

Evolutionary bi-stability in pathogen transmission mode

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Many pathogens transmit to new hosts by both infection (horizontal transmission) and transfer to the infected host's offspring (vertical transmission). These two transmission modes require specific adaptations of the pathogen that can be mutually exclusive, resulting in a trade-off between horizontal and vertical transmission. We show that in mathematical models such trade-offs can lead to the simultaneous existence of two evolutionary stable states (evolutionary bi-stability) of allocation of resources to the two modes of transmission. We also show that jumping between evolutionary stable states can be induced by gradual environmental changes. Using quantitative PCR-based estimates of abundance in seed and vegetative parts, we show that the pathogen of wheat, *Phaeosphaeria nodorum*, has jumped between two distinct states of transmission mode twice in the past 160 years, which, based on published evidence, we interpret as adaptation to environmental change. The finding of evolutionary bi-stability has implications for human, animal and other plant diseases. An ill-judged change in a disease control programme could cause the pathogen to evolve a new, and possibly more damaging, combination of transmission modes. Similarly, environmental changes can shift the balance between transmission modes, with adverse effects on human, animal and plant health.

Keywords: evolutionary stable strategy; *Phaeosphaeria nodorum*; real-time PCR; adaptive dynamics; animal disease; plant disease

1. INTRODUCTION

Pathogens depend on transmission to new hosts for their long-term survival. Therefore, transmission from infected to healthy hosts is a key component of pathogen fitness, and multiple transmission modes have evolved in many pathogens because they spread the risks associated with transmission. Two broad categories of transmission mode are horizontal transmission, in which the pathogen infects another host irrespective of relationship, and vertical transmission, in which the pathogen infects the host's offspring. Many pathogens of humans, animals and plants spread by a combination of horizontal and vertical transmission (Ewald 1987, 1994; Power 1992; Knell & Webberley 2004; Verdonck *et al.* 2007; Jestoi 2008). For example, the protozoan parasite *Ophryocystis elektroscirrha* of the monarch butterfly (*Danaus plexippus*) is transmitted horizontally when adult butterflies ingest spores on host plant leaves, and is transmitted vertically when spores are transmitted to the outside of the eggs (de Roode *et al.* 2008). Strains of the parasite that produce large numbers of spores can cause severe infections of the larvae and pupae, which considerably reduces vertical transmission because few larvae pupate successfully. These strains are, however, efficiently horizontally transmitted because they leave more spores on the leaves. In contrast to that, parasite strains that produce fewer spores are more successfully transmitted vertically, but have lower horizontal transmission success.

The virus causing cassava mosaic virus disease in cassava (*Manihot esculenta*) is transmitted horizontally by its whitefly vector and vertically through the use of cuttings to establish new crops (van den Bosch *et al.* 2007). Strains of the virus building up a high virus titre in the plant are easily acquired by the vector and thus efficiently horizontally transmitted. However, high virus titres produce severe symptoms and reduce vertical transmission because farmers avoid using such plants for cuttings. Conversely, strains with a low virus titre in the plant are efficiently vertically transmitted, but are less efficiently transmitted horizontally.

These two examples show that horizontal and vertical transmission modes require specific adaptations of the pathogen that can be mutually exclusive. This implies a trade-off between horizontal and vertical transmission modes, as described for a range of pathosystems (Dearsley *et al.* 1990; Bull *et al.* 1991; Ebert & Mangin 1997; Turner *et al.* 1998; Messenger *et al.* 1999; Kaltz & Koella 2003; van den Bosch *et al.* 2007).

In this paper, we show that such a trade-off between horizontal and vertical transmission modes can lead to evolutionary bi-stability. That is, under the same set of conditions (i.e. numerical values of the life-cycle parameters of host and pathogen), two evolutionary stable combinations of horizontal and vertical transmission (ESSs) can exist. We also show that in altered environments, one or the other of these ESSs may be lost.

Subsequently, we use a 160-year data series from a long-term experiment on the cultivation of wheat to investigate whether there is any evidence of such bi-stability in the balance between leaf-to-leaf (horizontal) and seed (vertical) infection by the fungal pathogen *Phaeosphaeria nodorum* (Bearchell *et al.* 2005; Shaw *et al.*

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2009.2211> or via <http://rspb.royalsocietypublishing.org>.

