Seasonality and selective trends in viral acute respiratory tract infections

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Abstract

Influenza and many unrelated viruses, including rhinovirus, RSV, adenovirus, coronavirus etc. share the same seasonality, since these viral acute respiratory tract infections (vARIs) are much more common in winter than summer. The lack of a viable explanation is a major problem for microbiology. Unfortunately, early investigations that used recycled "pedigree" virus strains seem to have led microbiologists to dismiss the common folk belief that vARIs often follow chilling, together with the scientific evidence that supports this idea. Today, incontrovertible evidence from polar, tropical and island-based studies, PCR-based surveys, and studies of the effects of outdoor dress and activities, shows that low ambient temperature and host chilling increase the incidence and severity of vARIs. This review considers four possible explanations of this link: (1) increased crowding in winter may enhance viral transmission; (2) lower temperatures may increase the stability of virions outside the body; (3) lower temperatures may increase host susceptibility; (4) chilling may activate dormant virions. There is little evidence for the first two explanations, the second of which is incompatible with tropical observations of vARIs. Explanation 3 is supported by a recent study that found that the immune response of chilled mouse airway cells was diminished. However, tropical observations and epidemiological anomalies such as the simultaneous arrival of vARIs over wide geographical areas, the rapid cessation of influenza epidemics in midwinter, and the low attack rate of influenza within families are compatible with explanation 4, but not 3 (at least not in its simple form). Explanation 4 is also compatible the natural temperature sensitivity of many wild and laboratory strains, and the frequent recovery of temperature-sensitive mutants from persistent infections. The evidence suggests that explanation 4 is the main driver of seasonality, but explanation 3 may also have an important role in seasonality.

Key index phrases

Respiratory tract infections, viral infections, temperature changes, temperature sensitivity, seasonality of the common cold, influenza seasonality, epidemiology of viruses.

The seasonality of colds and 'flu

The lack of an explanation for the seasonality of viral acute respiratory tract infections (vARIs) that can hold water [1, 3, 73, 78] is a major problem for microbiology, and it suggests that we do not fully understand the viruses involved. Other anomalous features of vARIs need to be explained too. For example, vARI epidemics increase dramatically and rapidly when ambient temperature drops, indeed they increase too rapidly to be the result of increased transmission [4] (Figure 1). Surveys also show that epidemics often occur simultaneously throughout wide geographical areas [4, 20, 22] (see Figures 1 and 2, and the discussion below). Moreover, influenza epidemics often cease very abruptly, even when a large number of susceptible individuals remain in the population [22].

The viruses that cause vARIs include many unrelated families such as double-stranded DNA viruses (e.g. adenovirus), positive-sense single-stranded RNA viruses (e.g. coronavirus), negative-sense single-stranded RNA viruses (e.g. respiratory syncytial virus (RSV), influenza, measles, mumps and parainfluenza virus), and positive-sense single-stranded RNA viruses (e.g. hand foot and mouth virus, rhinovirus, rubella virus). It is notable that the great majority of these diverse and often distantly-related strains share the same seasonality in temperate regions. For example, Hope-Simpson found in both 1954 and 1955 that the number of people suffering from "colds" in a sample of 380 volunteers was roughly 50 times greater in February than at the beginning of September [73] (Figure 3). The common cold is caused by over 200 serologically-distinct strains [74]. Not all of these strains share the same seasonality in temperate

regions, but it is clear that the great majority do, since colds in general show such strong seasonality. This has been confirmed recently by direct observation using modern diagnostic tests: a recent study at a children's hospital in Germany found that 7 out of 10 vARIs displayed normal seasonality, and they showed a significant correlation with ambient temperature (p-value <0.001) [78]. (There are a few clear counter-examples. Hope-Simpson isolated more type 3 parainfluenza viruses in the warm semester than the cold one, although parainfluenza type 1 and type 2 viruses showed normal seasonality [75].) This implies the existence of an important mechanism concerned with viral replication or transmission that is common to the majority of respiratory viruses, in spite of their widely-differing biochemistry and genetic mechanisms. It seems likely that a successful explanation would have far-reaching practical implications for treating and protecting humans and animals from vARIs.

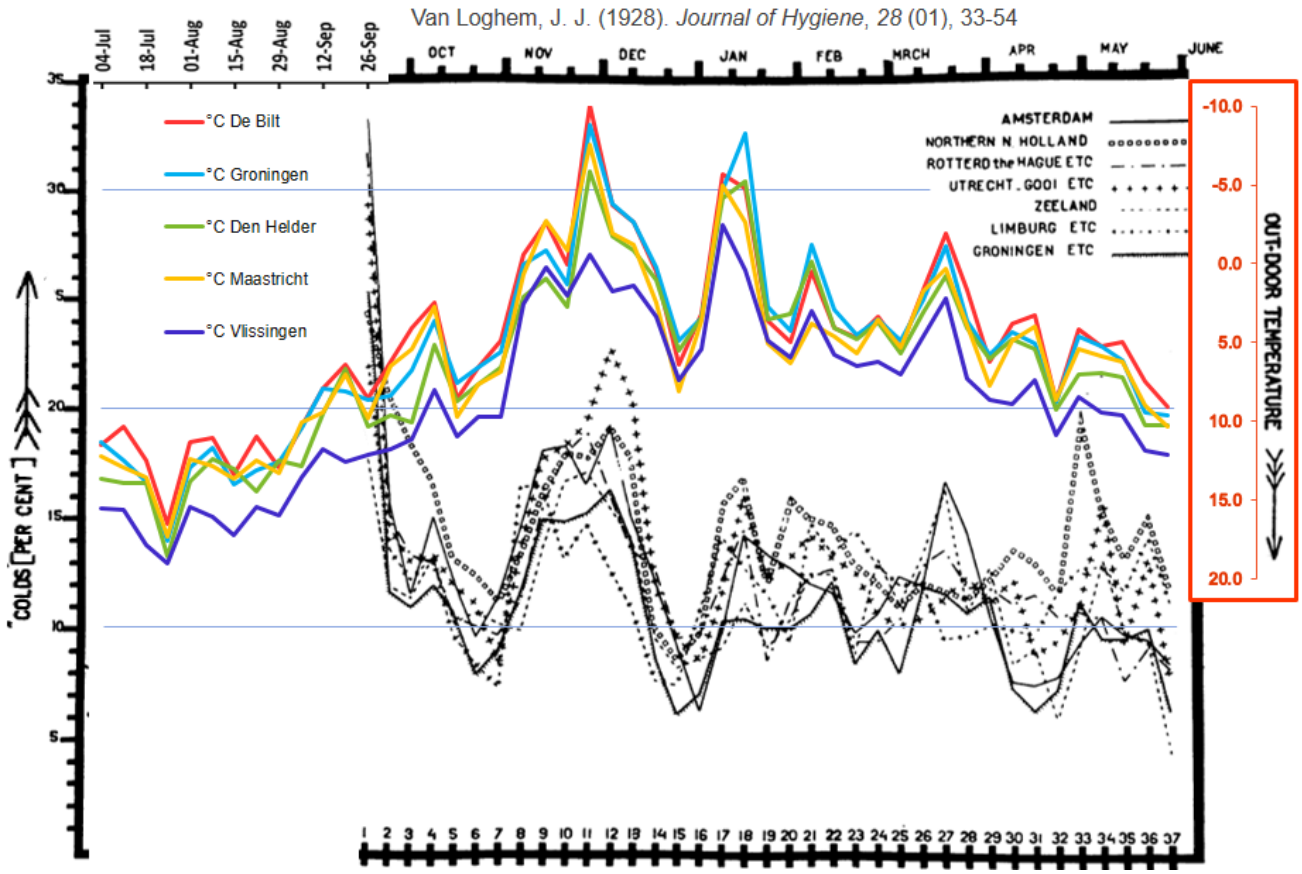

Figure 1. *Graph II from van Loghem's report [4] on the epidemiology of vARIs in the Netherlands in the winter of 1925/26, with ambient temperature superimposed*. The graph shows the percentages of persons with colds in seven regions of the Netherlands for 37 weeks. The data was compiled from the reports of 6933 correspondents that were submitted by post each week. Amsterdam had the largest number of informants (1159) and Noord-Holland the fewest (581). I have added the daily minimum outdoor air temperature (averaged over 7 days at weekly intervals) from five Dutch weather stations, with the temperature scale inverted (lowest temperatures at the top). Note that by far the highest rate of vARIs was at the beginning of the study (September 1925), and that vARIs in different regions are closely correlated with each other and with inverted temperature. These correlations are strongest in the first half of the cold season. ©1928, 2014. This figure was originally published in the *Journal of Hygiene*, *28*(01), 33-54.

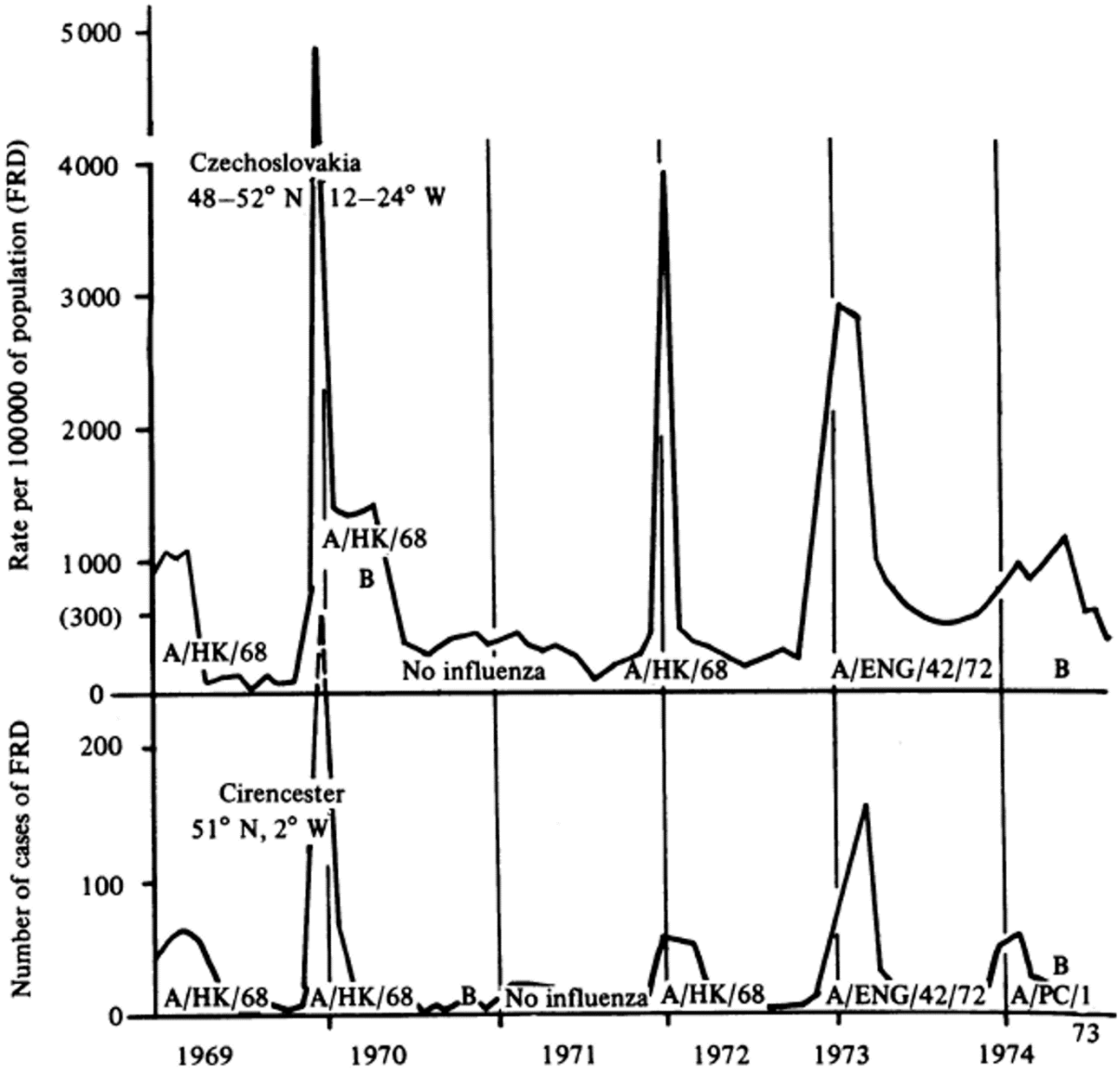

Figure 2. *The Cirencester (UK) acute febrile respiratory diseases at 51.430 N, 1.590 W, compared with notifications of such diseases in Czechoslovakia (Prague, 50.050 N, 14-250 E), 1969-74, taken from Hope-Simpson's investigation into the role of season in the epidemiology of influenza [22].* This remarkable figure demands theoretical explanation. The antigenic changes in influenza A virus (occurring at both sites) show clearly that novel influenza strains moved around Europe during the period shown. There is, however, no evidence of moving "waves" of influenza because epidemics at the two sites are very closely synchronized. Note that the shortest route between the two sites covers 1,400 km by sea and road, crosses four national boundaries, and passes through some of the most densely-populated regions of Europe. This suggests that the virus moved to both sites prior to its manifestation, and a stimulus that was present at both sites triggered the concurrent epidemics. These data are compatible with the fourth explanation discussed below, that virions can become dormant at some unknown location in the respiratory tract, and can subsequently be activated by host chilling. *Journal of Hygiene*, *86*(01), 35-47.

