Bugs and chills; the impact of temperature shifts on viral respiratory tract infections

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Abstract

Overdue weight seems to have been given to early volunteer experiments with viral respiratory tract infections (VRTIs) that may have used recycled viral strains that had accidentally lost their temperature-sensitive character. Perhaps for this reason, science has closed its eyes to detailed and clear-cut evidence that epidemics of VRTIs are closely correlated with ambient temperature, and that individuals are more likely to develop VRTIs after chilling. Moreover, the seasonality of colds and influenza is unexplained. In this review I note that diverse viral species cause very similar VRTIs, that the incubation periods of VRTIs may frequently be underestimated, and that colds and influenza frequently infect only a subset of the susceptible individuals who are exposed to them. Biochemical analysis may have been hindered by the use of laboratory respiratory viruses that were propagated in conditions that reduce temperature sensitivity. Nevertheless, many biochemical studies show decreased viral activity at elevated temperatures. I propose here the hypothesis that viruses may locate the upper respiratory tract by its lower temperature, that temperature changes are often used by viruses to synchronize their development at one or more steps in their life cycles, that temperature changes can allow temporary increases in the level of virulence of viruses, and that temperature changes provide a mechanism whereby a subset of susceptible individuals can become infected.

Key index phrases

Respiratory tract infections, viral infections, temperature changes, viral epidemiology, new hypothesis.

Introduction

Epidemiological anomalies that need to be explained

Before the discovery of influenza A virus in 1933, many physicians doubted that influenza was contagious. For example, in 1775 Thomas Glass of Exeter wrote of influenza, 'Nor does this distemper seem to arise ... from contagion. For in this city, in the year 1729, it was conjectured that two thousand persons at least were seized with it in one night' [1]. August Hirsch in 1883 doubted that influenza was communicable because it spread "quite independently of intercourse" [1]. Today, in spite of intense study and public interest, it has often been noted that influenza and other VRTIs spread in patterns that are difficult to predict or explain [2]. Hope-Simpson listed several unexplained features of the epidemiology of influenza A, including its reappearance after long absences, often explosively over wide areas at the same time, and also the low attack rate within households, often contrasting with a high attack rate within institutions [3]. For example, he found that during the "Hong Kong" (H3N2) epidemics of 1968/69 and 1969/70, 70% of infected households in Cirencester (UK) had only one case of influenza (out of a total of 134 households that were infected) [3]. This low attack rate within households could not be explained by the ages or susceptibility of patients; the H3N2 subtype arrived in Britain for the first time in 1968, and 70% of patients in the first two

epidemics in Cirencester were adults [98]. The low attack rate contrasts with e.g. measles, where up to 90% of susceptible persons who are exposed to the measles virus develop the disease [12]. Moreover, measles and similar diseases mainly infect young children, who have not been exposed to the disease and therefore have no immunity. Although children often have a higher infection rate than adults, influenza infects all ages. For example, 76% of patients infected by influenza A (including both H2N2 and H3N2 strains) in Cirencester between 1960 and 1976 were aged 16 or over [98].

Van Loghem found similar patterns of infection with common colds in a very large survey in Holland in the 1920s [4]. A more recent study in Chicago noted great variation in the transmission of respiratory viruses in a school that was unexplained [5]. Of the 14 viruses identified, seven spread to one or more children, and three of these spread quite extensively, infecting from 42% to 77% of the children. However the remaining seven viruses infected one child only. The difference was not explained by the characteristics of the associated illness, patterns of viral shedding, or levels of immunity.

Another puzzle is the seasonal appearance in temperate regions of colds and influenza each winter, so familiar that we take it for granted. Two recent reviews concluded that the seasonal occurrence of influenza cannot be explained by existing proposals [94, 95]. Tamerius *et al.* noted that differences in "crowding" between summer and winter are minimal since the amount of time people spend indoors in e.g. the USA varies by less than 10%, and that school term-times are not well-correlated with influenza prevalence. They also note that differences in viral survival outside the body e.g. due to changes in absolute humidity cannot explain influenza seasonality in the tropics [94]. Lofgren *et al.* also concluded that theoretical and empirical studies do not adequately explain influenza A seasonality, noting that no studies have been published that directly show that variations in crowding cause influenza seasonality, and also that a linkage between viral evolution and the wide assortment of other potential factors in influenza seasonality is lacking [95]. The inability of such proposals to explain simultaneously both the seasonality VRTIs and their occurrence in the tropics will be discussed in detail below. (Lofgren dismissed decreased ambient temperature as a cause of influenza seasonality since no direct biological justification for this effect has emerged.)

Since scientists have witnessed many instances of viruses jumping species barriers [9, 11, 26, 27], it is likely that all present-day viruses have gone through many cycles of increased and decreased virulence in their histories. This is discussed in section S1 of the Supplementary Information. One can therefore speculate that biochemical routes exist that allow the level of virulence to respond quickly to selective pressures. Viruses are relatively simple entities with high mutation rates, so it is likely that adaptation is by genetic mutation, but even one or two amino acid substitutions or small changes to RNA secondary structure can have substantial effects on temperature sensitivities [17, 28]. Moreover, it is clear that many RNA and protein sequence changes have relatively high probabilities of reversion. For example, substitutions of amino acids that are caused by single-nucleotides point mutations can be reversed by restoring the original nucleotide.

Some of the viruses that infect the human respiratory tract and are spread by coughing, sneezing, and runny noses are shown in table 1. It is striking that many unrelated strains have very similar lifecycles and produce indistinguishable symptoms, especially in the early stages of infection. Over 200 serologically distinct viruses

are responsible for human upper respiratory tract infections [29]. The clear seasonality of the common cold and most other VRTIs [4, 107] in spite of this variation suggests that respiratory viruses occupy a very successful and well-defined ecological niche. The number of different routes for human viral infection is limited, and most other routes (such as faecal-oral transfer, sexual transmission, transfer in saliva, transfer in blood - for example in fights, and the transfer of viruses causing warts and cold sores by skin contact) can be eliminated by modern preventative measures. It is difficult, however, to eliminate the transfer of VRTIs in aerosols, large droplets, or by physical contact [30].

Review

hSpecial cases: persistent viral infections in cell cultures and animals

In an interesting review of 1975 [15], Preble and Youngner suggested that viruses are subject to unusual selective pressures when they establish persistent infections in cultured cells, or when they become dormant in animals. These pressures often give a surprising result: temperature sensitive (*ts*) strains appear spontaneously, where the viruses involved are more active at lower temperatures. Viruses from persistent infections may also possess increased numbers of defective interfering particles (DIPs). (DIPs are viral particles with incomplete genomes that parasitize the virus, which is itself a parasite.) The authors suggest that both mechanisms can damp down an infection and allow a stable infection to become established (although they do not explain why *ts* mutations in particular should be selected, as opposed to other non-*ts* attenuating mutations). They reported the spontaneous generation of *ts* mutants in cell cultures with persistent infections of Newcastle disease virus, Western equine encephalitis virus, Sendai virus, measles virus (in both human and hamster cells), stomatitis virus, Sindbis virus, and a bacteriophage [for references, see 15]. Three more recent reports described the recovery of spontaneously-generated *ts* strains of influenza A from persistent infections of cell cultures [16, 17, 110].

Preble and Youngner noted in their review [15] that foot-and-mouth virus that was recovered from carrier cattle after several months was more *ts* than virus recovered from acute infections. Foot-and-mouth disease is an interesting example of a (partly respiratory) disease that often gives rise to persistent infections. It is often spread by aerosols and the early replication sites of the virus are typically the lungs, pharynx, and soft palate [18]. After causing a high fever for two or three days, the virus causes blisters on the lips, in the mouth and on the feet. Note that these are the parts of the body that tend to be at lower temperatures, especially if the animal cannot breathe through its nose. Profuse nasal discharge is a common symptom of the disease [19]. Infected animals frequently become disease carriers, and quantitative RT-PCR has indicated that the main sites of viral persistence are the tongue and feet [20]. As well as frequently being *ts* [21], viruses recovered from carrier animals show evidence of high rates of mutation with frequent amino acid substitutions and rapid antigenic variation [22]. The implications of these findings are discussed below.

Preble and Youngner also noted [15] that many stable infections of cell-cultures by viruses involved increased production of DIPs. This may be an alternative mechanism for reducing viral activity that is not related to temperature sensitivity. Several examples are mentioned in the Supplementary Information, as well as a case where loss of DIPs in a wild virus gave dramatically increased virulence in avian influenza.

The loss of naturally occurring ts phenotypes in conditions that select rapidly growing variants

Since *ts* mutations generally slow up the replication of viruses, we might assume that selective pressures when viruses replicate rapidly in previously uninfected cells will lead to the loss of *ts* mutations. This is the reverse of the selective process put forward by Preble and Youngner to explain the prevalence of *ts* strains in persistent infections. Remarkably, such selection has been observed by chance on at least two occasions. Chu *et al.* [23] found a naturally-occurring *ts* influenza A strain that was a subclone of the H3N2 strain Ningxia/11/72. When they passaged the strain three times through chicken embryos at 33°C, a non-ts strain was produced. Similarly, Oxford *et al.* [24] found that a naturally-occurring *ts* virus, A/Eng/116/78 (H1N1), progressively lost its *ts* character during five passages at 33°C. Both groups concluded that even at the permissive temperature (33°C) the *ts* character may confer a selective disadvantage in eggs. These studies are described in more detail in section S3 of the Supplementary Information.

Viral responses to temperature shifts – a hypothesis

I suggest that many respiratory viruses use temperature shifts to synchronize development at critical stages in their life cycle. The temperature shifts could in principle be upwards or downwards, and might involve one or more cycles (warm – cold – warm – cold etc). Most reported naturally-occurring mammalian *ts* strains are more active at lower temperatures. However, the opposite sensitivity has been reported in avian viruses, i.e. greater activity at higher temperatures [65, 111]. Sensitivity to both heat and cold may be important in the replication of e.g. human viruses. The biochemical evidence reviewed below suggests that entry into the cell, the initiation of transcription, and the initiation of genome replication are important steps that can be regulated by temperature.

Such temperature sensitivity might give respiratory viruses several important advantages: firstly, virus numbers can build up before a temperature shift, such that when the shift eventually occurs viruses move out of (or into) cells in rapid bursts that can overcome their hosts' immune defences. This argument is similar to the explanation given for the synchronized hatching of mayflies when they emerge from lakes in order to breed: by synchronizing their appearance they increase their chances of evading predators. Similarly, viruses may evade the host's immune system by e.g. emerging from cells in bursts. Secondly, since human family and community members have different roles and activities, they generally experience different patterns of temperature exposure. Therefore a subset of the group may become infected by a *ts* virus at a given time during an epidemic, which can allow the virus to linger for longer and leave some individuals as potential hosts in future epidemics. Thirdly, temperature sensitivity may allow a virus to increase its virulence temporarily. In particular, virulence may increase during an episode of chilling, allowing replication and transmission, but may decrease when the chilling ends, preventing its host from dying or becoming immobilised. A fourth advantage is that *ts* mutations may help viruses to establish persistent infections. We can speculate that viruses that cause diseases that become asymptomatic such as foot-and-mouth disease need to reduce their activity to evade detection by the host's immune system - less active viral clones may remain undetected and therefore be selected, while more active ones are destroyed. Any mutation that damps down activity could be used, but *ts* mutations have the advantage of encouraging the concentration of viruses in the coldest parts of the body. I suggest that *ts* forms of many respiratory viruses can readily be generated by limited nucleotide substitutions in as a result of their evolutionary

history, and that this explains why they crop up so frequently. The tropism of foot-and-mouth disease, discussed above, seems to be a clear example, but the propensity for many respiratory viruses to establish persistent infections in laboratory cell cultures using *ts* mutations suggests that many other viruses, including influenza, have similar tendencies. This point is also related to the first point, above: if viruses use *ts* mutations to colonize the coldest parts of the body, they may automatically possess a mechanism that allows them to be reactivated when their temperature changes (possibly rising or falling) and they can emerge in a burst, which may increase their chances of survival. To use another analogy, the establishment of persistent viral infections may be like the strategy followed by a plant that produces a batch of seeds that become dormant and germinate over several years. This can decrease the probability that the whole batch will be destroyed by a catastrophe after germination such as drought or late frost [31]. Similar reasoning suggests that dormancy may help viruses to achieve long-term transmission. (Human respiratory viruses may become dormant in the upper respiratory tract, but in principle they could become dormant in other parts of the body. This dormancy outside the respiratory tract may not benefit respiratory viruses in humans, but it may have allowed the survival or transmission of ancestral viruses in other species and been inherited when the viruses crossed to humans. This is discussed in section S8 of the Supplementary Information.)

