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The dynamics of a senescence plasmid in fungal populations

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Summary

Fungi normally do not senesce, but in some species mitochondrial plasmids are known to occur that induce senescence. In this paper models for the dynamics of a senescence plasmid in a fungal population are developed and analysed. In the first model it is assumed that total fungal biomass density is constant, while in the second model the resource dynamics and its effect on fungal growth is modelled explicitly. An additional death rate describes the effect of the plasmid on the senescent subpopulation. Plasmids can be transferred to non-senescent fungus. Criteria for the coexistence of the non-senescent and senescent fungal strains are derived, all of which have a clear biological interpretation. It is shown that coexistence is not possible in the first model, but is possible in the second model for a large range of parameter values. We show that the interplay between resource dynamics, fungal growth and plasmid transmission is crucial for coexistence. We develop a biological interpretation of how these mechanisms have to interact to promote coexistence. A numerical study of the second model further clarifies the relations between the numerical value of several parameters and coexistence of non-senescent and senescent fungal strains.

1. Introduction

Fungi normally do not senesce and are capable of unlimited somatic propagation, but senescence is observed in isolates of *Neurospora* and *Podospora*. Research on fungal senescence has recently been reviewed by Griffiths (1992). In contrast to the situation in *Neurospora*, where most isolates are non-senescent, all wild isolates of *Podospora* senesce and die through a process involving amplification of mitochondrial DNA (mtDNA) fragments (Griffiths, 1992, 1995). The best-studied examples of senescence of *Neurospora* isolates are in *N. intermedia* from Hawaii and *N. crassa* from India, in which senescence has been associated with the presence of the mitochondrial plasmids *kalilo* and *maranhar* respectively (Griffiths & Bertrand, 1984; Court *et al.*, 1991; Griffiths, 1992). The linear plasmids act as mutagens by inserting into the mtDNA, leading to abnormal mitochondrial physiology and ultimately to death of the culture (Griffiths, 1992). Analysis of the Hawaiian population of *N. intermedia* of the island of Kauai as sampled in 1972 and 1976 shows that approximately 40% of the isolates carried the *kalilo* plasmid (Debets *et al.*, 1995). Another mitochondrial plasmid not associated with senescence, Han-2, was detected in the same population in approximately 75% of the isolates. The distribution of this neutral Han-2 plasmid was found to be independent of that of the *kalilo* plasmid, and there is no significant difference between the Kauaian population of 1972 and 1976 (Debets *et al.*, 1995). There is thus evidence of coexistence of senescence-plasmid-carrying and plasmid-free cultures in natural populations. In the following we will use for these two cultures the terms 'senescent' and 'non-senescent', respectively.

The average lifetime of a senescent fungus is considerably shorter than that of a non-senescent fungus, but no other aspects of the life history of the fungus seem to be affected by the senescence plasmid.

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Table 1. *Definitions of the variables and parameters used in the model*

Variables	
$R(t)$	Resource density at time t
$F_a(t)$	Biomass density of non-senescent fungus at time t
$F_p(t)$	Biomass density of senescent fungus at time t
t	Time
Parameters	
θ	Resource supply rate
ω	Resource decay rate
β	Maximum resource consumption rate of one unit of fungal biomass
ψ	Shape parameter of the Michaelis–Menten equation
γ	Conversion efficiency of resource into fungal biomass
μ	Rate of fungal death due to all causes except senescence
α	Plasmid transmission efficiency factor
δ	Senescence-related death rate
C	Total fungal biomass, model I

A senescent individual therefore has a lower total reproductive output than a non-senescent individual, and in a situation of resource competition coexistence of the senescent and non-senescent fungal subpopulations is not to be expected. In such situations the non-senescent subpopulation is expected to drive the senescent subpopulation to extinction. How, then, can the non-senescent and the senescent subpopulations coexist under resource competition as is suggested by the Hawaiian population described above?

Horizontal transmission of the senescence plasmid has been demonstrated by Griffiths *et al.* (1990) and Debets *et al.* (1994). Transmission of the senescence plasmid transforms a non-senescent fungal individual into a senescent type and can thus be seen as an increase in the total reproductive output of the senescent mycelium. It might be feasible that the transfer of plasmids does increase the reproductive output to larger values than the reproductive output of a non-senescent individual. In this situation, however, the senescent would finally drive the non-senescent type to extinction, again leaving the question of coexistence unanswered.

In this paper we study the coexistence of non-senescent and senescent fungal types using simple analytically tractable models. We will show that the interplay between the population ecology and the population genetics of the system is the key to answering questions on coexistence of the non-senescent and senescent types. All variables and parameters are listed in Table 1.

2. Model I: population genetics only

In the first model we leave the population ecology of the fungus out of consideration, with no interaction with resources taken into account. Total fungal biomass density is assumed constant, allowing us to study the effect of a senescence plasmid dynamics in its bare essentials.