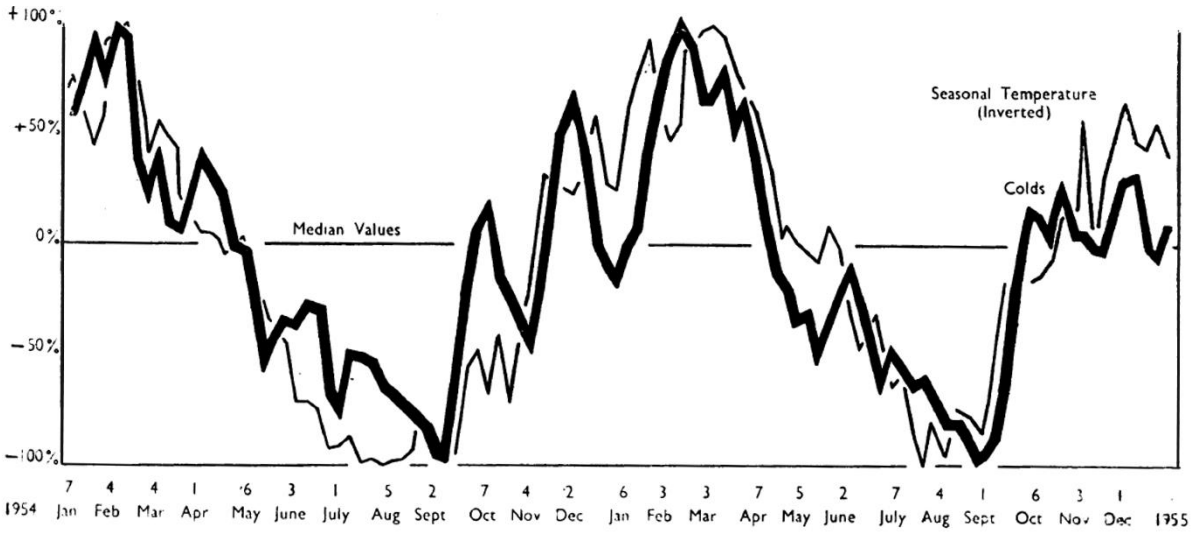

Figure 3. *Morbidity from colds in Cirencester, UK, 1954 and 1955, plotted alongside temperature [73].* Thick line - percentage of volunteers showing symptoms. Thin line - earth temperature (inverted). ©1958. This figure was originally published in the *Proceedings of the Royal Society of Medicine*, *51*(4), 267-271.

Microbiologists have put forward many explanations of the seasonality of vARIs. Proposed explanations of influenza seasonality, for example, include factors that change host contact rates (school closures, ambient temperature and precipitation), factors that may influence virus survival outside the body (relative humidity, absolute humidity, solar radiation and temperature), and factors that may change the immunity of hosts (humidity, photoperiodicity, temperature, viral interference, as well as deficiency of selenium, vitamin C, vitamin D and vitamin E) [1]. (Factors that may change the behavior of viruses at the biochemical level are seldom considered.) However, these well-known explanations are very difficult to reconcile with a straightforward observation: the vARIs in question are present in many tropical regions at intermediate levels throughout the year – much higher levels than in the summer in temperate locations. Almost all of the parameters listed above have more extreme values in the tropics throughout the year than in temperate summers. Therefore, according to these explanations, vARIs should not be present in the tropics at all [1]. Moreover, H3N2 influenza A (and presumably other influenza strains) circulates continuously in East and Southeast Asia, and spreads to temperate regions from this network [2], so we would expected it to have properties that allow it to be active during temperate summers. Note also that influenza and other vARIs shows clear seasonality in the tropics that does not coincide with fluctuations in temperature, humidity or solar radiation [1, 79]. The strange global and seasonal distribution of vARIs including influenza is shown schematically in Figure 4. These problems were noted by two recent reviews of influenza seasonality, both of which also noted the lack of a satisfactory explanation [1, 3].

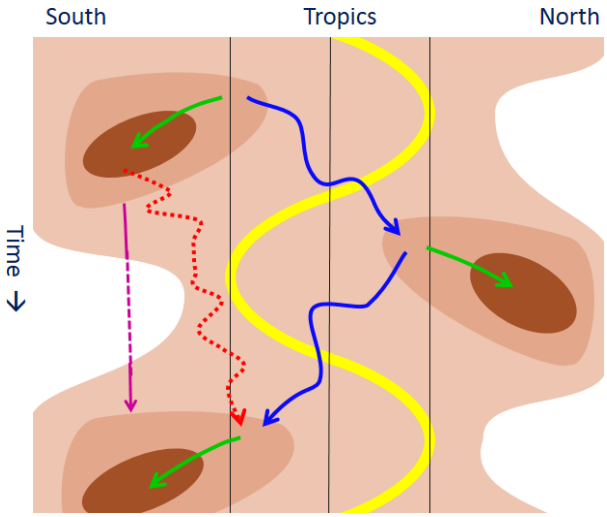

Figure 4. *The global distribution and seasonality of vARIs, shown schematically*. Levels of vARIs are indicated by brown shading, with dark brown showing the highest rates of infection, while the yellow curve shows the path of vertical solar radiation. The strange distribution of vARIs is shown, with more vARIs in the tropics throughout

the year than in temperate regions during the summer months [2, 3]. It is known that seed strains of influenza A (H3N2) circulate continuously in a network in East and Southeast Asia (blue arrows) and spread to temperate regions from this network (green arrows) [2]. Several lines of evidence suggest that personal chilling will increase the prevalence of vARIs [4 - 12], and, since travel away from the tropical regions is associated with a decrease in temperature, it is likely that vARIs spread more quickly from the tropics to the temperate regions (green arrows) than in the opposite direction (dotted red arrow). The degree to which viruses remain dormant during the summer in temperate regions (dotted purple arrow) is unknown.

This review will focus on vARIs in general, which are caused by a variety of viral species. If it were possible, more consistent conclusions might be arrived at by focusing instead on a single vARI, but unfortunately the relevant available data are very limited. This review will therefore consider influenza, the best-studied vARI, as well as colds and other vARIs, based on the assumption that it is not a coincidence that so many vARIs share the same seasonality, and that an unrecognized common thread runs through the replication or transmission of most respiratory viruses.

The effect of weather and host chilling on vARIs

Ambient temperature often has a dramatic effect on respiratory disease. In the UK, Hajat *et al.* found that general practitioner consultations for lower respiratory tract infections in one UK City (Norwich) increased by 19% for every degree that average temperature dropped below 5°C, observed 0 - 20 days before the consultation [7]. Absolute temperature, however, is not correlated with vARIs in a simple way. For example, we do not see a vARI that is limited to all global regions or seasons where temperatures remain below, say, 10°C (Figure 4). Evidence from many different sources, however, shows that vARI incidence is related to temperature *fluctuations*. For example, van Loghem conducted a very extensive survey of vARIs in the winter of 1925/26 with 6,933 participants from all regions of the Netherlands [4]. His data is shown in Figure 1, together with the temperatures recorded by five Dutch weather stations. Epidemics of vARIs in all seven regions were very closely synchronized with each other, and correlated with inverted temperature (i.e. lower temperatures were associated with increased vARIs). These correlations were strongest during the period when temperatures were generally falling, i.e. the first half of the cold season. Note also that the highest infection rate, which occurred at the start of the study, coincided with the highest temperature during the study. Just prior to the start of the study, however, the temperature declined after remaining steady during the summer months – again emphasizing that we need to consider temperature *fluctuations* rather than absolute temperature levels.

Milam & Smillie found similar patterns on the tropical island of St. John in the Virgin Islands in 1929, shown in Figure 5 [5]. Between mid-afternoon and midnight each day the temperature on the island dropped sharply by 5 - 7°C. When the temperature dipped in the autumn by 1.7°C (green bar) below the summer range, an epidemic of colds was triggered. More recently, Jaakkola *et al.* found that sudden declines in both air temperature and absolute humidity (in the three days that preceded the reporting of the sickness) increased the incidence of influenza A and B in military conscripts in Northern Finland [6]. Paradoxically, the incidence of influenza was lower at very low temperatures, and it was the sudden *decline* of temperature rather than low absolute temperature (and, the authors suggested, the decline of humidity) that increased the risk of influenza.

Historical studies have some advantages over modern investigations. The study by van Loghem was on a scale that would be difficult today, and he was able to collect data from multiple geographical locations within the Netherlands (Figure 1). The study by Milam and Smillie has the advantage that the island was very isolated, so that a limited set of viruses was studied (Figure 5). However, modern studies have the great advantage that they can identify the viral species involved. Two recent studies in Brazil and Germany

compared hospital admissions of children suffering from known vARIs with weather parameters [77, 78]. Both studies found that the number of cases for all pathogens was strongly inversely correlated with outside temperature. Viegas *et al.* plotted the frequencies of RSV, adenovirus, influenza A virus and parainfluenza virus alongside mean temperature. On all plots clear seasonality is apparent, but adenovirus and parainfluenza virus clearly lagged behind inverted temperature, peaking in early spring. Du Prel *et al.* presented similar plots and reported that RSV, influenza A and adenovirus were significantly correlated with temperature, while rhinovirus was correlated with relative humidity [78]. The study used Spearman's ranked correlation coefficients to find associations between meteorological parameters and hospitalizations for 12 viral and bacterial pathogens. This tabular information is difficult to interpret, however, because correlation coefficients dramatically *underestimate* the closeness of a relationship when one of the parameters lags behind another. It is therefore possible that associations were present for enterovirus, parainfluenza types 1 and 3, *Mycoplasma* and *Chlamydophila,* but not made evident by their correlation coefficients. For example, rhinovirus had a correlation coefficient with temperature of only -0.42, but close inspection of the plots shows a clear relationship, with the first rhinovirus epidemic of each season closely following the first major drop in temperature at the end of summer [78].

