INSURMOUNTABLE HEAT: THE EVOLUTION AND PERSISTENCE OF DEFENSIVE HYPERTHERMIA

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abstract

Fever, the rise in body temperature set point in response to infection or injury, is a highly conserved trait among vertebrates, and documented in many arthropods. Fever is known to reduce illness duration and mortality. These observations present an evolutionary puzzle: why has fever continued to be an effective response to fast-evolving pathogenic microbes across diverse phyla, and probably over countless millions of years? Framing fever as part of a more general thermal manipulation strategy that we term defensive hyperthermia, we hypothesize that the solution 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 to be able to function effectively at elevated temperatures, it disadvantages itself at infecting the next (thermonormative) host, becoming more likely to be thwarted by that host's immune system and outcompeted by wild ecotype conspecifics (a genetically distinct strain adapted to specific environmental conditions) that, although 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.

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UNDERSTANDING fever is critical to health and wellness. Suppression of fever in a human population increases disease prevalence and mortality by an estimated 5% (Earn et al. 2014). Most animals that have been studied raise their body temperature above its normal set point in response to infection (Bronstein and Conner 1984; do Amaral et al. 2002). Humans shiver, fish swim to warmer waters, and poikilothermic reptiles crawl to warmer surfaces to accomplish this. This fever response is always costly and often dangerous to the individual. In humans, maintaining a fever of just 2° Celsius requires a 20% increase in caloric consumption (Schumacker et al. 1987; Manthous et al. 1995). Fever is usually associated with fatigue, loss of appetite, and anemia (LeGrand and Alcock 2012). The higher temperatures damage body cells and reduce the effectiveness of organs. Rarely, fever can result in brain damage and death. Among men, fever leads to decreased sperm health and production (Carlsen et al. 2003). Some of the individuals most vulnerable to infection—pregnant women and the elderly—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 across species.

If the first puzzle concerns the phylogenetic breadth of fever, the second puzzle concerns its depth. The phylogenetic breadth of fever suggests that it has long been present in vertebrates. As we will discuss (see the section, Thermal Impairment of Microorganisms, below), most of the pathogens against which fever has utility are fast-evolving microbes such as bacteria and viruses. Although fever is imperfectly effective at combating infections, invasive microorganisms have failed to converge on any counteradaptation that is sufficiently effective as to render fever obsolete, perhaps even over hundreds of millions of years. The status of fever as a master strategy across phylogenetic space and time deserves explication. We propose that fever is a critical component of a broader immune strategy, defensive hyperthermia, and that it is taxonomically widespread and robust over eons because it leverages natural selection against pathogens by creating antagonistic demands during each of the two tasks all pathogens must accomplish: replicate inside the current host and infect the next one. Here, we explore 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 sign of disease, fever has been observed and recorded since antiquity. The oldest surviving word for fever is a sixth-century BCE Akkadian cuneiform inscription (Atkins 1982). In the Old Testament, fever is described as a punishment from God or other spiritual forces. Hippocrates, the father of medical science, was the first to demysticize fever around the fifth century BCE (Gensini and Conti 2004). He assigned it naturalistic, albeit erroneous, causes, namely an imbalance of the four purported bodily humors. Galen of Pergamon, a second-century Roman physician, believed that fever was itself the disease. Because of Galen's eminence, the lack of a scientific approach to fever, and the absence of a proper measuring apparatus (the clinical thermometer was not in common use until the mid-19th century), this view would go unchallenged for at least a dozen centuries (Gensini and Conti 2004).

In the 1840s the physician Ignaz Semmelweis noticed that women who gave birth outside of his clinic had a lower incidence of a deadly fever-causing illness, with the same holding true for their newborns (Semmelweis 1861, 1983). He deduced that the afflictions must have been caused by a transmitted infectious agent. Doctors who treated many patients and also performed autopsies were spreading the microbes that caused disease. Semmelweis' work was

largely ignored until Joseph Lister demonstrated the usefulness of antiseptic procedures in hospital settings and Louis Pasteur isolated streptococci from a woman with puerperal sepsis (Buchanan 1895).

In 1888, William 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, Welch demonstrated that heat itself did not cause the damage observed in autopsied remains of disease victims, and suggested then that fever might be beneficial, outlining the basic modern view of pathogenic febrile response (Atkins 1982). During the 20th century, much progress was made in identifying proximate mechanisms of fever. These included bits of pathogens that tend to elicit fever (exogenous pyrogens) and signaling molecules that ultimately lower body set temperature, such as prostaglandins. However, fever would not be evaluated in the context of evolutionary adaptation until late in the century.

