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Insurmountable heat: The evolution and persistence of defensive hyperthermia

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Insurmountable heat:

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by

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ABSTRACT OF THE THESIS

Insurmountable heat:
The evolution and persistence of defensive hyperthermia

by

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Master of Arts in Anthropology
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Professor Daniel M. T. Fessler, Chair

Fever, the rise in body temperature set point in response to infection or injury, is a highly conserved trait among vertebrates, and has also been documented in many arthropods. Fever is known to truncate the duration of infection and reduce mortality. These observations present an evolutionary puzzle: why has fever continued to be an effective response to fast-evolving pathogenic microbes over hundreds of millions of years and across diverse phyla? Framing fever as part of a more general thermal manipulation strategy that we term defensive hyperthermia, we hypothesize that the solution to this puzzle lies in the independent contributions to pathogen fitness played by virulence and infectivity. A host organism deploying defensive hyperthermia alters the ecological environment of an invading pathogen. To the extent that the pathogen evolves so as to be able to function effectively at both normal and elevated temperatures, it disadvantages itself in the task of infecting the next host – whose body temperature will be lower – becoming more likely to be thwarted by both that host's immune system and wild ecotype conspecifics that, though more vulnerable to elevated temperatures, operate more effectively at the host's normal temperature.

We evaluate this hypothesis in light of existing evidence concerning pathogen thermal specialization, and discuss theoretical and translational implications of this model.

The thesis of Edward King Clint is approved.

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Understanding fever is critical to health and wellness, as recent research has indicated that the suppression of fever in a human population can lead to a 5% increase in disease prevalence and mortality (Earn et al 2014). Most animals that have been studied raise their body temperature above its nominal set point in response to infection (Amaral et al. 2002; Bronstein and Conner 1984). Humans shiver, fish swim to warmer waters, and poikilothermic reptiles crawl to warmer and sunnier surfaces in order to accomplish this. This fever response is always costly and often dangerous to the individual. In humans, a fever of just two degrees Celsius requires a 20% increase in caloric consumption to maintain. It is usually associated with fatigue, loss of appetite, and anemia. The higher temperatures damage body cells and reduce the effectiveness of organs. In uncommon cases fever can result in brain damage and death. Among men, fever leads to a drastic decrease in sperm health and production volume (Carlsen et al 2003). Some of the individuals most vulnerable to infection — pregnant women and the very old — are least capable of benefitting from fever because elevated temperature during pregnancy can cause birth defects, while senescence reduces the ability to bear the burden of maintaining an elevated temperature (Gomolin et al. 2005). The costs of fever come on top of the stresses and symptoms directly caused by the infection. The first puzzle of fever is why such a costly, sometimes lethal, response is so common among animals tested.

If the first puzzle concerns the phylogenetic breadth of fever, then the second puzzle concerns its depth. The febrile response adaptation is no younger than vertebrates, spanning some 500 million years. Most of the pathogens against which fever has utility are fast-evolving microbes such as bacteria and viruses. While fever is imperfectly effective at combating infections, invasive

microorganisms have failed to converge on any counter-adaptation rendering fever obsolete, even over hundreds of millions of years. The status of fever as a master strategy across phylogenetic space and time deserves explication.

While often taken for granted, fever thus constitutes an important and puzzling phenomenon, as it, or its analogue, occurs in a vast number of species; many pathogens seem unable to evolve effective countermeasures that would obviate its utility; and, as a consequence, it plays a key role in contemporary public health. Here, we seek to provide an explanation for fever's taxonomic breadth, antiquity, and continued efficacy by exploring the spatiotemporal dynamics of pathogen evolution in a framework wherein host defenses select for derived ecotypes that fare poorly in competition with the wild ecotype.

History

As an obvious and perennial sign of disease, human observations about fever are as old as recorded history. The oldest surviving word for fever is an Akkadian cuneiform inscription dating back to the sixth century BCE (Atkins 1982). In the Old Testament of the Bible, fever is described as a punishment from God or other unseen spiritual forces. Hippocrates, father of medical science, was the first to demysticize fever around the 5th century BCE (Gensini and Conti 2004). He assigned it (and most medical conditions) naturalistic, albeit erroneous, causes, namely an imbalance of the four purported bodily humors. Galen of Pergamon, a 2nd century Roman physician, believed that fever was itself the disease. Because of Pergamon's eminence, this view would go unchallenged for at least a dozen centuries, with medical practice remaining beset by stubbornly lingering antiquated views regarding fever, compounded by the lack of a proper measuring apparatus (the clinical thermometer was not in common use until the mid-19th century (Gensini and Conti 2004)).

In the 1840's the physician Ignaz Semmelweis noticed that women who gave birth outside of his clinic (either on the street or under the care of midwives) had a substantially lower incidence of a deadly fever-causing illness, with the same holding true for the newborns. He carefully examined and documented the pathology of the afflicted and deduced that each must have been caused by a transmitted infectious agent. Doctors who treated many patients and also performed autopsies were spreading the microbes that caused the disease. While Semmelweis's work was largely ignored until proven experimentally by Louis Pasteur and Joseph Lister decades later, he was the first to provide scientific proof that febrile illness can be caused by transmissible microbes (Semmelweis 1861/1983).

In 1888, William H. Welch built on the work of Claude Bernard and Carl von Liebermeister, who had discovered and described thermoregulatory homeostasis in animals, by positing that body temperature, including during fever, was regulated by the central nervous system. Using rabbits, he demonstrated that heat itself did not cause the damage observed in autopsied remains of disease victims, and suggested then that fever might be beneficial. In effect, he outlined the basic modern view of pathogenic febrile response (Atkins 1982). During the twentieth century, much progress was made in identifying proximate mechanisms of fever. These included bits of pathogens that tend to solicit fever (exogenous pyrogens) and signaling molecules that ultimately lower body set temperature, such as prostaglandins. However, fever would not be located in a proper evolutionary framework and put to specific adaptation testing until quite late in the century.

Phylogeny and prevalence

In a series of animal experiments starting in 1975, Matthew Kluger and his colleagues demonstrated the phylogenetic antiquity of the febrile response while also showing its positive

impact on post-infection mortality and therefore its adaptive benefits (Kluger and Rothenburg 1979; Kluger et al. 1975). Once inoculated with killed bacteria *Aeromonas hydrophila*, the *Dipsosaurus dorsalis* lizard will behaviorally upregulate its body temperature by basking or otherwise seeking a warmer local environment. Kluger also infected the lizards with live *A. hydrophila* and kept individuals at different temperatures to observe the result. Within twenty-four hours, half of the lizards kept at 38° C were dead. Only 14% of those kept at 40° C, and none of those at 42° C, had died. Similar behavioral fever response has since been observed in arthropods (including cockroaches, grasshoppers, crickets, lobster, shrimp, horseshoe crabs, and crayfish), an annelid (leech), several more reptiles, four species of fish, and five amphibians (Kluger 1992; Cabanac 1989; Bernheim and Kluger 1976; Bronstein and Conner 1984; Cabanac and Rossetti 1987; Cabanac and Le Guelte 1980; Casterlin and Reynolds 1977; Casterlin and Reynolds 1979; D'alecy and Kluger 1975; Glassman and Bennett 1978; Amaral et al. 2002; Louis et al. 1986; Myhre et al. 1977; Reynolds 1977; Vaughn et al. 1974). Physiological and behavioral fever response has been observed in birds and many mammals (Boorstein and Ewald 1987). Honeybees exhibit a specialized behavioral response to the fungal brood parasite *Ascosphaera apis* (Starks et al. 2000). The bees always maintain a given temperature within a hive, generally higher than the ambient, but raise the temperature in a brood-comb when *A. apis* is detected; lower brood-comb temperatures are associated with infection and brood mortality.