A fungal mycelium has indeterminate growth. Therefore we quantify a fungal (sub)population by its total biomass, which can be considered roughly proportional to the number of individuals. Fungal biomass density of the fungus with and without the senescence plasmid is denoted by $F_a(t)$ and $F_p(t)$, respectively, where the subscript 'a' denotes absence and 'p' presence of the plasmid. The two 'fungal types' differ only in the effect of the plasmid on longevity and in the fact that plasmid transmission changes an F_a-type fungus into an F_p-type. The dynamics of the system are governed by the set of differential equations:

$$\left.\begin{aligned} \frac{dF_a}{dt} &= \begin{bmatrix} growth\ of \\ F_a\text{-}type \end{bmatrix} - [natural\ death] \\ &\quad - \begin{bmatrix} plasmid \\ transmission \end{bmatrix}, \\ \frac{dF_p}{dt} &= \begin{bmatrix} growth\ of \\ F_p\text{-}type \end{bmatrix} - [natural\ death] \\ &\quad + \begin{bmatrix} plasmid \\ transmission \end{bmatrix} - [senescence\ death]. \end{aligned}\right\} \quad (1)$$

The model can be completed by describing the various terms.

By the term 'natural death' we mean death due to all causes except senescence: for example by fungivory, fire or dehydration. Natural death is assumed proportional to the biomass density, giving

$$[natural\ death] = \mu F_i, \quad (2)$$

where subscript i is a or p, respectively.

The probability per unit time that F_a-type mycelium encounters F_p-type mycelium is proportional to the biomass density of both types. We assume that there

is a constant probability that during an encounter a senescence plasmid is transferred between the mycelia. We can thus write

$$[\textit{plasmid transmission}] = \alpha F_a(t)\, F_p(t). \tag{3}$$

The fungi carrying the plasmid have an additional senescence-related death rate, δ, giving

$$[\textit{senescence death}] = \delta F_p(t). \tag{4}$$

The assumption that the fungal types differ only in plasmid-induced characteristics yields

$$\left.\begin{aligned} &\begin{bmatrix}\textit{growth of}\\ F_a(t)\textit{-type}\end{bmatrix} = G\frac{F_a(t)}{F_a(t)+F_p(t)}\\ &\text{and}\\ &\begin{bmatrix}\textit{growth of}\\ F_p(t)\textit{-type}\end{bmatrix} = G\frac{F_p(t)}{F_a(t)+F_p(t)}, \end{aligned}\right\} \tag{5}$$

where G is the total population growth rate. The growth rate of $F_a(t)$ and $F_p(t)$ types can now be calculated using the assumption on constancy of total fungal biomass, giving

$$F_a(t)+F_p(t) = C. \tag{6}$$

This implies that

$$\begin{aligned}&\frac{\mathrm{d}(F_a(t)+F_p(t))}{\mathrm{d}t}\\ &= \begin{bmatrix}\textit{growth of}\\ F_a(t)\textit{-type}\end{bmatrix} + \begin{bmatrix}\textit{growth of}\\ F_p(t)\textit{-type}\end{bmatrix} - \begin{bmatrix}\textit{natural death}\\ \textit{of } F_a\textit{-type}\end{bmatrix}\\ &\qquad - \begin{bmatrix}\textit{natural death}\\ \textit{of } F_p\textit{-type}\end{bmatrix} - \begin{bmatrix}\textit{senescence}\\ \textit{death}\end{bmatrix} = 0. \end{aligned} \tag{7}$$

Substituting (2) to (5) into (1) we find that

$$G = \mu C + \delta F_p(t), \tag{8}$$

which can simply be interpreted as each unit of biomass removed from the population by 'natural' or 'senescence' death is replaced through growth of the fungal population.

The model becomes

$$\left.\begin{aligned} \frac{\mathrm{d}F_a(t)}{\mathrm{d}t} &= (\mu C + \delta F_p(t))\frac{F_a(t)}{C}\\ &\quad - \mu F_a(t) - \alpha F_a(t)\, F_p(t),\\ \frac{\mathrm{d}F_p(t)}{\mathrm{d}t} &= (\mu C + \delta F_p(t))\frac{F_p(t)}{C}\\ &\quad - \mu F_p(t) + \alpha F_a(t)\, F_p(t) - \delta F_p(t). \end{aligned}\right\} \tag{9}$$

(i) *Results*

In this section we analyse whether the senescent type, F_p, and the non-senescent type, F_a, can coexist. The analysis used is well known both in ecology, where it is known as determining *invasion criteria*, and in population genetics, where one analyses whether a *protected polymorphism* is possible.

Such an analysis proceeds in two steps. Assume that only non-senescent fungus is present. Now introduce an (infinitesimally) small amount of senescent fungus and calculate for which parameter values the senescent type invades the system. Next assume the system consists of senescent type only. Introduce an (infinitesimally) small amount of non-senescent fungus and calculate when this type can invade the system. When both fungal types can invade the system with only the other type present, the non-senescent and senescent coexist. Technical details of the analysis can be found in the Appendix.