Phylogeny and Prevalence

In a series of animal experiments starting in 1975, Matthew Kluger and colleagues showed that febrile responses to bacteria aided survival in a desert reptile and provided evidence of a beneficial effect of fever in rabbits (Kluger et al. 1975; Kluger and Rothenburg 1979). Once inoculated with killed bacteria *Aeromonas hydrophila*, the *Dipsosaurus dorsalis* lizard behaviorally increased its body temperature by basking or otherwise seeking a warmer local environment. Kluger also infected lizards with live *A. hydrophila*, keeping individuals at different temperatures. Within 24 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, lobsters, shrimp, horseshoe crabs, and crayfish), an annelid (leech), several more reptiles, four species of fish, and five amphibians (Vaughn et al. 1974; D'Alecy and Kluger 1975; Bernheim and Kluger 1976; Casterlin and Reynolds 1977, 1979; Myhre et al. 1977; Reynolds 1977; Glassman and Bennett 1978; Cabanac and Le Guelte 1980; Bronstein and Conner 1984; Louis et al. 1986; Boorstein and Ewald 1987; Cabanac and Rossetti 1987; Cabanac 1989; Kluger 1992; do Amaral et al. 2002). Physiological and behavioral fever response has been observed in birds and many mammals (Boorstein and Ewald 1987). Honey bees exhibit a specialized behavioral response to the fungal brood parasite *Ascosphaera apis* (Starks et al. 2000). The bees maintain a given temperature within a hive, generally higher than the ambient, raising 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 their surroundings. Many burrowing rodents raise their young in a den in which the temperature can be elevated and modulated with radiated body heat. The aforementioned honey bees climate control their brood comb against an invasive fungus; when their cousins, Japanese honey bees, are assaulted by large hornets, they defend their hive by swarming onto the hornets and vibrating their bodies rather than stinging, as their stingers are useless against the predator. They thereby raise the hornet's temperature to over 45°C, killing it. We can thus speak of thermal niche construction, the process whereby organisms control the temperature of their surroundings so as to benefit themselves, sometimes at the expense of other species that would otherwise exploit or compete with them.

When the invader organism or competitor is inside instead of out, the body is the pathogen's microecology, hence the host's adjustments of 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.

Defensive Hyperthermia

Within the range of approximately -10° to 120°C, no single temperature is universally harmful to all living things. Microbes in the Arctic flourish at subfreezing temperatures, while others at thermal vents replicate above the boiling point. A common lay understanding of "fever" is the metabolic raise in set temperature and human-typical symptoms such as body aches, fatigue, and loss of appetite. This conceptualization is too narrow to afford adequate understanding of how and why organisms increase their temperatures. Pathogen-infected fish swim to warmer waters to elevate their temperature. 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 thermoregulation; similarly, species differ as to whether they display associated symptoms typically observed in humans. For these reasons, we refer to protective metazoan fever responses collectively as defensive hyperthermia (DH).

Derived or Conserved?

Researchers have shown that DH is effective against infection in several classes of animals (Braude et al. 1960; Levy et al. 1969; Vaughn et al. 1974; D'Alecy and Kluger 1975; Casterlin and Reynolds 1977, 1980; Reynolds 1977; Kluger and Vaughn 1978; Louis et al. 1986; Small et al. 1986; Cabanac 1989; Whitrow 1990; O'Reilly and Zak 1992; Starks et al. 2000; Robert and Casadevall 2009; for cases in which elevating body temperature seems not to be effective against infection, see Eiseman et al. 1956; DuPont and Spink 1969; Arons et al. 1999; Schulman et al. 2005; reviewed in Eyers et al. 2010), but is the trait derived or conserved in these species? Since most motile animals thermoregulate for nonimmunological 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 (Cabanac and Rossetti 1987; Cabanac and Drolet 1991; Lefcort and Bayne 1991).

Among vertebrates, and especially among mammals, evidence for conserved DH is strong. There is no clear case of a vertebrate that lacks a febrile response. A striking example is the naked mole rat, which does not ordinarily endogenously thermoregulate, instead being a rare poikilothermic mammal. Naked mole rats usually maintain a body temperature about one degree above ambient, whether that is 14° or 28°C (Yahav and Buffenstein 1991). Once infected, however, they develop a fever metabolically (Urison et al. 1993). Similarly, the leech *Nephelopsis obscura* has a weak thermal preference unless inoculated with bacterial endotoxin or prostaglandins (Cabanac 1989).