2008). *Phaeosphaeria nodorum* (anamorph name *Stagonospora nodorum*, widely known by the earlier name *Septoria nodorum*), is a fungal pathogen causing leaf blotch disease on wheat. The pathogen was a key cause of yield loss in the UK from the 1950s until the 1980s, but severity has decreased since then and the pathogen is now considered to be of minor importance (Hardwick *et al.* 2001; Bearchell *et al.* 2005). It is transmitted within crops by spores (conidia) splashed between leaves and plants. It is seed-borne but may also survive the crop-free period on crop debris and volunteer plants, and as resistant, sexually produced, ascospores. Based on published experimental data, we argue that a trade-off between horizontal and vertical transmission operates in this plant pathogen, making it an ideal candidate to investigate evolutionary bi-stability in the long-term dataset.

2. MATERIAL AND METHODS

(a) The theory

To investigate the effects of a trade-off between horizontal and vertical transmission on the evolution of the pathogen transmission mode, we develop an epidemiological model, introduce the trade-off and analyse the evolutionary stable strategies.

(i) The epidemiological model

The model describes the flow between susceptible, *S*, and infected, *I*, crop host tissue, with both horizontal and vertical pathogen transmission (figure 1*a*). Removal from the *S* state occurs by death or harvest, at rate η . Removal from the *I* state occurs by death/harvest, η , and by pathogen-induced death at rate ω . Horizontal pathogen transmission is modelled with the classical linear transmission term βSI , with transmission rate parameter β . Since we are here interested in an agricultural cropping system, the planting rate is under control of the farmer and unrelated to the density of the pathogen and crop host. This leads us to assume that the total planting rate is a constant, σ .

Vertical transmission occurs by planting seeds infected with the pathogen. Seed-transmitted leaf-infecting fungal pathogens are generally dispersed onto the ears, preceding seed infection, through rain-splashed spores from leaf lesions (Agrios 1997), and *P. nodorum* is typical in this respect. The percentage of seeds infected is known to be correlated to pathogen severity on the crop (Cunfer & Johnson 1981). We thus assume in our model that the probability that a seed is infected is proportional to pathogen severity on the leaf, $I/(S + I)$, with proportionality constant θ . An infected seed is smaller than an uninfected one and often shrivelled. Seed producers and farmers select planting material by out-selecting small and shrivelled seeds (van Gasteren *et al.* 2002). We thus assume that an infected seed is out-selected with probability $1 - g$ (see §2*c*(i) for its relation to strain aggressiveness). The probability that a seed is infected through spores splashing from the leaf surface and is subsequently used for planting thus equals $g\theta I/(S + I)$ (see §2*a*(iv) for other forms of this vertical transmission term).

This leads to the model equations:

$$\left. \begin{aligned} \frac{dS}{dt} &= \sigma \left(1 - g(x) \frac{\theta I}{S + I} \right) - \eta S - \beta(x) SI \\ \text{and } \frac{dI}{dt} &= \sigma g(x) \frac{\theta I}{S + I} - \eta I - \omega(x) I + \beta(x) SI \end{aligned} \right\} \quad (2.1)$$

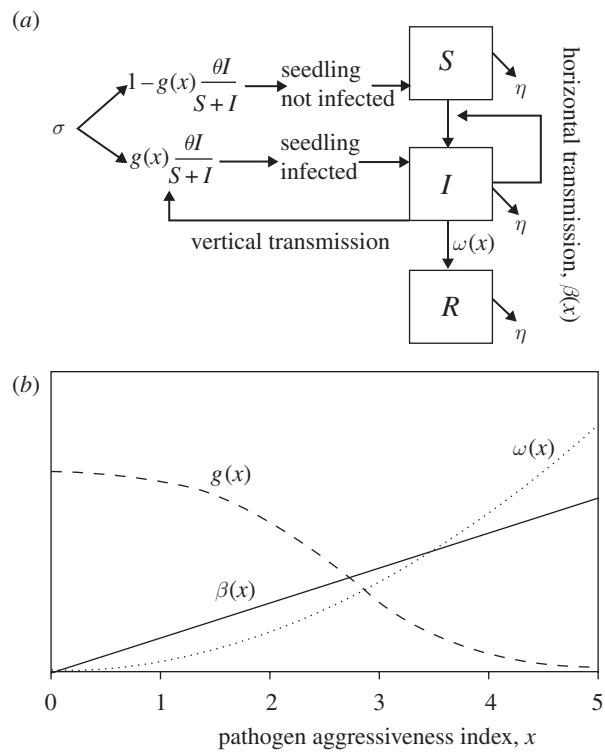


Figure 1. The model system of a pathogen with both horizontal and vertical transmission. (a) The epidemiology of the pathogen. The boxes represent states, with state variables densities of healthy, *S*, infected, *I*, and removed, *R*. Horizontal transmission, β , occurs through leaf to leaf fungal spore transmission. Vertical transmission through seed infection, governed by (i) the planting rate σ , (ii) the probability of ear infection ($\theta I/(S + I)$) and (iii) the probability of seed establishment when infected, $g(x)$. (b) Dependence of three epidemiological parameters on pathogen aggressiveness, x . $g(x)$ is the probability that an infected seed germinates and establishes an infected plant, $\omega(x)$ is the pathogen-induced leaf death rate and $\beta(x)$ is the horizontal transmission rate.