In addition, temperature can obviously be used to identify the respiratory tract during infection, since the temperature of the upper respiratory tract is below the general body temperature. For example, if a group of (heat-sensitive) *ts* virus particles are inhaled into an animal's lung they will remain inactive since the temperature of the lung in mammals is very close to body temperature. If they reach the blood stream (discussed below) they will also remain inactive, encouraging the host to remain active and potentially spreading the virus. Alternatively, they may be carried to the cooler [32] upper respiratory tract by the mucociliary escalator. There they may attach themselves and invade the cells of the lining of the respiratory tract, possibly forming a cluster. (Note that viruses that enter the watery "sol" mucus layer may not be carried out of the lungs but may remain close to the cells lining the respiratory tract. In fact it seems likely that viruses could spread via the sol layer.) Bear in mind that there is a temperature gradient within the respiratory tract, with temperatures in humans varying from around 24°C at the glottis to around 35.5°C at the subsegmental bronchi [32] (these measurements were made with 19°C air that was inhaled at a rate of 30 ventilations per minute). Mudd and Grant showed in 1919 that cooling the skin of human volunteers caused rapid cooling and constriction of blood vessels in the pharynx and tonsils [108]. Therefore chilling may allow activation of one or more clusters of *ts* viruses. This mechanism can work well if binding to cells (or entry into cells) and activation of viruses are both temperature-sensitive processes, but the binding temperature is the higher of the two. A model based on this mechanism is shown in Figure 1. It should be remembered that during the first few infective cycles the virus may mutate to produce progeny with less restrictive temperature sensitivity that can infect other areas. Note also that this mechanism can apply in the tropics since viruses may adapt to warmer temperatures by colonizing parts of the respiratory tract nearer the nose. Temperature dips below normal tropical temperatures can then allow viral activation in spite of warmer ambient temperatures. (Obviously viruses with higher *ts* transition temperatures may also evolve in the tropics.)

At the biochemical level, activation might involve any critical step in viral development that is *ts*. It could occur e.g. when previously-bound viruses enter cells, at the initiation of transcription, when the transition

from the production of viral proteins to viral genome replication takes place, or when virus particles are released from cells. Biochemical evidence for several *ts* steps is discussed below and in the Supplementary Information.

The economic impact and human cost of VRTIs

The direct medical costs of influenza in the USA have been estimated to be over $10 billion annually [33]. Projected lost earnings due to illness and loss of life from the disease amounted to over $16 billion. Worldwide, influenza epidemics result in about three to five million cases of severe illness each year, and about 250 000 to 500 000 deaths [34]. A 21st century estimate of the global mortality from the 1918-1920 "Spanish" influenza pandemic put the total at 50 to 100 million [35]. Other VRTIs also have high costs. The economic cost of lost productivity due to the common cold is close to $25 billion [36].

Factors that may have hidden respiratory tract viruses' sensitivity to temperature shifts from researchers

The importance of viral temperature sensitivity may have remained unrecognized by scientists for several reasons. Firstly, the growth of many viral strains may require several temperature shifts that are spaced out in time. For example, if a group of volunteers are inoculated with a respiratory virus and subjected to chilling, the number of colds may not be affected unless temperature is cycled several times over days or weeks.

Secondly, many researchers deliberately work with artificial *ts* mutants, either for biochemical studies or to make vaccines. This may have obscured the frequent temperature-sensitivity of wild-type viruses. This raises the intriguing possibility that some laboratory *ts* mutants may be closer to actual wild-type viruses than the corresponding lab strain that is regarded as "wild-type". Researchers have also focused on the factors that prevent viruses from transferring between species, including the temperature of sites where viruses replicate [37], rather than the effect of temperature *fluctuations.*

A third reason is the high mutation rate of viruses, which ensures that they adapt quickly to new growth conditions. Many laboratory viruses were isolated from wild strains more than 60 years ago, and have been passaged hundreds of times. For example, Chu *et al.* noted that the WSN virus strain in their lab had "undergone many passages in ferrets, mouse lung, mouse brain and chicken embryos" [23]. When the discovery of human coronaviruses was first published, the strain had already been passaged seven times in various human cells [38]. In influenza A, 22 codons in the HA1 segment of HA (hemagglutinin) have been identified that frequently mutate in embryonated chicken eggs [39]. Another study found that after the human influenza virus A/Fujian/411/2002 (H3N2) had passaged six times in eggs both the HA and NA (neuraminidase) protein sequences were altered, with four amino acid substitutions in HA and one in NA [40]. It is clear that the virus rapidly adapts to new hosts by the mutation of residues in critical positions in viral proteins. Moreover, changes to non-coding RNA and synonymous mutations that do not result in amino acid substitutions may change the secondary structure of RNA. The ordered RNA sequences that are the targets for such mutations are relatively large, which increases the probability of mutation. As discussed

below, it is likely that changes to RNA secondary structure can influence the temperature-sensitivity of viruses.

The prevalence of naturally-occurring ts viral strains and the temperatures that are used for virus isolation and propagation

If the model presented in Figure 1 is correct, it should be easier to propagate viruses sampled from patients by incubation at lower temperatures than at 37°C. This is in fact often the case. Rhinoviruses were first isolated at 35°C but a greater variety of rhinoviruses was discovered at 33°C [41], and this is the temperature that is recommended today for their isolation by the Clinical and Laboratory Standards Institute [42]. Coronaviruses were first isolated at 33°C [38] although laboratory strains are now frequently propagated at 37°C. Naturally occurring influenza strains are also frequently temperature-sensitive. For example, in 1962 Stern and Tippett [43] collected four viral specimens from patients with H2N2 "Asian" influenza, all of which were *ts*. All four isolates gave cytopathic effects in monkey cells and agglutination in eggs at 33°C but not at 37°C. Subcultures were able to adapt to culture at 37°C but grew more slowly than at 33°C. The authors also found that (in 1962) FM1 and PR8 also grew more slowly in monkey cells at 37°C than at 33°C. In 1977, Kung *et al.* found that nine of ten isolates of the newly emerged "Russian" H1N1 influenza were *ts* [44]. Oxford *et al.* found that 17 of 26 recent H1N1 isolates, and 2 of 11 recent H3N2 isolates were *ts*, producing at least 10 times more viral plaques at 34°C than at 38.5°C [24]. Chu tested seven H1N1 strains with varying degrees of temperature sensitivity in volunteers and found a correlation between temperature sensitivity and the severity of VRTI symptoms [23]. These studies are described in more detail in section S4 of the Supplementary Information.

Today, however, influenza A is frequently isolated from patients by propagation at 37°C [42]. This may be because the normal interactions with human cells that give temperature sensitivity are not present in cell cultures.

Evidence that exposure to cold can promote VRTIs.

Van Loghem carried out a very extensive survey of the common cold during the winter of 1925/26, with data from almost 7,000 participants who were distributed throughout Holland [5]. The data showed that (1) the development of cold epidemics in various part of Holland was synchronized, (2) the number of colds was closely and inversely correlated with outdoor air temperature (lower air temperature gave more colds) as shown in his Graph 1 (reproduced in Figure 2 of this paper), (3) a better correlation was observed in the months up to the end of January, (4) the various forms of "colds" (coryza, bronchitis, angina, influenza, laryngitis) were highly correlated with each other and inversely correlated with air temperature, and (5) the chance that family members would get colds was almost the same for small families as for large families. Van Loghem concluded the colds were not spread by contagion during the period of observation, but that microbes that were previously present as commensals were able to cause VRTIs because of disturbances to thermoregulation in their hosts [5].

Hope-Simpson carried out a similar survey in Cirencester (UK) in 1954 and 1955 [107]. Again a close negative correlation of colds with ambient temperature could be seen. Figure 1 of his paper is particularly striking. It

is noticeable in these data that cold epidemics are particularly related to temperature *drops*, with rapid drops followed one to three weeks later by increases in colds; constant low temperatures tend to be followed by a reduction in colds in Figure 1. It is again noticeable that the correlation is better before the end of January.

Mourtzoukou and Falagas reviewed the evidence that chilling increases the risk of developing VRTIs and dying from them, and concluded that the general public and public health authorities should reduce exposure to cold to prevent increases in morbidity and mortality due to VRTIs during the winter months [45]. Hajat *et al.* found [46] that both upper and lower respiratory tract infections were associated with cold weather, with general practitioner consultations for lower respiratory tract infections in one UK City (Norwich) increasing by 19% for every degree that average temperature dropped below 5°C, observed 0-20 days before the consultation.

Table 2 shows data generated by the Eurowinter Group [47]. The table shows the regression coefficients (R) and their significance (p-value), for indices of respiratory disease-related mortality on personal cold-exposure factors standardized at 7°C mean daily temperature with allowance for age and sex. These data are plotted in Figure 3. It can be seen that the indices for respiratory disease were positively related to the fraction who shivered while outside ($p<0{\cdot}001$) or who kept still for at least two minutes ($p<0{\cdot}04$). The indices were also positively related to wearing a sweater, overcoat or skirt, probably because these items are not usually worn with the more protective anorak and trousers. They were negatively related to heat stress sufficient to cause outdoor sweating ($p<0{\cdot}02$) and to the wearing of anoraks, trousers, and hats outdoors ($p<0{\cdot}004$ for all). Warm living rooms were also protective ($p<0{\cdot}001$). The observation that outdoor sweating is protective can be related to the proposed model shown in Figure 1: breathing cold air for a limited period may activate a small proportion of the viruses that are immobilized in the respiratory tract, while the heat stress ensures a plentiful blood supply to the respiratory tract and an efficient immune response (whereas chilling reduces the blood supply to the respiratory tract [54, 92]).

Several studies found that the induction of hypothermia in patients suffering from brain or other injuries increased the likelihood of also contracting pneumonia. Yanagawa *et al.* examined a group of patients who had suffered cardiopulmonary arrest but had a spontaneous return of circulation [48]. Eleven of 13 patients who were treated with mild hypothermia developed pneumonia, as compared to 6 of 15 controls who were maintained at normal body temperature ($p<0.02$).

The seasonality and transmission of VRTIs

Tamerius *et al.* reviewed the many mechanisms that have been put forward to explain human influenza seasonality [94], including factors that change contact rates (school closures, ambient temperature and precipitation), factors that influence virus survival outside the body and transmission rates (relative humidity, absolute humidity, solar radiation and temperature) and factors that change in the immunity of hosts (humidity, photoperiodicity, temperature, viral interference, and selenium, vitamin C, vitamin D [49] and vitamin E deficiency) [for references see 94]. However these mechanisms cannot explain seasonality in influenza while being at the same time compatible the moderate levels of influenza that are encountered in the tropics year-round [94]. (Moreover vitamin D deficiency cannot explain why colds and influenza

epidemics begin in the early autumn before vitamin D deficiencies can arise from lack of sunshine. For example, "Spanish" influenza appeared simultaneously in Alaska, Spitsbergen (Svalbard) and Kentucky during September to October 1918 [10], presumably as a result of temperature falls during those months.) Compared to summer conditions in temperate zones, all of the proposed factors have values that are either similar or more extreme in the tropics. If influenza is virtually eliminated in the summer in temperate zones by these factors it should not be present in the tropics at all. In reality there are as many cases of influenza in the tropics as in many temperate regions (for example, compare Singapore with Sidney, Australia, in Figure 1 of the publication by Tamerius, [94]), although in the tropics cases are often spread throughout the year. Explanations that suggest that viruses in the tropics differ from those in temperate and polar regions are incompatible with the prevalence of modern air travel, since travel to or from the tropics does not give dramatic increases in VRTIs. As already noted, school closures are not well correlated with influenza epidemics, either in tropical or temperate regions. Furthermore, Hajat, van Loghem, Hope-Simpson and others have shown that cases of VRTIs increase a few days or weeks after a drop in ambient air temperature, in a pattern that is both too fast [46] and too well-synchronized across geographical areas [4, 107] to be the result of increased *transmission.* This point needs to be emphasized. The incubation periods of VRTIs [58] are reported to be 2 to 10 days (although there is reason to believe that they may actually be greater, see below). It is therefore impossible for a VRTI to spread throughout a large geographical area with no apparent delay - unless incubation periods are much *longer* that suggested above and the virus is already present but not apparent. For example, van Loghem recorded a cold snap lasting from late October to late December in Holland in 1925 (his graph is reproduced in figure 2 of this report). About ten days after it began epidemics of VRTIs were recorded in all seven regions of Holland that paralleled the progress of the cold snap, with all regions being synchronized with each other to within roughly one week (see Graph II of van Loghem's report [4]). This suggests that the increased colds were triggered by the temperature fluctuations, which activated viruses that were already present. An important distinction here is that I am suggesting that *temperature changes* trigger VRTs, whereas all of the mechanisms reviewed by Tamerius are related to the *absolute values* of temperature and other parameters.