Thermal niche construction

Most organisms not only thermoregulate within a narrow temperature range, they also exploit or even modify the temperature of that niche. Many burrowing rodents raise their young in a den in which the temperature can be elevated and modulated with radiated body heat. The aforementioned honeybees climate-control their brood comb against an invasive fungus; their

cousins, the Japanese honeybees, offer an even more striking example of the defensive use of thermal manipulation: When assaulted by the much larger Japanese hornets, they defend their hive by swarming onto the hornets and vibrating their bodies rather than stinging, as their stingers are useless against the hornets. In this way, they raise the hornet's temperature to over 45 degrees, killing their would-be predator.

Thermal niche construction may not be limited to animals. Pine trees that grow in areas of frequent wildfires have adaptations that make them *more* flammable and spread a fire, should it occur, more quickly to the surrounding foliage. This is accomplished by way of thinner bark that increases flammability, and by failing to self-prune dead branches to help the fire reach the canopy. The cones of such pines are serotenous, meaning that the seeds survive wildfires and are activated to disperse by the fire itself. These fire-resistant pines benefit by spreading fire to nearby competitor tree species that are not nearly so resistant (Dylan Walker Schwilk diss., 2002).

When the invader organism or competitor is inside instead of out, the body is the pathogen's microecology, hence the host's adjustments to its own body temperature are not merely metabolic thermoregulation, but thermal microniche construction with regard to the pathogen. We define defensive hyperthermia as that form of thermal microniche construction wherein the host raises its own temperature in order to thwart an invader organism.

Defensive hyperthermia

Within the range of approximately -10 to 120 degrees Celsius, no single temperature is universally harmful to all living things. Microbes in the Arctic flourish at sub-freezing temperatures, while others at thermal vents replicate above the boiling point. The term "fever" is commonly understood to refer to the metabolic raise in set temperature and human-typical

symptoms such as body aches, fatigue, and loss of appetite. As discussed above, this conceptualization is too narrow to afford adequate understanding of how and why organisms increase their temperatures. Pathogen-infected fish have no option but to swim to warmer waters in order to produce a "fever". Poikilothermic reptiles move to a warmer patch of earth. Across species, the manifestation of this phenomenon varies based on the means each organism has for regulating its temperature; similarly, species differ as to whether they display associated symptoms typically observed in humans. For these reasons, it is appropriate to refer to metazoan fever responses collectively as defensive hyperthermia (DH).

Derived or conserved?

Kluger and his colleagues have shown that DH is effective against infection in several classes of animal, but is the trait derived or conserved in these species? Since most motile animals thermoregulate for non-immunological reasons, a capacity for DH could easily evolve if it conferred protection against infection. This would be true even if the ancestral lineage never had such a capacity. It is plausible that a costly feature such as DH could come and go many times over evolutionary time for any particular lineage if ecological and immunological factors varied sufficiently. This could explain why DH is not universal among animals studied to date.

Among vertebrates, and especially among mammals, evidence for conserved DH is strong. Among all of the species studied to date, there is no clear case of a vertebrate that lacks DH. A striking example is the naked mole rat, which does not ordinarily endogenously thermoregulate, instead being a rare case of a poikilothermic mammal. A naked mole rat will usually have a body temperature about one degree above the ambient temperature, whether that is 14 or 28 degrees Celsius (Yahav and Buffenstein 1991). Once infected, however, naked mole rats develop a fever metabolically (Umson et al. 1993) This is similar to observations of the leech *Nephelopsis obscura*,

which has a weak thermal preference unless inoculated with bacterial endotoxin or prostaglandins (Cabanac 1989).

There is evidence that the evolution of DH may predate vertebrates and chordates. Some genes and mechanisms for coping with infection are extremely old and highly conserved. Humans and the nematode *C. elegans* have very similar genes for Heat Shock Protein 1 (HSF-1). In both species, HSF-1 mediates an innate immune system pathway that requires temperature elevation to activate (Singh and Aballay, 2006). The protostome *C. elegans* and deuterostome *H. sapiens* diverged from bilateria approximately 670 million years ago (Ayala and Rzhetsky 1998). This is consistent with the findings that prostaglandin E_1 (PGE1) causes fever in humans as well as crustaceans, insects, and fish. Acetaminophen reduces fever in each (Cabanac and Rossetti 1987). It is thus likely that a primitive form of DH evolved early in animals, with some mechanisms strongly conserved across phyla. Subsequently, DH may have vanished in clades whose ecology made it unhelpful, and, conversely, DH may have further advanced and became more integrated into the physiology of other species.

Coordination or direct action?

Does DH directly impair or kill infectious microorganisms? It may be objected that autonomically-directed hyperthermia persists over evolutionary time because of its coordinative and pro-inflammatory features rather than its direct effects on infectious microorganisms. Temperature elevation does up-regulate the immune system and therefore helps coordinate the immune response to disease (Zhang et al. 2009; Hasday et al. 2000); however, it also directly impairs, slows, or kills invasive microbes (and tumors). Host mortality, disease severity, and duration of sickness are all curtailed by hyperthermia. Below we review three lines of evidence that support these conclusions.

Metabolic cost

A 2° degree rise in body temperature frequently causes a 20% increase in energy consumption among endothermic animals, and an equivalently substantial rise in metabolic rate has been documented in ectotherms such as amphibians (Kluger et al. 1998). As body temperature rises, many biochemical processes accelerate, consuming more energy. Cell membranes and other components sustain minor damage. Cells respond by diverting resources to coping strategies, including repair and the production of heat shock proteins. This expenditure of resources to create and cope with hyperthermia taxes the individual at the worst possible time, as the organism needs to muster a humoral immune response to the infection, which itself will require substantial resources. Lastly, for many mammals, there may be a further cost of fever: human semen produced during febrile states has 35% less concentrated sperm and contains over 20% more immotile sperm (Carlsen 2003), hence hyperthermia hits the body where, from the perspective of natural selection, it hurts the most – fertility.

Many means of signaling and coordination are available and used by metazoans besides temperature, so it seems unlikely that it would be necessary to maintain a metabolically costly fever for this reason. DH itself is coordinated by such a system of cytokines, interleukins, and prostaglandins. This is not to say that coordinative hyperthermia does not have any unique advantages compared to other methods of organizing immune response. It is global, signaling immune response everywhere in the body all at once, and, because the control system in the hypothalamus is behind the blood-brain barrier, it is more difficult for pathogens to undermine either locally or more globally (C.T. Bergstrom, personal communication). However, it is unclear that, in themselves, these advantages would outweigh the substantial costs of elevating body temperature.

Thermal impairment of microorganisms

Elevated temperatures can have many adverse effects at the cellular level. These include lesioning of organelles, damage to DNA, spontaneous membrane rupture, loss of mitochondrial tubules, diminished rates of protein production, and stress-induced apoptosis (Levy et al. 1969; LeGrand and Alcock 2012). In vitro studies have documented the deleterious effects of temperature elevation on several pathogenic species. Two-thirds of malaria parasites (*Plasmodium falciparum*) are destroyed after eight hours at 41° C, and none survive at sixteen hours (Oakley et al. 2007; Long et al. 2001). It is nearly impossible to cultivate infective-stage malaria parasites at 41° C because development nearly stops at that temperature and never accelerates, even after 24 hours. Typical human body temperature, around 37° C, is required for laboratory cultivation of malaria (Kwiatkowski 1989).

Salmonella typhimurium has been shown to be unable to produce iron transport compounds at 40° C or above. All eukaryotic cells require iron, but it is especially critical to growth and replication. Consequently, *Salmonella* ceases to grow at around 40° C, and this may be why birds, which have higher body temperatures than humans, are generally less susceptible to salmonellosis disease, even though poultry birds are often carriers (Garibaldi 1972).

Streptococcus pneumonia can cause many upper respiratory diseases in humans including meningitis, bronchitis, and pneumonia. *S. pneumonia* replicates steadily at 39° C, but dies quickly at 41° C (Small et al. 1986).