The internal steady state of this system exists if $R_0 > 1$, with $R_0 = \{ \theta g(x) \eta / (\eta + \omega(x)) \} + \{ \beta(x) \sigma / (\eta^2 + \eta \omega(x)) \}$ (see electronic supplementary material I). The stability of the internal steady state was checked numerically (see electronic supplementary material I, which also contains some additional information on the dynamic behaviour of this model).

(ii) Trade-off relationship and the effect of the environment

Horizontal and vertical transmission are both functions of environmental condition, ε , and the pathogen's aggressiveness, x (figure 1*b*; see §2*c* for a discussion on aggressiveness and the environmental variables in the case of *P. nodorum*). For figure 2, the specific relationships are modelled by $\beta(x) = \varepsilon x$ and $g(x) = (1 + c)/(1 + c \exp(k\varepsilon x))$ (but see §2*a*(iv)). By definition, a more aggressive pathogen kills host tissue more rapidly ($\omega(x)$, figure 1*b*). In this paper, we model this relationship with $\omega(x) = \omega_s x^2$ (but see §2*a*(iv)).

(iii) Evolutionary dynamics

Assuming that the resident pathogen population has aggressiveness index x^* , the fitness of an invader with aggressiveness index x , $W(x, x^*)$, is given by

$$W(x, x^*) = \sigma g(x) \frac{\theta}{S^* + I^*} - (\eta + \omega(x)) + \beta(x) S^*, \quad (2.2)$$

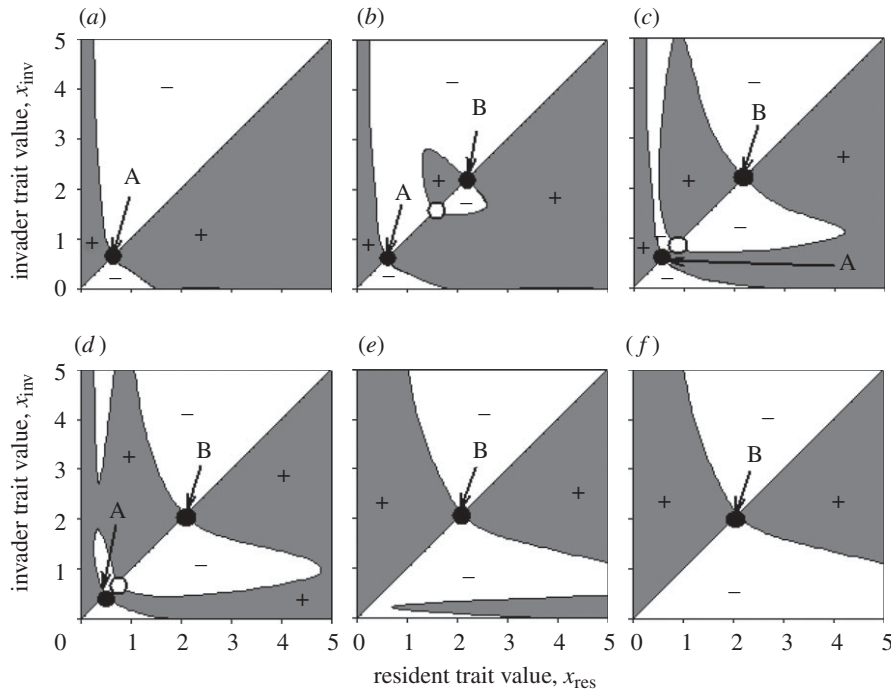


Figure 2. Pair-wise invasibility plots for the model system (figure 1). Each plot is for a value of the environment index, ε , quantifying the conduciveness of the environment for the pathogen. In the grey areas with a plus sign, the invader strain can invade the resident population. In the white areas with a minus sign, the invader strain cannot invade. Solid circles on the 45° line in each subplot show the evolutionary stable states, ESS, of pathogen aggressiveness, x_{ESS} . The open circle is an evolutionary repeller. Parameter values used are $\omega_s = 0.005$; $\theta = 0.8$; $c = 0.01$; $k = 2000$; $\sigma = 3$; $\eta = 0.025$. (a) $\varepsilon = 0.001$; (b) $\varepsilon = 0.0025$; (c) $\varepsilon = 0.004$; (d) $\varepsilon = 0.0055$; (e) $\varepsilon = 0.007$; (f) $\varepsilon = 0.015$.

