

Room for Thought: Of Mutations, Muscle, “Fitness,” and the Human Condition¹

HANSELL STEDMAN

Associate Professor of Surgery
University of Pennsylvania School of Medicine

ONE HUNDRED AND FIFTY YEARS AGO, when Franz Boas was born, the American Philosophical Society was already a few years out from its centennial. Mendel’s peas were still ripening on the vine, but across the continent, the ink was already drying on a draft version of Darwin’s *Origin*. We now know that the state of natural philosophy was poised for dramatic change. It’s curious that the contemporaneous concepts of gene and natural selection have become as intertwined as opposing strands of the celebrated double helix, yet one and only one of the two names has been glorified and/or stigmatized as a household word. I got a sense of this during a recent career day gig at my children’s school: “Didn’t he win the Academy Award or something for *Survival*?” “Oh yeah, some cool show about people who checked out along with their stupid ideas.” To which a teacher interjected, “You mean their not-so-intelligent designs,” recalling Pennsylvania’s recent role on the frontlines of the ID debate in the national news.

Now as far as I can tell, Darwin dealt head on with the real shock value of his synthesis only once in *Origin*—the rest is sugarcoated. He says in an introductory chapter, “Nothing is easier than to admit in words the truth of the universal struggle for life, or more difficult—at least I have found it so—than constantly to bear this conclusion in mind.” “We behold the face of nature bright with gladness . . . we do not see . . . that the birds which are idly singing round us . . .,” and then he trails off, only hinting at the predator-prey competition that the folks at *National Geographic* have built entire careers bringing to Technicolor.

I still remember the exact context of my first exposure to the word “fittest”: not a NOVA program or a creationist debate. In stark contrast, I was watching a teacher take on *The Origin of Species* in front of a

¹Read 25 April 2008.

group that happened to include two of “Jerry’s Kids”: very bright young brothers who had a severe form of muscular dystrophy. And later that year, I watched those same young boys at the annual Labor Day Telethon as the scientists talked about genes and mutations and research and prognosis. And not long after that, our entire community looked on with shock and awe as the older brother died and the younger carried on his memory in a project on the genetics of mice with muscular dystrophy. In short order, he gave a spellbinding valedictory speech that challenged everyone’s notion of empathy, and then he, too, succumbed to the inexorable disease.

So I’m interested in a few interrelated new developments that trace back to these iconic naturalists by way of the very colorful character Jack Haldane. His synthesis addressed the consequences of Mendelian genetics for Darwinian selection. His pioneering mathematical analysis of mutation rates eventually laid bare the nature of the muscular dystrophy genes and, in a more general sense, gave a snapshot of the core substrate for human evolution—the source of genetic variability. Using the example of hemophilia he incorporated the loss of reproductive fitness into a single algebraic factor. Pay attention not to the series of dry-looking equations, but rather to their enormous implication in his rhetorical bottom line.¹ His imagery conjures an explanation different from the traditional “arrow through the eye” account of King Harold’s death in the Battle of Hastings.

Now among Mendelian traits that affect fitness, few are as visually compelling as gradations in strength. For my purposes today, I should emphasize two things about muscle. First, muscles can generate enormous amounts of force from a small package. Take the Governor in his physical prime. The freshman biophysics student can see the lever arm analogy and calculate the mechanical disadvantage on the biceps here—each muscle must produce a contractile force on the order of one ton. Bring in some Newtonian mechanics and you can extrapolate to velocity and acceleration, both of which are important throughout the food chain. Now there’s an Achilles’ heel here; we’ll call it a constraint. Muscles, like all other tissues, are composed of cells, each of which is protected by a soap bubble–thin membrane. So the big questions have been these ones: how is this force produced in the first place? how does it get out of the cell without popping the soap bubble? (Bone for instance solves this latter problem by bearing all of the force in the calcified extracellular matrix.)

¹ “. . . in other words all Englishmen at the time of the Norman conquest would have been haemophilics!” J.B.S. Haldane in *J. Genet.* 31 (1935): 317–26; reprinted as a *J. Genet.* classic: <http://www.ias.ac.in/jgenet/Vol83No3/235.pdf>. See especially last full paragraph on p. 241 (i.e., p. 323 in the original).

Two muscle proteins are key: myosin, think “molecular *motor*,” and dystrophin—so named because it’s a novel protein found to distinguish dystrophic from normal muscle.