Among viral diseases, West Nile, yellow fever, vesicular stomatitis, and at least seven different pox viruses have optimal growth in vitro, or in chicken embryos, at or very close to 37° C. Beyond 37°, growth almost always diminishes quickly (Bedson and Dumbell 1961; Ruiz-

Gomez and Isaacs 1963). The same is true for rhinoviruses – the common cold is no exception (Stott and Heath 1970).

Nonhost resistance is asymmetrical for temperature

Nonhost resistance is a term that has been used to describe the inability of a pathogen to infect a species due to its specializations for infecting some other species, its de facto host (Heath 1981). Pathogens are generally unable to infect hosts that happen to have a body temperature range that, being different from the temperature range of the de facto host, is either too hot or too cold for the pathogen to function. Importantly, however, the direction of the difference appears to matter. The in vitro ability of most of the pathogens discussed above to proliferate falls quickly when the nonhost temperature is 3-5 degrees above the optimal temperature for growth, but the detrimental impact of cooler temperatures only increases more gradually. Growth is slowed, but continues for 10-20 degrees below optimal (Ruiz-Gomez and Isaacs 1963; Scholtissek and Rott 1969). At least fifty human pathogens respond to adverse environmental conditions by gradually reaching a torpid state. The most common impetus is cooler-than-optimal temperature, making such microbes resilient against relatively colder conditions (Oliver 2005; Oliver 2010). In contrast, increases in temperature appear to constitute a challenge that is more difficult for pathogens to overcome. For example, pathogenic *Helicobactor pylori*, for which humans are the natural host, survived fourteen days at 4° C but less than one day at 40° (Jiang and Doyle 1998). This may be a general phenomenon among many microbes, and, if so, then the thermal properties of DH alone would make it an effective adaptation.

This possibility is consistent with the observation that zoonotic infections tend to transfer between species by moving down a temperature gradient rather than up one, from higher body temperature animals to somewhat lower. Birds, pigs, cows, rats, and some insects are primary

vectors for a large number of diseases that infect humans, and each of them has a somewhat higher typical body temperature than humans. O'Shea and colleagues (O'Shea et al. 2014) recently proposed that this may be why bats transmit many diseases to humans, as bats experience substantial body temperature elevation during flight, hence pathogens that survive effectively in bats are readily able to affect humans. Additionally, Robert and Casadevall (2009) found that between 30° C and 40° C every degree of increase excludes 6% of possible fungal diseases. This puts most endothermic vertebrates into a "thermal exclusion zone". A hyperthermic boost of just a few degrees extends the nonhost resistance substantially with respect to potential fungal infections that plague plants and insects, and to which mammals with lower body temperatures, such as the duck-billed platypus and hibernating bats, are more susceptible (Obendorf et al. 1993; Blehert et al. 2009; Foley et al. 2011).

The effects of thermal characteristics are even apparent with regard to different body parts of the same animal. Rabbits are known to have a high resistance to *Cryptococcus neoformans*. Inoculations fail to lead to illness or mortality, but produce much more extensive effects on the testes, the organs that are naturally kept cooler than the rest of the body (Bergman 1967). The rhinoviruses that cause the common cold specialize in either the upper or lower respiratory tract in part because of the temperature differences between the two; such viruses are unlikely to be able to infect other parts of the interior body because the higher temperature would substantially limit their reproduction, even without DH (Stott and Heath 1970).

Why is hyperthermia sometimes defensively inadequate?

We have discussed how elevated temperatures can damage harmful microbes. Temperature range is so important that humans are effectively invulnerable to infection by many fungal species simply because of our higher natural body temperature. However, when studied outside the body,

medically important human pathogens, including *E. coli*, *Klebsiella pneumonia*, *Pasteurella multocida*, and *Staphylococcus aureus* remain viable and proliferate at both febrile and non-febrile temperatures (Mackowiak 1981; Jiang et al. 2000; Kuhn 1939; Enders and Shaffer 1936). Consider, for example, the constellation of features that, on the face of it, ought to make *K. pneumonia* a notable threat. Most people are exposed to the bacterium on a regular basis because it is commonly found in soil. Outside of the body, it can replicate at febrile temperatures and it is known to sometimes cause pneumonia and meningitis when it gets in the body. However, in the overwhelming majority of exposures, infection does not occur. Clearly, defensive hyperthermia, by itself, does not explain these observations. It is therefore important to understand DH as a critical component of a broader antipathogenic strategy.

In vivo, the features of a pathogen that make it a speedy infiltrator of host cells may necessarily make it comparatively vulnerable to the insult of environmental stresses, one of which is elevated temperature. Any pathogenic adaptation for increased rate of proliferation within the host may thus entail increased vulnerability to these stresses. Because the host's body is the environment for the pathogen, the host can induce such stresses, thermal and other kinds, as defensive microniche construction. LeGrand and Alcock's (2012) "immune brinksmanship" hypothesis details four ways that invading pathogens may be stressed and explains why these privations tend to disproportionately disrupt the pathogen compared to the host's own cells. This, in turn, may explain why DH is critical even when it is not decisive on its own, and why the logic of DH is such that it must necessarily be costly to the host.

Immune brinksmanship

The febrile response is sometimes dangerous and always costly to the host. It includes anemia (due to iron sequestration), anorexia leading to cachexic malnutrition, and, central to the

adaptation at issue, high caloric expenditure required to maintain an elevated body temperature. Locating this trait within a larger category of immune defenses, LeGrand and Alcock (2012) reason that such risky measures could only be adaptive if the cost to the host differs from the cost to the pathogen, with pathogens reliably paying the higher price. In their compelling framework, *immune brinksmanship* refers to processes whereby the host generates internal conditions that are harmful to itself because the harm inflicted on pathogens is greater than the harm inflicted on the host. They identify four reasons why virulent invaders are more vulnerable than their hosts to stresses induced by the immune system:

a) the host's targeted local inflammation works in synergy with acute stressors

b) the pathogen's proliferation/growth increases its vulnerability to stress

c) altered pathogen physiology results in pathogen stress or vulnerability

d) protective heat shock responses are partially abrogated in pathogens since their responses are utilized by the host to enhance immune responses

Local inflammation (a) turns a small area into a very hostile place by sequestering glucose, iron and oxygen, and generating concentrations of macrophages, digestive enzymes, and apoptosis-inducing ligands. Growing cells (b) are more vulnerable to reductions in the availability of materials, such as iron, zinc, and glutamine, and reductions in the availability of energy. When pathogens change from non-invasive to invasive mode (or in the case of a virus, the alterations made to the infected cell such as upregulating the cell's stress responses) (McInerney et al. 2005; LeGrand and Alcock 2012) (c), this alteration requires the production of many new proteins and enzymes; this shift in modality is therefore an extra stressor for the pathogens, one not confronted by most host cells. From microbes to human cells, most living things are capable of producing heat shock proteins (d) in order to prevent protein denaturation during high temperatures. However, the

immune system has evolved to seek and destroy cells displaying these proteins because infected cells are more stressed and therefore much more likely to produce them. This reduces the adaptive benefit of heat shock proteins for pathogens that might deploy them against fever, although such proteins are nonetheless still observed (Oakley et al. 2007). LeGrand and Alcock's model admittedly relies on some unproven claims (such as the relevance of zinc availability), and awaits confirmation that identified adaptive features are adaptations rather than side effects (e.g., fatigue and malaise could be caused by hypoferremia, and might therefore require no further explanation). Despite these limitations, at present, LeGrand and Alcock's model is the best explanation for the central paradox of the febrile response.