Some genes and mechanisms for coping with infection are extremely old and highly conserved. Humans and the nematode *Caenorhabditis elegans* have very similar genes for heat shock factor 1 (HSF1). The protostome *C. elegans* and deuterostome *Homo sapiens* diverged from bilateria approximately 670 million years ago (Ayala et al. 1998). In both species, HSF1 mediates an innate immune system pathway that requires temperature elevation to activate (Singh and Aballay 2006). HSF1 is also required for thermotaxis in *C. elegans*, although it is not known if the nematode changes its thermal preference during infection (Kimata et al. 2012). This is consistent with findings that prostaglandin E_1 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 con-

served across phyla. Subsequently, DH may have vanished in clades whose ecology made it unhelpful and, conversely, DH may have further advanced and become 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 proinflammatory features rather than its direct effects on infectious microorganisms. Temperature elevation does upregulate the immune system and therefore helps coordinate the immune response to disease (Hasday et al. 2000; Zhang et al. 2009); however, it also directly impairs, slows, or kills invasive microbes (and tumors, which resemble pathogens in many respects). Host mortality, disease severity, and duration of sickness are all curtailed by hyperthermia. Below we review three lines of evidence supporting these conclusions.

Metabolic Cost

A two-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 et al. 2003). Hence, hyperthermia may directly lower the reproductive component of natural selection.

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. Febrile responses, including DH, are coordinated by such a system of cytokines, interleukins, and prostaglandins. This is not to say that coordinative hyperthermia has no unique advantages compared to other methods of organizing immune response. Hyperthermia is global, simultaneously signaling immune response everywhere in the body (with the possible exception of the upper respiratory surfaces and testes) 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 globally (C. T. Bergstrom, pers. comm.). 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 on pathogens, including lesioning of organelles, damage to DNA, spontaneous membrane rupture, loss of mitochondrial tubules, diminished protein production, and stress-induced apoptosis (Levy et al. 1969; LeGrand and Alcock 2012; Jego et al. 2013; Blatch 2014). Although, in many cases, additional research is needed to distinguish between direct and indirect effects of thermal elevation (see, for example, O'Reilly and Zak 1992), nonetheless, the present literature contains numerous in vitro studies documenting deleterious effects of temperature elevation on multiple pathogenic species.

Two-thirds of malaria parasites (*Plasmodium falciparum*) in blood cells are destroyed after eight hours at 41°C, and none survive at 16 hours in vitro (Long et al. 2001; Oakley et al. 2007). Typical human body temperature, around 37°C, is re-

quired for laboratory cultivation of malaria (Kwiatkowski 1989).

Salmonella typhimurium has been shown in vitro 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 are often carriers (Garibaldi 1972).

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

Among viral diseases, West Nile, yellow fever, vesicular stomatitis, rhinoviruses (the common cold), 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°C, growth almost always diminishes quickly (Bedson and Dumbell 1961; Ruiz-Gomez and Isaacs 1963; Stott and Heath 1970).

Asymmetry of Pathogen Temperature Tolerance

Potential novel hosts with a body temperature significantly different from a pathogen's de facto host are partially or entirely immune to infection (Antonovics et al. 2013; Leggett et al. 2013). 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 temperature is three to five degrees above the optimal temperature for growth, but the detrimental impact of cooler temperatures 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 50 human pathogens respond to adverse environmental conditions by gradually reaching a torpid state (Oliver 2005). The most common impetus is cooler-than-optimal temperature, making such microbes resilient against relatively colder conditions (Oliver 2005, 2010). In contrast, increases in temperature appear to constitute a challenge that is more difficult for pathogens to overcome. For example, pathogenic *Helicobacter pylori,* for which humans are the natural host, survived in vitro 14 days at 4°C but less than one day at 40°C (Jiang and Doyle 1998). This may be a general phenomenon among many microbes and, if so, the thermal properties of DH alone would make it an effective adaptation.

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 relatively cooler humans; equivalent considerations likely explain the otherwise surprising finding that, despite not being highly social, hummingbirds—whose body temperature similarly soars during flight (Morrison 1962)—constitute a reservoir for avian influenzas (Williams et al. 2012).

Between 30°C and 40°C, every degree of increase thermally precludes growth of 6% of fungal species in a study of 4802 strains from 144 genera isolated from soils, plants, insects, and mammals (Robert and Casadevall 2009). 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 apparent with regard to different body parts of the same animal. Rabbits have a high resistance to *Cryptococcus neoformans.* Inoculations fail to lead to illness or mortality, but produce extensive effects on the testes, the organs that are 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 fever (Stott and Heath 1970). Indeed, some rhinoviruses specialize in the nasal cavity, a region where temperature is chronically lower than elsewhere in the body (Roth and Braitman 2008); when they do infect the lungs, these viruses may only be able to do so by virtue of the lower temperatures in the large airways of the lung relative to other tissues (reviewed in Foxman et al. 2015).

