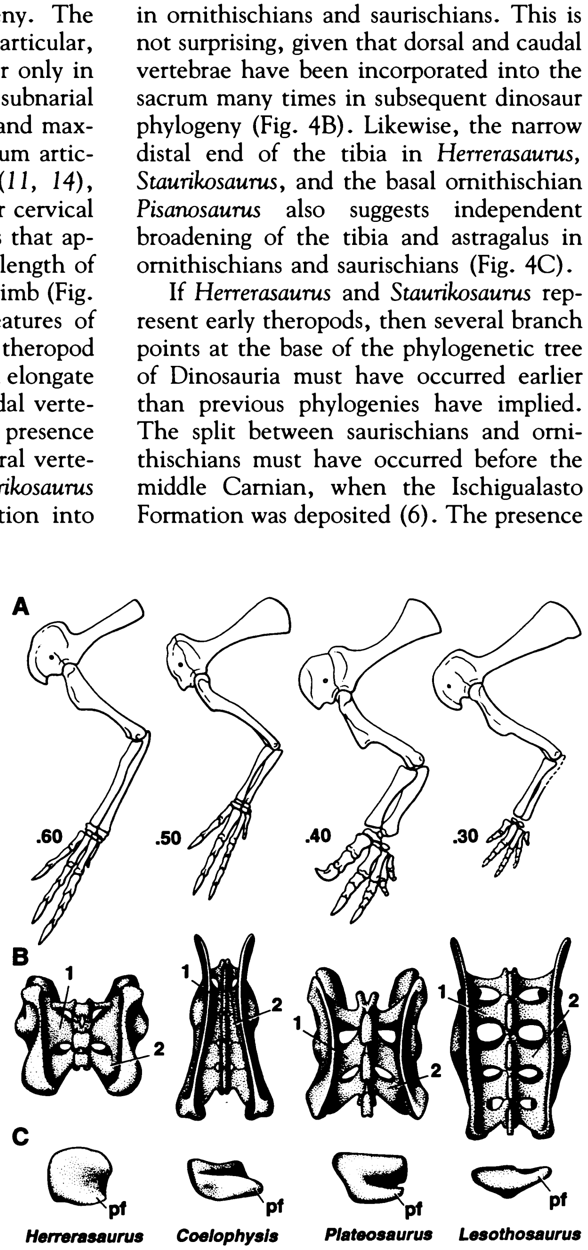

Fig. 4. Comparison of (**A**) the left pectoral girdle and forelimb in lateral view, (**B**) the ilia and sacrum in dorsal view, and (**C**) the left tibia in distal view in *Herrerasaurus ischigualastensis* (*10*), *Coelophysis bauri* (*25*), *Plateosaurus englehardti* (*26*), and *Lesothosaurus diagnosticus* (*27*), respectively. Fractional numbers in (A) indicate the length of the manus (digit III) as a proportion of the remainder of the forelimb (humerus + radius). In (B), 1 and 2 indicate the first and second primordial sacral vertebrae, respectively; in (C), pf indicates the posteromedial flange.

REFERENCES AND NOTES

1. E. H. Colbert, *Am. Mus. Novit.* **2405**, 1 (1970); P. M. Galton, *Palaeontol. Z.* **51**, 234 (1977); M. C. Barbarena *et al.*, *Natl. Geogr. Res.* **1**, 5 (1985).
2. O. A. Reig, *Ameghiniana* **3**, 3 (1963).
3. J. L. Benedetto, *ibid.* **10**, 89 (1973).
4. J. F. Bonaparte, *J. Paleontol.* **50**, 808 (1976).
5. Other dinosaur species reported from the Ischigualasto Formation (*2, 12*) [F. E. Novas, *IV Congr. Argent. Paleontol. Bioestratigrafía* **2**, 1 (1986)] all pertain to *Herrerasaurus ischigualastensis* (*10*).
6. J. F. Bonaparte, *J. Vertebr. Paleontol.* **2**, 362 (1982); P. E. Olsen and H.-D. Sues, in *The Beginning of the Age of Dinosaurs*, K. Padian, Ed. (Cambridge Univ. Press, Cambridge, 1986), pp. 321–351.
7. P. C. Sereno *et al.*, *J. Vertebr. Paleontol.* **8**, 26A (1988).
8. P. C. Sereno and F. E. Novas, *ibid.*, in press.
9. S. McDowell and C. Bogert, *Bull. Am. Mus. Nat. Hist.* **105**, 1 (1954).
10. F. E. Novas, *J. Vertebr. Paleontol.*, in press.