VTRIs in institutions

Hope Simpson pointed out that the attack rates of influenza in families are often low, whereas attack rates in institutions such as boarding schools and army camps are often high [1]. Consider a respiratory virus in a family setting that evolves increased virulence. The likely result is that extra family members will become sick and remain at home. Since these patients will not be at work or school there may be *fewer* opportunities for transmission than would be the case for a milder virus that allows hosts to carry on working. (Animals may show similar behaviour because they often keep away from other members of their species when they are sick. For example, cattle with foot-and-mouth disease often stand alone in a field and may be reluctant to move [19].) Contrast this with the situation in an institution. There, patients must remain close to their colleagues whether they are sick or well, and extra virulence may increase viral shedding and allow greater transmission. This may explain the sudden appearance of "Spanish" Influenza in the autumn of 1918 [99]. During World War I, unprecedented numbers of soldiers were stationed in military camps and trenches, many concentrated on the Western Front (for example, 1.1 to 1.2 million British and Dominion soldiers were there at any one time during the war [50]). Moreover, unlike most civilians, they engaged in *synchronized* activities and were therefore subjected to simultaneous temperature fluctuations, including fluctuations from weather. The high density of susceptible hosts, combined with high transmission

rates resulting in part from temperature fluctuations, may have given rise to non-equilibrium selection that increased virulence [6]. Once this high level of virulence was attained, similar selection may have maintained virulence and hastened the virus's transmission through civilian populations.

Studies with volunteers who were chilled

In an experimental study, Johnson and Eccles investigated whether acute cooling of the feet causes the onset of common cold symptoms [51]. When the total symptom scores for the first 4 or 5 days after chilling were analysed as dichotomous data, 26/90 (28.8%) of the chilled subjects and 8/90 (8.8%) of the control subjects were deemed to be suffering from a cold, and this difference was significant (p=0.001).

Several earlier studies of viral inoculation and chilling were reviewed by Eccles [54], including three influential experimental studies [55, 56, 57]. These studies failed to demonstrate any effect of chilling on susceptibility to infection with common cold viruses. In the study by Douglas *et al.* 44% of the volunteers who were chilled developed illness, whereas 28% became ill in the group that remained warm [57]. However, because the numbers were small (16 volunteers in all) and the results were not statistically significant, the authors reported a failure to demonstrate a "significant influence" on the incidence of infection. Eccles noted that the study by Dowling *et al.* [56] was complicated by the fact that 11% of the volunteers who had not been chilled developed colds, which casts doubt on the validity of the results. However, the real reason why these studies did not show a significant increase in colds after chilling may be that the investigators worked with viral strains that were not *ts*, even though the wild-type strains from which they were derived were. Serial passage experiments with animal parasites are known to produce rapid changes in the parasite, often increasing virulence [102]. The investigators recycled secretions from volunteers with colds to inoculate volunteers in experiments [56], and it would be natural to recycle strains that had shorter incubation times. While it is likely that they actively selected milder strains that prevented unnecessary distress to volunteers, they also wanted results to appear conveniently quickly. This may have resulted in the loss of temperature sensitivity in the early stages of infection. This suggestion is (again) the converse of the effect that was noted by Preble and Youngner with persistent infections of cell cultures [15]. They reported that selection for less active strains often produces strains that possess *ts* mutations. Andrewes, Douglas and Dowling may have demonstrated that selection for short incubation periods eliminates temperature sensitivity. These authors worked with recycled strains and saw no clear temperature effect, whereas Johnson and Eccles used "natural" strains that the participants were already carrying by chance, and saw an effect of chilling [51].

Eccles suggested that chilling the feet may cause reflex vasoconstriction of the blood vessels of the upper airways, thereby reducing host defences against infection [51, 54]. This explanation may be partly right since outdoor exercise is protective against VRTIs [47], presumably because of the increased blood flow during exercise. However, popular decongestants such as phenylephrine and pseudoephedrine alleviate the symptoms of colds and influenza by causing potent vasoconstriction of nasal blood vessels, which reduces mucosal oedema. If nasal vasoconstriction strongly exacerbated VRTIs these decongestants would not be effective cold remedies. Moreover it was the combination cold outdoor air with exercise in that was shown to be protective [47].

Viral Incubation periods and dormancy

Lessler *et al.* reviewed published articles on nine VRTIs [58], namely adenovirus, human coronavirus, severe acute respiratory syndrome coronavirus, influenza A, influenza B, measles, parainfluenza, respiratory syncytial virus, and rhinovirus, and they found median incubations periods that ranged from 0.6 days (influenza B) to 12.5 days (measles). The authors included 15 experimental studies and 24 observational studies. However, it can be argued both approaches are likely to underestimate incubation periods.

With experimental studies, everything depends on the choice of viral strain that is used to inoculate volunteers. As discussed above, experimenters may consciously or unconsciously choose strains that rapidly and consistently cause infection. The typical "wild" respiratory virus would probably give fewer colds in the time available.

Observational studies are likely to underestimate incubation periods for a different reason. Consider the following well-known incident from 1978 [59], which was included in Lessler's analysis: a Boeing 737 airliner was delayed on the ground for three hours because of engine failure, with the air-conditioning turned off. One passenger was in the early stages of influenza A, and within 72 hours 72% of passengers developed the symptoms of influenza. If, however, the disease had taken, say, a week to develop, it is likely that the incident would never have been reported, partly because it would not have been clear that the sufferers had been infected on the plane. A similar case was reported in 1918, when two doctors travelled from London to York in a railway compartment with an airman who had severe symptoms of "Spanish" influenza [60]. The doctors became ill in 41 hours and their families caught the disease from them in a similar period. The clear-cut origin and timing of the disease encouraged them to report the observed incubation period to the British Medical Journal [60].

In normal human societies, individuals are typically exposed to many different viruses every day, and it is difficult to trace the source of any particular infection. One of the few settings where it is possible to follow the progress of individual viral epidemics is in isolated Antarctic communities (in recent decades this isolation has been confined to the winter months). Such captive groups have limited and known contacts with outsiders and provide an excellent opportunity for epidemiological studies [61]. In their review of incubation periods, Lessler *et al.* reported a median incubation period for parainfluenza of 2.6 days [58]. However, Muchmore *et al.* reported persistent shedding of parainfluenza in healthy young adults throughout the 8½-month winter isolation period at Amundsen–Scott South Pole Station during 1978 [62]. Two episodes of respiratory illness were observed that year after 10 and 29 weeks of complete social isolation. Parainfluenza virus in this environment is unable to survive for more than 17 days either inside or outside of the polar station [109]. It is clear that (1) the virus was able to become dormant in asymptomatic carriers, (2) the incubation period (or the period of harbouring the virus before passing it to other individuals) was much greater than 2.6 days, and (3) the illness was able either to flare up in a dormant carrier, or to be passed from a dormant carrier to a new host who developed a VRTI. In another Antarctic study [61], Cameron and Moore reported the case of a man ("J.E.H.") who, after twelve months of isolation, picked up a virus from a visiting Russian field party. J.E.H reported minor abdominal discomfort a few days later, then, 17 days after leaving the field party, participated in an out-door activity that caused his hands and some outer clothing to become cold and damp. Thirty-six hours after that, J. E. H. noted the onset of

sore throat, mild rhinorrhea and muscle aches, as did two of the three men who worked out-doors with J.E.H. In spite of attempts, the virus involved was not isolated or identified. This case shows again that a respiratory virus could remain largely dormant for 18 days, during which period it was passed to other individuals, and also that the incubation period was far greater than Lessler reported for any respiratory virus. It is also a case of apparent "viral activation" by exposure to cold.

Studies in temperate regions have also identified asymptomatic carriers. In an experimental study [63], 81% of healthy volunteers inoculated in the nose with common cold viruses (three rhinovirus strains, a coronavirus and a respiratory syncytial virus strain) were infected and showed a clear increase in neutralizing antibody. However only 32% developed common cold symptoms in spite of the doses delivered being much higher than those expected to be encountered by natural transmission. In a prospective observational study of household members in Quebec City during the 2009 A/H1N1 influenza pandemic, approximately 10% of secondary patients in families affected were asymptomatic [64]. A serological study of the incidence and recall of influenza in a cohort of Glasgow healthcare workers found that at least 30% of infections were asymptomatic [93]. It is not clear how many of these asymptomatic individuals carried viruses that could be activated and infect other people.

A reasonable interpretation of the evidence above is that incubation periods are often close to those reported by Lessler [58], but that longer incubation periods and extended viral dormancy also exist and can have profound effects on the long-term survival of respiratory viruses and the re-emergence of VTRI epidemics.

Biochemical studies of VRTIs

For several decades virologists have found that maximum RNA transcription in influenza viruses occurs below normal body temperature. In 1977, Plotch and Krug [79] reported optimum activity of the RNA polymerase of WSN virus at 30 – 32°C. This is similar to the optimum of the polymerase of influenza C, which is 33°C [80, 81]. Ulmanen *at al.* [83] found that the rate of transcription by detergent-treated wild-type WSN viruses was about 10 times greater at 33°C than at 39.5°C, and that the binding of a cleaved primer cap, which they called the A13 fragment, to the viral cores was "unexpectedly" much weaker at 39.5°C than at 33°C. Once the heterologous RNAs were cleaved, the subsequent steps of transcription were temperature insensitive. This suggests the presence of one or more *ts* switches that initiate transcription.

Scholtissek and Rott [82] showed that the optimum for the polymerase of the Rostock strain of fowl plague virus was 36°C, five degrees below chickens' normal body temperature (41°C). Other avian viruses are cold-sensitive; the replication of two avian influenza A strains was delayed at 33°C compared to 37°C, with sensitivity to cold determined mainly be residue 627 of PB2 [111].

At least two reports show that temperature affects the balance between transcription and viral replication. Kashiwagi *et al.* looked at the effect of temperature on RNA production for five varied influenza A strains [84]. For all strains, vRNA unexpectedly decreased when the temperature was increased from 37°C to 42°C (cRNA production also decreased for two of the five strains.) The PA subunit of the viral polymerase caused

this thermal sensitivity. The temperature sensitivity identified may be a remnant of a switch that is useful in nature but is a disadvantage in the laboratory setting. In another interesting study, Dalton *et al.* showed that the production of mRNA by the PR8 influenza strain is favoured at high temperatures (41°C), with very little vRNA being produced. A plasmid-based recombinant system showed that as the incubation temperature increased from 31°C to 39°C the amount of replicative RNA products (c- and vRNA) decreased and a greater accumulation of mRNA was observed (their Figure 2). The cRNA that is used as a template to make the vRNA forms a complex with the polymerase that was particularly heat-labile, showing rapid dissociation even at 37°C. The authors suggested that the "switch" that regulates the transition from transcription to replication is dependent on temperature, but made no comments about how shifts in the host's body or respiratory tract temperature may influence this transition. They did suggest that this mechanism may have implications for the exchange of influenza between birds and man, given the different body temperatures of birds and mammals.