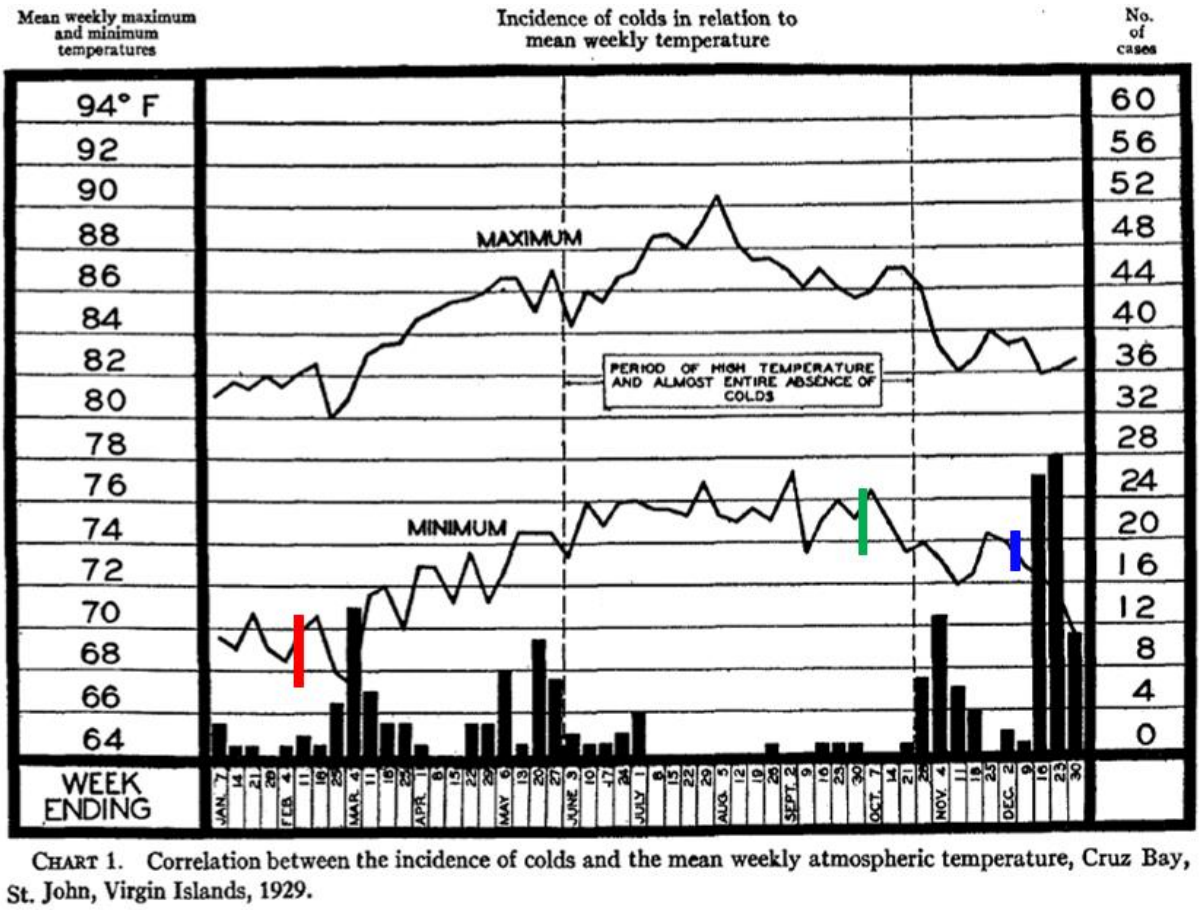

CHART 1. Correlation between the incidence of colds and the mean weekly atmospheric temperature, Cruz Bay, St. John, Virgin Islands, 1929.

Figure 5. *Chart 1 from Milam and Smillie's 1929 study of colds on an isolated tropical island.* The authors noted that outbreaks of colds often followed temperature drops, and were almost absent in the summer months. The red, green and blue bars indicate temperature fluctuations of 1.9, 1.7 and 1.0°C respectively. (The large outbreak in December seems to have been introduced to the island by a sailor on the mail boat.) ©1931. This figure was originally published in the Journal of Experimental Medicine. 53:733-752. doi: 10.1084/jem.53.5.733.

Studies at the individual level show that physical host chilling can increase the severity of vARIs. (The possible mechanisms involved will be discussed below.) The Eurowinter Group showed that shivering outside, and wearing inadequate winter clothing increased respiratory disease-related mortality, while outdoor exertion sufficient to cause sweating was protective [8]. Yanagawa *et al.* found that 11 of 13 patients recovering from cardiopulmonary arrest who were treated with mild hypothermia developed pneumonia, as compared to 6 of 15 controls who were maintained at normal body temperature (p-value <0.02) [9].

Costilla-Esquivel *et al.* found a relationship between weather and acute respiratory illnesses in Monterrey (Mexico), which they were able to model very accurately using only three weather parameters: weekly accumulated rainfall, minimum temperature in the week, and weekly median relative humidity [10]. Rainfall and relative humidity were positively correlated with respiratory illnesses, while temperature was negatively correlated. Both rainfall and low temperature can obviously cause personal chilling, while high relative humidity is a consequence of the other two.

In summary, the available evidence shows that both sudden weather changes and factors that cause individual chilling frequently bring on vARIs. This suggests that temperature sensitivity plays a role in seasonality, but the global patterns of vARIs rule out the possibility that viral activity is controlled solely by absolute temperature. Rather, respiratory viruses seems to adapt over a few weeks or months to the ambient temperature, such that temperature *fluctuations* outside the previous range trigger vARIs.

Relative (and absolute) humidity

Du Prel *et al.* explain the observed correlation of rhinovirus with relative humidity by pointing out that "rhinoviruses cannot survive in a dry environment", i.e. they suggest that the seasonality of rhinovirus is driven by changes in the survival rate of the virions outside the body. However other interpretations are possible: increased relative humidity is associated with rainfall, which can wet individuals' clothing and cause chilling. Moreover, and more fundamentally, rhinoviruses are mainly transmitted *indoors* in modern cities, and, as Hope-Simpson showed by direct measurement, *indoor* relative humidity is much *lower* in winter than in summer due to the effects of artificial heating in winter [73]. Changes due to indoor relative humidity would therefore generate the opposite seasonality. Low absolute and relative humidity have also been suggested as factors that increase the transmission of influenza [6, 13, 14]. However the evidence here is contradictory, and clear trends have not emerged. Animal experiments with guinea pigs show that, while transmission of influenza generally decreases with increasing relative humidity, it actually *increases* at 20°C when relative humidity moves from 50% to 70% [13] - and these are the typical values of indoor relative humidity in e.g. the UK in winter and summer respectively [73]. (Animal experiments with influenza are further discussed below.) Moreover, influenza outbreaks in the tropics often coincide with the rainy season [1, 79]. For example, Fortaleza in Brazil has a single large peak of influenza [1] that coincides with the rainy season (January to July). Clearly, correlations of vARIs with relative and absolute humidity are inconsistent.

Dormancy in vARIs

Viruses such as adenovirus [30], RSV [31], foot-and-mouth virus [32] and chickenpox virus - all of which can spread via the respiratory tract - are known to become dormant within their hosts. Other respiratory viruses show similar behavior on a shorter timescale. Morikawa *et al.* found human parechovirus, adenovirus, enterovirus, coronavirus 229E and HKU1, and rhinovirus in the gargle specimens of eight *asymptomatic* children using polymerase chain reaction (PCR) tests [76]. The tests required at least 100 copies of the genetic material, suggesting that the viruses in question had begun to replicate [76]. However, the authors noted that it is difficult to interpret PCR results because it is a very sensitive method, and the positive results may reflect an asymptomatic past or concurrent infection, or an imminent infection. The data suggest that at least some were imminent infections: four of the children

had positive results when they were asymptomatic that were followed four weeks later by a vARI that was caused by the same species (or a species that was indistinguishable due to the limitations of the testing method); rhinovirus was detected in a fifth child who was at first asymptomatic, but experienced the symptoms of a cold caused by that virus one week later.

Influenza viruses have been detected several times in the absence of symptoms or an immune response in the host, which indicates that dormant influenza virus is present. Foy *et al.* identified 10 asymptomatic individuals who were shedding influenza B virus but did not respond with antibody by any of the five test methods employed [33]. During the 2009 influenza A (H1N1) pandemic, Tandale *et al.* found that, of 65 asymptomatic individuals with PCR-confirmed H1N1, 12 had not seroconverted [34]. During the same pandemic, Papenburg *et al.* found two asymptomatic individuals with PCR-confirmed infections who had not seroconverted [35]. In Vietnam, Thai *et al.* found that, of 11 individuals shown by PCR to have been infected with pandemic H1N1 by other members of their household, one remained asymptomatic and had not seroconverted [36]. The authors commented that this "may indicate that viral RNA remained in the respiratory tract without being internalized and eliciting an immune response". These observations show the reality of influenza persistence in the respiratory tracts of asymptomatic individuals, and are compatible with the suggestion that virions can become dormant, and, later, be reactivated, for example by chilling.

Observations of vARIs in Antarctic stations after many months of complete isolation can provide evidence that is easy to interpret because only one or a few viral species are present at a time. For example, a geologist ("J.E.H.") at the Mawson station in 1966 picked up a respiratory virus from a visiting field party [11]. 17 days later he and three colleagues were exposed to cold and damp conditions, which brought on vARI symptoms including muscle aches and a sore throat in J.E.H. and two of his colleagues. Another study at Adelaide Island in 1969 found that after 17 weeks of complete isolation several men developed colds four days after the air temperature fell in one day from 0°C to -24°C [12]. Both studies suggest that chilling caused by particular activities or by weather changes can activate dormant virions, giving rise to vARIs. Similarly, Muchmore *et al.* reported parainfluenza shedding by healthy young adults throughout the 8½-month winter isolation period at Amundsen–Scott South Pole Station during 1978 [37]. The study recorded two episodes of respiratory illness caused by parainfluenza at the Station that year after 10 and 29 weeks of complete social isolation.

Taken together, the above observations strongly suggest that a variety of respiratory viruses can become dormant in human hosts and can subsequently be activated, giving rise to vARIs. This is discussed further below.

Mechanisms that would allow vARIs to respond to temperature changes

If we accept that host chilling (with various causes) triggers vARI epidemics and gives rise to vARI seasonality in both temperate and tropical regions, four possible mechanisms can be put forward: (1) low temperature increases crowding of human hosts, increasing transmission. (2) Colder conditions allows the virus to survive outside the body for longer, increasing transmission. (3) The susceptibility of hosts increases as a result of chilling. (4) Chilling increases the activity of viruses in the body. I will now consider the evidence for and against these four possibilities.

(1) Seasonal events may increase the crowding of human hosts during the winter

A popular explanation of vARI seasonality is that contact rates are lower in the summer when children are out of school, and when people spend more time outdoors. However, in the USA seasonal differences in "crowding" are minimal since the amount of time spent *indoors* varies by less than 10% between summer and winter [1]. In the UK, the number of school-days in the coldest six months of the year is less than 10% higher than in the warmest six months, but, like all

temperate countries, the UK has marked vARI seasonality. Moreover, one of the two peaks of influenza activity in Singapore [1] actually coincides with the school holidays in June. Another negative observation is that festivals and sporting events are not associated with increased vARIs. During football world cups people spend more time indoors and often crowd together in bars etc. to watch matches on television, but no significant increase is apparent in Google Flu Trends in any country in the Northern Hemisphere during the 2014 FIFA World Cup, and there was a downward trend in Argentina and Brazil during this period. Lofgren *et al.* agreed that theoretical and empirical studies do not adequately explain influenza A seasonality, noting in particular that no published studies show directly that variations in crowding cause influenza seasonality [3].

In summary, changes in the degree of human crowding may well play an important role in the progress of vARI epidemics, but there is no evidence that it is a general or the main driver of vARI seasonality.

(2) Colder conditions may allow virions to survive outside the body for longer

This is currently the most popular explanation of seasonality. The mechanism is, however, almost certainly not the main cause of seasonality, for several clear reasons.

Firstly, this explanation cannot explain why vARIs are present in many tropical regions all year round, but virtually absent from the temperate regions during the summer months. If respiratory virions can adequately survive outside the body in the tropics, they should certainly survive (according to this explanation) during temperate summers. (The suggestion that low absolute or relative humidity may increase viral survival does not help, because vARI epidemics occur during the rainy season in many tropical locations.)

Secondly, consider van Loghem's data [4] (Figure 1). While temperatures were generally decreasing (i.e. up to the end of January) changes in vARIs were very well-synchronized with changes in temperature, with no apparent lag. Since the average incubation period of seven common vARIs (excluding measles) reported in a recent review was 3.9 days [16], there is time for only one or two cycles of infection per week. The response of vARIs appears to be too fast to be the result of changes in viral transmission. It was also extraordinarily well-synchronized across the country, with no evidence of "waves" of infection moving between different locations, which would be expected if cold temperatures increase *transmission*. Hope-Simpson made similar observations when he compared influenza epidemics (1969-74) in the UK with epidemics in Prague, Czechoslovakia, showing the correspondence of epidemics at widely separated localities at a similar latitude [22] (see Figure 2). In another example, Magrassi was impressed by cases of influenza in 1948 among shepherds living in complete social isolation in open country in Sardinia, who developed the disease contemporaneously with the inhabitants of towns on the same island [20].