Historically, the most abundant proteins were the first discovered—putting myosin at the head of the list. In fact, you could call muscle a kind of support system for myosin. As a result, it is the primary source of protein in the diet of omnivores and carnivores, and the primary end-product of protein synthesis in herbivores. In head-to-head combat, the loser’s myosin is the prize and is generally recycled according to the winner’s blueprint (i.e., genome). In other words, the very structure of the motor protein can be viewed as being sculpted by natural selection. Like the Olympics, where it’s about that time in the four-year cycle for pundits to predict how the prizes will go out for stronger, faster, higher, etc. Or the Kentucky Derby, where there’s a more obvious role for directional selection. With the tools of modern genomics we can see exactly how the motors have been tweaked to achieve sprint vs. distance power on the one hand, and extreme precision on the other (like the muscles that move the eye back and forth, especially if you notice you can still read when your head is moving side to side). Better portfolio of myosin genes, better luck hunting on the Serengeti, and so on. It’s a competitive marketplace, a bit like the automotive showroom where more bells and whistles, each with a separate servo motor, might help in the head-to-head struggle for buyers.

Like Howard Hughes, as depicted in *The Aviator*, the field is going through a “show me all the blueprints” phase with the explosion of genomic DNA sequence information. This approach began with Sydney Brenner, whose behind the scenes account I first heard in this room, inspiring me to get back to the lab and get on with sorting this out in humans. Ironically, Professor Brenner had said he was interested in *behavioral* genetics and, by putting the food on one side of the Petri dish and the tiny worms on the other, thought he’d be able to make sense of the genes in worms that wouldn’t move. Although it didn’t work out exactly as planned, he eventually got a call from Stockholm for creating a system that instead identified genes for the moving parts of the molecular motor. In other words, his stagnant worms *couldn’t* move because their muscle was toast.

Fast forward twenty years and we now understand that humans have many, many more motor proteins than worms, and that human diseases *can* result from such mutations, but that the phenotypes are not what you’d expect. It also turns out that the most common and clinically severe forms of human (and murine, canine, and feline) muscle disease relate to other genes that would have been missed in the worm screening test because the phenotype is profoundly affected by the size of the animal.

Now even before he co-founded population genetics, Haldane authored another classic, entitled *On Being the Right Size*, which embodied the scaling concept that is now widely referred to as “Haldane’s principle.” To paraphrase the late Judah Folkman, if you’re a mouse and you have muscular dystrophy, we can take pretty good care of you. But Haldane realized the critical role of comparative biology in basic and translational research *and* realized that the blood vessel walls were stronger in larger animals. As a postdoc with Roy Schmickle, I was lucky enough to help define the genetic basis of muscular dystrophy in both the mouse and the dog, and—no surprise—establish that the “business ends” of the homologous genes, the part transcribed into messenger RNA and then translated into protein, are virtually identical. Oddly, the mice live an almost normal lifespan, but the dogs get into big trouble by the time they are a few months old.

A few observations: If you watch the dynamics in a brand-new litter, the seemingly normal dystrophic pups are neglected and quickly weakened beyond the pale, as if the dog-mum has a sixth sense—it’s a bit like battlefield triage in rationing her precious milk. Now this might have prompted some of the Darwinian sugarcoating, as for instance in the bottom line of *Origin*, chapter 3: “When we reflect on this struggle, we may console ourselves with the full belief that the war of nature is not incessant, that no fear is felt, that death is generally prompt, and that the vigorous, the healthy, and the happy survive and multiply.” If a human observer overrides this by hand feeding the runt pups for a few days, they quickly catch up and look pretty normal right up to weaning time. But then an uncanny thing happens that will make more sense when I wrap this up—the dystrophic pups all develop a form of lockjaw and have to be fed pureed food. This never happens in humans with Duchene muscular dystrophy. Nonetheless, this is a critically important theme in comparative biology, where one of our ultimate goals is to cure muscular dystrophy in the dog *and* human (or, as they say at the Vet School, Many Species, One Medicine™).

Haldane’s equations showed that the most common, single-gene lethal disease in man, Duchene muscular dystrophy, is associated with a reproductive success (i.e., Darwinian fitness) of absolute zero in the hemizygotes, and therefore had a high incidence that could be explained only by an astronomically high mutation rate. In other words, if you were looking for *present-day* evidence for the origin of *variation*, the substrates for evolution and speciation, you could not find a better place than the DMD “locus.” The simplest explanation was that the gene was huge—as we now know, it’s the largest in biology—a few percent of the X chromosome. Its product: a connecting rod that helps to convey the extraordinary force produced inside the muscle cell to the outside world without popping the soap bubble–thin membrane. The gene’s location means that boys uniquely bear the direct brunt of this one—born

perfectly healthy, but by the age of three displaying the tell-tale signs of “infant herculism.” Weightlifters all learn that muscle damage drives overcompensation, but in these boys a lifetime’s supply of spare parts, that is, myogenic stem cells, can be used up in just a few years.