Defensive Hyperthermia as One Weapon in the Immune Arsenal

Elevated temperatures can damage harmful microbes, and temperature range is so important that humans are invulnerable to many fungal species simply because of our higher natural body temperature. However, when studied outside the body, medically important human pathogens, including *Escherichia coli*, *Klebsiella pneumoniae*, *Pasteurella multocida*, and *Staphylococcus aureus*, remain viable and proliferate at both febrile and nonfebrile temperatures (Enders and Shaffer 1936; Kuhn 1939; Mackowiak 1981; Jiang et al. 2000). Consider, for example, features that, on the face of it, ought to make *K. pneumoniae* a notable threat. Commonly found in soil, most people are exposed to the bacterium on a regular basis. Outside of the body, it can replicate at febrile temperatures. Although it sometimes causes pneumonia and meningitis, infection does not occur in the overwhelming majority of exposures. Clearly, fever or defensive hyperthermia alone does not explain these observations. It is therefore important to understand DH as a critical component of a broader antipathogenic strategy.

Immune Brinksmanship

The febrile response is sometimes dangerous and always costly. It includes anemia (due to iron sequestration), anorexia leading to cachexic malnutrition and, central to the adaptation at issue, high caloric expenditure to maintain an elevated body temperature (Schumacker et al. 1987; Manthous et al. 1995). 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; and
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 hostile space 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 bacterial pathogens change from noninvasive to invasive mode or, in the case of viral pathogens, when infected cells are changed in a manner that upregulates the cell's stress responses (McInerney et al. 2005; LeGrand and Alcock 2012), (c), these alterations require the production of many new proteins and enzymes; these shifts constitute an extra stressor for the bacterial pathogens or virally infected host cells, stressors not confronted by most host cells. From microbes to human cells, all living things can produce heat shock proteins

(HSP) (d) in order to prevent protein denaturation during high temperatures (Morimoto 1998; Hasday and Singh 2000). However, the immune system has evolved to use many common HSPs as an activational alarm because infected (or neoplastic) cells are more stressed and therefore more likely to produce them. HSPs can activate natural killer cells, function as antigens, make their source cell a target of cytotoxic T cells, and induce macrophages to produce proinflammatory cytokines (Kol et al. 2000). LeGrand and Alcock's model admittedly relies on some unproven claims (such as the relevance of zinc availability), and awaits confirmation that the identified features are adaptations rather than side effects. Despite these limitations, their model is the best available explanation for the central paradox of the febrile response.

If correct, immune brinksmanship answers the first puzzle of fever listed earlier: 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. 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 recently killed more than 8000 people. Why does fever seem ineffective in these cases? Moreover, given such lack of efficacy, why have other pathogens not evolved over time to have similar thermal resiliency?

Evolutionary Dynamics and the Trade Off 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 goals in order to prosper: replicate in the host, and successfully reach and infect the next host. Selection acts at each of these stages, and many factors influence how these interact in the emergence of new pathogens. Seeking to add to factors recognized to date (see Leggett et al. 2012, 2013), we postulate that DH is 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 the presence of higher temperatures if it is to survive and reproduce within the host. However, while evolving to tolerate the elevated temperature makes the pathogen better at accomplishing its first goal, we propose that this necessarily makes the pathogen worse at accomplishing its second goal, for the most basic of Darwinian reasons: we postulate that, all else being equal, the ability to tolerate higher temperatures, via a broadening of the temperature range, or a shifting of the entire range upward, comes at the expense of efficiency in the normal-temperature host. When a fever-tolerant pathogen subsequently reaches a nonfebrile 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 host defenses prior to successful replication and transmission.

In our model, critically, at the population level, a pathogen strain that has an increased 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 infecting the nonfebrile host, they will outcompete the strain that has a high tolerance for fever. More precisely, regardless of the nature of the features that allow a strain to tolerate high tempera-

tures, the normal-temperature strain will spread faster than the fever-tolerant strain whenever the former's superior ability to infect 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—in Park et al.'s (2013) terms, despite having lesser fitness at the within-host scale, the normal-temperature strain ultimately has greater fitness at the between-host scale.

Rapidity of infection and transmission are critical in the competition between pathogen strains because a fever-tolerant strain will tend to be closely related to normal-temperature strains circulating at the same time in a given population of hosts. The corresponding phenotypic similarity between contemporaneous strains is such that, following an infection by whichever strain arrives and infects 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 infect, 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 (Keeling and Grenfell 2002; Riggs et al. 2007; Roberts et al. 2012). 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 that we propose will almost always be 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 outevolved by quickly mutating microbes because DH leverages evolution itself against them. 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.