11. R. T. Bakker and P. M. Galton, *Nature* **248**, 168 (1974).
12. D. B. Brinkman and H.-D. Sues, *Palaeontology* **30**, 493 (1987).
13. M. J. Benton, in *Third Symposium on Mesozoic Terrestrial Ecosystems*, W. E. Reif and F. Westphal, Eds. (Attempto Verlag, Tübingen, 1984), pp. 13–18; G. S. Paul, *ibid.*, pp. 175–180; *J. Vertebr. Paleontol.* **4**, 507 (1984); J. Gauthier and K. Padian, in *The Beginnings of Birds*, M. K. Hecht, J. H. Ostrom, G. Viohl, P. Wellnhofer, Eds. (Freunde des Jura-Museums Eichstätt, Eichstätt, 1985), pp. 185–197; F. E. Novas, *J. Paleontol.* **63**, 677 (1989); M. J. Benton, in *The Dinosauria*, D. B. Weishampel, P. Dodson, H. Osmólska, Eds. (Univ. of California Press, Berkeley, 1990), pp. 11–30; F. E. Novas, *Palaeontology* **35**, 51 (1992).
14. J. Gauthier, *Calif. Acad. Sci. Mem.* **8**, 1 (1986).
15. P. C. Sereno, *J. Vertebr. Paleontol.* **11**, 1 (1991); _____ and A. B. Arcucci, *ibid.*, in press.
16. Because the skull and forelimb are not preserved in the dinosaur precursors *Lagosuchus* and *Lagerpeton*, dinosaurian synapomorphies in these skeletal parts may eventually characterize a more inclusive group (pterosaurs and basal crurotarsal archosaurs show the primitive condition). The ungual on the fourth digit of the manus is absent in all dinosaurs except Cretaceous iguanodontian ornithischians such as *Iguanodon* [D. B. Norman, *Inst. R. Sci. Nat. Belgique Mem.* **178**, 1 (1980)], in which it constitutes an evolutionary reversal.
17. Because dinosaur polyphyly and saurischian paraphyly were often assumed at this time, the label "saurischian" did not commit one to a specific phylogenetic arrangement, as is evident in Bakker and Galton's (*11, 18*) more detailed discussion of the phylogenetic position of *Herrerasaurus* and *Staurikosaurus* and the origins of Ornithischia and Sauropodomorpha.
18. H.-D. Sues, in *The Dinosauria*, D. B. Weishampel, P. Dodson, H. Osmólska, Eds. (Univ. of California Press, Berkeley, 1990), pp. 143–147.
19. J. F. Bonaparte, *Opera Lilloana* **22**, 1 (1972). The tetanurian status of these fragmentary theropod remains is suggested by the flange-shaped anterior trochanter, slender fibula, and the apparently thin ascending process of the astragalus. Well-preserved ceratosaurians are also present by the late Carnian or Norian (*6, 25*).
20. The age of divergence between sister groups must be as early as, or earlier than, the first record of either sister group. The presence of Prosauropoda thus indicates that its sister taxon Sauropoda was also present. "Ghost" sister lineages may not yet have acquired all of the synapomorphies that unite known members of the sister taxon [W. Hennig, *Phylogenetic Systematics* (Univ. of Illinois Press, Urbana, 1966), pp. 163–169; M. A. Norell, in *Extinction and Phylogeny*, M. J. Novacek and Q. Wheeler, Eds. (Columbia Univ. Press, New York, 1992)].
21. J. F. Bonaparte, *Ameghiniana* **16**, 217 (1979); P. Stipanicíc and J. F. Bonaparte, *II Simp. Geol. Argent.* **1**, 523 (1979).
22. R. R. Rogers *et al.*, paper presented at the Fifth North American Paleontological Convention, Chicago, June 1992.
23. D. H. Erwin, *Hist. Biol.* **6**, 133 (1992).
24. Seasonality is suggested by marked growth variation in fossil conifer wood.
25. Modified from E. H. Colbert, *Mus. N. Ariz. Bull.* **57**, 1 (1989).
26. Based on F. von Huene, *Geol. Palaeontolog. Abh. N. F.* **15**, 129 (1926).
27. Based on R. A. Thulborn, [*Palaeontology* **15**, 29 (1972)] and P. C. Sereno, [*J. Vertebr. Paleontol.* **11**, 168 (1991)] with portions of the sacrum reconstructed as seen in *Hypsilophodon* and *Psittacosaurus*.
28. Supported by NSF research grant BSR 8722586, The David and Lucile Packard Foundation, National Geographic Society, and the Donors of The Petroleum Research Fund administered by the American Chemical Society (grant ACS-PRF 22637-G8) (P.C.S.). We thank W. Amaral, W. Simpson, and C. Vanderslice for fossil preparation and C. Abraczinskas for execution of the finished illustrations. We also thank A. Arcucci, R. Blob, M. Carrano, C. Forster, R. Rogers and H.-D. Sues for comments on the manuscript and A. Monetta and C. Treo for loan of fossil materials.