where I^* and S^* are the internal steady-state densities of equation (2.1) with the aggressiveness index x^* (see electronic supplementary material I). We study the effect of environmental variation on the evolution of pathogen aggressiveness by using pair-wise invasibility plots (PIPs), a technique borrowed from adaptive dynamics (Geritz *et al.* 1998) that we use here to study evolutionary stable strategies (Maynard Smith 1982). The invasibility plots (figure 2) are calculated numerically from $W(x, x^*) = 0$. Invader strains invade when $W(x, x^*) > 0$ and die out when $W(x, x^*) < 0$. Each point in the PIPs shows whether a rare strain can invade a uniform population with another trait value. They indicate what trait value cannot be replaced by another trait value differing slightly from it and is therefore evolutionarily stable.

(iv) *Robustness of the results*

How far are the results of our model analysis a consequence of the details of the model structure and the specific forms of the trade-off curves chosen, and how far does the bi-stability only occur in a narrow or unrealistic parameter range? First, we consider variants of the model, then we discuss parameter values. The results of the analysis of the model variants are presented in electronic supplementary materials II and III.

In the derivation of the vertical transmission term, we only included the out-selection and death of infected seeds before sowing as a function of the pathogen aggressiveness. As discussed in §1, there are two additional mechanisms operating, as follows.

(i) Infected seeds are lighter and smaller than uninfected seeds. Farmers may sow a fixed weight or volume of seeds per unit area. For this case, we interpret $g(x)$ as the ratio of

the weight of a seed infected with a strain of aggressiveness x and an uninfected seed.

(ii) An infected seed sown in the field has a smaller probability to germinate and produce an infected plant. In this case we interpret $g(x)$ as the probability that a seed infected with a strain of aggressiveness x will germinate and develop into an infected plant.

In electronic supplementary material II, it is shown that in case (i) the model reads

$$\frac{dS}{dt} = \sigma \left(1 - \frac{g(x)\theta I}{S + (1 + (1 - g(x))\theta)I} \right) - \eta S - \beta(x)SI$$

and

$$\begin{aligned} \frac{dI}{dt} = & \sigma g(x) \frac{\theta I}{S + (1 + (1 - g(x))\theta)I} - \eta I \\ & - \omega(x)I + \beta(x)SI, \end{aligned}$$

and in case (ii) it is

$$\frac{dS}{dt} = \sigma \left(1 - \frac{\theta I}{S + I} \right) - \eta S - \beta(x)SI$$

and

$$\frac{dI}{dt} = \sigma g(x) \frac{\theta I}{S + I} - \eta I - \omega(x)I + \beta(x)SI.$$

We analysed both these variants of model equation (2.1) using the same trade-off functions and parameter values. The results are presented in electronic supplementary material II and are consistent with the main model.

We also analysed, using model equations (2.1), the effect of different specifications of the trade-off relationships.

Electronic supplementary material III presents the PIPs for each of the variants:

$$\beta(x) = \frac{\varepsilon x}{1 + \delta x},$$

$$\omega(x) = \rho(\exp(\omega, x) - 1)$$

and

$$g(x) = \frac{a}{x_{\text{lim}}} \frac{x - x_{\text{lim}}}{x - a},$$

$$x_{\text{lim}} = x_{\text{max}} \exp(-b\varepsilon).$$

These trade-off relationships have different shapes, but conform to the general requirement that increasing aggressiveness, x , increases horizontal transmission, decreases vertical transmission and increases pathogen-induced death rate.

Next, we consider whether the range of parameter values we examined was reasonable and relevant for our key example to be introduced in the next section. Field and experimental observations report *P. nodorum* seed infection percentages to range from 30 to 96 per cent (Cunfer & Johnson 1981; Gilbert *et al.* 1995; Shah & Bergstrom 2000). We use a default value for the maximum fraction of seeds infected (at 100% severity), θ , of 0.8.

The mean lifetime of an uninfected host unit, $1/\eta$, is approximately 40 days in our parameterization, which corresponds well to wheat leaf lifespans of 30–50 days in the field (Milne *et al.* 2003). The mean infectious period of an infection, $1/(\omega + \eta)$, for our default parameters at x_{ESS} ranges from 22 to 36 days. These quantities correspond well to the published infectious periods of *P. nodorum*. Leonard (1988) presents data that show an infectious period of 30 days, and Djurle & Yuen (1991) use 20–45 days, depending on temperature, in their model. Our value corresponds well to the general range of infectious periods, between 10 and 50 days, of fungal wheat leaf pathogens (Madden *et al.* 2007).

