Fossil Horses--Evidence for Evolution

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from 12 patients with recessive dystrophic epidermolysis bullosa. They show convincingly that susceptibility to developing invasive SCC, both clinically and experimentally, depends strictly on the retention of part of the collagen VII protein. Keratinocytes from patients carrying mutations that abrogate the deposition of collagen VII do not develop into invasive SCC, whereas those from patients with mutations that result in deposition of a crucial fragment of collagen VII do become cancerous.

Collagen VII is produced primarily by keratinocytes, with perhaps a small contribution from dermal fibroblasts. The collagen VII molecule has a characteristic central glycine-rich, triple-helical collagenous domain, with noncollagenous domains at its amino and carboxyl ends. Keratinocytes from patients with mutations that specifically leave intact the amino-terminal noncollagenous domain (NC1) of collagen VII, and more specifically the fibronectin III–like repeats within the NC1 domain (FNC1) that bind to laminin 5, developed into invasive SCC. Furthermore, introduction of either the NC1 or FNC1 domains into patient keratinocytes deficient in collagen VII restored a predisposition to tumorigenesis, whereas introduction of NC1 without the fibronectin repeats did not. Interestingly, antibodies that specifically recognized the FNC1 domain of collagen VII either prevented tumor development or suppressed tumor invasion when administered to mice with SCC tumors caused by Ras/IκB-transformed keratinocytes from normal individuals. Invasion studies in vitro confirmed the in vivo findings and further revealed that interaction of FNC1 with laminin 5 was required for the invasive phenotype to develop.

What do these results tell us about epidermolysis bullosa and SCC? First, they suggest an explanation for why chronic wounds seldom develop into SCC in patients with mutations in adhesion complex proteins that are closer to the epidermis (for example, laminin 5, hemidesmosomal proteins, and intermediate filament proteins). Keratinocytes harboring such mutations lack an intact adhesion complex between the NC1 domain of collagen VII and laminin 5 and the hemidesmosomes. Hence, these keratinocytes are not tethered to the dermis and may not receive the stromal signals that they would need to migrate to and invade the dermal layer. Laminin 5 is the ligand for $\alpha_6\beta_4$ integrin, a signaling receptor on the surface of basal keratinocytes. Hence, interactions between collagen VII and laminin 5 may be the conduit for stromal signals that direct the migratory and invasive behaviors of epidermal tumors (*6*).

Ortiz-Urda *et al*. also show that boosting production of NC1 enhances the invasiveness of transformed keratinocytes from normal individuals, and of keratinocytes from patients with other skin diseases. A central regulator of collagen VII expression is transforming growth factor–β (TGF-β) (*7*), which enhances invasion and metastasis of established squamous cell tumors and other epithelial neoplasms (*8*). The new work suggests that the relationship between collagen VII and TGF-β is worth exploring further. There are also two possible clinical applications of the current study. Attempts to restore collagen VII locally using gene therapy in patients with dystrophic epidermolysis bullosa are under active investigation (*9*). The authors caution that for certain patients, restoration of collagen VII containing the NC1 domain could increase their risk of developing SCC, particularly in those who lack production of collagen VII. On the other hand, the good news is that the NC1 domain could be a therapeutic target for treating invasive SCC and other cancers. However, a therapeutic molecule that binds to the NC1 domain must block the molecular interactions required for tumor invasion while leaving intact those required for anchoring the epidermis to the dermis. We are faced with a possible Pyrrhic victory as we contemplate the epithelial-stromal interface: perhaps winning the battle against SCC but losing the battle against the disfiguring skin defects of dystrophic epidermolysis bullosa.

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EVOLUTION

Fossil Horses—Evidence for Evolution

Bruce J. MacFadden

Thomas Huxley, an early advocate of Darwinian evolution, visited the United States in 1876 on a lecture tour. Huxley had planned to talk about evidence for evolution based on a fragmentary sequence of fossil horses from Europe. One of Huxley's first stops was at Yale, where he studied the fossil horse collection assembled by the paleontologist O. C. Marsh during expeditions to the western territories. Huxley was so taken with the definitive evidence provided by Marsh's fossil horse collection that he used this evolutionary sequence as the focal point for his subsequent talk to the New York Academy of Sciences (*1*).

Since the late 19th century, the 55-million-year (My) phylogeny of horses (Family Equidae)—particularly from North America—has been cited as definitive evidence of long-term "quantum" evolution (*2*), now called macroevolution. Macroevolution is the study of higher level (species, genera, and above) evolutionary patterns that occur on time scales ranging from thousands to millions of years. The speciation, diversification, adaptations, rates of change, trends, and extinction evidenced by fossil horses exemplify macroevolution.