So here’s where the wires get crossed between molecular biology and surgery. All medical students aspire to become healers through their newly acquired knowledge. In surgery the training’s a bit longer to enable a focus on durable “one shot” therapies, but always in the context of general medical care for more chronic diseases. We are confronted here with the mother of all drug delivery problems—the ultimate drug is a gene, about one million times the size of most over-the-counter remedies, and the target tissue is the muscle mass, about half the body. So surgeons are hard-wired to think in terms of anatomy, physiology, and the systematic elimination of fear, in particular that of blood. I’m going to lay it all on the line here: building on the work of the giants in this field, we think we may have just been able to catch a glimpse of how this might work out: a sort of high tech vaccination against a genetic disease.

The gene is three million base pairs of DNA in size. Translation: 1/1,000th of the cell’s total DNA and two billion times the molecular weight of hydrogen. Most drugs are under 1,000. However, the “business end,” the mRNA, is a mere three million, and this can be pared down to one million by preserving the absolutely essential parts. This will just fit into the safest and most efficient Trojan horse we have, a so-called recombinant adeno-associated virus vector that will bind the muscle cell surface and shuttle the DNA payload past all of the cell’s obstacles into the nucleus. So this part works well in the Petri dish and the living mouse, but to scale to a dog or a human you need the surgical step: briefly run the circulation backward to force the vector particles through the back door, which was left open; that is, the permeable walls of the so-called post-capillary venules. This can be done safely if you obey all of the rules of circulatory management, as worked out over the last fifty years of general surgery and its many branches. We’ve done this in the isolated limb of the dystrophic dog, *but* not in the entire body of the normal dog, so the near-term goal is to do this safely and effectively in the entire body of the dystrophic dog. We’ve published about half of this story, and are writing up the other half now between experiments. Perhaps through my gracious mentor, Dr. Barker, I can keep you posted on how this works out over time.

Why go to this length, and what of the inherent risks? For openers: the disease is common, disabling, disheartening, and lethal! Not overnight—but fast enough.

And then there’s the human condition. Well, whatever the biological basis of altruism—a preoccupation of both Haldane and his student John Maynard Smith over the concept of kin selection—one thing that

also unifies us, as able-bodied members of the species, is that we simply cannot walk away from, for example, a photograph of the disease in a set of two-, ten-, and twelve-year-old brothers—hence our trillion dollar health care industry. However old this behavioral trait may be (this business of protecting the weak and infirm in one's own midst), I suggest you consider its role in the fixation of the bizarre RFT mutation ("Room for Thought"—stay tuned).

I would be remiss if I did not wrap things up by also explaining why we think these dogs get lockjaw. It relates to a serendipitous finding that came out of our hunt for muscular dystrophy genes. We were perplexed by a deletion in one of the human myosin genes that would have been predicted to cause a devastating muscle disease. We quickly found the homologue of this gene in all the nonhuman primates and were eventually able to trace it all the way back to selected invertebrates. The problem was, when we searched for the normal control or "wild type" copy of this particular gene in humans, we discovered that those closest at hand (that's code for every member of my lab) had the mutation on both chromosomes—and the lab membership looked like the model United Nations. So what was this muscle disease that was unique, if you will, to the human condition? Well, we brought out our CSI Philadelphia magnifying lenses and my lab sleuths figured out that this myosin is ordinarily made only in the jaw-closing muscles, where it can be responsible for 99 percent of the power in an aggressive dog or gorilla bite. If you look at the structure of the myosin genes in the lowly bay scallop, the version that closes the shell or "jaw" is identical only with the human gene with the mutation, not the other ten. If you compare identical muscles from human to non-human primate under the microscope, they're indistinguishable until you look at the jaw-closer just outside of the skull. You might not even be aware you have this little remnant sliver of a muscle unless you can remember chewing gum in the back of the classroom as a kid. If you happen to be one of our closest living cousins, the chimpanzee, this muscle can be as big as your quadriceps. So a key question becomes, how might this mutant version of the gene have been selected to take over—eclipsing an entire gene pool of "normal," functioning versions in the population at large?

So just as evolution sculpts the myosin genes, muscle sculpts bone to suit its needs. Now, skulls from species as divergent as rats, chimps, and gorillas look very similar from *this* point of view. We're the odd men out. But what is that silly Mercedes Benz-looking symbol that comes into view only on the human skull?² Those are the open sutures:

² See figure on next page. Also see cover photograph, *Nature* 428 (6981) (25 March 2004): <http://www.nature.com/nature/journal/v428/n6981/index.html> or fig. 3g: <http://www.nature.com/nature/journal/v428/n6981/pdf/nature02358.pdf>.