The senescent type can invade a population consisting of non-senescent fungus when

$$\delta < \alpha C. \tag{10}$$

This inequality has a clear biological interpretation. The factor αC is the probability per unit time that a plasmid is transferred from a senescent type to a non-senescent type in an environment where only an infinitely small number of senescent type biomass units are present. The inequality (10) thus states that the senescent type can invade a non-senescent system when the probability per unit time of dying due to senescence is smaller than the probability per unit time of transferring a plasmid. It goes without saying that when plasmid transfer rates overshadow plasmid-induced senescence death, the senescent type can invade. It can furthermore be shown (see Appendix) that when inequality (10) holds the senescent type will drive the non-senescent type to extinction. The final state of the system will thus be a fungal population consisting of senescent fungus only.

The non-senescent type can invade a population consisting of senescent fungus when

$$\delta > \alpha C. \tag{11}$$

This inequality is the reverse of inequality (10), and its biological interpretation is similar. It can furthermore be shown that when this inequality holds the non-senescent type will drive the senescent type to extinction. The final state of the system is thus a fungal population consisting of non-senescent fungus only.

Summarizing the above we can conclude that: *Coexistence of non-senescent and senescent fungal types is* ***not*** *possible in this model.*

3. Model II: population genetics and ecology

In the second model we take resource dynamics and the dependence of fungal growth rate on resource density into consideration, in a system where the growth of the fungus depends on a limiting resource, with density denoted $R(t)$. Fungal biomass increases

due to consumption of resource. Growth is the same for both the fungus without the plasmid, $F_a(t)$, and with plasmid, $F_p(t)$, this assumption allowing us to study the interplay between plasmid dynamics and the fungus–resource interaction. The basic model now becomes

$$\left.\begin{aligned}
\frac{dR}{dt} &= \begin{bmatrix}resource\\dynamics\end{bmatrix} - \begin{bmatrix}consumption\\by\ F_a\text{-}type\end{bmatrix} - \begin{bmatrix}consumption\\by\ F_p\text{-}type\end{bmatrix},\\
\frac{dF_a}{dt} &= \begin{bmatrix}growth\ of\\F_a\text{-}type\end{bmatrix} - [natural\ death] - \begin{bmatrix}plasmid\\transmission\end{bmatrix},\\
\frac{dF_p}{dt} &= \begin{bmatrix}growth\ of\\F_p\text{-}type\end{bmatrix} - [natural\ death] + \begin{bmatrix}plasmid\\transmission\end{bmatrix}\\
&\quad - [senescence\ death].
\end{aligned}\right\} \tag{12}$$

To allow straightforward comparison we use for the natural death, plasmid transfer and senescence death the same formulation as in model I.

For the resource dynamics we assume that resource becomes available to the fungus at a constant rate, θ (DeAngelis, 1992), which can be interpreted as the mineralization rate of the ecosystem. Due to other organisms using this resource, and through leakage, etc., the resource 'decays' at a rate proportional to resource density (DeAngelis, 1992). The resource dynamics term thus takes the form

$$[resource\ dynamics] = \theta - \omega R. \tag{13}$$

We assume Michaelis–Menten kinetics for the resource consumption by the fungus. The resource consumption terms thus take the form

$$[consumption\ by\ F_i\text{-}type] = \frac{\beta R}{1+\psi R} F_i, \tag{14}$$

where the subscript i is a for the F_a-type and p for the F_p-type, respectively.

Consumed resource is converted to new fungal biomass with constant efficiency, γ. The fungal biomass growth terms thus equal (14) multiplied by γ.

Putting all the pieces together, model equations (12) become

$$\left.\begin{aligned}
\frac{dR}{dt} &= \theta - \omega R - \frac{\beta R}{1+\psi R} F_a - \frac{\beta R}{1+\psi R} F_p,\\
\frac{dF_a}{dt} &= \gamma \frac{\beta R}{1+\psi R} F_a - \mu F_a - \alpha F_a F_p,\\
\frac{dF_p}{dt} &= \gamma \frac{\beta R}{1+\psi R} F_p - \mu F_p + \alpha F_a F_p - \delta F_p.
\end{aligned}\right\} \tag{15}$$

(i) *Analytical results*

In this section we analyse the conditions under which the senescent type, F_p, and the non-senescent type, F_a, can coexist. We first analyse when the senescent type and the non-senescent type are able to build up a population in an environment where no fungus is present. We will loosely call such an environment a virgin environment.

The non-senescent type, F_a, can build up a population when

$$\frac{\beta(\theta/\omega)}{1+\psi(\theta/\omega)} \gamma \frac{1}{\mu} > 1. \tag{16}$$

The senescent type can build up a population when

$$\frac{\beta(\theta/\omega)}{1+\psi(\theta/\omega)} \gamma \frac{1}{\mu+\delta} > 1. \tag{17}$$

Both criteria have a clear biological interpretation. In an environment without these fungi the resource density is θ/ω. Consider in this situation one unit of fungal biomass. Using (14) we see that the rate of resource consumption of this unit of fungal biomass at this resource density equals the first term on the left-hand side of (16), and multiplying by the conversion efficiency parameter γ yields the increase in biomass per unit time of one unit of fungal biomass. A unit of non-senescent fungal biomass survives on average $1/\mu$ time units. Thus the left-hand side of (16) can be interpreted as the total number of biomass units produced by one unit of biomass during its lifetime, and obviously when this number is larger than unity a population can build up. Equation (17) has the same interpretation but the average lifetime of a senescent unit of biomass equals $1/(\mu+\delta)$.