While exploring factors influencing the evolution of antibiotic resistance, Rodríguez-Verdugo et al. (2013) kept 115 *E. coli* populations at 42.2°C for 2000 generations. These populations originated from clones of a strain that had previously been kept at 37°C for 2000 generations. When the ancestral and daughter strains were placed together at 37°C, the daughter strains suffered a fitness penalty. This penalty did not occur at 42.2°C. Consistent with expectations of the brinksmanship hypothesis, the daughter strains 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 that had not previously been exposed to high temperatures, the modified mutant gained a large fitness advantage over the nonmodified strain in a 42.2°C low-glucose environment. We propose that these patterns are representative—in most cases, the better a 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 evolve improvements in those abilities thus maroon themselves in the given host by virtue of reductions in their ability to compete with unmodified strains for access to new hosts.

The Evolution of Resistance and Compensatory Mutations

Our model is premised on the assumption that pathogens face an intrinsic, largely insurmountable cost to the ability to tolerate higher-than-normal host temperatures. Readers may object that, as illus-

trated by the many well-studied examples of the evolution of antibiotic resistance (Levin et al. 2000), it is possible for pathogens to evolve countermeasures to specific insults, adaptations that, although initially costly, are then complemented by subsequent compensatory mutations that appear to reduce or eliminate their fitness costs. Moreover, there is evidence that even when compensatory mutations are incomplete (such that antibiotic-resistant strains suffer a fitness decrement in vitro), resistance still spreads, arguably because the use of antibiotics creates a preponderance of resistant types at bottleneck points in the infection-transmission chain (Levin et al. 2000).

A recent review (MacLean and Vogwill 2015) suggests that the conventional belief that compensatory mutations obviate the costs of evolved antibiotic resistance may be overstated, as the evidence, primarily derived from laboratory experiments, is contradicted by findings from clinical studies. Consistent with our predictions regarding pathogen microevolution in the face of DH, the authors argue that the disparity between laboratory and clinical studies owes to incomplete eradication of antibiotic-sensitive strains that then outcompete the resistant strains when the antibiotic is no longer present, and to the existence of costs of compensatory mutations that are unobvious or absent in vitro but not in vivo. Compensatory mutations tend to confer adaptive benefits in one environment, which explains some of the experimental evidence. DH may then help explain why such mutations are less likely or less effective in vivo: DH is part of the way the host changes the environment (the host's body), privileging a sensitive wild-type strain that is not paying the costs of resistance or compensatory mutations in the infiltration stage, and then selecting against that sensitivity in the replication stage.

Although the evolution of antibiotic resistance may be more complex than has hitherto been generally appreciated, nonetheless, the fact remains that, in the wild, antibiotic resistance both evolves and spreads. Hence, given that antibiotics are not ubiquitous in the host population, it is clear that, at least in the short term, the fitness disadvantages of resistance do not suffice to prevent resistant strains from succeeding in colonizing untreated hosts. Why then have pathogens been unable to similarly evolve both the ability to apparently resist DH and compensatory mutations that reduce the fitness costs of such resistance, a process that, paralleling the abandonment of older antibiotics, would ultimately have resulted in the disappearance of DH as a weapon against pathogens? We suggest that the key lies, in part, in the options that are available to pathogens as they seek to battle DH, and the liabilities each entails.

It is possible for pathogens to tolerate a range of thermal environments through the combination of robusticity and selective inactivity, such that they revert to an inert or low-activity phase outside of the preferred temperature and simply weather the storm. Alternately, it is possible for pathogens to actively deploy mechanisms to cope with the challenge of a suddenly elevated temperature. The weather-the-storm strategy comes at the cost of transmissibility, as inert or slowly reproducing pathogens are, by virtue of low numbers, less likely to achieve transmission to a new host. Although the active-coping strategy avoids the costs of retreat into inactivity, it entails a formidable liability, namely generating cues that attract and upregulate host defenses. For example, HSP60, a common variant produced by *Legionella*, *Borrelia*, *Treponema*, and *Mycobacterium* organisms, marks them as targets for cytotoxic T cells, and is also highly antigenic. The leukocyte receptor for HSP60 known as CD14 is the same high-affinity receptor of lipopolysaccharide, suggesting that HSP is a reliable cue of infection exploited by the immune system (Retzlaff et al. 1994; Hasday and Singh 2000; Jiang et al. 2000; Kol et al. 2000). Many heat shock proteins also spur macrophages to release proinflammatory interleukins. Fever thus turns heat shock response, a ubiquitous cellular stress response employed by all living things, into an alarm that activates host immune responses.