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                10      20      30      40      50      60      70      80
WOOLLY MONKEY GAGCAGCTGAACAAGCTGATGACCACCCCTCCACAGCAGGGTACCCCATTTTGTCCGCTGTATTGTGCCAATGAGTTAAGCAATCGG
PIGTAIL MAC  GAGCAGCTGAACAAGCTGATGACCACCCCTCCATAGCACGGCACCCCATTTTGTCCGCTGTATTGTGCCAATGAGTTAAGCAATCGG
RHESUS        GAGCAGCTGAACAAGCTGATGACCACCCCTCCATAGCACGGCACCCCATTTTGTCCGCTGTATTGTGCCAATGAGTTAAGCAATCGG
ORANGUTAN     GAGCAGCTGAACAAGCTGATGACCACCCCTCCATAGCACGGCACCCCATTTTGTCCGCTGTATTGTGCCAATGAGTTAAGCAATCGG
GORILLA       GAGCAGCTGAACAAGCTGATGACCACCCCTCCATAGCACGGCACCCCATTTTGTCCGCTGTATTGTGCCAATGAGTTAAGCAATCGG
HONOBO        GAGCAGCTGAACAAGCTGATGACCACCCCTCCATAGCACGGCACCCCATTTTGTCCGCTGTATTGTGCCAATGAGTTAAGCAATCGG
CHIMPANZEE    GAGCAGCTGAACAAGCTGATGACCACCCCTCCATAGCACGGCACCCCATTTTGTCCGCTGTATTGTGCCAATGAGTTAAGCAATCGG
              E Q L N K K L M T T L H S T A P H F V R C I I P N E F K Q S
HUMAN
AFRICA (PYGMY) GAGCAGCTGAACAAGCTGATGACCACCCCTCCATAGC--CGCACCCCATTTTGTCCGCTGTATTATCCCAATGAGTTAAGCAATCGG
SPAIN (BASQUE) GAGCAGCTGAACAAGCTGATGACCACCCCTCCATAGC--CGCACCCCATTTTGTCCGCTGTATTATCCCAATGAGTTAAGCAATCGG
ICELAND        GAGCAGCTGAACAAGCTGATGACCACCCCTCCATAGC--CGCACCCCATTTTGTCCGCTGTATTATCCCAATGAGTTAAGCAATCGG
JAPAN          GAGCAGCTGAACAAGCTGATGACCACCCCTCCATAGC--CGCACCCCATTTTGTCCGCTGTATTATCCCAATGAGTTAAGCAATCGG
RUSSIA         GAGCAGCTGAACAAGCTGATGACCACCCCTCCATAGC--CGCACCCCATTTTGTCCGCTGTATTATCCCAATGAGTTAAGCAATCGG
SOUTH AMERICA GAGCAGCTGAACAAGCTGATGACCACCCCTCCATAGC--CGCACCCCATTTTGTCCGCTGTATTATCCCAATGAGTTAAGCAATCGG
              E Q L N K L M T T L H S R T P F C P L Y Y P Q * V * A I
    
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EXON 18



Mutation in the highly conserved jaw-closing muscle “MYH16” gene depicted above the morphological difference in skulls. The topmost lines show DNA sequences from several species of non-human primate corresponding to a critical site in the gene for the main myosin contractile protein that powers closure of the jaw. Just below this appears the sequence of DNA from human individuals tracing their recent ancestry to multiple continents. Note the presence of a shared mutation: a two-base deletion that by “frameshift” alters the encoded protein sequence below, resulting in a premature STOP as indicated by the asterisk. The largest muscle that makes this particular myosin is the temporalis, situated on the outside of the skull. In the chimpanzee and gorilla skulls at right, the anchoring ridge for this powerful muscle is readily appreciated, as well as the enlarged hole through which it attaches to the jaw. In humans, there are no such ridges. Instead, there is the clearly visible growth plate, which allows for continued expansion of the cranial vault.

the growth plates for the braincase. In Olduvai Gorge about two million years back two close cousins left behind their distinctive remains. The structure of the human and chimpanzee myosin genes suggests that the most likely time for this mutation to have occurred was just before the dawn of our own genus. That's why we now only half jokingly refer to this as the RFT or Room for Thought mutation in the lab. So here's one question to leave you with: could this have been a gain of function mutation that was ultimately "fixed" in the ancestral gene pool because it lifted an evolutionary constraint on encephalization? The anatomy and evolution of introspection, with the skull and its contents at the center ever since "the seat of the soul" shifted from the sacrum to the CNS.

Now back to a "myocentric" view of survival: if you are a muscle cell your engine has to be matched to the rest of the power train right on down to where the rubber meets the road. So if you have a gerbil under the hood, you might be able to limp to the next gas station on a razor-thin tire, but if you are packing a turbocharged 454, these same tires are toast as soon as you take your foot off the clutch. This is pretty much what we think happens in the dystrophic canine, but not human, jaw muscles, where the mutant myosin is protective and partially compensates for the missing dystrophin. This system provides us a unique opportunity to learn more about both proteins at the same time, while working out some basic issues in both human evolution and gene therapy for genetic disease.