Much recent attention has focused on the role of RNA secondary structure in influenza A. Clearly ordered RNA is thermally sensitive and could be used for biochemical switching. Little secondary structure is predicted in vRNA outside the untranslated terminal ends of the vRNA strands that form the "panhandle" structure [89]. However the positive-sense RNA is predicted to have extensive secondary structure, which is conserved, in segments 1, 2, 5, 7 and 8. It is interesting that the most stable (+)RNA sequences tend to be avian, followed by swine, then human [90]. This sequence corresponds to the temperature of the site of replication in these strains: the avian gut is at 42°C, while the swine and human respiratory tracts are at roughly 37°C and 33°C respectively. It seems likely that RNA structures must be stable enough, but must not be too stable, to perform their functions at each temperature.

More details of these and other biochemical studies are given in the Supplementary Information, sections S6 and S7.

Ts entry into cells, sites of infection and viremia

One of the suggestions made in this review is that temperature dips can allow viruses to attach themselves to cells. Clearly respiratory viruses have preferences for particular cell-types and the specific sialic acid groups that influenza virus particles bind to have been studied in great detail [27]. However, temperature may also influence binding, since we can speculate that binding to sub-optimal receptors may be possible at lower temperatures.

Entry into cells may also be affected by temperature. In an interesting microbiological study, Russell measured the uptake of two influenza viruses and Newcastle disease virus into canine cells [65]. The uptake of Newcastle disease was sensitive to cold, remaining at low levels from 0 to 30°C, then increasing rapidly as temperature rose. The influenza virus A/Jap/Bel gave a result that Russell described as "unexpected". Uptake of the virus increased steadily from 0°C, with 100% of the virus entering the cells at 30°C. However, at 34°C and 38°C less A/Jap/Bel was taken up than at 30 °C (his Figure 2). This was repeated on two separate occasions using a chicken anti-H2 serum when 100% of virus escaped neutralization at 30°C, compared to 50% at 38°C, suggesting that viral entry into cells was *ts*.

Takashita *et al.* found that, in influenza C (C/Ann Arbor/1/50), roughly half the amount the hemagglutinin-esterase-fusion protein (HEF) was found on the cell surface at 37°C compared to 33°C [66]. (HEF in influenza C carries out the functions of both hemagglutinin and neuraminidase in influenza A or B.) Moreover, membrane fusion mediated by HEF was observed at 33°C but not at 37°C. This was found to be due to instability of the trimeric form of HEF at 37°C.

Obviously the cells that line the upper respiratory tract are typically at a lower temperature than the general body, and temperature-dependent binding or entry to cells may allow viruses to target these tissues. This raises the interesting question of whether human respiratory tract viruses can enter cells in other organs that happen to be at low temperatures. Avian influenza infects the gut of birds, but oedema and purple discoloration of the colder extremities including the head, eyelids, comb, wattles, and hocks are signs of severe avian influenza infection in birds [67]. More virulent strains can cause haemorrhage of the legs and feet [25]. In humans, measles and rubella spread to the skin, presumably via the blood. Moreover, mumps, measles, and rubella virus can frequently be found in the urine without any associated symptoms in the urogenital system [68]. Urine samples from patients with mumps collected in the first five days of facial swelling revealed mumps virus in 72% of cases [69]. Measles virus was found in the urine of 6 of 8 patients just before or after the rash appeared [70]. Hand, foot and mouth disease causes a skin rash in parts of the body that are likely to be colder – the feet (especially the soles), the hands and, in young children, around the lips [71]. As noted above, foot-and-mouth disease may be another VRTI where the virus becomes localized in the coldest parts of the body [20].

Human VRTIs that do not usually cause skin rashes or blisters may also involve viremia. There are several reports of viremia from rhinovirus [72] and human influenza [73 - 76], including three children who were infected by H1N1 influenza and presented with petechial rashes [103]. Influenza A caused hemorrhagic cystitis in 33 patients who were infected by the H3N2 strain [77]. Khalpour *et al.* [78] found influenza virus by chance in the blood of an asymptomatic patient who subsequently developed influenza, suggesting that viremia may exist only in the very early stages of the disease when it might not normally be noticed. Note that even very low and transient levels of virus in the blood might allow the virus to reach tissues other than the respiratory tract. Deposits of viruses in other parts of the body may provide reservoirs that can be activated later on (in animals if not in humans).

Viral selection in laboratory cultures

Many of the arguments in this paper hinge on the differences that may exist between laboratory viral strains and the corresponding wild-type strains. This in turn depends on how laboratory strains are propagated. Serial passages of viruses in cell cultures may select the fastest-growing variants, and, as discussed in section S1 of the Supplementary Information, this selection would be expected to reduce temperature-sensitivity if it exists. Loss of temperature sensitivity has been observed in the first few passages of newly isolated viruses [23, 24]. The entities being selected here are virus particles, and the selection may be similar to that experienced by emerging infectious diseases in nature [6] with “colonizers” having the greatest reproductive success [97]. Such selection would be expected to predominate, for example, in an embryonated egg inoculated with an egg-adapted laboratory influenza strain. In a typical experiment, the virus would grow rapidly before being harvested (in part to provide a viral stock for future experiments). Here the host cells either cannot reproduce, or cannot reproduce quickly enough to influence selection. By contrast, medium-

term selection in cell-cultures that contain viruses tends to reduce viral activity and virulence and increase temperature-sensitivity [8]. Here selection may predominantly be of cells (containing viruses), and cellular repair mechanisms and cell division must keep up with viral activity. The viruses selected may be "competitors" that are less virulent but can outcompete colonizers in co-infected cells. This type of selection is likely to dominate when persistently infected cell lines are established [15]. Some of these proposed selective trends are summarized in section S9 of the Supplementary Information.

Temperature-sensitive mechanisms may act at several stages in viral development, including viral attachment, entry into cells, transcription, genome replication, exit from cells, and release from the surface of cells. It seems likely that a few strategically placed *ts* viral processes can allow respiratory viruses to be activated in bursts, to infect a subset of susceptible individuals, to increase their virulence temporarily, and to establish persistent infections.

Conclusions

A hypothesis describing the effect of temperature on VRTIs

No satisfactory explanation is available for the close inverse correlation of VRTIs with ambient temperature [5, 107] or the seasonal appearance of VRTIs [94, 95]. Moreover, the low attack rate of influenza in families [3] and the tendency for personal chilling to increase mortality from VRTIs [47, 48] are unexplained.

However these trends can be explained by the following hypothesis:

1. Respiratory viruses may establish infections in the respiratory tracts of new hosts by refraining from developing at normal body temperature and confining development to tissues at lower temperatures.
2. Temperature fluctuations may provide a signal that activates many viruses simultaneously, thereby overcoming the hosts' immune defences.
3. Transient temperature fluctuations may allow viruses to increase their activity for a limited period, allowing transmission without increasing overall virulence to the point where the hosts become immobilized and therefore less efficient transmitters of respiratory viruses.
4. Since members of families and communities are often subject to different patterns of chilling and heating, temperature changes provide a mechanism whereby a subset of susceptible individuals can become infected.

Recommendations for research into VRTIs

The following steps may give a better understanding of VRTIs:

1. Study the biochemistry, genetics and infectiousness of respiratory viruses using recently isolated strains. Avoid selective conditions that may alter temperature sensitivity, the level of defective interfering particles or RNA secondary structure.

2. Find naturally-occurring *ts* strains, and equivalent non-*ts* strains, and sequence them. Identify sequence differences including those in HA, NA and viral RNA polymerase (which are known to provide spontaneous *ts* mutants).
3. Investigate the response of viral processes to temperature shifts and temperature cycling in animals, tissue cultures and *in vitro.*
4. Use temperature shift-up and shift-down experiments to investigate the effect of temperature on the synthesis of viral proteins.
5. Use temperature shift-up and shift-down experiments to investigate the effect of temperature on the synthesis of viral mRNA, cRNA and vRNA.
6. Using a wide variety of virus strains, look for immobilized and dormant viruses in cold parts of the bodies of humans and animals, including the upper respiratory tract, lips, ears, feet, toes and fingers.

List of abbreviations used

VRTI or VRTIs: Viral respiratory tract infection or infections
Ts or *ts:* temperature sensitive
DIPs: defective interfering particles
HA: hemagglutinin
NA: neuraminidase
HEF: hemagglutinin-esterase-fusion protein

Competing interests

I declare that I have no competing interests.

Author's information

I am one of the two founders and Directors of Douglas Instruments Ltd, a small UK company that manufactures automatic systems for protein crystallization. I am the inventor of the earliest microfluidic system that I know of, see UK patent GB2097692 "Combining chemical reagents". I worked with Professor David Blow in the 1990s, and have published several papers about protein crystallization (some of which have been cited over 100 times). However, 18 months ago I knew very little about viruses and I am effectively an amateur and outsider in this field.

Supplementary Information

S1. Patterns of viral evolution

Hope-Simpson and others have been puzzled by the patterns of respiratory infections, but it can be seen that they may give the virus a long-term selective advantage. I will attempt to divide viruses into four rough categories (A to D) based on their level of activity and virulence: (A) some viral diseases (such as myxomatosis) infect and kill a high proportion of the host population. The viruses involved have often crossed recently to the current host from another species, and may spread rapidly. Both theoretical work on virulence evolution during disease emergence [6] and empirical studies [7] suggest that at high host densities

and in conditions that favour transmission, selective pressures may increase virulence. Such viruses are in danger of killing all of their hosts that do not become immune, and they risk extinction unless the host population is large (such as the population of European rabbits in Australia). Once the density of the host population goes down, or a proportion of the host population becomes immune, more virulent virus strains may spread less efficiently because infected individuals die before they can infect many other potential hosts [8]. Less virulent variants may therefore arise, a trend that is consistent with the well-known "trade-off" model [6, 7]. During the first year after its introduction, myxomatosis in Australia is estimated to have killed 99.5% of the rabbits that it infected [9]. However, even this high rate allowed around 0.5% to recover and breed. By the third year the mortality rate was down to 65%, probably due to a combination of increased host resistance and decreased viral virulence [9]. (B) Once equilibrium is approached, viral species that are highly infectious but rarely fatal may arise [10]. For example, measles is highly infective, predominantly infecting children because most adults are immune. Measles is a fairly recent human VRTI, having diverged from the formerly widespread rinderpest virus, which infects cattle, roughly 1000 years ago [11]. Deaths from measles are comparatively rare (about 1 death in 1000 cases). Both categories A and B need relatively large host populations to survive, so that enough susceptible progeny can be born to allow the virus to replicate (it is estimated that measles requires aggregations of over 500,000 people to become endemic [13]). (C) Influenza and colds use more subtle patterns of infection, where a low rate of attack [3 - 5] allows the virus to linger in a community for longer, and to return to the same community in subsequent seasons. To use a wildfire analogy, myxomatosis is like a forest blaze that burns very fiercely and rapidly consumes its fuel, while measles burns more gently, allowing most trees in the forest to survive and propagate. Influenza is like a fire that has the unusual property of burning only some trees, leaving others intact. (D) Many viruses, including influenza, can also "smoulder" by generating asymptomatic infections [14, 93]. Which of these strategies is followed by a particular viral strain depends on its history and the selective pressures that it experiences.

Note that the tendency for higher host densities and transmission rates to give increased virulence [6, 7] discussed above suggests a timing effect, since the viral strains that are shed early on during an infection are more likely to be transmitted in conditions of high host density. Laboratory serial passage experiments with animal parasites are likely to use the parasite samples that are shed early for reinoculation of animals, and such experiments tend to increase parasite virulence [102]. Cooper *et al.* (working with the nuclear polyhedrosis virus of the gypsy moth) showed that the time of transmission altered viral virulence within nine cycles of transmission, with early transmission favouring the evolution of greater virulence [96]. In another study, after observing that an RNA virus clone in cell culture diversified into two subpopulations with differing virulence, Ojosnegros *et al.* introduced a competition–colonization trade-off model of infection [97]. They suggested that natural viral populations include "colonizers" (fast-spreading virulent strains) and "competitors" (less-virulent variants that can outcompete the colonizers in co-infected cells). If these trends are typical, wild viruses may constantly adjust virulence by rapidly evolving strains with increased or decreased virulence in response to changing opportunities for transmission.