If correct, immune brinksmanship answers the first puzzle of fever listed in the introduction: it is costly because it has to be, and because death is a higher cost. If and where elevated temperatures disproportionately advantage the host versus the pathogen, and the host is not killed by the febrile state, such an adaptation will enjoy positive selection pressure. This is true across diverse clades, and would explain the phylogenetic breadth of DH. However, by itself, immune brinksmanship does not directly answer the second puzzle. Why has DH persisted or evolved in multiple lineages over many millions of years? Consider again malaria, which is not cured by fever; the H5N1 influenza virus that has killed 60% of those infected; or Ebola, which has recently returned to the headlines after more than five thousand deaths. Why has DH proven ineffective in these cases? Moreover, given such lack of efficacy, why have other pathogens not evolved over time to have similar DH-defeating features?

Evolutionary dynamics and the tradeoff between virulence and infectivity

We propose that the key to the durability of DH as an effective defense over vast stretches of evolutionary time lies in the manner in which this adaptation exploits natural selection that

operates on pathogens at two different stages, namely initial infection and subsequent reproduction. Pathogens must accomplish two basic goals in order to prosper: they must replicate in the host, and they must successfully reach and infiltrate the next host. Selection acts at each of these stages, and many factors beyond DH influence how these interact in the process of emergence and evolution of new pathogens (see discussion in (Park et al. 2013). We postulate that DH is one part of this complex interaction. Viewed as a source of selection pressure operating on successive generations of a given pathogen with which it has become infected, a host deploying DH effectively changes the environment confronting the pathogen, forcing it to adapt to a broader temperature range if it is to survive and reproduce within the host. However, while evolving in order to tolerate the elevated temperature makes the pathogen better at accomplishing its first goal, it necessarily makes it worse at accomplishing the second goal, for the most basic of Darwinian reasons: we propose that, all else equal, the ability to tolerate a broader temperature range comes at the expense of efficiency in a narrower temperature range, specifically that of the normal-temperature host. When a fever-tolerant pathogen subsequently reaches a non-febrile host, it will tend to have its efficacy and virulence impaired. Hence, the more that the pathogen evolves to tolerate fever, the more that it is in danger of being thwarted by the host defenses prior to successful replication and transmission. Critically, at the population level, a strain with a high tolerance for febrile temperatures is in constant competition with strains that, by virtue of being less able to tolerate high-temperature environments, are more competitive at the host's normal body temperature; because the latter will generally be superior at infiltrating the non-febrile host, they will out-compete the strain with a high tolerance for fever. More precisely, the normal-temperature strain will spread faster than the fever-tolerant strain whenever the former's superior ability to infiltrate normal-temperature hosts ultimately facilitates transmission to a greater extent than does

the latter's ability to resist DH – a configuration that, we propose, is common. Rapidity of infiltration and transmission are critical in the competition between pathogen strains because a fever-tolerant strain will necessarily be closely related to normal-temperature strains circulating at the same time in the given population of hosts, the latter being either the direct ancestor of, or cousins of, the former. The corresponding phenotypic similarity between contemporaneous strains is such that, following an infection by whichever strain arrives and infiltrates first, the host will develop antibodies that will often also be effective against whichever strain arrives second, precluding infection by the latter. In the race to reach naïve hosts, the strain that is better able to infiltrate, reproduce, and achieve transmission before being destroyed will win, and will thus dominate the population of pathogens. Importantly, fast-replicating pathogens can achieve transmission to a new host prior to the deployment of full-blown host defenses (Roberts et al. 2012; Riggs et al. 2007; Keeling and Grenfell 2002). Hence, even holding aside the possibility that adaptations for tolerating high temperature will often compromise the ability to resist other early-stage host defenses, the ability to thrive at normal host temperatures – a characteristic reduced by adaptations for high temperature – is a key determinant of success in the competition between strains. Because fever-tolerant strains perform less well during the initial stages of infection due to the costs of their specialized adaptations, they lose the race to reach naïve hosts. Thus, despite the fact that pathogens can generally evolve much faster than their hosts, except for the most sophisticated of pathogens, DH cannot be out-evolved by quickly mutating microbes because DH leverages evolution itself against them. Importantly, this novel hypothesis is supported by existing evidence, as investigators pursuing other questions have demonstrated that pathogens that adapt to the febrile condition suffer a fitness penalty at normal host body temperature.

In the course of an experiment exploring factors influencing the evolution of antibiotic resistance, Rodríguez-Verdugo et al. (2013) kept 115 separate *E. coli* populations at 42.2° C for 2000 generations. These populations originated from clones of an *E. coli* strain (REL1206) that had previously been kept at 37° for 2000 generations. When the ancestral and daughter strains were placed together at 37°, the daughter strains suffered a fitness penalty. This penalty did not occur at 42.2°. Consistent with expectations of the brinksmanship hypothesis, the evolved strain had the largest fitness advantage in a low-glucose high-temperature environment. The strains adapted to the higher temperatures exhibited more mutations on the rpoB gene. When a single rpoB mutation for higher thermal tolerance was artificially inserted into a strain (REL606) that had not previously been exposed to high temperatures, the modified mutant gained a large fitness advantage over the non-modified strain in a 42.2° low-glucose environment. We propose that in most cases, the better the pathogen is at prospering in febrile host temperatures, the less competitive it is at normal host temperatures. Pathogens that manage to survive in the face of host thermal defenses for a long enough period as to be able to evolve improvements in those abilities thus maroon themselves in the given host by virtue of reductions in their ability to compete with unmodified strains when seeking to infect a new host.

Exceptions that prove the rule

DH is one part of the coordinated immune system response to infection that includes not only other brinksmanship strategies, but also many other critical mechanisms, such as proper antibody generation and production, cytotoxic T-cell activation, and stimulation of natural killer cells. By itself, DH is rarely sufficient to cope with dangerous pathogens because infection must be recognized in order for DH to be activated and because some brinksmanship effects only work if immune system cells are competent to recognize infected body cells and pathogens in the body.

With the understanding of the adaptive trade-offs forced by DH, the logic of the brinksmanship hypothesis, and DH as just one part of the coordinated immune system response, we can return to the question of why hyperthermia is sometimes defensively inadequate, and why some pathogens are able to tolerate febrile temperatures. Below, we consider just a few of the human-infecting pathogens that are not severely reproductively impaired in vitro by human-febrile temperatures.

K. pneumonia

The soil bacterium *Klebsiella pneumonia* is unaffected by human febrile temperatures, but, in spite of this, virtually never infects humans unless they are immunocompromised (Podschun and Ullmann 1998). This is because *K. pneumonia* is not an obligate human pathogen, and has not specialized in infecting us, even though it can, and therefore is unable to overcome immune system defenses at any temperature. The ability of some pathogens to prosper in a wide range of thermal environments – making them largely immune to the effects of DH – comes at the expense of specialized adaptations that allow human-specific pathogens to combat non-thermal features of our immune defenses. Hence, we prose that in conjunction with the full suite of defenses, DH constitutes a significant weapon – those thermal-generalist species against which DH is ineffective are more vulnerable to other immune defenses, while those pathogens that are able to resist many of the latter do so using costly mechanisms developed in parallel with cost-saving thermal specialization, which, in turn, makes them vulnerable to DH.