No estimates of transmission rates suitable for our model framework are available for *P. nodorum*. We can however calculate the basic reproduction number, R_0 , for the ESS aggressiveness values (as these are the ones that will be observed in the field). The basic reproduction number for the horizontal transmission route is given by $R_0 = (\varepsilon x \sigma) / (\eta(\varepsilon x^2 + \eta))$. At low environmental conduciveness to epidemic development, $\varepsilon = 0.002$, we find $R_0 \approx 6.1$ and $R_0 \approx 10.7$ for the high- and low-aggressiveness ESS, respectively. At high environmental conduciveness to epidemic development, $\varepsilon = 0.008$, we find $R_0 \approx 24.5$ and $R_0 \approx 42.7$ for the high- and low-aggressiveness ESS, respectively. No estimates of R_0 are available for *P. nodorum*, but estimates published for other leaf-infecting fungal plant pathogens are 3.2 for *Peronospora farinosae* on spinach (van den Bosch *et al.* 1988); between 0.5 and 23, depending on temperature and leaf wetness duration, for *Puccinia striiformis* on wheat (Papastamati & van den Bosch 2007); 55.4 for *P. striiformis* on wheat (van den Bosch *et al.* 1988); and 383 for *Puccinia lagenophora* on *Senecio vulgaris* (Frantzen & van den Bosch 2000).

PIPs comparable to figure 2 for a range of different parameter values are presented in electronic supplementary material IV.

(b) General notes on seed-transmitted plant pathogens

(i) The evolving trait

We use aggressiveness, x , as the evolving quantity. In a plant disease system, aggressiveness would be experimentally

measured as the disease severity (% leaf area affected) caused per infecting pathogen propagule on a standard host in a standardized environment (Pariaud *et al.* 2009a). Pariaud *et al.* (2009a) show that for a range of fungal plant pathogens (i) there is quantitative variation for aggressiveness in pathogen populations, (ii) this variation is polygenically determined and (iii) it can be selected for both experimentally and in the field. We therefore take aggressiveness as our evolving trait in this paper.

(ii) The trade-off

It has been shown for several fungal plant pathosystems that spore production from a single disease lesion is proportional to the severity of the lesion (Pariaud *et al.* 2009a,b). Horizontal pathogen transmission is therefore proportional to spore production with a proportionality constant that is the product of the mean probability that a spore is deposited on a host and the mean probability that a spore germinates and produces a lesion. This therefore establishes that a greater aggressiveness results in a greater horizontal transmission rate in these pathogens. Vertical pathogen transmission equals the product of the probability that a seed is infected and the probability that this infected seed is used for planting and germinates to develop into an infected plant. It has been shown that severe infestation with a pathogen reduces seed size and weight. Smaller infected seeds are less likely to be used as planting materials and are less likely to germinate and develop into an infected host plant (Hewett 1975; Cunfer & Johnson 1981; Doohan *et al.* 1999; Fox *et al.* 1999; Argyris *et al.* 2003; Rashid 2005; Bergum *et al.* 2008). This thus establishes that greater aggressiveness of the pathogen decreases the probability of successful vertical transmission. Experimental data thus show that there is a trade-off between horizontal and vertical transmission related to aggressiveness in these fungal plant pathogens.

(iii) The environment

Environmental conditions (climatic conditions, host physiological status, pathogen physiological status) affect the expression of aggressiveness (Schoeneweiss 1975; Laine 2008; Pariaud *et al.* 2009a). We therefore also consider the effect of long-term changes in environmental conditions on the evolution of the balance between horizontal and vertical transmission.

(c) The *Phaeosphaeria nodorum*–wheat pathosystem

(i) Trade-off

For *P. nodorum* specifically, Jeger *et al.* (1984) showed a positive correlation between aggressiveness and spore production. This establishes the relation between aggressiveness and horizontal transmission for *P. nodorum*. Hewett (1975) and Cunfer & Johnson (1981) showed an inverse relation between aggressiveness and vertical transmission in *P. nodorum*. Therefore, based on the published data on a range of plant pathogens and the published data on *P. nodorum* specifically, we conclude that it is very likely that a trade-off between horizontal and vertical transmission operates in *P. nodorum*.