Importantly, as long as DH retains some utility despite the initial evolution of patho-

gen countermeasures, hosts will continue to deploy DH, pathogens will deploy their countermeasures, and the latter will then provide a target for the evolution of host counter-countermeasures. This configuration likely obtains in most forms of DH, and certainly obtains in vertebrates, in which DH serves the additional function of coordinating a wide variety of immune responses. However, at each step in this arms race, any burden to the DH-resistant pathogen strain entailed by its added adaptations will increase the fitness decrement that it faces when competing with strains that are not resistant to DH to infect the normal-temperature host.

The above considerations indicate that the pathogen-host evolutionary dynamics that obtain in DH differ markedly from the evolution of antibiotic resistance. Pathogens and hosts have been locked in a coevolutionary arms race for a very long time. By virtue of their short generation span, pathogens can rapidly evolve countermeasures against host defenses; when such countermeasures are employed consistently over time (as they will be whenever a given countermeasure initially greatly increases pathogen fitness, and therefore spreads within a pathogen population), they present a possible target for the (much slower) evolution of additional host defenses that exploit the existence of the given countermeasure. Humans have employed antibiotics on an extensive scale for only three to five host generations, and this has coincided with the rise of medical technology that greatly constrains the scope of natural selection acting on our species. As a consequence, humans have not evolved additional defenses that exploit changes in bacteria that endow the latter with antibiotic resistance, thus restricting the fitness costs to pathogens of antibiotic resistance to a much narrower range than will be true of countermeasures to DH.

Defensive Hyperthermia and the Pathogen's Primrose Path

Unlike antibiotics, DH targets a ubiquitous vulnerability, the need to maintain biochemical compatibility with the temperature range of the ecology in which the organism competes for survival and reproduction (or, for some clades such as mammals, to thermoregulate near to a phylogenetically moored minimum). As noted, all living things share some basic thermal coping mechanisms, such as the use of heat shock proteins. Possible adaptations to cope with thermal stress may be placed, roughly, into two classes. The first is relatively quick and easy, upregulate or bolster the thermal coping mechanisms that already exist and do not need to be evolved de novo, and perhaps subsequently develop compensatory mutations. Experimentally, these sorts of adaptations in microbes are commonly observed as the environment gets warmer (Rodríguez-Verdugo et al. 2013). A second class includes more substantial changes that permit a pathogen to flexibly withstand and prosper in the higher and lower temperature conditions. This is certainly possible, as demonstrated by several species of malaria (Kwiatkowski 1989). However, such strategies require many more mutations working in concert. In malaria, the sexual morph alone expresses 315 proteins not produced by any other form (Bousema and Drakeley 2011). By manipulating a coping mechanism that a pathogen is assured to have, DH leads it down the primrose path of that first class of coping strategies. Since the hyperthermia is not a consistent feature of the host, or one generally present during initial contact and infiltration by the pathogen, the second class of superior but slower-to-evolve coping strategies is much less likely. The readily evolved upregulations of mechanisms for coping with heat are thus a trap—they both afford the evolution of host counter-countermeasures and, we conjecture, reduce the pathogen's ability to infect the next normal-temperature host.

How do the postulated tradeoffs between thermal generalism and thermal specialization, and between the ability to resist DH and the ability to infect normal-temperature hosts, play out at the microevolutionary level? Below, we consider two pathways whereby selection may operate in a manner that preserves the utility of DH.

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 nonfebrile hosts. As noted, Rodríguez-Verdugo et al.'s results (2013) reveal the evolution of specialization for elevated temperature in *E. coli* after 2000 generations at 42.2°C. The authors, who aimed to explore antibiotic resistance rather than thermal specialization, 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 six to seven 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. (2001) obtained *E. coli* and *Salmonella enterica* from natural populations of turtles, repeating the process over 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 to generate cyclical changes in the prevailing pathogen phenotype. If this interpretation is correct, then such selection would be unlikely to operate over the time course of DH during infections by pathogens subject to equivalent combinations of strength of selective pressure, generation time, and duration of selection.

At present, the possibility that thermal flexibility in pathogens is disfavored due to selection during DH for high-temperature strains remains speculative. An alternative is 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. (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 1000–2000 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 (possibly because heat shock is addressed through changes in membrane lipids, alterations that, in turn, reduce protein production and secretion; see Yuk and Marshall 2003). The latter pattern reveals that thermal flexibility comes at a cost, such that the better equipped the pathogen is to tolerate febrile temperatures, 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 may be importantly constrained by selection for infectivity, limiting thermal flexibility in a manner that preserves the utility of DH.