Malaria

Hosts afflicted with malaria may suffer through multiple cycles of fevers. In some cases, they may experience relapse, even if they are not re-infected by a mosquito. Malarial parasites such as *Plasmodium falciparum* have multi-stage life cycles that are among the most complex and

specialized of any known organism. DH leverages evolution by creating two host ecologies in which adapting to one usually entails becoming maladapted to the other. However, malaria uses a remarkable strategy to cope with DH, namely producing several different phenotypes that exist simultaneously in a single host. The sporozoite phenotype addresses infiltration, the trophozoite phenotype undertakes replication, and the gametocyte phenotype serves transmission. The specialized forms fit predictions that might be made about the trade-offs required to combat DH. The infiltrator sporozoites, newly arrived from a mosquito's bite, hide quickly in liver cells. They are low-virulence, creating packets of copies inside a few host cells rather than infecting a large number of host cells. These can evade the immune system and withstand fevers. The trophozoite form follows: the packets burst and trophozoites invade red blood cells en masse. This virulent form triggers a quick and substantial immune response, including fever. Trophozoites are quite vulnerable to both DH and other immune defenses, but tend to succeed by sheer numbers and speed. The trophozoites become or produce gametocytes that will live only hours if they do not get into a mosquito. Trophozoites and gametocytes are not resistant to fever. At 40°, trophozoite phase parasites cease activity, while sporozoites are almost unaffected, and remain active (Kwiatkowski 1989). By manifesting in multiple specialized phenotypes, the malaria parasite thus fights a prolonged ebb-and-flow battle with host defenses – low-virulence sporozoites reproduce slowly in the liver, eliciting minimal host reactions, and able to weather waves of febrile temperature; highly virulent trophozoites flood the body during the period when the mosquito vector is active, eliciting massive febrile response; many die, but gametocytes result, playing the odds that transmission will occur before death at the hands of the host. High-virulence forms are destroyed within hours, the fever response dissipates, and low-virulence forms slowly prepare the next wave in anticipation of the succeeding transmission window. Hence, through a division of

labor among multiple phenotypes, the parasite cuts the Gordian knot of DH. Importantly, however, this strategy only works because the parasite is able to time waves of temperature-sensitive high-virulence forms to coincide with the appearance of the vector through which transmission occurs, greatly enhancing opportunities for transmission – a circumstance that, in conjunction with the remarkable complexity of the multi-phenotype strategy, precludes the evolution of similar tactics in most other pathogens.

The influenzas

The 1918 pandemic, sometimes called Spanish flu, is estimated to have killed 3-5% of the global population (Taubenberger and Morens 2006). However, while in absolute terms the losses were staggering, viewed in relative terms, the mortality rate was comparatively low. In the United States, just 2.5% of those infected died. The virus was very good at spreading from person to person, infecting an estimated 28% of Americans. In contrast, H5N1, an avian flu subtype with widely reported outbreaks in 2008, has killed around half of those infected with it, but there is no strong evidence that it can transmit from person to person at all. The most prolifically infectious influenza viruses, sometimes called seasonal influenza, are also the least virulent, with a mortality of 0.019% in the US (Thompson 2003). Within the influenza viruses, each form that excels in one host environment does not succeed as well in the other. The effective infiltrator/highly infectious pathogen, such as seasonal flu, is not usually able to overwhelm host defenses (including DH). The deadly viruses are better equipped to overcome host defenses, but are comparatively poor at achieving transmission to other hosts. Granted, such comparisons oversimplify the complexities of host-pathogen interaction and do not differentiate the effects of DH from those of other important components of the immunoresponse. Under the right circumstances, it is possible for a pathogen to be highly contagious and lethal. Nonetheless, we believe that the overarching pattern

of virulence and transmissibility features for influenzas and other diseases is consistent with, and partly explicable in terms of, DH as part of the coordinated immunoresponse.

The Black Death

The most famous plague is caused by the bacterium *Yersinia pestis*. The Black Death featured high mortality and high rates of transmission. However, like malaria, the Black Death relied on ubiquitous zoonotic vectors and reservoir species, in this case fleas and rodents. It did not usually spread from person to person. There are three forms of plague depending on the site of infection: bubonic, septicemic, and pneumonic, affecting, respectively, the lymphatic, circulatory, and respiratory systems. The most common, by far, is bubonic plague. It is also the lowest mortality of the three, at 50% (untreated)(Putzker et al. 2001; Kool 2005). Pneumonic plague usually kills all victims that develop it, and it is the only form of the three that can spread directly from person to person. While there have been outbreaks of primary pneumonic plague, and in some nations continues to be, this form of contagion is probably substantially slower than animal-born *Y. pestis* (Gani and Leach 2004; Stenseth et al. 2008). Only a fifth of plague victims ever develop the pneumonic form and those who do are not infective until the final hours or day while coughing "copious amounts of bloody sputum" (Kool 2005; Alsofrom et al. 1981). *Y. pestis* has existed as a pathogen to humans and other animals for at least several thousand years. The airborne form existing since at least the 14th century has failed to evolve into a serious pandemic. Part of the reason could be that DH is among the obstacles to *Y. pestis* evolving the superior transmissibility enjoyed by less virulent pathogens.

While multiple factors may be responsible for the patterns described above – and extensive research is needed to map out the proximate mechanisms at issue – nevertheless, we expect that DH will prove to be part of the reason these and other pathogens are not able to simultaneously

maximize their transmissibility and their virulence. If so, this may both inform clinical treatment and contribute to the growing literature on how fitness trade-offs between transmission and within-host competition shapes how these organisms may evolve in the future.

Why most pathogens do not evolve either extensive thermal plasticity or separate phenotypes for higher and lower temperatures

In principle, microbes could evolve phenotypic expression for multiple temperature ranges. From hibernating mammals to deciduous plants, many macro-organisms clearly possess adaptive plastic responses to dramatic seasonal variation in temperature. Moreover, temperature-specific morphs can evolve in macro-organisms when temperature varies regularly across generations (reviewed in (Fusco and Minelli 2010)). Many species of bacteria are indeed commonly observed to revert to a hibernative form, retreating to a torpid, high-resilience state when their environment is too cold or in other ways inhospitable. It is therefore reasonable to ask why pathogens would not evolve either more robust plastic responses or temperature-specific morphs for febrile and non-febrile host conditions.

One possibility is that selection operates within the febrile host, such that thermal flexibility, being costly, is disfavored relative to thermal specialization. Fever-adapted strains could arise during the febrile state, displacing thermal generalists, only to then subsequently lose out to strains that specialize in the host's normal body temperature during competition for transmission to non-febrile hosts. As noted earlier, results reported by Rodríguez-Verdugo et al. (2013) reveal the evolution of specialization for elevated temperature in *E. coli* after 2,000 generations at 42.2° C. The authors, who aimed to explore antibiotic resistance rather than thermal specialization per se, do not report changes along the latter dimension at intermediate points in their experiment. However, inspection of their findings regarding the development of antibiotic

resistance reveals evidence of substantial evolutionary change after only a few hundred generations. Given that, in their experiment, antibiotic resistance correlated with heat tolerance, and given that the model organism produces 6-7 generations per day, this suggests that changes in a prevailing pathogen thermal phenotype could conceivably occur within a timespan approximately equivalent to an extended DH response. (*E. coli* infections do not typically produce high fevers, nor does infection typically last several weeks; rather, the utility of the results lies in the demonstration of principle). However, such conclusions are contradicted by observations of pathogen populations sampled from seasonally-varying thermal environments. Bronikowski et al. (Bronikowski et al. 2001) obtained *E. coli* and *Salmonella enterica* from natural populations of turtles, repeating the process over the course of two years. Although the body temperature of the ectothermic host animals varied systematically by season, when the growth rates of the sampled pathogens were tested at multiple laboratory temperatures, no evidence of thermal specialization was found, leading the authors to suggest that the seasons were not sufficiently long as to generate cyclical changes in the prevailing pathogen phenotype, i.e., within-season selection does not create season-specific thermal specialists. If this interpretation is correct, then, clearly, such selection is can be unlikely to operate during the time course of DH during some infections.

At present, the possibility that thermal flexibility in pathogens is disfavored due to selection for fever-adapted strains operating during DH remains a highly speculative conjecture. Rather, it is likely that the evolution of such flexibility is constrained not by competition in the febrile host, but rather by competition in the normal-temperature host. Cooper et al. (Cooper et al. 2001) demonstrate that when *E. coli* is maintained at a constant temperature of 37° C there are sustained improvements in growth rates (experimentally evident within the first 1,000-2,000 generations) and a corresponding increase in impairment at 41° C. This suggests that the wild ecotype does

indeed possess some capacity for thermal flexibility, but that this capacity is selected against when the environment is thermally invariant. The latter pattern reveals that thermal flexibility comes at a cost, such that the better equipped the pathogen is to weather the storm of DH, the slower its growth rate at the host's normal body temperature, and thus the more that it will lose out to thermal specialists in the race to infect new hosts. Hence, the evolution of thermal flexibility is likely constrained primarily by selection for infectivity, limiting thermal flexibility in a manner that preserves the utility of DH.