Thirdly, it seems unlikely that virus transmission is acutely sensitive to small temperature fluctuations (about 4°C) over such a large range (about 20°C). In van Loghem's data, a drop from 10°C to 5°C causes a vARI epidemic, as does a subsequent drop from 5°C to 0°C, and a drop from 0°C to -5°C. The earlier drop from 15°C to 10°C in August also seems to have had dramatic effect; substantial cold epidemics are often observed in early autumn [5, 21, 78], and 1925 was clearly no exception, since *33%* of the population of Amsterdam suffered from colds at the end of September. If each of these epidemics were caused by increased viral transmission, this mechanism would imply a change in viral transmission of several orders of magnitude. A similar argument applies to the data of Milam & Smillie (Figure 5), which also showed fast-acting sensitivity to small temperature drops, and these events occurred at a range of temperatures throughout the year [5]. Jaakkola *et al.* reported that "sudden declines" of around 5°C preceded the onset of influenza in Northern Finland [6], occurring at temperatures above 15°C and also below -15°C. This implies that transmission needs to vary over roughly 30°C.

Changes in viral transmission as a result of temperature fluctuations may well significantly influence the progress of many vARI epidemics, but, unless new evidence comes to light, we need to look elsewhere for a general explanation of vARI seasonality.

(3) Chilling may increase the susceptibility of hosts

Eccles suggested that physical chilling may cause reflex vasoconstriction of the blood vessels of the upper airways, thereby reducing host defenses against infection during the winter [17-19]. This hypothesis can explain the results of Eccles' own study where the chilling of volunteers' feet increased the number of vARIs in the following 4 to 5 days [18], and it can, for example, explain the occasional simultaneous appearance of vARIs throughout a wide geographical region [20], when a "subclinical" epidemic that is present in the region is suddenly converted to an epidemic of "clinical" infections with severe symptoms. Eccles' suggestion (at least in its simple form) may therefore partly explain the observed seasonality of vARIs, but it has difficulty in explaining much of the reported scientific data.

The difficulty with scales that was noted above for explanation (2) above also applies to this explanation. If innate human susceptibility does not vary much in different climates – and it is hard to find reasons that are compatible with evolutionary theory why it should - the proposed mechanism needs to act over a wide range of temperatures, applying in different climates and at different times of year. It is difficult to reconcile this with the observed sensitivity to small temperature drops. Consider again, for example, Chart 1 of Milam & Smillie's paper [5] (Figure 5). Every night in the summer the temperature dropped by 5 - 7°C. In the autumn the temperature fell by an extra 1.7°C, which triggered an epidemic of colds. Can we believe that the inhabitants' immune systems could cope well with a regular 6°C drop but succumbed after a 7.7°C drop? Note that the absolute temperature after the dip - about 23°C at night - was still very comfortable, and would certainly not cause a vARI epidemic in, say, Helsinki.

Note also that there is often a peak of vARIs in the early autumn [4, 5, 21]. This can be seen, for example, in Google Flu Trends for Germany [29], especially away from coastal regions. (Flu Trends in Germany is unusual because it models "acute respiratory illness", which includes all vARIs whether or not they cause fever.) Another example is the very high level of colds at the beginning of the study by van Loghem, which began on 19 September, 1925 [4]. vARI levels were much higher at the start of this study than in the rest of the cold season. It is difficult to explain why the human immune system should be less efficient in early autumn than in, say, March.

Another problem with this idea is the abrupt cessation of influenza epidemics. Hope-Simpson noted that all the major influenza epidemics that he recorded in Cirencester, UK, (1951, 1957, 1959, 1969 and 1973) rose rapidly to a single peak within four weeks, then abruptly ceased in the following 4 - 5 weeks [Figure 1 in ref. 22]. In at least one case it was clear that this was not due to a lack of susceptible persons: the H2N2 subtype arrived explosively for the first time in Cirencester in September 1957, with over 100 individuals suffering from acute febrile respiratory diseases by the third week of October. This epidemic abruptly ceased after only six weeks. It is known for certain that many susceptible individuals remained in the population at that time because there was a second major H2N2 epidemic 16 months later [22]. The abrupt cessation of the first epidemic is therefore unexplained. The other four major epidemics listed above were in midwinter, when (according to this view) the immune system should be at its weakest, suggesting that the virus should spread and the epidemic should continue for more than nine weeks.

An interesting and ingenious recent review looked directly at the seasonality of immune responses in humans by investigating antibody responses following vaccination [24]. Although the authors found seasonal variation in immunity, it could not explain vARI seasonality: seven of the studies of vaccines reviewed reported a stronger immune response in winter than in summer, with only 1 showing the opposite trend. There was no clear trend with regard to the dry and

rainy seasons in tropical regions and several studies showed no trend at all. These data therefore suggest that variations in host susceptibility do not fully explain the seasonality of vARIs.

There is little evidence for changes in host susceptibility at the epidemiological level, but new evidence has been found at the biochemical level. Foxman *et al.* found that mouse airway cells infected with mouse-adapted rhinovirus 1B exhibited significantly lower expression levels of type I and type III interferon genes and interferon-stimulated genes at 33°C relative to 37°C [80]. This is a very interesting but puzzling result, and the authors offer no explanation of the possible benefits to the mouse. If airway cells possess mechanisms that can reduce infections, it seems strange that they should be down-regulated at lower temperatures. (One explanation is that the interferons cause damage to the cells, and the mouse prefers to tolerate the virus. A related proposal is that mice permit the growth of viruses in their respiratory tracts in order to raise antibodies against them before the virus can reach the internal organs.) We need to wait for equivalent results in the cells of other species, both *in vitro* and *in vivo,* before we can fully interpret these data.

A study by the Eurowinter Group may shed light on how host susceptibility to vARIs varies with temperature. This group found that exposure to cold outdoor air can alter host susceptibility in opposite directions: shivering outside greatly increased respiratory disease-related mortality (p-value=0.001), while outdoor exertion sufficient to cause sweating reduced it (p-value=0.02) [8]. The protective effect of outdoor exertion is presumably the result of physiological changes in the host, but the increase in mortality following chilling may be the result of the temperature-sensitive responses of either the host or the virus.

Together, this evidence suggests that changes in host susceptibility may influence vARI epidemics considerably, and might contribute towards seasonality, but that they are not the main driver of seasonality.

(4) Chilling may increase the activity of respiratory viruses as a result of their natural temperature sensitivity

Lwoff proposed in 1959 that the degree of virulence of viruses is related to their level of temperature sensitivity, i.e. greater sensitivity to heat is correlated with reduced virulence [25]. In 1979, Richman & Murphy confirmed this association and reviewed its implications for the development of live virus vaccines [26]. They noted that the replication of temperature-sensitive (*ts*) influenza, parainfluenza, RSV, and foot-and-mouth viruses was consistently more restricted in the lungs of a variety of animals than in their nasal cavities. (In Richman & Murphy's paper, and in this review, *ts* refers to viruses that are *less* active at higher temperatures.) They also found that both naturally-occurring and synthetic *ts* viruses were very frequently less virulent than their non-*ts* counterparts in humans and animals, noting several cases (including influenza and vaccinia virus) where the loss of the *ts* phenotype resulted in the restoration of the virulence or growth capacity of the virus, both *in vivo* and *in vitro* [26]. Chu *et al.* later tested seven H1N1 strains with varying degrees of temperature sensitivity in volunteers and found a correlation between temperature sensitivity and the severity of symptoms, with more *ts* strains being less virulent [50]. It is reasonable to conclude that the *ts* phenotype facilitates the transmission of the wild virus because it prevents or reduces multiplication of the virus in the lungs or internal organs, which might result in the death or immobilization of the host. One possible explanation of vARI seasonality is therefore that the lower temperatures of winter increase the activity and virulence of viruses, as a side-effect of their tropism.

It is known that there is a temperature gradient in the human respiratory tract, from around 24°C at the glottis to around 35.5°C at the subsegmental bronchi [27]. The temperature in the respiratory tract drops rapidly when the air being breathed is cold, when the host breaths rapidly, or – significantly - when the host is chilled [27, 28]. Putting these observations together, a simple explanation of the seasonality of vARIs and the anomalous behavior of respiratory viruses

described above might be proposed along the following lines:

1. One or several steps in the life-cycle of most respiratory viruses are *ts.* These *ts* steps might include the binding of virions to cells, and their entry into cells, or any subsequent step in their replication (see the biochemical evidence reviewed below).
2. *Ts* virions that bind to cells lower down the respiratory tract (therefore at relatively high temperatures) may remain dormant at some unknown cellular location (which might be on extracellular material, on the surface of, or within, the cells that line the respiratory tract).
3. Virions that bind to cells higher up the respiratory tract (therefore at relatively low temperatures) will become active, but will not normally cause a vARI because they are active in low numbers at any one time and can be removed by the host's immune system.
4. Each day the temperature of the respiratory tract varies, and this variation clears certain populations of virions from certain regions of the respiratory tract, leaving other populations intact.
5. If the temperature of the respiratory tract drops suddenly below its normal range, batches of virions that were previously dormant may be activated simultaneously, giving rise to a vARI.

Moreover, if the binding of virions to cells is *ts* a mechanism can be envisaged that allows virions to become concentrated in particular regions of the respiratory tract: the virions might move (carried e.g. by the mucociliary escalator) up the respiratory tract until they reach colder regions where they can bind. (The virions' binding sites in the respiratory tract might also reflect the correlation, noted by Richman & Murphy, that more virulent strains tend to be less *ts* [26]. For example, virulent pandemic influenza strains might be expected to bind lower down the respiratory tract than typical seasonal influenza, because they are less *ts*.)

An important point is that this mechanism is sensitive to temperature *changes*, not absolute temperature, because the temperature sensitivity of viruses may vary as a result of selective pressures, and they can bind in different regions of the respiratory tract. This can explain the data of van Loghem, Milam, Jaakkola and others [4 – 6, 10 - 12]. It can also explain the seasonality of vARIs, because chilling outside of the range of the previous few days or weeks becomes more likely when the seasonal temperature drops in the autumn. In the spring, by contrast, exceptional chilling becomes less frequent as the seasonal temperature steadily rises.

The proposed mechanism can explain the association of vARIs with wind and rain in the tropics, since these weather events often result in the chilling of individuals, even when the ambient temperature remains constant. It also provides an explanation for the strange epidemiology of influenza, including the low attack rate of some epidemics within families [23, 48] and the rapid cessation of epidemics when many susceptible individuals remain in the population [22]; bear in mind that each member of a family has a different history of exposure to viruses and of chilling. For example, some family members may take regular exercise outdoors, which (I suggest) clears virions from the respiratory tract in small batches (outdoor exercise reduces mortality from vARIs [8]). Other family members may be exposed to a virus for the first time during particularly cold weather (e.g. in midwinter), so that virions bind and become dormant relatively low down the respiratory tract, and are therefore not activated unless even colder weather follows. Still others, who remain indoors much of the time, but are chilled occasionally (e.g. waiting for a bus in the rain - standing still outside in cold weather has been shown to be a dangerous activity [8]) may be prime candidates for infection. These trends can therefore explain the low attack rates and lack of transmission within families that are sometimes observed [23, 48], as well as the rapid cessation of epidemics [22].

Table 1. Four general mechanisms that might explain the seasonality of vARIs.