Next we turn to the invasion criteria. The senescent type can invade a system where only non-senescent type is present when

$$-\delta + \frac{\alpha\theta\gamma}{\mu} - \frac{\alpha\omega\gamma}{\gamma\beta - \psi\mu} > 0. \tag{18}$$

The non-senescent type can invade a system with only senescent type when

$$\delta - \frac{\alpha\theta\gamma}{\mu+\delta} + \frac{\alpha\omega\gamma}{\gamma\beta - \psi(\mu+\delta)} > 0. \tag{19}$$

When both criteria (18) and (19) are met the two fungal types can coexist. Both criteria again have a clear biological interpretation that becomes more transparent if we first rewrite the criteria.

Criterion (18) can be written as

$$\frac{\beta \hat{R}_1}{1+\psi \hat{R}_1} \gamma \frac{1}{\mu+\delta} + \alpha \hat{F}_a \frac{1}{\mu+\delta} > 1, \tag{20}$$

where R_1 is the equilibrium resource density in the system when only the non-senescent type is present and F_a is the equilibrium density of the non-senescent type in this system. Criterion (20) can now be interpreted as the total amount of senescent biomass produced by one unit of senescent biomass during its

lifetime in a system where only the non-senescent type is present. First consider the left-most term on the left-hand side of (20), the first part of which is the rate of resource consumption of a unit of senescent fungal biomass in an environment where only non-senescent type is present. Multiplying by the conversion efficiency, γ, and the average lifetime of a senescent fungus yields, as in (16) and (17), the total senescent biomass produced by one unit of senescent biomass during its entire lifespan through resource consumption. The right-most term on the left-hand side of (2) has to do with plasmid transfer. One unit of senescent biomass will transfer the plasmid to αF_a units of non-senescent biomass per unit time. This term is multiplied with the average lifetime of a senescent biomass unit. The second term in (20) can thus be interpreted as the total number of non-senescent biomass units that receive a plasmid from the senescent biomass unit during its entire life. It is obvious that the senescent type can invade a system with non-senescent type only when the sum of biomass growth through resource consumption and plasmid transfer to non-senescent fungus during its lifetime is larger than unity. The left-hand side of (20) is known in ecology as the 'net reproductive number' or the 'lifetime reproductive output'. Note that for larger resource densities and larger densities of non-senescent fungal biomass the lifetime reproductive output of the senescent type is larger. Resource dynamics and its effect on fungal biomass density (the ecological factors built into model II) are thus major factors in the invasion criterion.

Criterion (12) can be written as

$$\frac{\beta \hat{R}_2}{1+\psi \hat{R}_2}\gamma\frac{1}{\mu}-\alpha\hat{F}_p\frac{1}{\mu}>1, \tag{21}$$

where R_2 is the equilibrium resource density in the system when only the senescent type is present and F_p is the equilibrium density of the senescent type in this system. Once again criterion (21) can be interpreted as the total amount of non-senescent biomass produced by one unit of non-senescent biomass during its lifetime in a system where only the senescent type is present. The left-most term on the left-hand side of (21) is again interpreted as the growth due to resource consumption, and the right-most term is now the number of biomass units that receive a plasmid from the existing population of senescent fungus. When the difference between gain due to resource consumption and loss due to plasmid transfer turns out to be larger than unity the non-senescent can invade the senescent population. Again note that resource density and density of senescent biomass are major determinants of the invasion criterion.

We conclude that: *Contrary to the results of model I, in the present model coexistence of non-senescent and senescent fungal types is possible*. In the numerical simulations to be discussed we show that the steady state with both types present is stable. No periodic population oscillations occur.

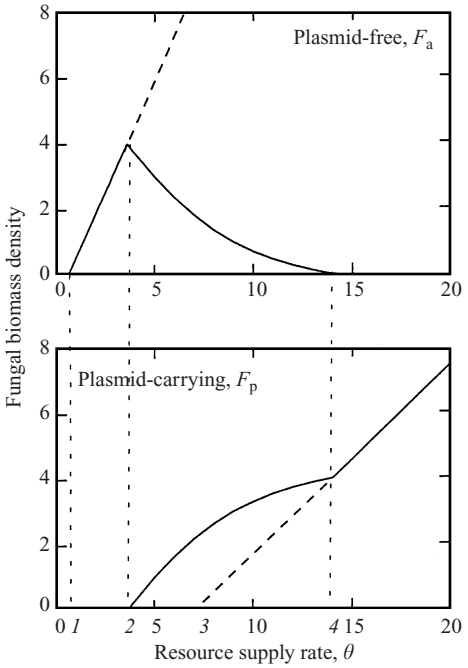

Fig. 1. Fungal biomass density of the non-senescent type, F_a, and senescent type, F_p as a function of the resource supply rate, θ. Continuous lines are biomass densities in the situation where both fungal types can be present. The long-dashed lines are the fungal biomass density when the fungal type is the only one present in the system. The x-axis of this figure corresponds to the dashed line in Fig. 2. The numbers *1* to *4* on the x-axis mark the relevant transitions of the system. Parameter values are $\omega = 1{\cdot}0$, $\beta = 2{\cdot}0$, $\psi = 1{\cdot}0$, $\gamma = 1{\cdot}0$, $\alpha = 1{\cdot}0$, $\mu = 0{\cdot}8$ and $\delta = 1{\cdot}0$.