(ii) Trait selection

Halama *et al.* (1999) showed that components of aggressiveness (leaf necrosis and spore production) in *P. nodorum* are under polygenic control and there is heritable variation in the population for aggressiveness (Engle *et al.* 2006; Ali & Adhikari 2008). No data are published that specifically

study the inheritance of aggressiveness as defined here (lesion size owing to one infecting spore). The remaining component of aggressiveness is infectivity per spore. However, even if this were invariant, heritable variation in the other components would still provide sufficient variation in aggressiveness to allow selection to work. Furthermore, although selection of altered aggressiveness has not been directly shown in *P. nodorum*, it has been shown in a range of other fungal pathogens (Pariad *et al.* 2009*a,b*). We conclude that it is very likely that selection for aggressiveness traits takes place in *P. nodorum*.

(iii) The environment

Phaeosphaeria nodorum is greatly influenced by the environment. Bearchell *et al.* (2005) showed a close correlation between *P. nodorum* epidemic severity and SO₂ levels in the atmosphere over a 160-year period. Both the increased SO₂ emission from the 1900s to the 1960s and the decreasing SO₂ emission from the 1960s till the present were closely correlated to epidemic severity (Shaw *et al.* 2008). Whether SO₂ has a direct impact on crop and pathogen physiology or whether SO₂ is a surrogate for a combination of air pollutants is not known. Initial experiments suggest that SO₂ or sulphate fertilization might be involved in alterations in plant defenses (M.W.S. & P. Chandramohan 2010, unpublished results).

(d) The experiment

The Broadbalk experiment at Rothamsted Research (Harpenden, UK) on the cultivation of winter wheat has run continuously since 1843. A series of plots receive distinct fertilizer inputs (Goulding *et al.* 2000), but otherwise the agronomic practice at Broadbalk follows the common practice in this part of the UK. After each harvest, samples of (i) the grain and (ii) the stem and leaves are dried and stored. This was originally done to enable future nutrient analysis.

The development of PCR-based techniques has made it possible to quantitatively estimate the DNA content of wheat-infecting pathogens in these samples. This paper uses the data series described in Bearchell *et al.* (2005) and Shaw *et al.* (2008); quantification used fluorescent minor groove binder-conjugated TaqMan probes and was described in the original papers. The data on *P. nodorum* DNA span a period between 1844 and 2003.

The relationship between *P. nodorum* DNA in seed and leaf samples and the transmission mode was determined as follows. *Phaeosphaeria nodorum* DNA content of the leaf and stem correlates well with national average estimates of *P. nodorum* severity at the end of the growing season (Fraaije *et al.* 2001; Bearchell *et al.* 2005). The PCR analysis yields an estimate of the amount of amplifiable pathogen DNA per unit total DNA extracted from the sample. The vast majority of the DNA extracted is wheat DNA, so the results can be interpreted as the amount of pathogens per unit leaf or seed. Measurements on both seed and vegetative parts are the end product of pathogen multiplication during the season. In a favourable season, both infection of foliage and transfer of inoculum to the ear will be high; in a less favourable season, both will be low. However, the ratio between the concentration of DNA in the leaf and the seed must represent the ratio between horizontal and vertical transmission. To obtain a measure, we rely on averaging over decades in order to remove the annual variation in the suitable summer weather for the spread of the pathogen (Shaw *et al.* 2008). The average leaf population of *P. nodorum*

then represents the horizontal transmission capacity of the population in the average environment of that period, the product of aggressiveness and the average environmental suitability.

3. RESULTS AND DISCUSSION

The axes of figure 2 represent the resident and invader aggressiveness, x , which is the evolving trait in our model. The solid circles (marked A and B) on the 45° line in each of the subplots represent evolutionary stable strategies, ESS, of pathogen aggressiveness, x_{ESS} . The open circle is an evolutionary repeller. Our results thus show (figure 2*b–d*) that there are two evolutionary stable states of aggressiveness, x , separated by an evolutionary repeller for a wide range of environmental conditions, ϵ . That is, our results show evolutionary bi-stability in aggressiveness. Given the trade-off relation, this implies that we have found evolutionary bi-stability in the balance between horizontal and vertical transmission. Our analysis of the model variants shows that evolutionary bi-stability in the transmission mode is robust to changes in model specification and exists over larger biologically relevant parameter ranges (electronic supplementary materials II, III and IV). There is a single evolutionary stable state when environmental conditions are very unfavourable to epidemic development (figure 2*a*) or very conducive to it (figure 2*e,f*). Our model thus shows that bi-stability can arise as the result of a trade-off between horizontal and vertical transmission efficiency and that jumping from the high-aggressiveness to the low-aggressiveness state can be induced by environmental changes.