(1) Seasonal events may increase crowding during the winter, increasing transmission
- *The simultaneous arrival of vARI epidemics throughout wide geographical regions [4, 20, 22] is a problem for this explanation.*
- *School holidays are not well-correlated with vARI epidemics [1].*
- *There is little evidence for this mechanism [1, 3].*

(2) Colder conditions and low relative humidity may allow virions to survive outside the body for longer, increasing transmission
- *The prevalence of vARIs year-round in the tropics [1, 3, 79] is incompatible with this explanation.*
- *The simultaneous arrival of vARI epidemics throughout wide geographical regions [4, 20, 22] is also a problem.*
- *Cannot explain why vARIs respond to temperature dips rather than sustained low absolute temperature [4-6, 73].*
- *The rapid cessation of influenza epidemics in mid-winter when many susceptible individuals remain in the host population [22] is difficult to explain.*

(3) Chilling may increase the susceptibility of hosts
- *This mechanism is supported by a study using cultured mouse airway cells [80].*
- *The prevalence of vARIs year-round in the tropics [1, 3, 79] seems to be incompatible with this explanation.*
- *The (repeated) simultaneous arrival of vARI epidemics [4, 22] throughout geographical regions is also a problem.*
- *Cannot explain why vARIs respond to temperature dips rather than sustained low absolute temperature (unless viral dormancy is accepted) [4-6, 73].*
- *This mechanism is incompatible with the published results of numerous studies of the common cold in the 1950s and 60s that used "pedigree" strains [39–41], and with vaccination studies [24].*
- *The peak of colds in the early autumn, when temperatures have dropped only a few degrees from their summer highs [4, 78] is difficult to explain.*

(4) Chilling may increase the activity of respiratory viruses as a result of their natural temperature sensitivity
- *This mechanism is compatible with all of the above observations, including the sensitivity of vARIs to temperature dips [4-6, 73].*
- *In addition, the low attack rate of influenza within families [23], the increase in mortality from respiratory disease following outdoor shivering [8], and the decrease following outdoor sweating [8] are compatible with the mechanism.*
- *Explains the benefit of temperature sensitivity [25, 26, 44] to the virus, and provides an explanation for viral dormancy [11, 12, 31-37, 76].*
- *Compatible with the recovery of* ts *strains from persistent infections of tissue cultures [26, 44-47, 49], and the generation of non-*ts *strains in conditions that allow rapid viral replication [50, 51].*
- *Supported by studies using "pedigree" strains [39 – 41], and also Antarctic studies [11, 12, 37].*

Note that the natural temperature sensitivity of respiratory viruses contributes to mechanism (4) in two steps; in a first step, it allows virions to become dormant; in a second step, it provides viral activation as a result of chilling. Step 2, however, could also be the result of the depression of immunity in the host – mechanism (3) above. There is no reason to rule this out, and without further experimentation, the relative contributions of changes in host physiology and virus biochemistry remain unknown. I said above that Eccles' suggestion in its simple form has difficulty explaining much of the published data. Eccles mentions "subclinical" infections, which might suggest that viruses are actively replicating in the host, but at a low level. In this case we would expect the host to raise antibodies against the virus, so that the infection would not be stable for many weeks or months. This makes it hard to see how vARIs could arrive year after year simultaneously in midwinter in wide geographical areas (Figures 1 and 2), since they would not be truly "dormant". The same argument applies to colds that appear in the early autumn (Figure 1) - in this case the subclinical infections would have to be active over the summer months. If, however, virions can remain dormant in that they are able to remain "in the respiratory tract without being internalized and eliciting an immune response" (the wording of Thai *et al.* [36]), and, moreover, if they become concentrated in certain parts of the respiratory tract, then host chilling (due to ambient temperature *dips*) that lowered the temperature of those parts might cause a local infection to develop, followed by a vARI.

There is extensive biochemical evidence for the temperature-sensitive activation of viruses, discussed below, while evidence for the temperature-sensitive depression of immunity in a single species, the mouse, has emerged recently [80].

The evidence therefore suggests that the most important contribution to seasonality comes from mechanism (4), or possibly from changes in host susceptibility combined with viral dormancy (that is, a combination of mechanisms 3 and 4).

Animal experiments with human influenza virus

Experiments with guinea pigs suggest that the transmission of influenza A is more efficient at lower temperatures and lower relative humidity [13]. Lowen *at al.* found a 3.5-fold increase in the transmission of human influenza A between guinea pigs at 5°C compared to at 20°C [13] (in fact this was true only at 50% relative humidity; at higher and lower humidity, transmission rates were either similar at both temperatures or higher at 20°C). These differences are in agreement with measurements of the stability of influenza A virions generated in cell cultures in air of different temperatures and humidities [14]. This suggests that variations in transmission due to weather changes might contribute to the seasonality of influenza (and other vARIs) in temperate regions. The results were, however, inconsistent; for example, transmission of influenza at 20°C was higher 50% relative humidity that at 70% [13]. A more recent study found that the transmission of influenza between guinea pigs by medium-range aerosol was eliminated at 30°C, although transfer between animals in the same enclosure by short-range aerosol or direct contact was as efficient at 30°C as at 20°C [15]. The authors postulate that the normal mode of influenza transmission varies depending on climate: in temperate regions aerosol transmission may predominate, while in the tropics short-range and contact transmission may be more important. Epidemics of H3N2 influenza in the temperate regions are, however, seeded each year from a network of temporarily overlapping epidemics in East and Southeast Asia [2]. There is therefore no reason why influenza cannot be transmitted by the contact route in the summer months into and within the temperate regions. Lowen *et al.* recognize this difficulty, and they postulate the existence of unknown "additional factors, other than warm temperature and high relative humidity, which suppress influenza transmission by all routes during the summer months" in temperate regions [15]. Although the authors may have identified the routes of influenza transmission at different latitudes, they have therefore not provided a robust explanation of its seasonality.

Early studies where volunteers were chilled

It is widely believed by doctors and scientists that chilling does not affect vARIs, and that this idea is "an old wives' tale" [38]. This belief seems to come from numerous studies from the 1950s and 1960s where volunteers who had been inoculated with respiratory viruses were chilled, including three influential reports by Andrewes, Dowling and Douglas [39 - 41]. Unfortunately, these studies generally used "pedigree" strains of cold virus that were recycled by being collected from volunteers and used to inoculate the following batches of volunteers in subsequent experiments. (Steps were taken to avoid using natural "wild" strains that the volunteers happened to be carrying [39].) In these early studies it seems likely the researchers consciously selected strains that very quickly caused mild vARIs in a significant proportion of the volunteers (but without causing dangerous infections). This may well have removed some aspects of their natural temperature sensitivity, since temperature sensitivity might give rise to dormancy and thereby delay infection. This suggests that the results may have differed from those that would have been obtained using wild viruses, which might have shown an effect of chilling. One study, however, by Jackson *et al*., did use wild viruses that the volunteers were carrying [42]. In some of their experiments, volunteers in scant dress were exposed to 15.5°C air for four hours. In others, warmly-dressed volunteers breathed air at -12°C for two hours. Of those who were chilled, only 10% developed colds in the next 7 days, whereas 12% who were not chilled developed colds. However, the authors do not tells us what proportion of volunteers were chilled by breathing cold air and what proportion by wearing scant clothing; breathing cold air while remaining warm can be protective [8], presumably because viruses are activated but they can be removed by the immune system.

More recently, Johnson & Eccles used wild strains that the participants were carrying by chance, and saw an effect of chilling by immersing the participants' feet in cold water [18]. Of the chilled subjects, 28% developed colds, whereas only 9% of the control subjects who were not chilled did.

Simple experiments along similar lines need to be carried out to resolve these apparent contradictions.

The recovery of *ts* viruses from persistent infections

In an interesting review of 1975 [44], Preble & Youngner noted that *ts* strains often appear spontaneously in persistent infections of cell cultures with a variety of unrelated insect-transmitted and respiratory viruses (including Newcastle disease virus, Western equine encephalitis virus, Sendai virus, measles virus, stomatitis virus, and Sindbis virus). Similarly, Richman & Murphy found that persistent infections of cell-cultures with mumps virus and vesicular stomatitis virus consistently yielded *ts* virus, although they noted that persistent infections could also be established or maintained by non-*ts* mutations [26]. Three more recent reports described the recovery of spontaneously-generated *ts* strains of influenza A from persistent infections of cell cultures [45 – 47]. Similar tendencies are seen in persistent infections of animals; foot-and-mouth viruses recovered from carrier animals are frequently *ts*, and show evidence of high rates of mutation with frequent amino acid substitutions and rapid antigenic variation [49].

Preble & Youngner pointed out that since *ts* strains tend to be less virulent they may allow persistent infections to become established, because a balance between viral and cell replication is required [44]. They do not, however, explain why *ts* mutations in particular should be selected in persistent infections, as opposed to non-*ts* attenuating mutations. In many cases the cells were grown in the laboratory at 37°C, so temperature sensitivity should be a *disadvantage.* If, however, the wild virus from which the laboratory strain was derived was *ts*, the probability of reverting to the *ts* phenotype may be relatively high. For example, a protein might lose its *ts* character by a point mutation caused by a single nucleotide change, so that the *ts* character could be restored by a

restoring the original nucleotide. This may be more probable than other mutations that would attenuate the virus without conferring temperature sensitivity. Changes to RNA secondary structure (discussed below) may have similar effects. Bear in mind that many virus families include species that cause both systemic and respiratory tract infections, and strains may have jumped between these sites many times in their evolutionary history. For example influenza often infects the gut in birds, but it usually infects the respiratory tract in mammals. These considerations suggest that genetic pathways may exist that allow the rapid elimination and reintroduction of temperature sensitivity.

If this interpretation is correct, it may explain how respiratory viruses such as influenza are able to infect hosts throughout the world. A tropical virus might cause serious illness if it arrives in colder parts of the world, because it might infect sites lower down the respiratory tract. Since too much virulence may *reduce* viral transmission (patients become bed-ridden), subsequent selection in the temperate site for reduced virulence may increase temperature-sensitivity to a level that is appropriate to the virus's new surroundings. A similar argument suggests that viral temperature-sensitivity and virulence may be adjusted within a single season by natural selection as the temperature changes with the seasons.

The converse trend: the loss of the *ts* phenotype in conditions that allow the rapid replication of viruses

Since *ts* strains are generally less virulent *in vivo* [25, 44], and are associated with persistent infections both *in vivo* and *in vitro* [26, 44], it might be anticipated that the *ts* character would be lost in *in vitro* conditions that allow rapid replication of viruses, and this has indeed been observed. Chu *et al.* found a naturally-occurring *ts* influenza A strain that was a subclone of the H3N2 strain Ningxia/11/72 [50]. When they passaged the strain three times through chicken embryos at a *low temperature* (33°C), a non-*ts* strain was unexpectedly produced. Similarly, Oxford *et al.* [51] found that a naturally occurring *ts* virus, A/Eng/116/78 (H1N1), progressively lost its *ts* character during five passages at low temperature (33°C). Both groups concluded that even at the permissive temperature (33°C) the *ts* phenotype may confer a selective disadvantage in eggs.

The unexpected loss and gain of temperature sensitivity (in a wide variety of viruses) when increased or decreased viral activity is selected is shown schematically in Figure 6.

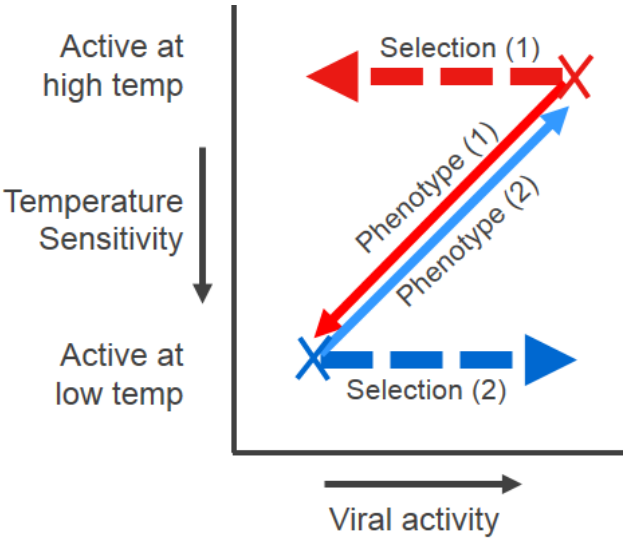

Figure 6. *The observed effect on temperature sensitivity of selection for increased and decreased viral activity.* Selective pressures are indicated by dotted arrows, while the resulting changes to viral phenotype are indicated by solid arrows. The establishment of persistent viral infections of cell cultures generally requires reduced viral activity so that viral and cell replication can be in balance [26, 44]. The corresponding selective pressure is indicated by the dotted red arrow. Unexpectedly, reduced activity is often accompanied by the spontaneous appearance of temperature (heat) sensitivity. This is indicated by the solid red arrow. See the main text for examples [45 - 47]. The converse trend is equally surprising: when *ts* viruses are propagated in conditions that allow rapid growth (thereby selecting the most active mutants, dotted blue arrow), heat sensitivity has been lost (solid blue

arrow) *even when selection takes place at low temperatures* (see main text [50, 51]).