S2. Observations of persistent viral infections in cell cultures and animals

Preble and Youngner collected together and reviewed many examples where cell cultures with persistent viral infections yielded virus that was *ts* [15]. These included cell cultures infected by Newcastle disease virus that was both less virulent and possessed less HA activity than the parent strain. The virus recovered

had *ts* variants of HA, NA and RNA polymerase. *Ts* mutants were also recovered from cell cultures with Western equine encephalitis virus, Sendai virus, measles virus (in both human and hamster cells), stomatitis virus, and Sindbis virus, and from other systems including a bacteriophage that had a polymerase that was defective at higher temperatures. Conversely, an artificially generated *ts* strain of reovirus that was injected into rats caused a slowly progressing encephalitis, while the wild-type strain caused acute encephalitis [for references, see 15].

More recently, spontaneous *ts* persistent infections of cell cultures by influenza A have arisen several times. Frielle *et al.* recovered *ts* influenza A strains from persistent infections of the WSN (H1N1) strain in hamster cells [16]. The *ts* virus recovered from the infection could not be amplified in eggs or several cell-lines, and expressed larger amounts of NP and dramatically reduced amounts of matrix protein in comparison to the parental strain. Similarly, Liu *et al.* established a persistent infection of influenza A (E61-24-P15) in canine cells, and recovered *ts* virus with an M1 protein that had two amino acid substitutions and was defective at 38°C [17]. In another study, during another persistent infection of canine cells with the influenza A/Victoria/35/72 virus (H3N2), after 158 days the population consisted entirely of ts, inhibitor-resistant small plaque clones [110]. Less than 0.05% of the cells contained the influenza virus antigen. Multiple ts mutations were found in the genes coding for P2, NP, NA, M and NS proteins.

Preble and Youngner [15] found many examples where stable infections of cell-cultures by viruses involved increased production of DIPs. These include infections with vesicular stomatitis virus, measles in various cell-types, Western equine encephalitis virus, lymphocytic choriomeningitis, Newcastle disease virus, Parana virus, and Sindbis virus. As they pointed out, increased DIPs and *ts* mutants can contribute simultaneously to reduction of viral activity in a system. A more recent report showed that the loss of DIPs can cause a dramatic increase in virulence [25]. In April 1983, an H5N2 influenza A virus appeared in chickens in Pennsylvania and caused low mortality. In October of that year, virulent influenza appeared in chickens in the state, with a mortality of up to 80% after 5 days. This disease spread to chickens, turkeys, guinea fowl and partridges, and caused the destruction of 15 million birds. Oligonucleotide mapping of the individual genes of the virulent and avirulent strains showed that they were very closely related, but no consistent differences could be correlated with pathogenicity. However analysis of the viral RNA showed that the early (avirulent) strains possessed DIPs that could be seen on polyacrylamide gels, which the virulent strain lacked. This is consistent with the suggestion that selection in conditions that give high rates of viral transmission may increase virulence, including by the loss of DIPs.

S3. The loss of naturally occurring *ts* phenotypes in conditions that select rapidly growing variants

Chu *et al.* [23] identified a naturally occurring *ts* influenza A strain, Xia-*ts,* which was a subclone of the H3N2 strain Ningxia/11/72. In trying to generate a cold-adapted strain, they treated Xia-*ts* with dimethyl sulfate and passaged it through chicken embryos three times at 25°C, and once at 33°C. To their surprise, a non-*ts* mutant, Xia-*ts*+, was selected by this process. They later found that the infective titres at 33°C of Xia-*ts* in both eggs and hamster cells were one to two logs lower than Xia-*ts*+, and this lower replication rate of the *ts* strain may explain the unexpected appearance of Xia-*ts*+. They also tested the replication of both strains in

the lungs of live hamsters, and found that Xia-*ts* was much less active, replicating more slowly and with a maximum titre that was two logs lower than that of Xia-ts+.

Oxford *et al.* made similar observations [24] when they also investigated naturally occurring *ts* influenza A strains that had been isolated from patients shortly before the study. They noticed that a *ts* virus, A/Eng/116/78 (H1N1), progressively lost its *ts* character during five passages at 33°C. The authors then looked carefully at a similar virus, A/Eng/772/78 (H1N1), that had minimal *ts* properties, and found that it contained a mixture of *ts* and non-*ts* viruses. The authors reached a similar conclusion to Chu: even at the permissive temperature (33°C) the *ts* character may confer a selective disadvantage in eggs. .

S4. The prevalence of naturally-occurring ts viral strains and the temperatures that are used for virus isolation and propagation

In 1963 Stern and Tippett [43] collected five viral specimens from patients with H2N2 "Asian" flu. One could not be propagated, but the remaining four were *ts*, showing cytopathic effects in monkey cells at 33°C but not at 37°C. Inoculation of eggs showed the same pattern, with agglutination at 33°C but not 37°C. Limited growth was observed in some of the cell cultures at 37°C, and subcultures of isolates obtained from them were able to grow at 37°C, albeit more slowly that at 33°C, confirming that the viruses could adapt to culture at 37°C. The authors also looked at older egg-adapted strains including FM1 (H1N1, 1947) and PR8 (H0N1, 1934). Both strains (in 1962) grew much more strongly and rapidly on both cynomolgus and rhesus kidney cells at 33°C than at 37°C.

In 1977, Kung *et al.* found by accident that nine of the ten isolates of the newly emerged "Russian" H1N1 influenza that they looked at were *ts* [44]. These isolates had been passaged one to five times in eggs. The "cut-off" temperature (at which at least 90% of virus replication at inhibited) was determined for six of the nine *ts* isolates, and it was found to be roughly 38°C for three isolates, and 39°C for the remainder. These relatively high temperatures may be due to the adaptation of isolates to growth in eggs. Note also that viral temperature-sensitivity in the human respiratory tract may be finely tuned, and that viral interactions with chicken cells may give different results to those with human cells, possibly having higher cut-off temperatures.

Around the same time, Oxford [24] tested 26 recently isolated H1N1 influenza strains, and found that 17 were *ts*, producing at least 10 times more virus at 34°C than at 38.5°C. Two of eleven H3N2 isolates from that period were also *ts* (H3N2 was then in circulation concurrently with H1N1). These isolates were obtained from the World Health Organization and had been passaged two to five times in eggs or cell cultures. These results were compared to those from standard laboratory strains. Of 17 older laboratory H1N1 strains originally isolated between 1947 and 1963, only three were *ts.*

Today, however, influenza A is frequently isolated from patients by propagation at 37°C [42].

As noted in section S3 above, both Chu and Oxford observed that *ts* strains may lose their *ts* character when they undergo multiple passages through cells and eggs [44, 24]. Chu also tested seven H1N1 strains with

varying degrees of temperature sensitivity in volunteers and found a correlation between temperature sensitivity and the severity of VRTI symptoms [23]. Four with cut-off temperatures of 38°C or below gave milder fevers when administered to volunteers, and were variable in eliciting an antibody response. Those with cut-off temperatures of 39°C or above gave fevers above 38.6°C in eight volunteers and generally gave strong antibody responses.

S5. Studies of transcription in influenza

Scholtissek and Rott conducted a study [82] with the Rostock strain of fowl plague virus, which infects chickens. Figure 3 of their report shows a maximum activity of viral RNA polymerase at 36°C, five degrees below chickens' normal body temperature (41°C). Polymerase activity falls off rapidly above 36°C. At 4I°C the virus RNA polymerase was unstable in vitro as well as in vivo. The synthesis of virus HA and NA, however, was unimpaired at 4I°C.

The genome of influenza A consists of eight single-stranded RNA segments, referred to as viral RNA (vRNA). These segments are negative-sense, meaning that they must be transcribed by the viral polymerase to produce viral messenger RNA (mRNA). This mRNA is translated to produce the 11 viral proteins. However the mRNA is not a complete copy of the vRNA. In the early stages of viral infection, a second batch of positive-sense RNA is also produced called cRNA, which is an exact copy of the vRNA, and serves as a template to generate the vRNA. Ulmanen *at al.* looked at the first step in influenza viral mRNA synthesis [83]. This is the endonucleolytic cleavage of heterologous RNAs containing cap 1 (m7GpppNm) structures to generate capped primers that are 10 to 13 nucleotides long, which are then elongated to form the viral mRNA chains. When they investigated these steps using detergent-treated wild-type viruses they found that the rate of transcription was about 10 times greater at 33°C compared to 39.5°C (their Figure 1A). Since they wanted to use artificial *ts* mutants at 39.5°C, they did not use the detergent-treated material. Instead they used purified "viral cores" (presumably ribonuclearprotein complexes) that were more stable at 39.5°C. (Even using the purified material, activity was reduced by about 20% at 39.5°C in comparison to 33°C, Figure 1B in their report.) The unpurified material contained "an undefined nuclease", which was more active at 39.5°C than at 33°C and which degraded the primer fragments generated by the viral cap-dependent endonuclease. This temperature dependence was not investigated further, and purified material lacking the nuclease was used in the studies. The authors later investigated the binding of a cleaved primer cap, which they called the A13 fragment, to the viral cores. "Unexpectedly", the wild-type viral cores bound much more weakly to A13 at 39.5°C than at 33°C (their Figure 5). (This difference was eliminated by adding ATP, GTP and CTP to the mixture so that work further could be carried out with *ts* mutants.) Once the heterologous RNAs were cleaved, the subsequent steps of transcription were temperature insensitive (their Figure 4, focusing on the first five minutes). This suggests the presence of one or more *ts* switches that initiate transcription.

Kashiwagi *et al.* looked at the effect of temperature on RNA production for five influenza A strains [84]. These strains came from a wide variety of sources, including an H1N1 strain that was isolated in 1933, 1968 H3N2 ("Hong Kong flu"), 2009 H1N1 ("swine flu"), and two strains of H5N1 "bird flu". For all strains, mRNA production increased with increasing temperature. While vRNA production increased when the temperature was increased from 33°C to 37°C, it unexpectedly decreased for all five strains when the temperature was

further increased from 37°C to 42°C (cRNA production also decreased for two of the five strains.) These data are shown in Figure 1B and Table 1 of that report. By creating artificial hybrids containing PA subunits from different influenza strains, the authors showed that the PA subunit of the viral polymerase caused this thermal sensitivity. Since only three temperatures (33°C, 37°C and 42°C) were examined, the study did not show at what temperature the maximum vRNA production would have occurred. The maximum may have been below 37°C. Moreover, the viral strains used are typically propagated at 37°C and can be presumed to have adapted to this temperature. All of the strains except for A/Kurume/K0910/2009 had been maintained in a laboratory environment for at least five years prior to the experiments (in the case of A/WSN/33 around 76 years). The temperature sensitivity seen may be a remnant of a switch that is useful in nature but is a disadvantage in the laboratory setting.

Working with the PR8 influenza A strain (isolated in Puerto Rico in 1934) [85], Dalton *et al.* showed (their Figure 1) that the production of mRNA is favoured at high temperatures (41°C), with very little vRNA being produced. A plasmid-based recombinant system that recreates functional influenza virus RNPs in cells was also used in the study. Using this, it was clear that as the incubation temperature increased from 31°C to 39°C the amount of replicative RNA products (c- and vRNA) decreased and a greater accumulation of mRNA was observed (their Figure 2). mRNA is produced by the viral polymerase using vRNA as a template. The complex of the polymerase with vRNA degrades very slowly at 37°C (their Figure 7), allowing efficient production of mRNA. However, the cRNA that is used as a template to make the vRNA forms a complex that is particularly heat-labile, showing rapid dissociation even at 37°C. The authors suggest that a *ts* "switch" regulates the transition from transcription to replication.

Some steps of viral development were not affected by higher temperatures. Scholtissek found that the synthesis of virus HA and NA was unimpaired at 4I°C [82]. Kashiwagi and Dalton both found that mRNA production increased above 37°C [84, 85]. Ulmanen found this to be the case from the point where transcription was initiated by the cleavage of the heterologous RNAs [58].