Is such evolutionary bi-stability and jumping between states also observed in the Broadbalk experiment? Since SO₂ levels have changed drastically over the 160 years of the experiment, and since it has been shown that SO₂ levels affect the dynamics of *P. nodorum*, it is an ideal candidate system to look for evolutionary bi-stability and jumping between states.

As argued above and in Bearchell *et al.* (2005), the concentration of pathogen DNA is a measure of pathogen population density. There are therefore clear long-term trends in (i) *P. nodorum* density in the leaf and stem (figure 3*a*), and (ii) the ratio of leaf and stem pathogen density to seed pathogen density (figure 3*b*). As motivated above, we interpret the ratio of pathogen DNA in the leaf and stem to that in the seed (y -axis, figure 3*c*) as a measure of the balance between horizontal and vertical transmission of the pathogen. Plotting this ratio against the leaf and stem pathogen DNA content (i.e. epidemic severity; figure 3*c*) shows two clearly distinct groups of data points, each grouped around a straight line. The data grouped around the lower line are all from a single historical period and have a lower ratio of horizontal to vertical transmission than the data grouped around the upper line.

Two initial hypotheses to explain these groupings are: (i) that they represent some change in crop management at the Broadbalk experiment that leads to changes in the sampling of the pathogen in seed and vegetative parts; and (ii) that they represent a replacement of the population by another with an incidentally different pattern of infection on the seed and stem. For the first hypothesis, the extensive archives on the Broadbalk experiment

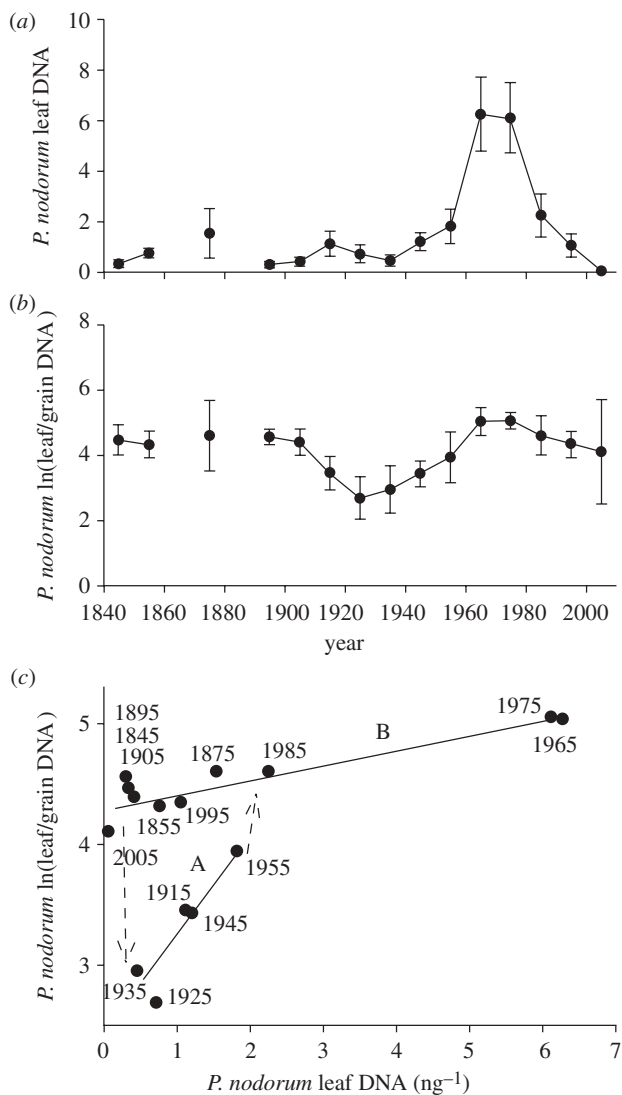


Figure 3. Time series for pathogen DNA from the Broadbalk experiment (Harpenden, UK) from 1844 to 2003. (a) Ten-year mean and standard error of amplifiable *P. nodorum* DNA in wheat leaf and stem samples. (b) Ten-year mean and standard error of the ratio of amplifiable *P. nodorum* DNA in leaf/stem to seed samples. (c) The ratio of amplifiable *P. nodorum* DNA in leaf/stem to seed samples as a function of amplifiable *P. nodorum* DNA in samples of the leaf and stem.

(e.g. Anon 1969) allowed us to work systematically through a range of possible factors that might explain the changes we observed. In electronic supplementary material V, we present all information available. We conclude there that it is very unlikely that changes in crop management or harvest technique cause the existence of two groups in the data through sampling effects. The second hypothesis can never be ruled out entirely, but is completely *ad hoc*, without explanatory content. Instead, we suggest that the two groups of data points in figure 3c are an example of the phenomenon shown in our model analysis, and represent alternative stable states of the pathogen's transmission dynamics, with a jump to the lower state in the early twentieth century and a jump back in mid-century.