Temperature-specific morphs can evolve in macroorganisms when temperature varies regularly across generations (reviewed in Fusco and Minelli 2010), and many species of bacteria are 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. Why then do pathogens not generally solve the problem of DH using a strategy of multiple temperature-specific forms? As noted above, unlike the limited thermal plasticity evident in *E. coli,* malaria possesses multiple thermally specialized

morphs. Critically, however, the two organisms differ 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. 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 that 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 completes sexual reproduction only after transmission to the mosquito vector. Therefore, selection is strongly acting on malaria parasites that have passed through all of the host ecologies: the febrile and nonfebrile human as well as the mosquito. This is a sharp contrast to fast-reproducing bacteria or viruses on which selection is acting across multiple generations inside a single host.

Hypothermia

If DH is effective because it creates a host microecology that is thermally inhospitable to pathogens, we can 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 *hypo*thermia 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 subsequently resisted 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 fewer such benefits, and could even advantage the pathogen, as many pathogenic microbes are less impaired by below-optimal versus above-optimal temperatures. However, although hypothermia cannot serve as a primary defense, 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 (bodywide inflammation) following the 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 bumble bees, and cold-seeking behavior has been observed in septic human patients, mice, and bees (Blair et al. 1964; Habicht 1981; Müller and Schmid-Hempel 1993; Romanovsky and Székely 1998). In all cases, hypothermia reduced mortality rates. On the basis of such evidence, Romanovsky and Székely (1998) propose a bimodal model of thermoregu-

latory inflammation management. They argue that fever is adaptive when general health and nutrition are adequate, and the infection is of a manageable scale. However, if an individual is starved or in an already subnormal temperature environment, 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 require less oxygen. Romanovsky and Székely demonstrate that rats given small or moderate doses of bacterial lipopolysaccharide (1 or 10 micrograms) develop fever, but rats given 1000 micrograms develop hypothermia and diminished motor activity. Presumably, the salutary effects of hypothermia constitute another (albeit last-ditch) form of immune brinksmanship in which the costs to the pathogen exceed those to the host, but it is not entirely clear what effect cooler temperatures have on pathogens in vivo.

Interhost Dynamics: Positive Externalities and Superinfection by Related Strains

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 nonfebrile hosts. For this to be true, either some transmission must occur prior to the destruction of all normal-temperature variants within the host via DH (i.e., prior to complete within-host selection for heat tolerance), 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 both. 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 bearer's social group. 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 generates a positive externality for conspecifics. This aspect is not required for DH to be subject to positive selection, as it is in the 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 by related strains occurs with the proper timing, might the tables 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, although 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 hostwide 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 a fever), 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 latecomer 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. Although this can aid the fever-tolerant type in the short run by eliciting a fever 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.

Fever Suppression

Inadequate attention to the nature and dynamics of DH may undermine clinical efforts (Cannon 2013). In a sample of children with severe pneumonia, those who did not exhibit fever were more likely to die (Shann et al. 1989). Antipyretic drug treatment has been reported to prolong influenza and *Shigella* infection, as well as chicken pox in a randomized, double-blind, placebo-controlled study (Doran et al. 1989; Plaisance et al. 2000). Aspirin and acetaminophen treatment increased the duration of rhinovirus shedding in a randomized, double-blind, placebo-controlled study (Graham et al. 1990). Schulman et al. (2005) terminated their randomized study comparing outcomes for intensive care unit patients given either aggressive antipyretic (drug and mechanical) treatment or not when the first interim analysis revealed a trend toward high mortality in the antipyresis group. A model based on epidemiological data indicates that use of antipyretic drugs leads 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). Indeed, to the extent that, as Cooper et al.'s *E. coli* results discussed above suggest, DH selects for a limited degree of thermal generalism at the expense of maximal infectivity, the widespread use of antipyretics may actually be favoring the evolution of greater infectivity in a variety of pathogens. It remains common practice for physicians to regard fever as a detriment calling for alleviation, even though there is little clinical evidence that this improves patient prognoses (Mackowiak 2000a,b; Blomberg et al. 2003; Best and Schwartz 2014). 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.

Many cultures have not only recognized the beneficial effects of fever, but treated fever and a variety of ailments with thermotherapy, artificially raising body temperature by external means (Bierman 1942; Atkins 1985). This includes the Greeks, Romans, and Egyptians from the fifth century BCE on, precolonial Native Americans, and the ancient cultures of Japan and China (Bierman 1942). 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.