(ii) *Numerical results*

What combinations of mechanisms causes coexistence in model II and why does this not operate in model I? This question can be answered by combining the invasion criteria with a plot of the steady-state densities of the fungal types as a function of resource supply rate, θ (Fig. 1). In Fig. 1 the continuous line is the density of the fungal types at a given resource supply rate; the dotted line is the fungal density that would have been attained in a system where the other fungal type is not present, and the numbers *1* to *4* on the x-axis mark the relevant transitions of the system. Consider a system with a resource supply rate smaller than *1*. Resource densities in such a system are so small that both criteria for the existence of a fungus, (16) and (17) do not hold, so no fungal population can build up. Increasing resource supply rate above 1, criterion (16) is met and the non-senescent type can build up a population; but since all other criteria are not met there will only be non-senescent fungus. Further increasing the resource supply rate

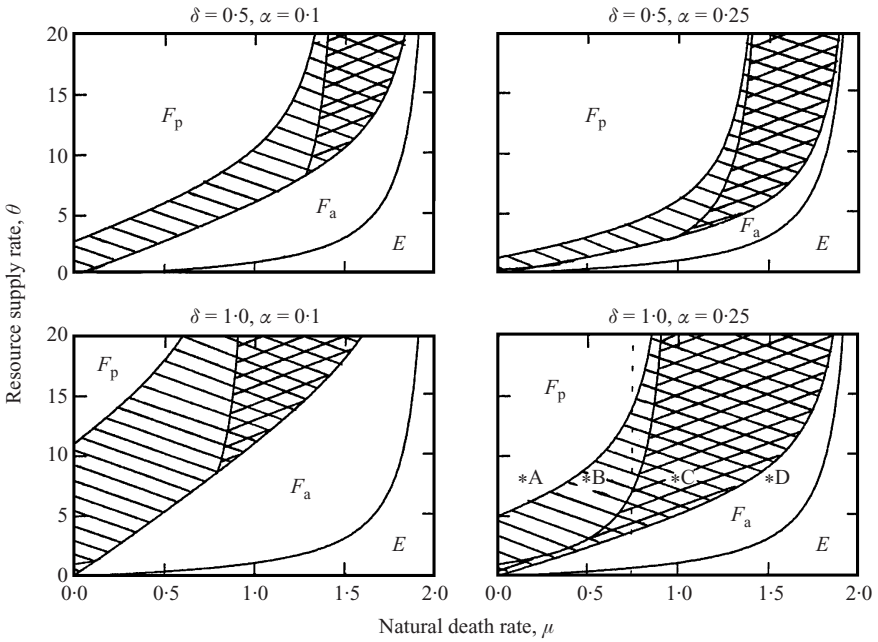

Fig. 2. Resource supply rate, θ, versus natural death rate, μ, parameter planes for four combinations of the senescence-related death rate, δ, and the plasmid transmission efficiency, α. In each of the four parts of the figure the parameter areas where the various states of the system exist are depicted. In the area marked E no fungal biomass is present. In the area marked F_a only non-senescent fungus is present. In the area marked F_p only senescent fungus is present. In the hatched areas both fungal types coexist. In the single-hatched area the senescent fungal type can build up a population in an environment where the non-senescent type is not present. In the double-hatched area the senescent fungal type can not build up a population in an environment where the non-senescent type is absent. The asterisks in the bottom-right of the figure indicate the parameter combinations of the simulations in Fig. 3. The dashed line serves as the x-axes in Fig. 1.

the biomass density of non-senescent type increases. Beyond resource supply rate *2*, criterion (20) is met because fungal density is so large that plasmid transmission becomes an important factor in the lifetime reproductive output of the senescent type. Further increasing resource supply rate beyond *4*, the fungal densities are so extremely large that plasmid transmission starts to overshadow the growth rate of the non-senescent type. Criterion (21) no longer holds and the non-senescent type will disappear from the system. (Below we will discuss resource supply rate *3*.) Clearly the addition of resource dynamics to our model caused fungal densities to vary with parameter values. Consequently plasmid transmission rates vary with parameter values since plasmid transmission rates depend on fungal biomass densities (equation (3)). From the above discussion we conclude that: *Coexistence is caused by the interaction between resource dynamics, fungal biomass density and plasmid transmission rates.*

The conditions for coexistence in the previous section were shown to have a clear biological interpretation, opening the possibility for a more detailed analysis of the conditions leading to co-existence in order to understand which biological and/or physical characteristics of the system are crucial to coexistence. To this end we analysed the model and the invasion criteria numerically. To simulate the model and to make graphs of the criteria one has to fix several parameter values. We studied the system extensively for a wide range of parameter values and found that the qualitative behaviour of the system is to a large extent independent of the precise parameter values used, though quantitative differences exist.