Unlike the limited thermal plasticity evident in *E. coli,* malaria possesses multiple thermally specialized morphs. Critically, however, the two organisms differ markedly in the relationship between their generational timescales and the relevant selective environments, allowing malaria to circumvent the processes that normally limit the evolution of thermal flexibility in pathogens. As noted, malaria has a complex life cycle that features distinct morphs for each phase, and each morph is specialized to its particular task. The parasite can survive the fever it elicits because it has a dormant form that does not replicate or invade body cells during a fever. Conversely, the form that virulently replicates in red blood cells is vulnerable to fever, with none surviving beyond 16 hours (Oakley et al. 2007)– a period sufficiently long as to afford transmission prior to destruction. One of the likely reasons malaria was able to evolve this specialization is that the time spent in a human host is but one half of one life cycle. Malaria sexually reproduces only after transmission to the mosquito vector. Therefore, selection is strongly acting on particular genetic "individual" malaria parasites that have passed through all of the host ecologies. This is a sharp contrast to fast-reproducing bacteria or viruses on which selection is acting across multiple generations inside a febrile host.

Hypothermia

If DH is effective because it creates a host microecology that is thermally inhospitable to pathogens, it is reasonable to ask why these defenses involve raising the temperature rather than lowering it. If obligate pathogens specialize in a narrow range of temperatures, then, ceteris paribus, cooling down should work as well as heating up. A hypothetical defensive hypothermia would have the advantages of conserving rather than expending energy at a time when the host needs to employ resources to fight the infection via other avenues. One possibility is simply evolutionary inertia/path dependence: once hyperthermia became a component of the coordinated immune response at some point in a lineage, it would subsequently resist selective pressure for hypothermia. However, this could not explain the phylogenetic breadth of DH, as at least a few species would be expected to break the trend, particularly among ectotherms that are much more stringent about conserving energy than endotherms. The brinksmanship hypothesis suggests a better answer: a raise in temperature gives comparative benefit to the host versus the pathogen, but a temperature decrease may offer no such benefit, and could even advantage the pathogen somewhat. This is consistent with the observation that several pathogenic microbes are less impaired by below-optimal versus above-optimal temperatures. However, while hypothermia cannot serve as a primary defense, there is evidence that it may nonetheless be an adaptation selectively deployed when particular circumstances make it a better option than DH.

The energy-intensive thermal elevation aspect of the brinksmanship strategy can work because it differentially advantages the host. However, the advantage is lost if the host does not have energy reserves sufficient to produce and withstand its own fever. This could be true under conditions of malnutrition or the host's environment being sufficiently cold that maintaining an elevated temperature would be exceptionally costly. Additionally, DH would fail the

brinksmanship cost-benefit analysis in one other important condition: when it has already been deployed against a given pathogen and has utterly failed to halt its progress. Under such circumstances, defensive hypothermia may constitute a last-ditch effort at brinksmanship.

Septic shock is a general term meaning severe infection and sepsis (body-wide inflammation) following the total failure of the immune system to combat an infection. In cases of experimentally induced septic shock, hypothermia has been observed in dogs, rats, mice, and bumblebees, and cold-seeking behavior has been observed in septic human patients, mice, and bees (Blair et al. 1964; Romanovsky and Székely 1998; Habicht 1981; Müller and Schmid-Hempel 1993). In all cases, hypothermia reduced mortality rates. On the basis of such evidence, Romanovsky and Székely (1998) propose a bimodal model of thermoregulatory inflammation management utilizing the same evolutionary framework as Kluger, LeGrand and Alcock, and others. They argue that fever is adaptive when general health and nutrition are adequate, and the apparent infection is of a manageable scale. However, if an individual is starved or in an already sub-normal temperature environment, then a fever may be metabolically unsustainable or lethal. Similarly, in the event of septic shock, fever has either already failed to control infection, or is unlikely to do so. In either case, hypothermia allows for the conserving of highly limited energy reserves. A lower body temperature results in less strain on the heart and lungs because the diminished activity levels requires less oxygen. In their own study, Romanovsky and Székely demonstrate that rats given small or moderate doses of bacterial lipopolysaccharide (1 or 10 micrograms) developed fever, but rats given 1000 micrograms developed hypothermia and diminished motor activity instead. Presumably, the salutary effects of hypothermia constitute another form of immune brinksmanship in which the costs to the pathogen exceed those to the

host, but it is not entirely clear yet what precise effect cooler temperatures have on pathogens in vivo.

Positive externalities and superinfection

Our model of the evolutionary persistence of defensive hyperthermia holds that pathogen strains that are more fever-tolerant are consistently outcompeted by strains that are better optimized for normal body temperature and are thus more successful at infecting non-febrile hosts. For this to be true, either a) some transmission must occur prior to within-host selection for heat tolerance, b) either within or between hosts, such selection must be incomplete, leaving some less-fever-tolerant pathogens to be transmitted prior to complete clearance by other immune defenses, or c) both. Either way, at any one time, a given infected host may be shedding one type, the other type, or both types.

Focusing for the moment only on transmission of the highly fever-tolerant type, a welcome side effect of DH is the protective impact it has on the social group of the bearer. A pathogen that evolves to be better suited to a wide range of thermal environments does so by sacrificing efficacy at the host's normal body temperature, and thus will be less able to invade the next potential host. When this occurs, and when infection is intraspecific, other members of the host's social group thus enjoy benefits from DH without having to do anything. Hence, not only is DH good for the bearer, it also generates a positive externality for conspecifics. This aspect is not required for DH to be subject to positive selection, as it is in the individual host's own interests to employ brinksmanship in shifting the microecology of its body against an invading pathogen whether conspecifics are present or not. However, in those circumstances in which selection operates to enhance the welfare of those around the focal actor – as, for example, whenever relatedness and

propinquity are positively correlated – this positive externality will enhance inclusive fitness, potentially augmenting selection for DH.

Next, it is important to consider more closely the dynamics of infection on which our model is premised. At the heart of our model, the more fever-tolerant type suffers a competitive disadvantage relative to the wild ecotype when infecting naïve hosts. However, if sequential superinfection occurs with the proper timing, the tables can be turned in this regard. Specifically, if the fever-tolerant type is transmitted to a host who is currently employing DH to combat the wild type, then, all else being equal, the fever-tolerant type could enjoy a competitive advantage, having found itself in a thermally hospitable environment, while the wild type struggles with what, for it, is a thermally inhospitable environment. Importantly, however, all else will not be equal in most such cases. Specifically, first, while the thermal environment will be hospitable from the perspective of the newly-arriving fever-tolerant type, the same will not be true of other aspects of the host, since DH accompanies a storm of other immune responses such as upregulated levels of cytokines, interferons, antibodies, phagocytic T-cells and natural killer cells, as well as host-wide anemia (Jiang et al. 2000; LeGrand and Alcock 2012). Because the new arrivals are few in number relative to the variant causing the ongoing infection (as it is the latter's proliferation that elicited DH), each loss to the fever-tolerant type's ranks due to the ongoing storm of immune responses has a greater impact in slowing its rate of reproduction than is true of the wild type, reducing the competitive advantage that the fever-tolerant type enjoys by virtue of thermal considerations. Second, the thermal benefits of superinfection for fever-tolerant types erode rapidly, as, due to similarity between the types, the late-comer will often find itself confronting a host who is already building an arsenal of antibodies that are effective against both types. Third, at the time of initial superinfection, the superior numbers of the wild type afford them greater likelihood of

transmission during the same period when the fever-tolerant type is only beginning to replicate. While this can aid the fever-tolerant type in the short run by eliciting DH in new hosts, thus creating a hospitable thermal environment for superinfection, nevertheless, iterated over many hosts, the successive head starts enjoyed by the wild type will be such that there will be a progressive increase in the number of hosts who have had time to develop antibodies, and even to clear the initial infection and end the febrile state, before the fever-tolerant type arrives.