Temperature sensitivity in wild and laboratory viruses

Numerous studies have found that it is easier to propagate respiratory viruses when they are freshly collected from patients by incubation at temperatures below 37°C. Rhinoviruses were first isolated at 35°C but a greater variety of rhinoviruses was discovered at 33°C [52], and this is the temperature that is recommended today for their isolation by the Clinical and Laboratory Standards Institute [53]. Coronaviruses were first isolated at 33°C [54] although laboratory strains are now frequently propagated at 37°C. Naturally occurring influenza strains are also frequently *ts*. For example, in 1962 Stern & Tippett [55] propagated four viral specimens from patients with H2N2 "Asian" influenza, all of which were *ts*. All four 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 (in 1962) that the FM1 (H1N1, 1947) and PR8 (H0N1, 1934) strains 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* [56]. Oxford *et al.* found that 17 of 26 recent H1N1 isolates, and 2 of 11 recent H3N2 isolates were *ts*, producing cultures that gave at least 10 times more viral plaques at 34°C than at 38.5°C [51].

Membrane fusion and *ts* entry into cells

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 [65]. (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.

In an interesting study, Russell saw an "unexpected" result when he measured the uptake of the triple reassortant influenza virus A/Jap/Bel into cells [66]. 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 [Figure2 of ref. 66]. 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*.

Biochemical studies of the temperature sensitivity of respiratory viruses: transcription

Most laboratory respiratory viruses are propagated at 37°C, which may result in the rapid loss of *ts* characters, especially since viruses mutate very rapidly when they are introduced to new hosts. If, however, temperature sensitivity is a common feature of wild respiratory viruses, we might expect to see remnants of temperature sensitivity in the biochemistry of laboratory strains. It turns out that such remnants are in fact quite common.

For several decades virologists have found that maximum RNA transcription in influenza viruses occurs below normal body temperature. In 1977, Plotch & Krug [57] reported that the greatest activity of the RNA polymerase of WSN virus was at 30 – 32°C. This is similar to the optimum temperature of the polymerase of influenza C, which is 33°C [58, 59]. Ulmanen *at al.* [60] found that the rate of transcription by detergent-treated WSN viruses (influenza A) was about 10 times greater at 33°C than at 39.5°C, and also that the binding of a cleaved primer cap (the "A13 fragment") to the viral cores was "unexpectedly" much weaker at 39.5°C than at 33°C. Scholtissek & Rott [61] 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). 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 [62]. For

all strains, vRNA unexpectedly decreased when the temperature was increased from 37°C to 42°C. The PA subunit of the viral polymerase caused this thermal sensitivity. In another interesting study, Dalton *et al.* showed that the production of mRNA by the PR8 influenza strain is favored at a higher temperature (41°C), with very little vRNA being produced at that temperature [63]. 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. The cRNA that is used as a template to make the vRNA formed 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.

The secondary structure of RNA

Much recent attention has focused on the role of RNA secondary structure in influenza A, although discussion of its role in temperature sensitivity has been limited. "RNA thermometers" are RNA segments (found in both microorganisms and higher organisms) that respond to temperature changes with three-dimensional conformational changes that alter gene expression [71]. They are frequently (but not always) found in the 5'-untranslated regions of mRNA, and can act in both directions – translation can be augmented or suppressed at high temperatures [71]. Chursov *et al.* used bioinformatic techniques to search for pronounced differences between mRNA from cold-adapted *ts* influenza strains and the corresponding wild-type sequences [72]. Pronounced differences were found in the mRNAs of four viral proteins. The authors suggest that temperature-induced structural changes of mRNA may constitute an unappreciated molecular mechanism of cold adaptation and temperature sensitivity [72]. Little secondary structure is predicted in influenza vRNA outside the untranslated terminal ends of the vRNA strands that form the "panhandle" structure [64]. However, the positive-sense RNA is predicted to have extensive secondary structure, which is conserved, in segments 1, 2, 5, 7 and 8 [64]. Since ordered RNA is intrinsically *ts*, and since individual base changes may have a cumulative effect on the overall secondary structure of RNA, it is likely that viral temperature sensitivity can be fine-tuned by small changes to untranslated regions of RNA. For example the secondary structure of vRNA, cRNA and mRNA might all affect temperature sensitivity in influenza. (Changes to protein sequences may also be in involved of course.)

Temperature sensitivity and the evolution of viral tropism

We can speculate that temperature sensitivity might have profound effects on viral tropism. Many or most respiratory viruses possess temperature sensitivity, and we can suggest that a respiratory virus that loses its temperature sensitivity might infect the lungs, gut or other internal organs. (Some method of limiting virulence other than temperature sensitivity might then be necessary to ensure the long-term survival of the virus.) Conversely, viruses originating in the internal organs that develop temperature sensitivity could safely cause severe local infections that would be limited to the upper respiratory tract, without greatly incapacitating the host (incapacitation would limit opportunities for transmission of the virus). Obviously the resulting irritation of the respiratory tract might cause coughing, sneezing and runny noses, all of which might help to transmit the virus – in other words a respiratory virus has been generated. Influenza infects the gut of water fowl but the respiratory tract of mammals (and birds), and is presumably able to move between these two ecological niches. Viruses that are transmitted via skin rashes and blisters that burst, such as chickenpox, measles, smallpox, and hand, foot and mouth disease in humans, and foot-and-mouth disease in cloven-hoofed mammals, could also benefit from temperature sensitivity that might allow them to infect the skin preferentially and so to spread by direct contact. (In all those diseases, and others, contact transmission from blisters coexists with aerosol transmission.) More virulent human influenza strains can cause viremia [67, 68], and three children who

were infected by pandemic H1N1 influenza in 2009 ("swine flu") presented with petechial rashes [69]. H3N2 influenza A caused hemorrhagic cystitis in 33 patients who were infected by the strain [70], suggesting that what we think of as respiratory viruses can occasionally cause systemic infections.

Conclusions and suggestions for experimental verification

Many suggestions have been put forward to explain the seasonality of vARIs, especially influenza. Changes in crowding (mechanism 1), and changes in the survival rate of viruses outside the body (mechanism 2) cannot be ruled out, but they fall short in at least one important respect: they cannot explain why vARIs including influenza are transmitted in the tropics, especially during wet weather, but are almost absent from temperate regions in the summer months. The alternative suggestion (3), that chilled hosts are more susceptible as a result of temperature-sensitive changes associated with the immune mechanisms of cells, is a better candidate because a biochemical study using cultured mouse cells supports the idea [80]. However, (if we leave aside the possibility that respiratory viruses frequently become temporarily dormant, discussed below) evidence from vaccination studies, epidemiological evidence that vARIs respond to small temperature changes throughout a wide range of absolute temperatures (Figures 1 and 3), and evidence that vARIs frequently arrive simultaneously throughout large geographical regions (Figures 1 and 2), seem to rule out changes in host susceptibility as the main driver of vARI seasonality.

This leaves the explanation that might seem most intuitive to the lay-person – (4) that viruses can become temporarily dormant, and they can become active again as a result of chilling, which changes in the behavior of viruses at the biochemical level. This explanation is seldom considered by microbiologists, who seem to have ruled it out on the basis of historical experiments from the 1950s and 1960s that purported to show that chilling does not bring on vARIs. However, there is now so much clear evidence that chilling *does* increase vARIs that we need to look more closely at those historical experiments. I suggest above that they were flawed because they used "pedigree" viral strains which were passed by the investigators from one group of volunteers to the next. Obviously the investigators would have thought carefully about the choice of strains that they used; they certainly didn't want to put volunteers at risk, so mild strains needed to be selected. However they naturally wanted their experiments to fit into the time available – Andrewes worked with volunteers who each stayed at his unit for 10 days, and his experiments did not start until the volunteers had been in quarantine for three days. The incubation period of the strain that he used was much the most frequently two to three days. By selecting such a strain he and his colleagues may have eliminated the virus's natural temperature sensitivity (predicted by mechanism 4) during the early stages of infection– a flaw in the design of his experiments with far-reaching implications.

Note that explanation (3), based on changes in susceptibility brought on by low temperatures, cannot easily explain the negative results of the host-chilling experiments using pedigree strains.

Leaving aside these early experiments, what is the evidence for or against the idea that temperature fluctuations can activate respiratory viruses and give rise to vARI seasonality? Epidemiological evidence shows that (1) surges in vARIs often follow a few days after temperature dips [3 - 7, 78, 77]; (2) it is temperature *drops* that are generally associated with vARI epidemics rather than sustained low absolute temperature [4 - 6]; (3) the attack rate (of influenza) and the rate of transmission within families is often low [23, 48]; (4) epidemics often appear simultaneously throughout wide geographical areas, with no evidence of waves of transmission that move between neighboring localities [4, 20, 22] (Figures 1 and 2); and (5) influenza epidemics often occur in midwinter but cease while many susceptible individuals remain in the population [22]. The first two points can perhaps be explained by the idea that temperature dips depress host immune defenses but points (3) - (5) can only be explained by some variation of the fourth mechanism considered above – that

chilling somehow increases the activity of respiratory viruses that were present before the epidemic began. (As discussed above, this does not mean that changes to the susceptibility of hosts have no effect. For simplicity I have considered only four mechanisms, but many other combinations are possible. If it is accepted that virions can become dormant, then increased host susceptibility at lower temperatures, possibly combined with biochemical changes in the behavior of viruses, may encourage vARIs. This is a combination of elements of mechanisms 3 and 4 above.)

The idea that chilling activates dormant viruses is compatible with data showing that tropical vARI epidemics often follow or coincide with wind and rain [1, 3]. The idea is also supported by data showing that standing still in cold weather and wearing inadequate clothing both increase mortality from respiratory disease, while outdoor exercise is protective [8].

Observations of the timing of vARIs suggest that respiratory viruses can become dormant, a suggestion that is confirmed by the occasional presence of vARIs in polar communities after many weeks or months of complete isolation [11, 12, 37]. Biochemical tests of asymptomatic individuals who shed influenza A and B without seroconversion [33 – 36] are compatible with dormancy, and PCR tests showed directly that individuals harboring a variety of viral pathogens subsequently developed vARIs that were (or seemed to be) caused by the same strains [76].

We also need to explain the clear trend in the laboratory for persistent infections of cells to yield *ts* strains of a variety of viruses in the absence of obvious selective pressures [26, 44], and also the converse observation, that temperature-sensitivity is lost when viruses are grown in cells at low temperature in conditions that allow rapid replication [50, 51] (Figure 6). Moreover, biochemical studies show that many steps in the replication of laboratory strains of influenza and other viruses have residual natural temperature sensitivity [57-66], in spite of many generations of replication of laboratory viruses, typically at 37°C.