In Fig. 2 the criteria (16) to (19) are plotted as a function of the resource supply rate, θ, and the natural death rate, μ, for various values of the plasmid transmission coefficient, α, and the senescence-related death rate, δ. Fig. 3 shows numerical solutions of the model. The parameter values used in the simulation in Fig. 3*a* to *d* correspond to the asterisks in Fig. 2, where the θ-μ-parameter plane is divided into five parameter areas.

In the area E both the non-senescent and the senescent fungus go extinct because both criteria (16) and (17) do not hold. This implies that resource levels are too low and/or the natural death rate is too large for the fungus to build up a population even in a virgin environment. From a biological viewpoint the shape of this area is plausible: at high natural death rate the resource level has to be high for fungi to grow, while at low natural death rate resource levels can be too low for fungal growth to occur.

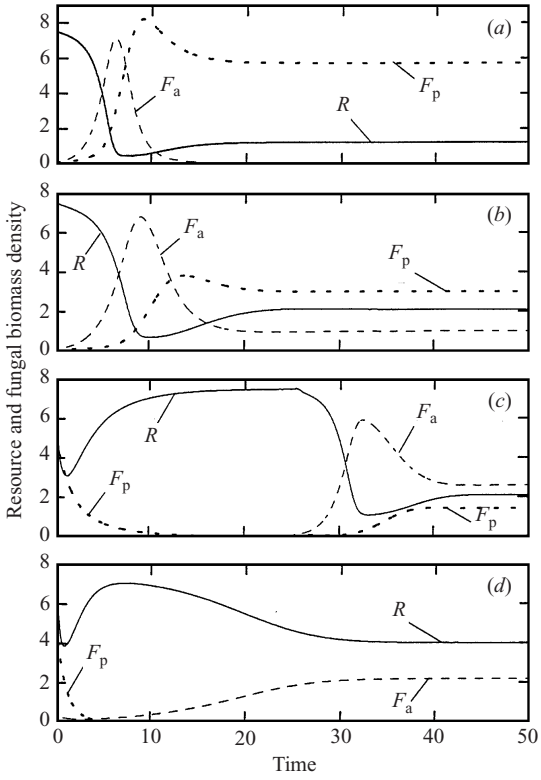

Fig. 3. Simulation runs of model equations (15). On the x-axis is the time, on the y-axis the resource, R, and fungal biomass densities. F_a is the density of non-senescent type and F_p is the senescent type. Parameter values are $\theta = 1{\cdot}0$, $\omega = 1{\cdot}0$, $\beta = 2{\cdot}0$, $\psi = 1{\cdot}0$, $\gamma = 1{\cdot}0$, $\alpha = 1{\cdot}0$ and $\delta = 1{\cdot}0$ for all runs. (*a*) A run for $\mu = 0{\cdot}1$, (*b*) for $\mu = 0{\cdot}5$, (*c*) for $\mu = 1{\cdot}0$ and (*d*) for $\mu = 1{\cdot}5$. All runs were started with the resource at density 7 and both fungal types present, except for (*c*) where the simulation was started with no F_a-type and at time 25 a small amount of F_a-type was added to the system.

In the parameter area F_a the non-senescent type can invade a virgin environment. In this area the resource levels are too low and/or natural death rate too high for the senescent type to invade the virgin environment, resulting in a system with non-senescent fungi only. A simulation of this situation is shown in Fig. 3*d*. The system was started with senescent fungus present and only a very small density of non-senescent fungus. The senescent type went extinct within a short time while the non-senescent type built a population. For these parameter values the final density of non-senescent fungus is small compared with that in the other simulations.

In the hatched parameter areas in Fig. 2 coexistence of the non-senescent and senescent type is possible, but there is a marked difference between these two parameter areas. In the single-hatched area both the non-senescent and the senescent type can invade a virgin environment, as obviously the resource and natural death rate levels allow growth of both types. Furthermore criteria (18) and (19) are fulfilled, implying that neither of the types can drive the other to extinction. Fig. 3*b* shows a simulation of this situation, in which the system was started with low densities of both fungal types. The simulation shows that after a temporary overshoot the densities of the fungal types settle at their final value. Since in the single-hatched area all four criteria are satisfied it is not surprising to find coexistence. The situation in the double-hatched area is essentially different from the situation in the single-hatched area. Criteria (16), (18) and (19) are satisfied, but criterion (17) is not satisfied. The senescent type can thus not invade a virgin environment, yet it can invade an environment where the non-senescent type is present. This situation is shown in Fig. 3*c*. At time zero the simulation is started with senescent fungus only, which goes rapidly towards extinction. At time 25, when the senescent fungus has decreased to extremely low densities the system is inoculated with non-senescent fungus. After this inoculation both types build up a population, so existence of senescent type in this parameter area depends on the transfer of plasmid to non-senescent fungi. In view of criterion (2) we can conclude that the plasmid transmission term on the left-hand side causes the inequality to hold.