The sequence from the Eocene "dawn horse" eohippus to modern-day *Equus* has been depicted in innumerable textbooks and natural history museum exhibits. In Marsh's time, horse phylogeny was thought to be linear (orthogenetic), implying a teleological destiny for descendant species to progressively improve, culminating in modern-day *Equus*. Since the early 20th century, however, paleontologists have understood that the pattern of horse evolution is a more complex tree with numerous "side branches," some leading to extinct species and others leading to species closely related to *Equus*. This branched family tree (see the figure) is no longer explained in terms of predestined improvements, but rather in terms of random genomic variations, natural selection, and long-term phenotypic changes (*3*).

The Equidae, a family within the odd-toed ungulate Order Perissodactyla (which includes rhinoceroses, tapirs, and other closely related extinct groups), consists of the single extant genus *Equus*. Depending upon interpretation, it also includes several subgenera, 8 to 10 species, and numerous subspecies (*4*). On the basis of morphological differences, *Equus* is separated into two or

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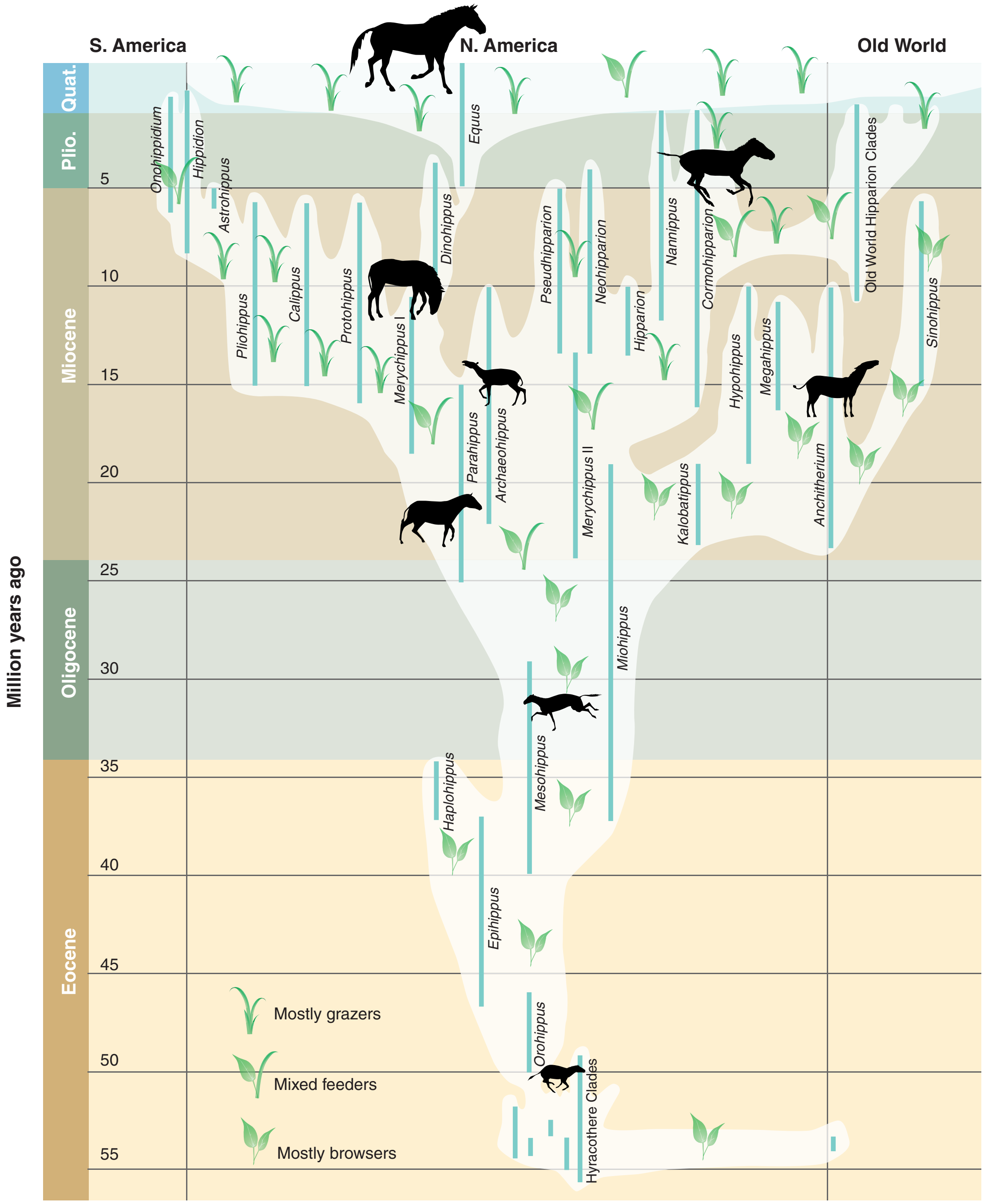