Lwoff (1959), and Richman & Murphy (1979) suggested that the temperature sensitivity of respiratory viruses allows them to target the respiratory tract, and to avoid the lungs [25, 26]. This idea, and the related idea that less *ts* strains tend to be more virulent, seems to be widely accepted by microbiologists. However, the corollary, that ambient temperature dips can activate viruses that were previously inactive, is less popular. It is, however compatible with the well-known "trade-off" model of virulence. This model suggests that the benefits of virulence (in particular the increased rate of virus production and shedding) are balanced against the reduction of the time during which shedding takes place (and also changes in the behavior of the host) if virulence is too great. The implication is that mechanisms are required to moderate virulence, and temperature sensitive mechanisms can clearly achieve this in the case of respiratory viruses. An analogy in the form of a joke may be helpful here. Two scientists were out hiking, when they met an angry-looking bear. "It's no use," said one scientist to the other, "you can never outrun a bear". "I don't need to outrun the bear," replied his companion, "I only need to outrun you". It is often assumed that an "arms race" exists between viruses and the host immune system, with each making a series of small improvements to gain an advantage over the other [43]. This assumption may not be correct – it may be that most viruses could easily overcome the defenses of hosts (especially if they lack specific immunity), but that selective pressures reduce the virulence of well-adapted viruses, as predicted by the trade-off model. Some of the evidence for this comes from viruses that have recently jumped from one host species to another, such as myxomatosis, HIV and Ebola, which are often unusually virulent, in spite of (or because of) having little time to adapt to their hosts. The arms race may not be between viruses and hosts, but between individual hosts, where each needs to reduce its susceptibility to outcompete other members of the same species. (The analogy is not perfect, because we also need an immune system to protect us from opportunistic infections, such as tetanus.)

…

It is unlikely that either evidence gleaned from studies that were designed to investigate other aspects of viral biochemistry or epidemiological observations can determine the causes of vARI seasonality with certainty. Instead it will be necessary to investigate temperature sensitivity directly *in vivo* and *in vitro*, working with viruses that are as close as possible to wild viruses. As a start, wild and laboratory viral samples should be "deep sequenced" (i.e. the relative proportions of different sequences in a sample should be established at multiple genetic sites) to determine the mix of *ts* and non-*ts* sequences in wild samples, and to establish the impact on temperature sensitivity of propagating wild viruses in the laboratory. This analysis needs to include consideration of RNA secondary structure. The information gained can be applied at many levels, from observations and experiments with living organisms to experiments with cell cultures and in solution. It may be possible to image the distribution of virions in the respiratory tracts of animals, and to see e.g. differences in animals that were housed at high and low temperatures prior to the investigation. Virions might be released from tissues or cells by raising the temperature, or captured by lowering the temperature. (Note that the genetic information remains attached to the chemical probe, in a manner analogous to the phage display technique.) The entry of virions into cells can be investigated by measuring the escape rate of pre-adsorbed virus from neutralization by antibody in temperature shift experiments. In tissue cultures, transcription and the production of genetic material can be followed during temperature shifts (for example the production of mRNA, cRNA and vRNA can each be studied in influenza). Similarly, the production of viral proteins can be followed during temperature-shift experiments. Experiments in solution can also yield valuable information. For example, the thermal stability of mutants of hemagglutinin and neuraminidase from influenza virus can be investigated in solution by thermal shift assays, and similar experiments can be undertaken with other respiratory viruses. The thermal stability of secondary structures in wild-type and laboratory viral RNA and RNA/protein complexes can be measured in solution. Bioinformatics can be applied to the problem. For example the sequences of hemagglutinin, neuraminidase and other viral proteins from wild and laboratory strains, and from the viruses obtained from different animal hosts and laboratory procedures can be analyzed. This analysis can consider the impact of mutations on the structures of viral proteins and complexes that have been determined by x-ray crystallography and other techniques. For example the effect of changing a particular residue can be anticipated or investigated experimentally. Secondary structure prediction techniques can be applied to viral RNA, DNA and protein/nucleic acid complexes. Yet another approach is to investigate and model viral epidemiology with these ideas in mind. Finally, experiments can be performed at the whole organism level. For example, chilblains and chapped lips can be examined for the presence of respiratory and other viruses. Other, very simple, experiments can be performed with human volunteers. For example, groups of volunteers can be subjected to chilling in the autumn or midwinter (which are the seasons when individuals are particularly susceptible to vARIs) and compared to control groups who are kept warm. The number of vARIs suffered by both groups can then be compared. This approach would make use of dormant viruses that the participants were already carrying by chance. These experiments can be extended by subjecting the volunteers to cyclical chilling, which may activate viruses that require more than a single temperature shift.

List of abbreviations used

HEF: hemagglutinin-esterase-fusion protein
RSV: respiratory syncytial virus
Ts or ts: temperature-sensitive
vARI or vARIs: Viral acute respiratory tract infection or infections

In this article temperature-sensitive or *ts* refers to viruses that are more active at *lower* temperatures, i.e. they are heat-sensitive.

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 worked with Professor David Blow in the 1990s, and have published 15 papers about protein crystallization that have together been cited over 700 times. A few years ago I began to think about respiratory viruses when a friend bet me that I couldn't find biochemical evidence that chilling could trigger vARIs. I started to write a short note, but everything fell into place so neatly that it grew into the current document. A longer version is available at viXra.org.

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Figures

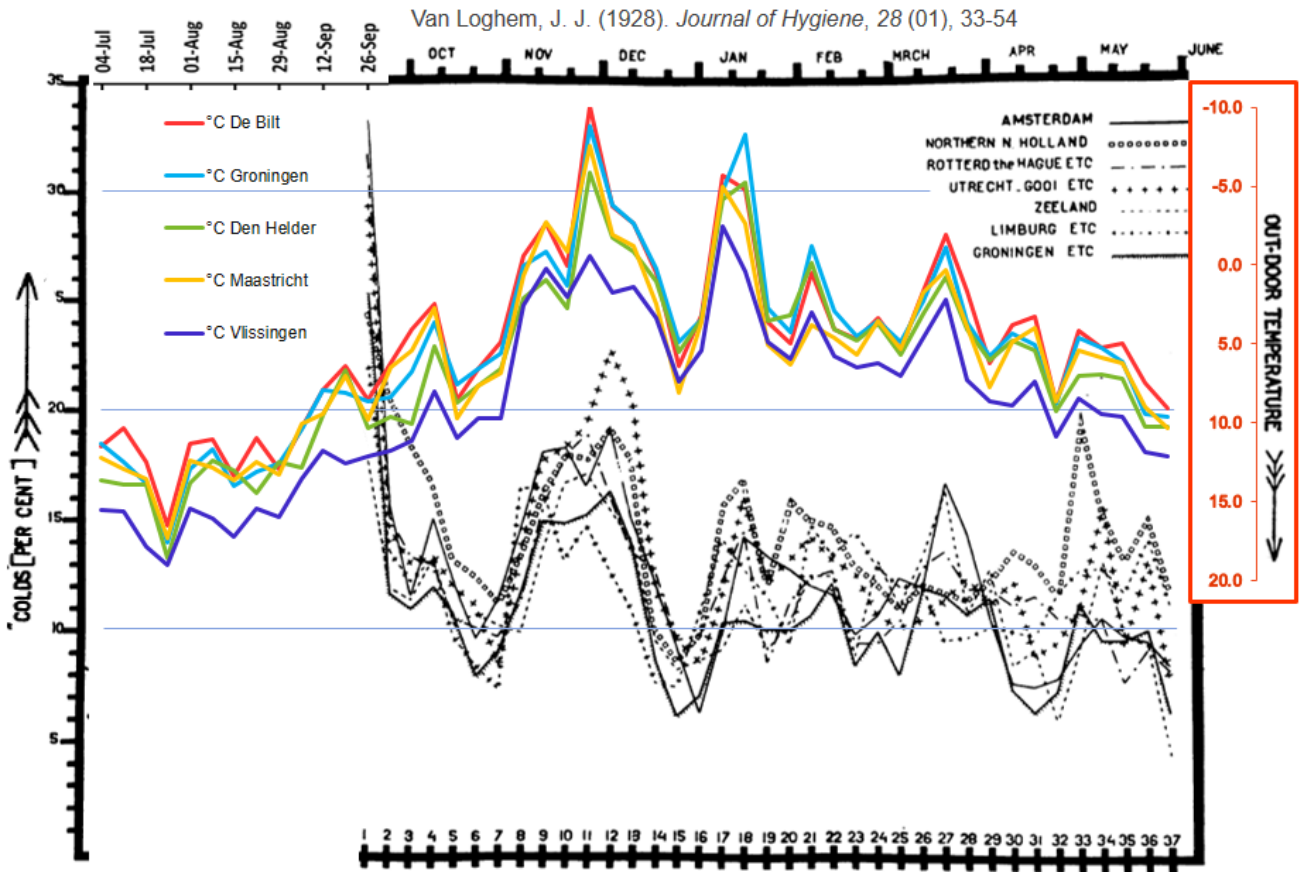

Figure 1. *Graph II from van Loghem's report [4] on the epidemiology of vARIs in the Netherlands in the winter of 1925/26, with ambient temperature superimposed*. The graph shows the percentages of persons with colds in seven regions of the Netherlands for 37 weeks. The data was compiled from the reports of 6933 correspondents that were submitted by post each week. Amsterdam had the largest number of informants (1159) and Noord-Holland the fewest (581). I have added the daily minimum outdoor air temperature (averaged over 7 days at weekly intervals) from five Dutch weather stations, with the temperature scale inverted (lowest temperatures at the top). Note that by far the highest rate of vARIs was at the beginning of the study (September 1925), and that vARIs in different regions are closely correlated with each other and with inverted temperature. These correlations are strongest in the first half of the cold season. ©1928, 2014. This figure was originally published in the *Journal of Hygiene*, *28*(01), 33-54.