S6. RNA secondary structure

Brinson *et al.* studied infectious salmon anemia virus (ISAV), which is a member of the family *Orthomyxoviridae*, and distant cousin of the influenza viruses (like influenza A and B, but not C, the virus has eight genomic segments of single-stranded RNA with negative polarity) [86]. They predicted a "temperature-dependent switch in RNA secondary structures between 15°C, the temperature of the North Atlantic and the optimal temperature for ISAV replication, and 37°C", noting that the virus does not replicate above 25°C. The authors also tested the temperature dependency of RNA structures using high-resolution NMR spectroscopy and thermal melts.

The secondary structure of the RNA of influenza A is conserved in both negative-sense (vRNA) and positive-sense RNA (mRNA and cRNA). The untranslated 3' and 5' ends of vRNA strands contain complimentary sequences that can hybridize to circularize the strand forming a panhandle structure both *in vitro* and *in vivo* [87]. The structure formed creates the promoter that is needed for vRNA to be copied to make positive-sense RNA. On binding the viral polymerase, both termini form hairpins, a rearrangement referred to as the "corkscrew" structural model. A 2010 study suggests that virus-encoded short RNA transcripts that are

complimentary to the 3' termini of cRNA may allow viral RNA synthesis to switch from transcription (production of mRNA) to replication (production of vRNA) [88]. All these interactions between complimentary RNA sequences provide opportunities for changes in temperature to switch viral biochemistry on or off.

The secondary structure of influenza A segment 7 mRNA, which encodes the M1 matrix protein and the M2 ion channel, has been studied in detail [89]. This region can fold as either a hairpin or a pseudoknot, and (if either interacted with a protein that would increase stability) a *ts* switch could presumably be created.

S7. Viral functionality that is affected by naturally occurring temperature-sensitivity

It may be helpful to compare the viral functionality that was affected by *ts* mutations in the studies of influenza discussed above. The uptake into cells of the triple assortment virus A/Jap/Bel (H2N2/H1N1/H1N1) used by Russell was *ts* [65]. Ulmanen *at al.* found that the very first steps of influenza A mRNA synthesis were *ts*, but that subsequent steps of transcription were not sensitive to temperature [83]. Liu *et al.* recovered *ts* virus with defective M1 protein from a persistently-infected cell culture [17]. Frielle *et al.* recovered *ts* virus from a persistently infected cell culture that expressed larger amounts of NP and dramatically reduced amounts of matrix protein in comparison to the parental strain [16]. Chu *et al.* looked at naturally occurring *ts* strains and found lesions in the NP gene of two H3N2 strains and in a matrix protein gene of two H1N1 strains [23]. Oxford *et al.* found that the *ts* lesions in naturally-occurring *ts* H1N1 strains were not located in the NA or HA proteins [24]. Priore *et al.* showed that the positive-sense RNA in influenza A is predicted to have extensive conserved secondary structure in segments 1, 2, 5, 7 and 8 and that the predicted thermal stability of (+)RNA is correlated with the temperature of the site of viral replication in different species [90]. Kashiwagi *et al.* found that, for five diverse influenza A strains, mRNA production was insensitive to temperature, but that vRNA production was *ts* [84]. They found that the PA subunit of the viral polymerase caused this thermal sensitivity. Dalton *et al.* showed that the production of mRNA is favoured at high temperatures (41°C), but that with very little cRNA and vRNA are produced at high temperatures [85]. They also showed that the cRNA that is used as a template to make the vRNA forms a complex that degrades rapidly at 37°C. Finally, Takashita *et al.* found that less hemagglutinin-esterase-fusion protein in influenza C (C/Ann Arbor/1/50) was found on the cell surface at 37°C compared to 33°, and less membrane fusion was observed [66].

S8. Possible sites for persistent viral infections resulting from local chilling

Viruses such as foot-and-mouth virus seem to locate the tissues that they will infect using temperature, including the lips, tongue, soft palate, pharynx and feet [18]. VRTIs may also affect tissues outside the respiratory tract that are colder than the individual's general body temperature, such as the feet or head in birds. Possible benefits to the virus include (1) the provision of a reservoir of virus that can become activated when the temperature of those tissues subsequently rises, and (2) the transmission of viruses from, for example, blisters on feet. Human respiratory viruses may also affect sites outside the respiratory tract, although it seems unlikely that they are frequently spread by viral shedding from these sites. Moreover, tissues may be infected "accidentally". For example viruses that normally infect both the

respiratory tract and the skin of other species might be transferred to humans where they might continue to infect sites outside the respiratory tract.

Chilblains are a poorly understood tissue injury that can occur when a predisposed individual is exposed to cold [100]. They most often affect the toes, but many parts of the body that tend to be cold can be affected, including the fingers, ears and nose. Chilblains can occur in one geographical area but not in another that has the same general characteristics of temperature and humidity. Occasionally the illness persistently affects one side of the body [100]. These observations are compatible with a viral involvement in the disease, suggesting that chilblains may be analogous to the blisters generated by foot-and-mouth disease in cloven-hoofed mammals (although they are probably "accidental" in that they may not increase transmission of the virus involved).

Chapped lips (cheilitis) can be caused by cold weather, often when the individual has a cold, but they are also associated with high fevers, including fevers caused by VRTIs [101]. The conventional explanation is that breathing through the mouth when the nose is blocked causes the lips to become "dried out". However, it is not clear how the extra humidity of breath can cause "drying". An alternative explanation is that the virus itself is immobilized in the lips and causes chapping, since the lips tend to be cold, especially when breathing through the mouth.

An interesting parallel may be drawn between the study by Johnson and Eccles where volunteers' feet were chilled and a study by Baerheim and Laerum, who found that chilling of the feet was associated with the subsequent development of bacterial symptomatic lower urinary tract infection in a sample of cystitis-prone women [52]. This is consistent with the widespread belief in the Norwegian lay population that cystitis may be induced by having cold feet. Respiratory viruses including adenoviruses and influenza A virus can cause, or play a role in, cystitis [53, 76].

S9. The fever response

Many vertebrates raise their body temperature in response to microbial infection, either by seeking a warmer environment or by expending energy [91]. However the usefulness of this ancient response is controversial, and studies have failed to show any benefit in helping recovery from infection in humans, or any undesirable effects of treating fever with antipyretics [91]. One possible benefit is that higher body temperature does not aid recovery but may prevent new acute viral infections by keeping the body permanently above the range of temperatures where other existing persistent infections can be activated. This may partly explain one of the features of influenza that perplexed Hope-Simpson [3]: the tendency for each new epidemic of influenza to replace all previous strains with very little overlap. (I would also argue that fever may reduce the risk of predation etc. by speeding up recovery. A higher body temperature will in principle speed up the metabolism of both the host and the pathogen – if anything the pathogen can more easily adapt to higher temperatures since it is less complex and can mutate much more rapidly. However the outcome is likely to be arrived at faster if the body temperature of a sick animal rises, reducing the likelihood of discovery by a predator or competitor.)

S10. Summary of proposed selective trends

Table S1 shows some of the selective trends that are described in the main text. Many other outcomes are possible depending on the timing and causation of transmission, and the details of the biochemical and physiological interactions of the virus with the host. While it is difficult to predict the effects of competition between viral strands within cells it may result the loss of temperature sensitivity (*TS*) (since *TS* may slow replication), and the formation of increased DIPs (since abundant levels of viral proteins provide opportunities for parasitisation by incomplete viral particles). Typical growth conditions of viruses in laboratory systems (eggs or cell cultures) remove the need for the transmission step of the viral life-cycle, instead selecting only on the basis of the replication rate. Since *TS* tends to reduce growth, it is typically lost or reduced in these conditions. DIPs may increase as a result of selection within the cell. The stable propagation of dividing cells that contain viruses may, however, give very different results. Rapid growth of viruses may kill the host cell. In order to establish a persistent infection, viral activity must be reduced until equilibrium is reached. This can increase both *TS* and DIPs. Serial passage experiments where human volunteers or animals are inoculated with virus isolates derived from other volunteers or animals again removes the need for transmission, and may decrease *TS.* Finally, selection of wild viruses in animal or human communities can give different results depending on host densities and virus transmission rates. In conditions of high density and rapid transmission, both *TS* and DIPs may decrease. At lower host densities and transmission rates, the level or *TS* and DIPs tend to increase until equilibrium is reached.

S11. Some natural history

The scientist and writer Rupert Sheldrake once said to me, “All science starts with natural history” – i.e. with disinterested observation of natural phenomena. I would like to include some anecdotes about VTRIs, not to draw definite conclusions, but to suggest opportunities for experimentation.

- In February 2011, I travelled to Mumbai, and woke up on the first morning with few bedclothes covering me, but with the air conditioning on, and I found that I had a distinct sore throat. Since steam inhalation had previously prevented a VRTI from developing when I used it as soon as the infection became detectable, so I used inhalation for ten minutes in my hotel room each morning for five days, which made my throat feel much better for about 30 minutes. However the cold progressed to a fever which lasted for ten days and seemed unusually severe.
- In September of that year, I noticed a scratchy throat. I used steam inhalation which removed all symptoms of a VRTI. Four weeks later I again had a scratchy throat, which was this time not cured by steam inhalation, and was followed by feverish cold.
- In September 2012 I developed a cold and sore throat. This time I did not use steam inhalation and instead I avoided hot drinks. I completely recovered within two days, which was faster than I had expected.

My interpretation of these events is: (1) in the early stages of a VRTI, heat can suppress either the entry of viruses into cells, the generation of virus particles, or their release. (2) Once a substantial VRTI has developed heat can spread the infection or hinder the immune system in eliminating the infection. (3) Heat may sometimes suppress a VRTI but not eliminate it.

S12. Conversations with virologists

I have heard the statements below from virologists or medics. All need to be examined carefully.

"Viruses cause colds and flu, not exposure to cold." This assertion, which appears in medical textbooks [104], is based on a false dichotomy. Very few educated people believe today that colds and flu are not viral diseases that can be caught from infected individuals. However many people (whether ignorant or educated) believe that chilling can cause the symptoms of VRTIs to appear by activating viruses that are already present. This distinction was made very clearly by van Loghem in 1928 [4].

"Influenza viruses can't become dormant." It is difficult to generalize about the behavior of viruses because they have a very high rate of mutation and sometimes jump species barriers. It is known that many viruses, including foot-and-mouth virus, chickenpox virus, coronavirus, HIV, and Epstein-Barr virus, can become dormant. Avian influenza, including the highly pathogenic H5N1 strain, can be transmitted by ducks that are themselves asymptomatic [14], and in human influenza, even during an epidemic of an influenza strain that is likely to be more virulent than normal, 10% of secondary patients were asymptomatic [64]. It is not known whether asymptomatic human carriers can transmit influenza [105].

"Viruses can't survive in the bloodstream." Many viruses cause viremia, including those causing mumps, rubella and measles, all of which can also be found in the urine of patients [68-70]. Avian influenza infects most organs of the chicken [67], and human influenza can infect mice, again invading multiple organs [106]. Several reports describe viremia in human influenza A [73 - 77], including the study of Khakpour *et al.* who found viremia during the incubation period of the virus in one patient [78]. The presence of viruses in the blood was picked up by chance and would not normally have been noticed, so it is difficult to know how common this is.

"Bugs, including viruses, can be very sensitive to temperature". This is true, but one needs to ask why. A virus could very quickly develop more stable proteins by mutation. This might, of course, interfere with the regulation of viral processes. In a sense, this paper is an exploration of the consequences of such interference.

To any readers who doubt the hypothesis put forward in this review, I would ask the following question: would you go swimming in cold water if you noticed the symptoms of a cold or influenza that was just beginning?

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Tables and figures

Table 1. Characteristics of virus families and strains that cause human VRTIs.