Adaptive radiation of a beloved icon. Phylogeny, geographic distribution, diet, and body sizes of the Family Equidae over the past 55 My. The vertical lines represent the actual time ranges of equid genera or clades. The first ~35 My (Eocene to early Miocene) of horse phylogeny are characterized by browsing species of relatively small body size. The remaining ~20 My (middle Miocene until the present day) are characterized by genera that are either primarily browsing/grazing or are mixed feeders, exhibiting a large diversification in body size. Horses became extinct in North America about 10,000 years ago, and were subsequently reintroduced by humans during the 16th century. Yet the principal diversification of this family occurred in North America. Although the phylogenetic tree of the Equidae has retained its "bushy" form since the 19th century [for example, see (*2*, *3*)], advances in knowledge from fossils have refined the taxonomy, phylogenetic interrelationships, chronology, and interpretations of the ancient ecology of fossil horses.

three deep clades within the genus. These include caballines (domesticated horse, *E. caballus*); zebras (three species recognized); and asses, donkeys, and related species. Recent studies of mitochondrial DNA indicate two deep clades within *Equus*, namely, the caballines and the zebras/asses (*5*). These deep clades split ~3 million years ago (Ma) in North America and subsequently dispersed into the Old World. *Equus* became extinct in the New World ~10,000 years ago, probably as a result of multiple factors including climate change and hunting by early humans. In the Old World, although its range contracted, *Equus* persisted and was then domesticated in central Asia about 6000 years ago from a stock similar to Przewalski's wild horse, *E. caballus* (sometimes considered its own species, *E. przewalskii*) (*4*).

The single modern genus *Equus* stands in marked contrast to a highly diverse adaptive radiation of the Family Equidae over the past 55 My that resulted in some three dozen extinct genera and a few hundred extinct species (*3*). Although the overall branched pattern of horse phylogeny (see the figure) has remained similar for almost a century, new discoveries and reinterpretation of existing museum fossil horse collections have added to the known diversity of extinct forms. Recent work reveals that Eocene "hyracothere" horses, previously known as "eohippus" or *Hyracotherium*, include an early diversification of a half-dozen genera that existed between 55 and 52 Ma in North America and Europe (*6*). New genera have recently been proposed for the complex middle Miocene radiation (*7*), although the validity of these genera is still debated.

Horse teeth frequently preserve as fossils and are readily identifiable taxonomically. They serve as objective evidence of the macroevolution of the Equidae. Horse teeth have undergone considerable changes over the past 55 My. The tempo of this morphological evolution has sometimes been slow and at other times rapid (*2*, *3*). Primitive Eocene through early Miocene (between 55 and 20 My) horses had short-crowned teeth adapted for browsing on soft, leafy vegetation. During the later Miocene (between 20 and 15 Ma), horses underwent explosive adaptive diversification in tooth morphology. Shorter crowned browsers,

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which inhabited forests and open-country woodlands, declined in diversity during this time (*8*). In contrast, many other clades of horses evolved high-crowned teeth adapted for grazing on the extensive grasslands of more open-country biomes, which spread during the Miocene (25 to 15 Ma). Once high-crowned teeth evolved, some clades underwent a secondary adaptation, that is, they went from being grazers to being mixed feeders with diets consisting of grass and some leafy plants (*9*). Studies of carbon isotopes preserved in fossil horse teeth indicate that before ~7 Ma, early tropical and temperate grasslands of the world consisted primarily of grasses that used the C_3 photosynthetic pathway, whereas today these grasslands consist mostly of C_4 grasses (*10*).