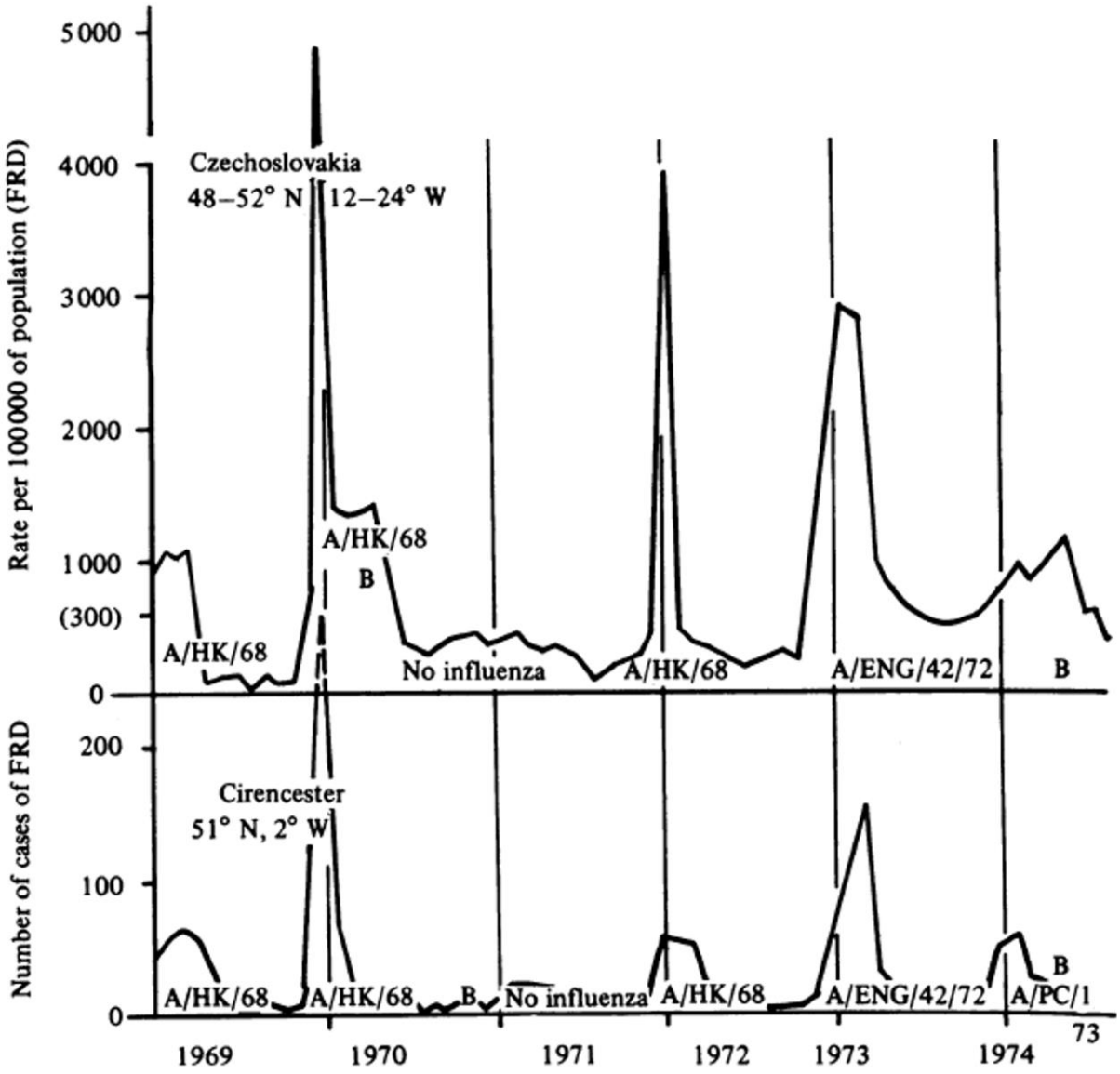

Figure 2. *The Cirencester (UK) acute febrile respiratory diseases at 51.430 N, 1.590 W, compared with notifications of such diseases in Czechoslovakia (Prague, 50.050 N, 14-250 E), 1969-74, taken from Hope-Simpson's investigation into the role of season in the epidemiology of influenza [22].* This remarkable figure demands theoretical explanation. The antigenic changes in influenza A virus (occurring at both sites) show clearly that novel influenza strains moved around Europe during the period shown. There is, however, no evidence of moving "waves" of influenza because epidemics at the two sites are very closely synchronized. Note that the shortest route between the two sites covers 1,400 km by sea and road, crosses four national boundaries, and passes through some of the most densely-populated regions of Europe. This suggests that the virus moved to both sites prior to its manifestation, and a stimulus that was present at both sites triggered the concurrent epidemics. These data are compatible with the fourth explanation discussed below, that virions can become dormant at some unknown location in the respiratory tract, and can subsequently be activated by host chilling. *Journal of Hygiene*, *86*(01), 35-47.

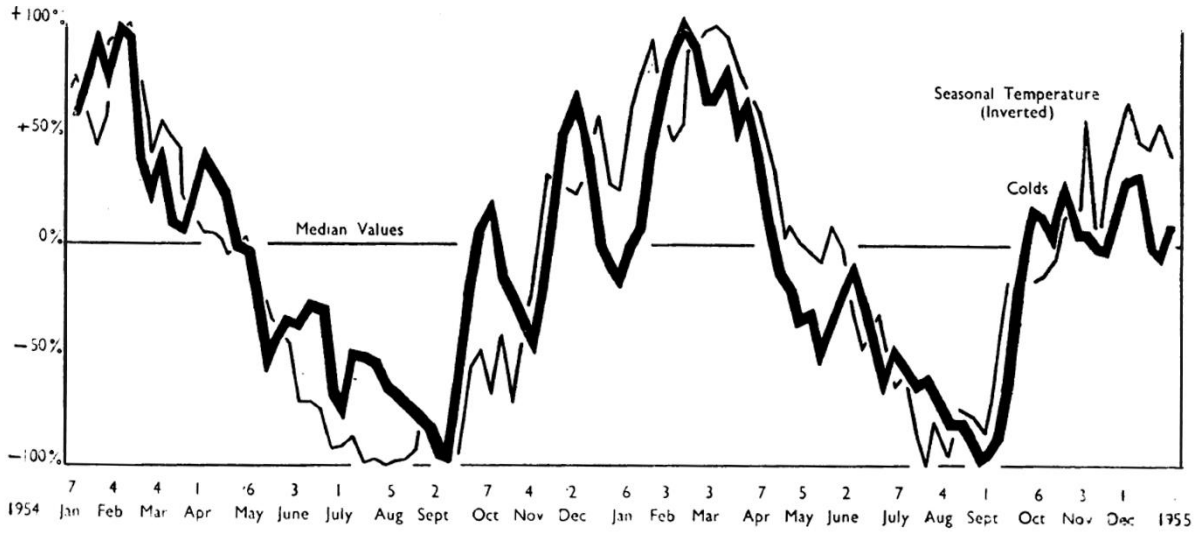

Figure 3. *Morbidity from colds in Cirencester, UK, 1954 and 1955, plotted alongside temperature [73].* Thick line - percentage of volunteers showing symptoms. Thin line - earth temperature (inverted). ©1958. This figure was originally published in the *Proceedings of the Royal Society of Medicine*, *51*(4), 267-271.

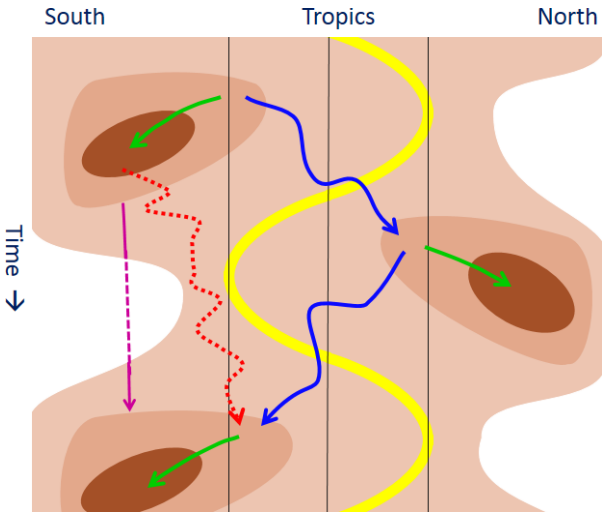

Figure 4. *The global distribution and seasonality of vARIs, shown schematically*. Levels of vARIs are indicated by brown shading, with dark brown showing the highest rates of infection, while the yellow curve shows the path of vertical solar radiation. The strange distribution of vARIs is shown, with more vARIs in the tropics throughout the year than in temperate regions during the summer months [2, 3]. It is known that seed strains of influenza A (H3N2) circulate continuously in a network in East and Southeast Asia (blue arrows) and spread to temperate regions from this network (green arrows) [2]. Several lines of evidence suggest that personal chilling will increase the prevalence of vARIs [4 - 12], and, since travel away from the tropical regions is associated with a decrease in temperature, it is likely that vARIs spread more quickly from the tropics to the temperate regions (green arrows) than in the opposite direction (dotted red arrow). The degree to which viruses remain dormant during the summer in temperate regions (dotted purple arrow) is unknown.

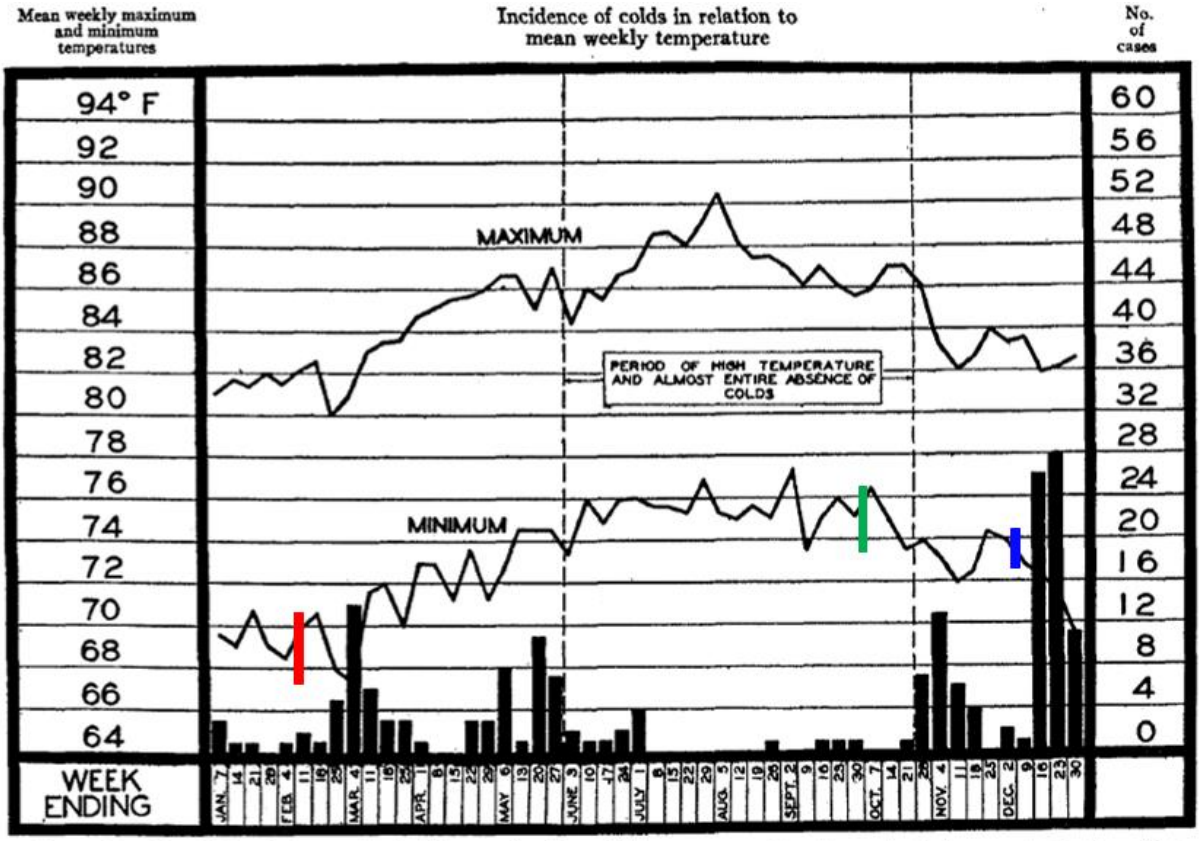

CHART 1. Correlation between the incidence of colds and the mean weekly atmospheric temperature, Cruz Bay, St. John, Virgin Islands, 1929.

Figure 5. *Chart 1 from Milam and Smillie's 1929 study of colds on an isolated tropical island.* The authors noted that outbreaks of colds often followed temperature drops, and were almost absent in the summer months. The red, green and blue bars indicate temperature fluctuations of 1.9, 1.7 and 1.0°C respectively. (The large outbreak in December seems to have been introduced to the island by a sailor on the mail boat.) ©1931. This figure was originally published in the Journal of Experimental Medicine. 53:733-752. doi: 10.1084/jem.53.5.733.

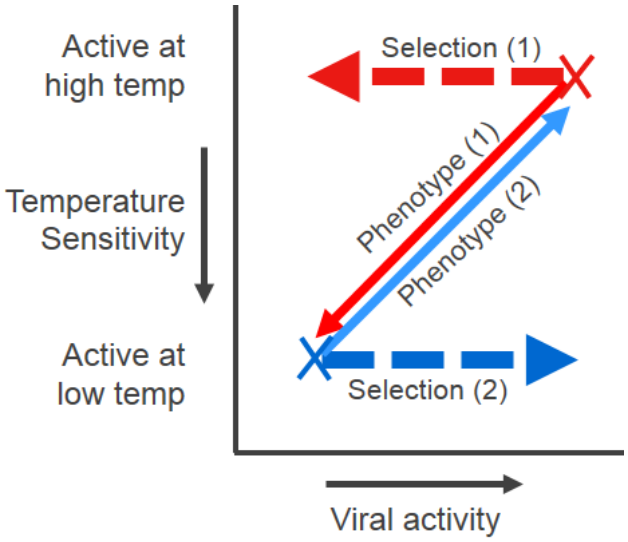

Figure 6. *The observed effect on temperature sensitivity of selection for increased and decreased viral activity.* Selective pressures are indicated by dotted arrows, while the resulting changes to viral phenotype are indicated by solid arrows. The establishment of persistent viral infections of cell cultures generally requires reduced viral activity so that viral and cell replication can be in balance [26, 44]. The corresponding selective pressure is indicated by the dotted red arrow. Unexpectedly, reduced activity is often accompanied by the spontaneous appearance of temperature (heat) sensitivity. This is indicated by the solid red arrow. See the main text for examples [45 - 47]. The converse trend is equally surprising: when *ts* viruses are propagated in conditions that allow rapid growth (thereby selecting the most active mutants, dotted blue arrow), heat sensitivity has been lost (solid blue arrow) *even when selection takes place at low temperatures* (see main text [50, 51]).