In the parameter area F_p only the senescent type survives; a simulation is shown in Fig. 3*a*. Although the non-senescent type can build up a population the senescent type finally drives the non-senescent type to extinction. Due to high fungal densities in this situation the plasmid transfer to non-senescent fungi is so successful that no non-senescent type remains. In view of criterion (21) the loss of growth potential due to plasmid transfer overshadows the growth of the non-senescent fungus from resource consumption.

The dashed line in Fig. 2 is the line that serves as the x-axis in Fig. 1. The resource supply rates where the transitions of the system take place (*1* to *4*) are also marked on the dashed line in Fig. 2. Resource supply rate *3* in Fig. 1 is seen to correspond with the boundary of the single-hatched and the double-hatched region, as expected.

Comparing the four graphs in Fig. 2 provides an impression of the effect of the plasmid transmission parameter, α, and the senescence-related death rate, δ, on the five parameter areas. For larger senescence-related death rate the parameter area where only the non-senescent type survives is larger, and the parameter area where only the senescent type survives is smaller, both these effects of senescence-related death rate being biologically plausible. It is remarkable that as a result of increasing senescence-related death rate the parameter area of coexistence increases. Increasing the plasmid transmission parameter decreases the area where only the non-senescent type survives and increases the area where only the senescent type survives. Again this is biologically plausible. As a

result of increasing transmissibility of the plasmid the area of coexistence increases. Fig. 2 shows that the combination of a high senescence-related death rate and a high transmissibility of the plasmid results in large parameter areas where the non-senescent and senescent fungus coexist.

4. Discussion

In this paper we have developed and analysed two simple models for the dynamics of senescence plasmids in fungi. To keep the models tractable to analysis several simplifying assumptions were made and several, potentially important, aspects of the biology of this system were not taken into consideration. To describe resource dynamics, for example, we assumed simple supply and decay rules. Resource dynamics in real-life situations is much more complex than this, so we performed a similar analysis with logistic-type resource dynamics, which gave qualitative results in agreement with those presented here. We therefore have the impression that the precise formulation of resource dynamics will not affect the qualitative conclusions. There are, however, factors with effects on the dynamics that are not clear from the present model study. For example, it is assumed in this study that plasmid transfer is equally likely between any two fungal individuals. In reality plasmid transfer is restricted, though not completely blocked, by the phenomenon of vegetative incompatibility (Debets *et al*., 1994). Two individual strains are incompatible if they differ at one or more vegetative incompatibility loci vs (*het*-loci). Natural populations of *Neurospora* are highly polymorphic for *het*-loci (Mylyk, 1976; Debets *et al*., 1994), so that in general randomly taken isolates will be incompatible, thus limiting the horizontal spread of the senescence plasmid. Other important features possibly resulting in population structure with respect to plasmid transfer are differences between mating types, a meta-population structure of the fungal population and possible frequency-dependent plasmid transfer. A different mechanism not incorporated in the model is the loss of plasmids. Debets *et al*. (1995) show that the senescence plasmid can be lost at a low rate during the sexual cycle.

Further research is needed on the effects of such additional mechanisms before general conclusions can be drawn on the dynamics of senescence-related plasmids in fungal populations. We do, however, believe that the qualitative relation between coexistence of non-senescent and senescent fungal types as found in the present model will be robust against such additional mechanisms.

It is interesting to consider the question why these plasmids induce senescence. The proximate causation of the senescent phenotype is in all likelihood the accelerated induction of mitochondrial mutations due to insertions of plasmids into mitochondrial genes. However, in *Neurospora* resistance to senescence plasmids has been documented (Yang & Griffiths, 1993), indicating that senescence is not an unavoidable consequence of the plasmid infection. In the present model senescence is assumed to be a pleiotropic effect of the plasmid on the fungal phenotype. With regard to animal senescence it has been proposed that the occurrence of senescence is actually a pleiotropic negative effect of genes having positive effects on fitness early in life (Williams, 1957). Because the force of natural selection declines with age, the early positive effects easily outweigh the late-occurring negative effects, and such genes will be selected. A rather similar proposal is the so-called disposable soma theory, which proposes that a balance between investment in reproduction and in somatic repair is selected, implying submaximal somatic maintenance (Kirkwood & Holliday, 1979). Moreover, because natural selection is less effective in old age, many deleterious late-acting genes may reach high frequencies at mutation–selection balance. For a review, see Kirkwood and Rose (1991). The hypotheses of Williams and of Kirkwood would predict that senescent fungal types would show more vigorous growth or higher reproduction at young stages. Another possibility is that the senescent phenotype itself is an adaptation of the plasmid, contributing to its spread. This is not a wholly unrealistic suggestion, because senescence in its late stages involves cell lysis, which presumably could favour horizontal transmission of the plasmid (at that stage present in very high copy numbers), provided uninfected strains are growing in close vicinity. Further experimental work to test the implications of these ideas would be interesting.