Clinical and public health implications

Superinfection

While the dynamics of overlapping infection by two related strains of pathogens is important to our theoretical account of the enduring nature of DH's efficacy, the relationship between superinfection and the utility of DH may also have direct implications for clinical practice. When a fever-tolerant pathogen encounters a host who is currently battling assault by a different pathogen species, the host's DH again creates a hospitable thermal environment for the newcomer. However, unlike the case of superinfection by related strains, in this case, antibodies produced to counter the initial infection will be ineffective against the secondary infection. Superinfection may thus pose a significant risk to the host not simply because the host must battle multiple pathogens at the same time, but, moreover, because, under the right circumstances, the secondary pathogen will be less susceptible to DH, a key component in front-line defense. Similarly, whereas, by virtue of shared antigens, the temporal dynamics of transmission will constrain the spread of fever-tolerant pathogens when superinfection involves two strains of the same pathogen species, this constraint is relaxed when superinfection involves two unrelated pathogens. Epidemics characterized by high transmission rates and extensive saturation thus conceivably allow for the spread of fever-tolerant secondary pathogens. Moreover, this can occur even absent epidemics

when human actions place many febrile individuals in proximity with one another. Hospitals and similar settings in which patients infected with different pathogens are housed together may thus constitute breeding grounds for fever resistance. In both scenarios, the fever-tolerant strain will likely lose in competition with the normal-temperature type outside of the context that supports the evolution of the former, and hence the utility of DH is preserved in the long run. However, in the short run, mortality rates may be elevated by the diminished efficacy of DH.

Fever suppression

Housing febrile patients infected with different pathogens together is merely one example of how failure to understand the nature and dynamics of DH can significantly undermine clinical efforts. Suppression of fever has been shown to increase viral shedding and slow the clearance of malarial parasites (Brandts et al. 1997; Graham et al. 1990). Use of antipyretic drugs is estimated to lead to a 1% increase in instances and mortality of pandemic influenza and a 5% increase in instances and mortality of seasonal influenza (Earn et al. 2014). It remains common practice for physicians to regard DH as a detriment calling for alleviation, even though there is little clinical evidence that this improves patient prognoses (Best and Schwartz 2014; Mackowiak 2000a; Blomberg et al. 2003; Mackowiak 2000b). Lingering notions of fever as a harmful condition, rather than an important weapon against infection and contagion, may be taking a great toll in human lives, suffering, and economic losses. Interestingly, the common endorsement of these notions seems to be relatively recent.

Many cultures have not only recognized the beneficial effects of fever, but treated fever and a variety of ailments with thermotherapy, artificially raising the body temperature by bathing, steam inhalation, blanketing, and other means (Bierman 1942; Atkins 1985). This includes Greeks, Romans, and Egyptians from the 5th century BCE on, pre-colonial Native Americans, and the

ancient cultures of Japan and China. It is not clear precisely when and why common attitudes reversed. Successes and innovations in medical science may have precipitated the decline of popular and medical interest in thermotherapies.

In 1927, the Nobel Prize for medicine was awarded to Julius Wagner von Jauregg for the development of malariotherapy, the practice of infecting a patient suffering neurosyphilis with malaria specifically to induce repeated hyperthermic states. At the time, 10-20% of inmates in mental institutions were patients with a form of syphilis infecting the brain and causing paralysis, dementia, and, in most cases, death within five years (Snounou and Pérignon 2013). There was no cure for syphilis, but there was a cure for malaria; after multiple bouts of fever, the patient was treated for malaria. For forty years, this was a standard treatment for syphilis. However, after the discovery of antibiotics that could cure syphilis without the risks associated with malariotherapy (15% mortality), the treatment was abandoned, and interest in thermotherapy research waned. The absence of consideration of the effects of temperature is most notable in the case of von Jauregg and his contemporary, William Coley. Coley demonstrated that a bacterial cocktail administered to stimulate the immune system by way of repeated infection could be effective in treating cancer. Although it is clear in his own data, Coley never noticed that remissions correlated strongly with intensity and frequency of fevers of the patients, nor did he suspect the temperature itself was curative against tumors, something that the ancient Greeks and Romans knew (van der Zee 2002). (Because malignant tumor cells replicate faster than normal cells, the logic of brinksmanship applies to them just as it does to pathogens). In contrast, von Jauregg did recognize the clinical importance of fevers and noted that their severity predicted likelihood of remission among the syphilitic; nonetheless, like Coley, he suspected that the curative mechanism was a result of toxins produced by the bacteria or parasites, not the temperature (Whitrow 2012). Coley and von Jauregg

notwithstanding, many physicians of the nineteenth and early twentieth centuries knew or suspected that hyperthermia itself was the treatment. A 1936 editorial in the journal *California and Western Medicine* called hyperthermia "an established therapeutic procedure" for treatment of such diseases as syphilis, gonorrheal arthritis, and epididymitis, and stated that any means of temperature elevation, be it malaria or electric blankets, worked equally well (Epstein 1936). Coley died the same year and interest in antineoplastic thermotherapy largely died with him. Further clinical testing in humans would not begin again until the 1970's (Baronzio and Hager 2006).

Thermotherapy may also have faded from prominence due to other advances that provided more treatment alternatives: vaccines, chemotherapy (itself a form of brinksmanship), more sophisticated surgical techniques, and advances in the understanding of disease transmission. Palliative substances have long been used to treat pain and suffering, but only in the last 150 years were many of them isolated, chemically synthesized, concentrated, and made readily available to most people. In a time when newly-produced vaccines and antibiotics were astonishing wonders, the pills that took away pain would benefit from the medical science halo effect. With fever's connection to decreased mortality and morbidity obscured by revolutionary new treatments, analgesics had no apparent down side.

In addition to widespread misunderstanding of the virtue of DH for health and wellness that had taken root by the 1950's, over-the-counter medications may exacerbate the harm to public health caused by interfering with DH. Even an informed consumer who understands that DH is important to getting well sooner may inadvertently take an antipyretic drug because all over-the-counter products designed to relieve cold or influenza symptoms, including all pain relievers, are also antipyretics: acetylsalicylic acid (aspirin) and all other salicylates, and the class of drugs that includes ibuprofen, and paracetamol, also known as acetaminophen, the active ingredient in

Tylenol. There are presently no pain relievers available in the drugstore that do not also reduce fever because the regulatory pathways for fever, inflammation, and pain sensitivity are closely connected.

While thermotherapy may yet enjoy a renaissance, lessons learned from studies of the evolution of drug resistance suggest that caution is in order in this regard. Consider the results of experimental evolution of antibiotic resistance in *E. coli*: 115 populations evolved for 2000 generations at 42.2 C showed convergent evolution for a small number of genes that conferred not only heat tolerance, but antibiotic resistance (Rodríguez-Verdugo et al. 2013; Tenaillon et al. 2012). This can occur because the brinksmanship stressors ultimately succeed by slowing replication and by causing apoptosis. Mutations that help a pathogen cope with one type of stress, like thermal stress, increase the relative level of total stress the host must inflict before apoptosis occurs. Similarly, drug-resistant malaria strains depend on heat-shock protein genes such as HSP-90 and HSP-110 to survive hyperthemic conditions, but, because they are chaperone genes that facilitate protein folding under most types of duress, they also convey drug resistance (Muralidharan et al. 2012; Ramdhave et al. 2013). When considering applications of thermotherapy, it is therefore prudent to consider these possible unintended consequences so as not to repeat mistakes such as the over-prescription of antibiotics.