In many fossil groups, the trend toward larger body size in ancestral-descendent sequences has been termed "Cope's rule." Early Eocene hyracothere horses classically have been compared in size to a small dog (~10 to 20 kg), although house-cat–sized species have been discovered more recently. At the other end of the evolutionary spectrum, wild modern *Equus* attains a body size of ~500 kg (*3*, *4*). Although the 55-My-old fossil horse sequence has been used as a classic example of Cope's rule, this notion is now known to be incorrect. Rather than a linear progression toward larger body size, fossil horse macroevolution is characterized by two distinctly different phases. From 55 to 20 Ma, primitive horses had estimated body sizes between ~10 and 50 kg. In contrast, from 20 Ma until the present, fossil horses were more diverse in their body sizes. Some clades became larger (like those that gave rise to *Equus*), others remained relatively static in body size, and others became smaller over time (*3*).

Fossil horses have held the limelight as evidence for evolution for several reasons. First, the familiar modern *Equus* is a beloved icon that provides a model for understanding its extinct relatives. Second, horses are represented by a relatively continuous and widespread 55-My evolutionary sequence. And third, important fossils continue to be discovered and new techniques developed that advance our knowledge of the Family Equidae. The fossil horse sequence is likely to remain a popular example of a phylogenetic pattern resulting from the evolutionary process.

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PHYSICS

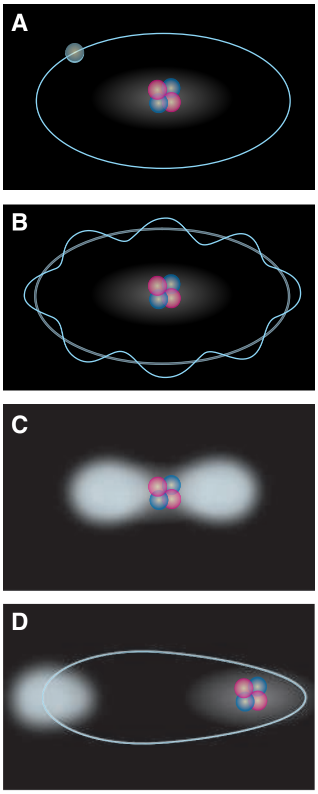
Toward Creating a Rutherford Atom

David M. Villeneuve

The early 20th century saw a rapid evolution in the concept of the atom (see the first figure). In 1911, Ernest Rutherford proposed that the atom resembled a tiny solar system, with most of the mass concentrated in a nucleus, and electrons revolving around it in planetary orbits (see the first figure, panel A). Although his model has been supplanted by quantum mechanics, Maeda *et al.* show on page 1757 of this issue (*1*) that it is possible to make Rutherford atoms in the laboratory.

Doubts about the Rutherford model were raised soon after he proposed it. The emission spectrum of hydrogen was known to have a regular progression of lines, and Johannes Rydberg had shown in 1888 that these lines could be fit to a simple algebraic formula. The Rutherford model could not explain this behavior.

In 1913, Niels Bohr postulated that the angular momentum of the electrons must be quantized. This quantization led to discrete orbits (see the first figure, panel B), which were labeled by an integer quantum number and explained Rydberg's formula. When Bohr's model was supplanted by quantum mechanics as we know it today, the concept of the electron as a planet was replaced by a mathematical wave function that was not directly observable. The electron orbits became fuzzy clouds (see the first figure, panel C).



Evolution of the concept of atomic structure. (**A**) In the Rutherford model, an electron orbits a massive nucleus in a planetary orbit. (**B**) In the Bohr model, the electron is partly a wave that can only go in discrete orbits. (**C**) In the quantum mechanical picture, the electron is spread out within an orbital. (**D**) In the Rydberg atom, the electron is localized into a small volume and follows an almost classical orbit, as demonstrated by Maeda *et al.* (*1*).

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Quantum mechanics has been highly successful in predicting the structure of atoms and molecules, giving birth to notions such as quantum teleportation and quantum computing. It is so precise that experiments have been proposed to look for tiny changes in the fundamental constants as the universe ages over a period of years (*2*). Yet we know that objects in the macroscopic world that we inhabit are not fuzzy clouds. Electrons are real particles that travel through wires and form images on television screens. So where does the quantum world end and our everyday classical world begin?

This question is being addressed by scientists who try to construct atoms that resemble classical objects. Maeda *et al.* (*1*) show that this approach can yield atoms that behave like Rutherford's miniature solar system.

To make a Rutherford atom, one must first localize the electron cloud, that is, confine it to a small volume. In quantum mechanics, this is achieved by creating a coherent superposition of states, called a wave packet (see the second figure). For example, a femtosecond laser pulse is a superposition of many sine waves that add constructively only in

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