Family	*Viral strains that cause human VRTIs*	*Primary genetic material*	*Replication site in the cell*	*Presence of lipid envelope*	*Virion shape*
Adenoviridae	Adenovirus	Double-stranded DNA	Nucleus	lacking envelope	icosahedral
Coronaviridae	Coronavirus, severe acute respiratory syndrome virus	Positive-sense single-stranded RNA	Cytoplasm	enveloped	spherical with projections
Orthomyxoviridae	Influenza virus	Negative-sense single-stranded RNA	nucleus	enveloped	spherical or filamentous
Paramyxoviridae	Measles, mumps, parainfluenza and respiratory syncytial viruses, human metapneumovirus	Negative-sense single-stranded RNA	Cytoplasm	enveloped	spherical or variable
Picornaviridae	Hand foot and mouth virus, rhinovirus	Positive-sense single-stranded RNA	Cytoplasm	lacking envelope	icosahedral
Togaviridae	Rubella virus	Positive-sense single-stranded RNA	Cytoplasm	enveloped	icosahedral

Table 2. Data from the Eurowinter Group [47]. Regression coefficients (R) and their significance (p), for cause-specific indices of respiratory disease-related mortality on personal cold-exposure factors standardised at 7°C mean daily temperature.

Cold exposure factor	**R**	**p-value**
Mean duration of going out	0	0.922
Living room temperature	-1.8	0.001
Frequency of going out	-1.9	0.116
Whether going out	-2	0.623
Clothing area (fraction of body surface)	2.2	0.183
Bedroom heating	-2.8	0.053
Long underpants	-3.8	0.022
Gloves	-3.9	0.065
Long-sleeved vest	-4.5	0.072
Hat	-4.7	0.004
Long trousers	-6.6	0.005
Anorak	-6.7	0.001
Overcoat	7.3	0.002
Skirt	8.3	0.005
Sweater	9.5	0.001
Stationery >2 mins	13.2	0.04

Sweating outside	-17.5	0.02
Shiver	23.8	0.001

Table S1. Some proposed effects of selection on temperature sensitivity and DIPs based on the site of selection, the entities that are subject to selection and the class of selective agent involved.

Site of selection	*Entities that are subject to selection*	*Selective agent*	*Effect on temperature sensitivity (TS)*	*Effect on defective interfering particles (DIPs)*
Cell	Viral strands	Spontaneous	Neutral or decrease *TS*	Increase
Chicken egg	Virus particles or DIPs	Spontaneous	Decreased *TS* gives rapid growth	Variable – DIPs may increase if infection takes place at high multiplicity
Culture flask (with rapid batch propagation followed by rapid virus harvesting)	Virus particles or DIPs	Spontaneous	Decreased *TS* gives rapid growth	Variable – DIPs may increase if infection takes place at high multiplicity
Culture flask (with continuous propagation allowing persistent infections)	Cells that contain viruses	Spontaneous	*TS* increases to an optimum	DIPs increase to an optimum
Laboratory	Virus isolates	Human	Decreased *TS* gives rapid growth	Depends on scientific objective
Community with high host densities	Virus strains	Spontaneous	Decreased *TS* gives rapid growth	Decrease DIPs for rapid growth
Community with low host densities	Virus strains	Spontaneous	*TS* increases to an optimum	DIPs increase to an optimum

Figure 1. *A model suggested by this hypothesis for the typical infective route of VRTIs.* It is known that there is a temperature gradient in the respiratory tract of mammals, with lower temperatures at the nose and higher temperatures in the lungs [32]. I propose that many viruses possess *ts* properties that allow them to be activated only below a lower transitional temperature (LTT). In addition, I propose that viruses may possess other ts properties that cause them to become attached to cells (or to enter into them) only below an upper transitional temperature (UTT) that is above the LTT. For example, the LTT of a respiratory virus might be 32°C and the UTT a few degrees higher, say 34°C. This defines a transitional band of temperatures, which corresponds to one or more areas - shown in green in the figure - where the viruses will be immobilized (on the surface of, or inside, cells) but not activated. One larger and two smaller areas are shown in the figure. If a virus particle that is inhaled lands in a part of the tract (shown as pale blue) that is at a temperature below the LTT, it will be immobilized and activated. If the strain is not highly virulent or if the dose of viruses received is not great, the virus is likely to be destroyed by the immune system. This is indicated by the purple arrow. If a virus particle is inhaled and lands in an area of the tract (shown as yellow) that is above the UTT, it will not be immobilized, and so may be carried by the mucociliary escalator to the transitional band (green), where it binds. This route is indicated by the red arrows. If the particle is delivered in a larger droplet it may land in a cooler area, but may subsequently be inhaled again (solid red line). Alternatively it may be inhaled in a fine aerosol and so travel straight to the warmer parts of the tract (dotted red line). If the host is chilled the tract will also cool [108] and the position of both the LTT and the UTT will move further down the tract (dotted green lines). The result is that any viruses that were previously immobilized in the green area (or areas) will be activated in a burst. Respiratory viruses in the tropics may colonize regions of the respiratory tract that are closer to the nose.

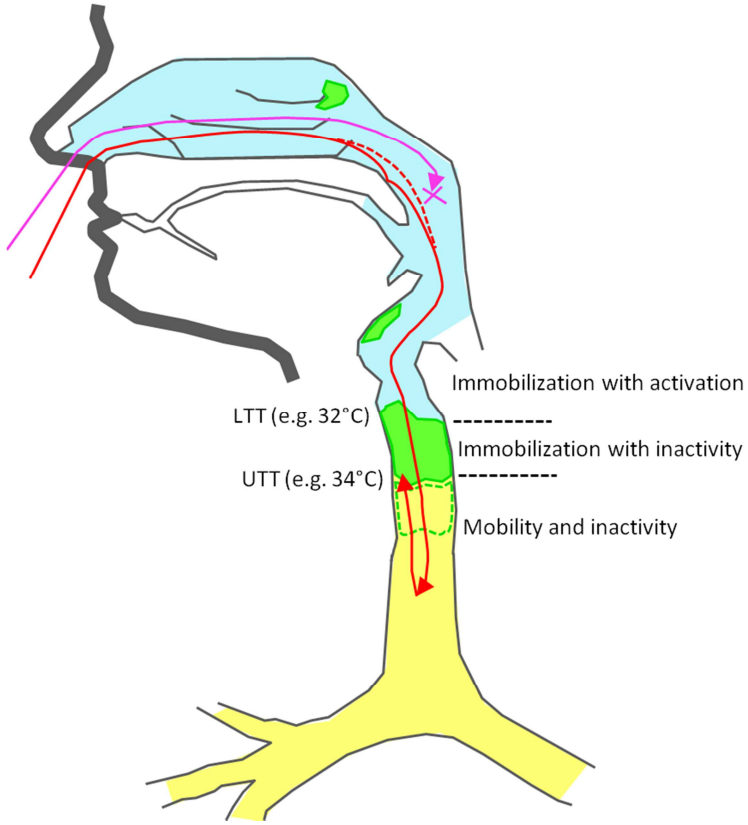


Figure 2. *Graph I from van Loghem's report on the epidemiology of respiratory diseases in Holland in the winter of 1925/26* [4]. The graph shows the percentages of persons with colds in Amsterdam (1159 informants) and the remaining Netherlands (5774 informants) for 37 weeks, compared with the average outdoor air temperatures in the Netherlands.

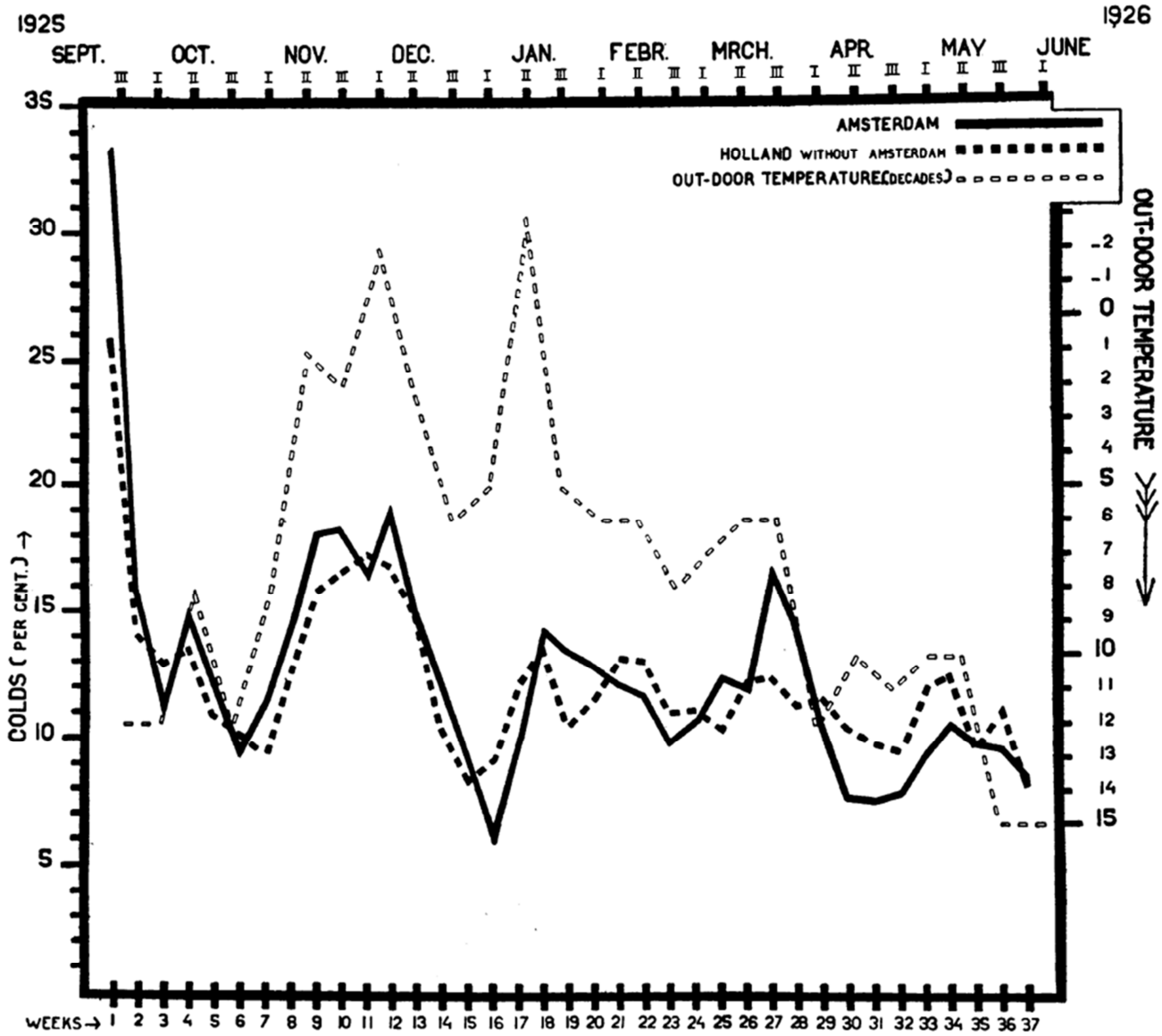
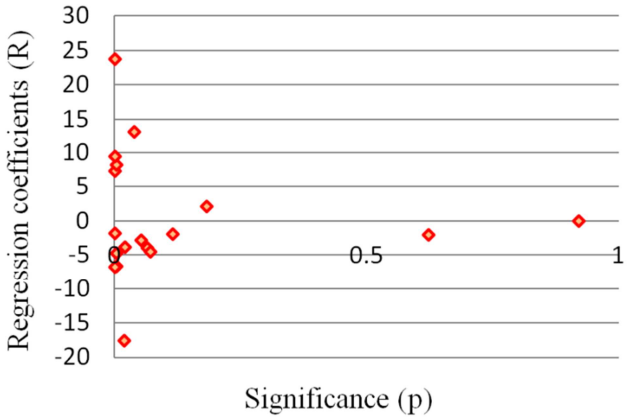


Figure 3. *Data from the Eurowinter Group* [47]. The regression coefficients of Table 1 are plotted against their significance (p).