Common infection

Infants

Over-reliance on powerful, modern medical advances may also unduly prejudice considerations of DH among the youngest and oldest people generally most vulnerable to disease. Compared to adults, infants are more prone to bouts of hyperthermia and somewhat higher fevers

(Rehm and Kris P. MD 2001). The lack of a well-developed adaptive immune system may make DH more important to fighting infection in infants. Relative to their body size, infants have a large quantity of brown adipose tissue compared to adults (Dawkins and Scopes 1965). Brown adipose tissue is thermogenic and necessary to thermoregulation in infants, but it also plays a role in immunoresponse (Cannon and Nedergaard 2004). Exogenous pyrogens, such as lipopolysaccharides comprising the outer membrane of many pathogenic bacteria, induce brown adipose tissue to up-regulate expression of a gene (UCP1) associated with thermogenic respiration in brown adipose tissue (Cannon et al 2006). The tissue also produces cytokines interleukin 1 beta and interleukin 6, indicating that it plays a role in regulating immunoresponse. Together, these observations suggest that selection has equipped infants with enhanced DH capabilities that partially compensate for the immaturity and lack of exposure to antigens of other parts of the immune system.

Senescence

In the elderly, the quantity of brown adipose tissue associated with thermogenesis is markedly reduced. More broadly, presumably reflecting general patterns of senescence, the ability to mount an effective DH response to infection declines in the elderly (Dawkins and Scopes 1965). In turn, this reduced ability may contribute to the increased mortality observed among elderly patients infected by relatively common pathogens such as influenza.

Speculative future directions

Plant thermotherapy parallels to DH

Clinical applications of thermotherapy are not limited to humans and other animals. A review of thermotherapy used to treat infected plants by Panattoni et al (2013) described such

treatments in terms that closely parallel DH in animals. Temperatures between 35° and 54° C but within the tolerance limits of the plant species are recommended and it is noted that, while the thermal tolerance of some viruses exceeds that of the host they infect, it is easier to reverse tissue damage caused by heat than damage caused by the virus. The higher temperatures cause physical impairments of the viruses, such as rupture of hydrogen and disulfide bonds of capsid proteins. Higher-than-normal temperatures stimulate antiviral immune responses, known as virus-induced gene silencing (VIGS), in some plants, proving that in these species "fever" stimulates immunoresponse just as it does in many animals. Such plants may require a certain minimum temperature in order to metabolically facilitate VIGS (Szittya et al. 2003).

The relationship between temperature and pathogen defense in plants is apparently similar to that of many animals in many regards:

- Elevated temperature advantages the host against pathogens, even while being costly to both
- Elevated temperature stimulates immune response
- Pathogens are maladapted to and/or physically injured by elevated temperature for initially unclear reasons
- Hypothermia is a viable form of antipathogenic thermal defense
- Elevated temperature against infection is effective in a phylogenetically broad range (Panattoni et al. 2013)

It remains unclear why plants that do not thermoregulate, and likely had no recent ancestors that did, could benefit from temperature elevation or require it to mount an effective immune response. The explanation that only at those temperatures is the response metabolically practical

does not account for the lack of evolution of a similar defense that functions at lower temperatures. Such an adaptation must be possible because the narrow temperature range that is currently optimal would have varied substantially over evolutionary time, and because VIGS is common in many phyla where optimal temperatures vary.

Additionally, there are a small number of plants that do thermoregulate above ambient temperatures. The skunk cabbage (*Symplocarpus renifolius*) can maintain a temperature at least 25° C above ambient, and the sacred lotus (*Nelumbo nucifera*) can maintain its preferred temperature range against higher or lower ambient temperatures consistently for several days. Plants thermoregulate to function in colder climates, volatilize scents or otherwise encourage pollinators, and to metabolically facilitate inflorescence. However, plant DH is possible in some cases. Tobacco plants resistant to tobacco mosaic virus respond to tissue infection and injury with production of the plant hormone salicylic acid. In addition to promoting acute defenses against infection in plants, salicylic acid also induces a rise in temperature at the sites of infection. It thus appears that forms of DH may have evolved, or at least could evolve, in a number of plant species. However, this leaves unanswered the question of why a number of plant defenses are triggered by elevated temperatures.

Anticipatory DH in advance of pathogen exposure risk

Psychogenic fever, sometimes called stress-induced fever, has been reported in humans, rats, rabbits, and cheetahs (Kluger et al. 1987). Rodent body temperatures rise when handled by humans or when confronted with a novel open field environment. Human psychogenic fever has been documented in a wide variety of stressful situations, such as in athletes before a competition. Like fever associated with infection, these hyperthermic episodes are produced and

mediated by a rise in hypothalamic set point. They are associated with increased production of prostaglandins, and the effects can be blocked with standard antipyretic drugs. More recently, it has been found that the post-hunting rise in cheetah body temperature previously thought to be caused directly by the act of sprinting is unrelated to the thermal effects of high speed running. These psychogenic fevers are generally attributed to stress response. While it is possible that hyperthermia is simply a nonfunctional (or even dysfunctional) side effect, explicable in terms of constraints on the optimality of the proximate systems central to the stress response, it is important to note that stress responses often occur in situations that entail elevated risk of injury or pathogen exposure. We speculate that these fevers may be anticipatory DH. A review of 300 papers on the relationship between stressors and immune response concluded that acute stressors upregulate innate immunity while downregulating specific immunity (Segerstrom and Miller 2004). Since innate immunity is the generalized front-line defense against infections, this is substantial evidence that the stress response in humans is partly immunological in nature, and consistent with the hypothesis that psychogenic fever is a form of DH.

Conclusion

To summarize our position, there is now substantial evidence that infection-induced rise in body temperature is a critical component of the immune response in humans and many animals. Suppressing fevers by any means is associated with increased mortality, morbidity, duration of infection, and duration of infectivity. Antipathogenic hyperthermia is a master strategy notable for its efficacy in many different phyla of animals (and perhaps some plants) and for its evolutionary heritage extending at least 380 million years into the past. The question of why this strategy has persisted so long and in so many different lineages in the face of invasive

microbes that reproduce and mutate much faster than their hosts has not been addressed previously.

We term this master strategy defensive hyperthermia, rather than fever, to place it within a broader evolutionary and ecological context necessary to addressing the question of its persistence, to benefit the understanding of host-pathogen coevolution and disease transmission, and to help inform public health policy.

We posit that DH has endured, and likely evolved independently multiple times, because it leverages evolution against pathogens. Disjunction between any pathogen's optimal temperature for growth and reproduction and that of the host disadvantages it in its critical contests against the host immune system. By presenting the pathogen with two radically disparate thermal environments, that of the normal body temperature and that of the DH state, DH forces pathogens into a competitive dilemma to which there is no perfect solution. Because thermal flexibility comes at a cost, to the extent that they evolve to tolerate both normal and elevated body temperatures, pathogens reduce their ability to infect normal-temperature hosts. Competition among variants racing to infect new hosts thus constrains the evolution of thermal flexibility, thereby preserving the efficacy of DH as a host tactic. We acknowledge that, while consonant with a range of existing empirical observations, ours is but an informal verbal model of the postulated processes. Building on existing mathematical models of the evolution of thermal specialists and thermal generalists and the determinants of thermal reaction norms (see Gilchrist 1995; Angilletta et al. 2003)), it should be possible to test our account using formal models.

Beyond the immediate questions of the evolutionary persistence and near-ubiquity of DH, we believe DH can serve as an important nexus of research in the consilience of ecology,

epidemiology, zoology, medicine, and public health that lies at the heart of the emerging field of evolutionary medicine. Public health policy, is it relates to widely-used antipyretics and clinical disposition toward treating fever, is of particular importance. Finally, we suggest that some hyperthermias, such as psychogenic fevers in humans, post-hunt feeding in cheetahs, and some thermogenesis in plants, may reveal the manner in which adaptations can evolve to deploy DH in an anticipatory manner.

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