Based on this model, we suggest a tentative interpretation of the sequence of events at Broadbalk as follows. The variable we want to explain, plotted on the y-axis in figure 3c, is the ratio of horizontal to vertical transmission.

In the model this is $\beta(x)/g(x)$, which is an increasing function of aggressiveness, x (figure 1b). In the early part of the experiment (1844 to around 1900), the system was in the evolutionary stable state with the higher ratio of horizontal to vertical transmission; that is, at a higher aggressiveness index, x (upper line, B, in figure 3c; point B in e.g. figure 2b). A gradual decrease in conduciveness to epidemic development during the later nineteenth century, for which several explanations can be put forward, led to the disappearance of this evolutionary stable state (going from figure 2b to a) and the system jumped to the evolutionary steady state with lower aggressiveness (lower line, A, in figure 3c; point A in figure 2a). A gradual improvement of the conditions for epidemic development, correlating well with SO₂ emission (Beauchell *et al.* 2005), between 1915 and 1955 then led the system to move along the lower line in figure 3c (point A in the sequence figure 2b → c → d) until this evolutionary stable state was lost (figure 2e) and the pathogen jumped back to the evolutionary stable state with a higher ratio of horizontal to vertical transmission (point B in figure 2e). The pathogen has lived in this evolutionary stable state ever since (the sequence figure 2f → e → d → c → b). The reason for the improving conditions for epidemic development might be either varietal susceptibility changes—introduction of new varieties and the extent of changes in their phenotype have been increasingly rapid since the early twentieth century—or SO₂ emission effects (Beauchell *et al.* 2005).

As discussed, the published experimental evidence strongly suggests a trade-off between horizontal and vertical transmission in the fungal plant pathogen *P. nodorum*. A comprehensive set of experiments for a representative sample of pathogen isolates would be needed to specify the exact shape of the trade-off and the joint inheritance of the characters involved. To test the specific interpretation put forward here, the relation between the pathogen DNA content of the leaf and seed and the transmission mode, as well as the precise effects of SO₂ on *P. nodorum*, need further experimental work. However, our key finding is that it is very probable that in the Broadbalk experiment *P. nodorum* shows evolutionary bi-stability in the transmission mode, evidenced by distinct jumps between states. This is unaffected by the details of the mechanisms proposed to drive the changes.

We thus conclude that we have shown, both in a long-term experiment and in a theoretical model, evolutionary bi-stability in the transmission mode of a pathogen. The bi-stability appears to be a generic feature of models with a similar model structure and trade-offs we investigated (electronic supplementary materials II, III and IV), although clearly the range of environments and parameter values giving rise to the bi-stability varies. The key qualitative aspects of models with bi-stability and the loss of bi-stability in extreme environments are the combination of (i) a decreasing vertical transmission rate for larger aggressiveness and (ii) a decreasing vertical transmission rate in environments more favourable to horizontal transmission, with the potential for efficient vertical transmission at low aggressiveness or in unfavourable environments. These conditions are appropriate to a range of human, animal and crop pathogens in which aggressive strains kill tissue rapidly so that young individuals are particularly unlikely to survive infection.

4. CONCLUDING REMARK

Existing model studies on the evolutionary consequences of multiple transmission modes concentrate on the evolution of virulence (defined as pathogen-induced host death rate). These studies show that the virulence of a pathogen is greatly influenced by the type of transmission mode used. Ewald (1987, 1994) hypothesized that pathogens with both horizontal and vertical transmission should fall along a continuum with primarily horizontally transmitted pathogens evolving high virulence and primarily vertically transmitted pathogens evolving towards low virulence. In their model study, Lipsitch *et al.* (1996) show that vertical transmission does select for low virulence, but that horizontal transmission might or might not select for high virulence depending on circumstances. Other studies have considered the effect of free-living infectious stages, water-borne transmission and seasonal host dynamics on the evolution of virulence (van Baalen & Sabelis 1995; Bonhoeffer *et al.* 1996; Ewald & De Leo 2002). No studies have primarily concentrated on the evolutionary consequences of the trade-off between transmission modes, which is the key topic of this paper. We have shown how such trade-offs can lead to bi-stable evolutionary states. Jumps resulting from such bi-stability could have practical consequences for management strategies.

This work was funded by the Biotechnological and Biological Sciences Research Council (BBSRC) of the UK. We are grateful to Paul Poulton for his help and suggestions concerning the Broadbalk agronomy and crop management.

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