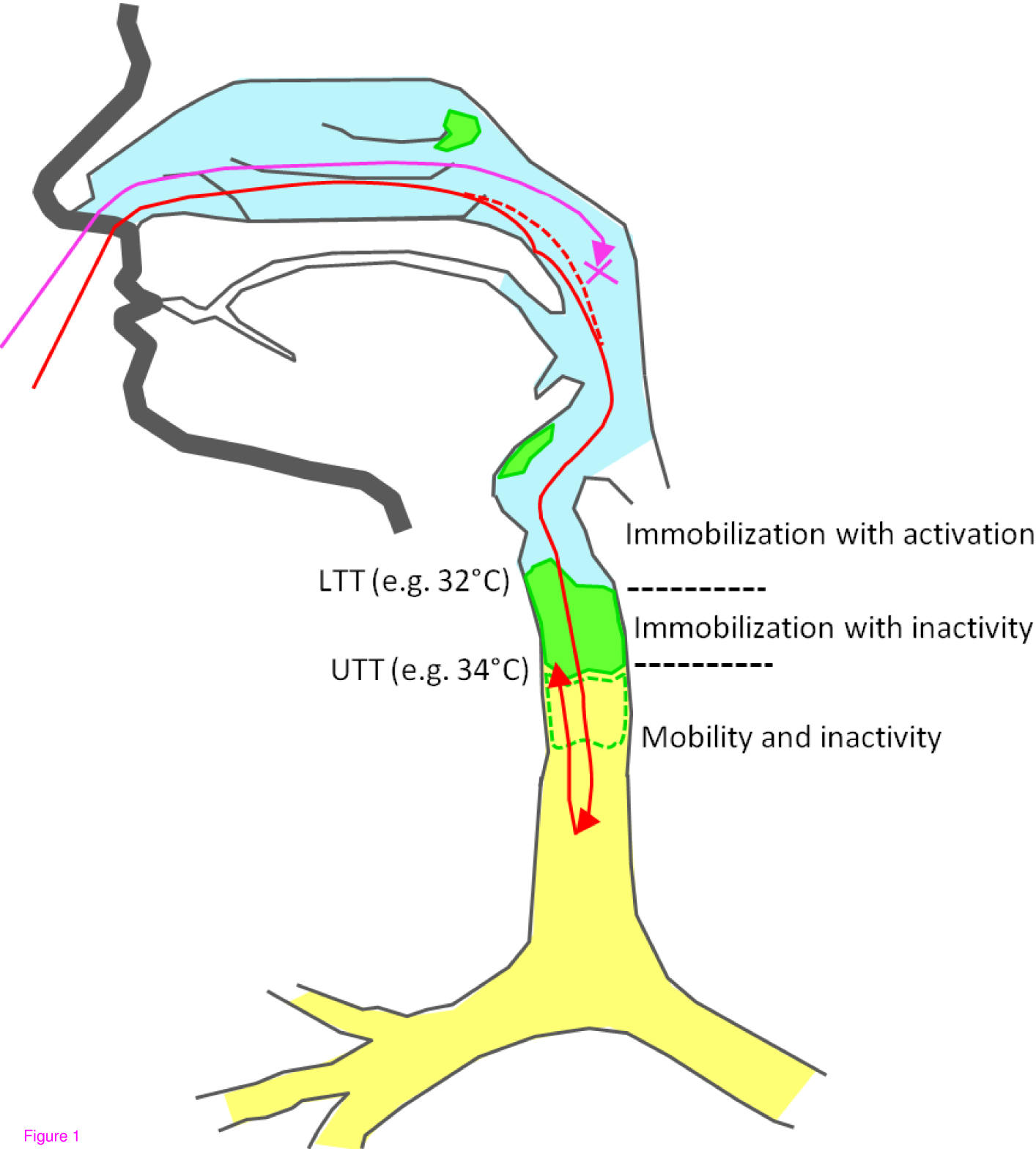
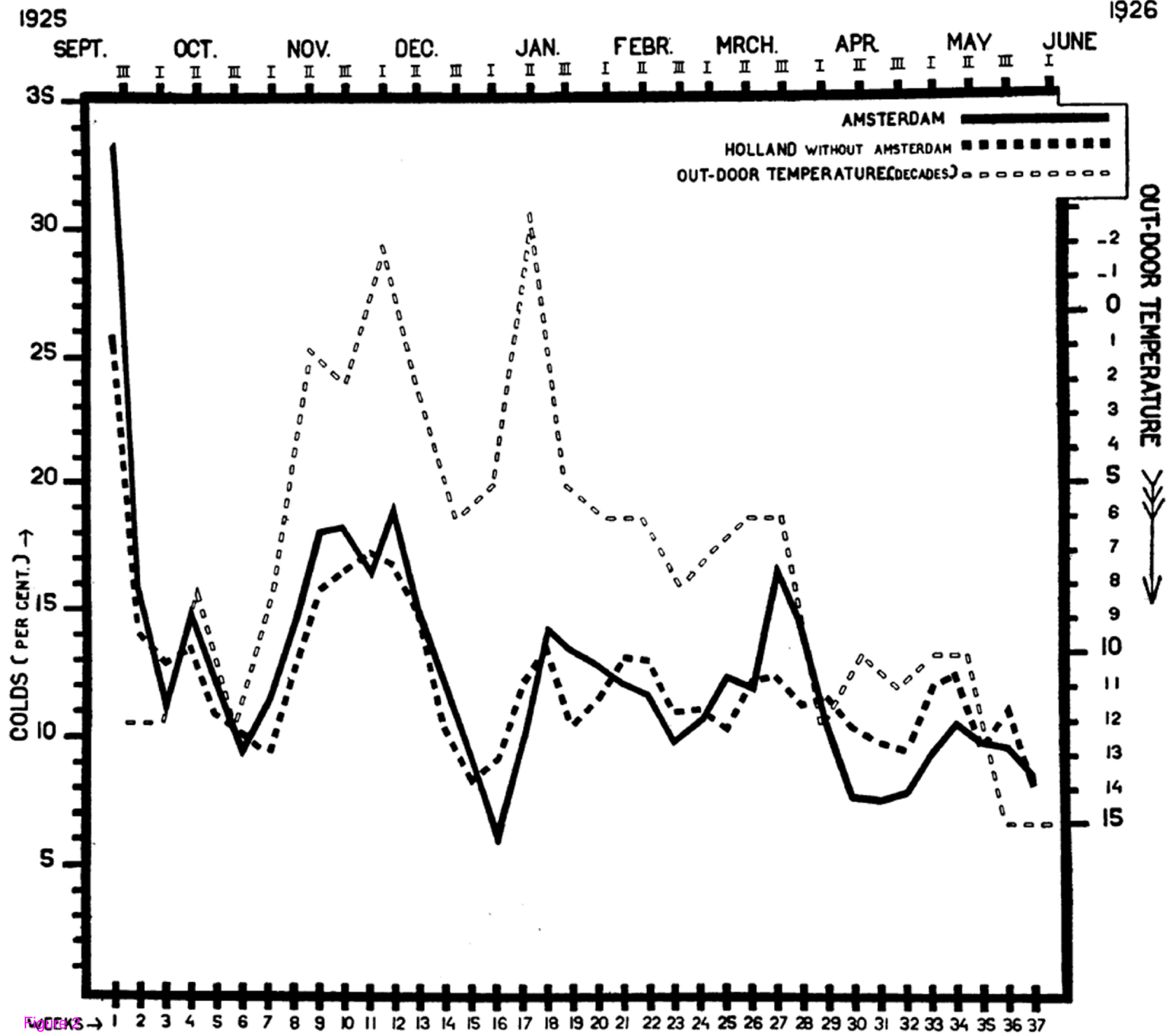

Immobilization with activation
LTT (e.g. 32°C)
Immobilization with inactivity
UTT (e.g. 34°C)
Mobility and inactivity

Figure 1


1925
1926
SEPT.
OCT.
NOV.
DEC.
JAN.
FEBR.
MRCH.
APR.
MAY
JUNE
AMSTERDAM
HOLLAND WITHOUT AMSTERDAM
OUT-DOOR TEMPERATURE(DECADES)
COLDS (PER CENT.) →
OUT-DOOR TEMPERATURE
WEEKS →

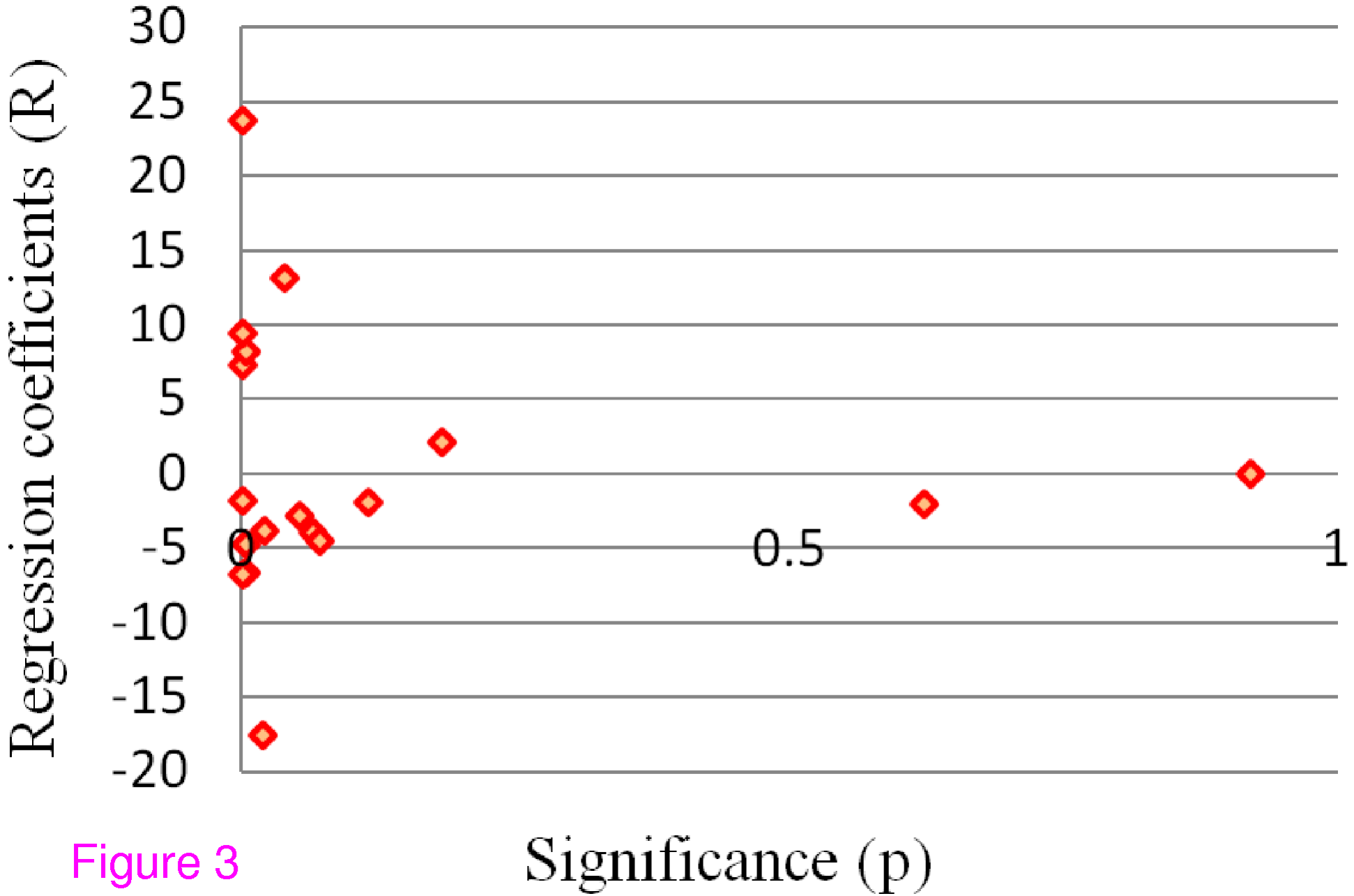

Regression coefficients (R)
30
25
20
15
10
5
0
-5
-10
-15
-20
0
0.5
1
Significance (p)

Figure 3

Additional files provided with this submission:

Additional file 1: Bugs and chills.pptx, 3607K
http://www.biologydirect.com/imedia/1615084921109942/supp1.pptx