The 1927 Nobel Prize for medicine was awarded to Julius Wagner-Jauregg for the development of the practice of infecting a patient suffering neurosyphilis with malaria

to induce repeated hyperthermic states. At the time, 10–20% of inmates in mental institutions were patients with syphilis infecting the brain, 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 40 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 waned. The absence of consideration of the effects of temperature is notable in the case of Wagner-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 (because malignant tumor cells replicate faster than normal cells, the logic of brinksmanship applies to them just as it does to pathogens). Although it is clear in his own data, Coley never noticed that remissions correlated strongly with the intensity and frequency of fevers in his patients, nor did he suspect that temperature itself was curative against tumors, something that the ancient Greeks and Romans believed (van der Zee 2002). In contrast, Wagner-Jauregg recognized the clinical importance of fevers and noted that their severity predicted remission among the syphilitic; nonetheless, like Coley, he suspected that the curative mechanism was a result of toxins produced by the pathogens, not the temperature (Whitrow 1990). Coley and Wagner-Jauregg notwithstanding, many physicians of the 19th and early 20th centuries suspected that hyperthermia itself was the treatment. A 1936 editorial in *California and Western Medicine* called hyperthermia "an established therapeutic procedure" (Epstein 1936:357) 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 1970s (Baronzio and Hager 2006).

Thermotherapy may also have faded from prominence due to advances that provided alternatives: vaccines, chemotherapy (itself a form of brinksmanship), sophisticated surgical techniques, and advances in understanding 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, synthesized, and made readily available. With fever's connection to decreased mortality and morbidity obscured by new treatments, analgesics had no apparent downside.

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 may inadvertently take an antipyretic because all over-the-counter products designed to relieve cold or flu symptoms, including all pain relievers (aspirin and all other salicylates, and the class of drugs that includes ibuprofen and acetaminophen), are also antipyretics. There are presently no pain relievers available in the drugstore that do not reduce fever because the regulatory pathways for fever, inflammation, and pain sensitivity are closely connected.

Although thermotherapy may yet enjoy a renaissance, lessons learned from studies of the evolution of drug resistance suggest that caution is in order. Consider that the previously discussed experiments with *E. coli* (Tenaillon et al. 2012; Rodríguez-Verdugo et al. 2013) reveal convergent evolution for a small number of genes conferring not only heat tolerance, but antibiotic resistance. This can occur because brinksmanship stressors ultimately succeed by slowing replication and 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 HSP90 and HSP110 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 possible unintended consequences so as not to repeat such mistakes as the overprescription of antibiotics.

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). Rodents' temperatures rise when handled by humans or confronted with a novel open field environment. The posthunting rise in cheetah body temperature is not caused by the act of sprinting, as previously believed (Hetem et al. 2013). Human psychogenic fever has been documented in a wide variety of stressful situations. 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. These psychogenic fevers are generally attributed to a stress response. Although 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 frontline defense against infections, this is evidence that the stress response in humans is partly immunological in nature, and is consistent with the hypothesis that psychogenic fever is a form of DH.

Conclusion

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. We have reviewed evidence from human and animal models, controlled clinical experiments, and biochemical mechanisms that suggest that suppressing fevers 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 for its evolutionary heritage likely extending back to early forms of life capable of thermoregulation. 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 to place it within a broader evolutionary and ecological context, to foster understanding of its persistence, 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 the host's temperature 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 febrile 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; likewise, pathogens that specialize in higher temperatures suffer a reduced capacity to infect healthy hosts. Competition among variants racing to infect new hosts thus constrains the evolution of both thermal flexibility and specializa-

tion in elevated temperature, thereby preserving the efficacy of DH as a host tactic.

Our account of DH is testable via a number of avenues. First, our informal verbal model can be tested using formal mathematical models by building on existing models of the evolution of thermal specialists and thermal generalists and the determinants of thermal reaction norms (see Gilchrist 1995; Angilletta et al. 2003). Second, the postulated process of the evolution and rapid extinction of pathogen strains able to tolerate febrile temperatures can be tested experimentally in laboratory animals, perhaps accelerating the process by artificially elevating host temperature for prolonged periods using exogenous pyrogens, then reintroducing the wild type in competition for infection of naïve hosts. Third, examination of hospital settings may reveal that, when febrile patients suffering different diseases are insufficiently isolated from one another, the presence of naïve high-temperature hosts allows for the evolution and transmission of pathogen strains specializing in elevated temperature. Fourth, although we have focused on systemic temperature elevation, the logic of DH also applies to increases in temperature confined to the vicinity of an infection. However, to reap the benefits of DH at a lower cost than systemic temperature increases, local temperature increases must encompass an area larger than the site of the infection to prevent the escape of pathogens into normal-temperature tissue. It should be possible to model the optimal size of the requisite buffer region, then compare this with experimental and clinical observations. Lastly, our model suggests that important new insights into the dynamics of host-pathogen coevolution may derive from studies examining tradeoffs in ecological succession between pioneering species and steady-state species (e.g., Connell and Slatyer 1977).

Beyond the immediate questions of the evolutionary persistence and near-ubiquity of fever, 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 as 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, may reveal the manner in which adaptations can evolve to deploy DH in an anticipatory manner.

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