22 June 1992; accepted 15 September 1992

Cooperativity Induced by a Single Mutation at the Subunit Interface of a Dimeric Enzyme: Glutathione Reductase

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When glycine^{418} of *Escherichia coli* glutathione reductase, which is in a closely packed region of the dimer interface, is replaced with a bulky tryptophan residue, the enzyme becomes highly cooperative (Hill coefficient 1.76) for glutathione binding. The cooperativity is lost when the mutant subunit is hybridized with a wild-type subunit to create a heterodimer. The mutation appears to disrupt atomic packing at the dimer interface, which induces a change of kinetic mechanism. A single mutation in a region of the protein remote from the active site can thus act as a molecular switch to confer cooperativity on an enzyme.

Many enzymes are oligomeric; of these, some exhibit standard Michaelis-Menten kinetics and others display homotropic or heterotropic cooperativity in ligand binding and a characteristically sigmoidal dependence of reaction velocity on substrate concentration. The two principal models advanced to explain cooperative behavior (*1, 2*) rely on ligand-induced conformational changes in the protein, the effects of which are transmitted across a domain or subunit interface. Some of these ideas have recently been tested by directed mutagenesis (*3, 4*). Cooperativity can be eliminated: For example, replacement of key residues involved in the binding of fructose 6-phosphate in the active site of *Escherichia coli* phosphofructokinase leads to loss in cooperative behavior in the enzyme tetramer (*5*). Similarly, the elimination of certain interactions between the aspartate and carbamoyl phosphate domains of the catalytic subunit of *E. coli* aspartate transcarbamoylase causes it to lose both catalytic activity and cooperativity in binding aspartate (*6*).

Conferral of cooperative behavior on an enzyme has also been reported. Replacement of an active-site Arg residue with Gly in *E. coli* ornithine transcarbamoylase creates an enzyme of reduced catalytic efficiency but one that exhibits cooperative responses to both substrates (*7*). Likewise, replacement of an active-site Arg residue with Gly in the noncooperative aspartate transcarbamoylase of *Bacillus subtilis* converts it into a cooperative enzyme (*8*).

We have been able to confer cooperativity on an enzyme, *E. coli* glutathione reductase (E.C. 1.6.4.2), by manipulation of the subunit interface away from the active site. This dimeric enzyme is a member of the important family of flavoprotein disulfide oxidoreductases (*9, 10*). Glutathione reductase catalyzes the following reaction:

$$\mathrm{H^+ + NADPH + GSSG = NADP^+ + 2GSH} \qquad (1)$$

where NADPH is the reduced form of nicotinamide adenosine dinucleotide phosphate (NADP$^+$) and GSH and GSSG are the reduced and oxidized forms of glutathione, respectively. The crystal structure of the human enzyme at 1.5 Å resolution (*11*) serves as the reference point for the rest of the family (*12–15*). The primary (*16*) and three-dimensional (*17*) structures of *E. coli* glutathione reductase are similar to those of the human enzyme, enabling the cloned gene (*gor*) for *E. coli* glutathione reductase to be used for protein engineering experiments, notable among them the insertion of an intersubunit disulfide bridge (*18*), investigation of residues critical to the catalytic mechanism (*19–21*), and switches of the coenzyme specificity from NADPH to NADH (*22*) and the substrate specificity from glutathione toward trypanothione (*23*).

The enzyme normally exhibits a ping-pong kinetic mechanism (*9, 24, 25*), but replacement of a single residue (Tyr^{177}) in the NADPH binding site changes it to ordered sequential (*19*). No homotropic or heterotropic cooperativity has been observed in the binding of NADPH or glutathione. The binding sites for NADPH and glutathione are physically distinct and are separated by the isoalloxazine ring of the enzyme-bound flavin-adenine dinucle-

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