With some imagination the senescence-related plasmid can be seen as an infectious disease, the susceptible individuals being the non-senescent fungus and the infected individuals the senescent fungus. Viewed in this way the model has some relations with the theoretical epidemiology literature, the growth of the senescent fungus then being the so-called vertical disease transmission, and the plasmid transfer the horizontal disease transmission. Lipsitch *et al*. (1995) studied a model for the population dynamics of vertically and horizontally transmitted diseases that has some resemblance to the model described in this paper. They show that when population growth and horizontal disease transmission are large, the infection will take over the whole population leaving no individual uninfected, closely agreeing with our observation that at high resource levels the senescent type drives the non-senescent type to extinction. Lipsitch *et al*. (1995) also found disease extinction and coexistence of infected and healthy populations comparable to the observations in the present model.

The model can be used to generate hypotheses that can be tested experimentally or by field studies. From Fig. 2, for example, one would expect that along a resource gradient the senescent type will be absent at low resource level, present together with the non-senescent type at intermediate resource levels and the only type present at high resource levels. Another testable hypothesis would be that an increased natural death rate (e.g. releasing fungivores) at a site where both types coexist will cause the senescent type to go extinct leaving a population of non-senescent fungus only.

Finally we would like to stress that for a proper understanding of the population genetics of fungal senescence plasmids we need tools other than classical population genetics in which population size fluctuations are ignored. In our analysis the incorporation of ecological aspects in the form of resource dynamics appeared to be an essential ingredient of the model.

Appendix. Analysis of the models

(i) *Model I*

Model equations (9) can, after some rearrangements, be written as

$$\left.\begin{aligned}\frac{dF_a}{dt} &= \frac{\delta}{C}F_a F_p - \alpha F_a F_p,\\ \frac{dF_p}{dt} &= \frac{\delta}{C}F_p^2 + \alpha F_a F_p - \delta F_p.\end{aligned}\right\} \tag{A 1}$$

Using the fact that $F_a + F_p = C$, (A 1) can be written as

$$\left.\begin{aligned}\frac{dF_a}{dt} &= \left(\frac{\delta}{C} - \alpha\right)F_a(C - F_a),\\ \frac{dF_p}{dt} &= \left(-\frac{\delta}{C} + \alpha\right)F_p(C - F_p).\end{aligned}\right\} \tag{A 2}$$

Both equations (A 2) are mathematically equivalent to the standard logistic growth equation and it is immediately clear that when inequality (10) holds the non-senescent type will approach density C and the senescent type goes extinct. When inequality (11) holds the reverse is true.

(ii) *Model II*

In the absence of any fungal biomass the differential equation for the resource becomes

$$\frac{dR}{dt} = \theta - \omega R. \tag{A 3}$$

Equation (A 3) has a globally stable steady state, $R = \theta/\omega$. We will refer to this as the resource density in a virgin environment. Substituting this steady state in the equation for the non-senescent type, assuming the senescent type is absent from the system, we find

$$\frac{dF_a}{dt} = \left\{\gamma\frac{\beta(\theta/\omega)}{1 + \psi(\theta/\omega)} - \mu\right\}F_a. \tag{A 4}$$

From (A 4) we conclude that the non-senescent type can invade a virgin environment when the term between brackets is larger than zero. Rearranging this expression yields criterion (16). Substituting the resource density in a virgin environment in the differential equation for the senescent type, assuming the non-senescent type is absent from the system, we find

$$\frac{dF_p}{dt} = \left\{\gamma\frac{\beta(\theta/\omega)}{1 + \psi(\theta/\omega)} - \mu - \delta\right\}F_p. \tag{A 5}$$

From (A 5) we conclude that the senescent type can invade a virgin environment when the term between brackets is larger than zero. Rearranging this expression yields criterion (17).

In a system where the senescent type is absent the resource and the biomass of the non-senescent type settle at the steady-state densities:

$$\left.\begin{aligned}\hat{R}_1 &= \frac{\mu}{\gamma\beta - \mu\psi},\\ \hat{F}_a &= \frac{\theta\gamma}{\mu} - \frac{\omega\gamma}{\gamma\beta - \mu\psi}.\end{aligned}\right\} \tag{A 6}$$

Substituting this steady state in the differential equation for the senescent type we find

$$\frac{dF_p}{dt} = \left\{\frac{\alpha\theta\gamma}{\mu} - \frac{\alpha\omega\gamma}{\gamma\beta - \psi\mu} - \delta\right\}F_p, \tag{A 7}$$

and the term within brackets justifies criterion (18).

In a system where the non-senescent type is absent the resource and the biomass of the senescent type settle at the steady-state densities

$$\left.\begin{aligned}\hat{R}_2 &= \frac{\mu + \delta}{\gamma\beta - (\mu + \delta)\psi},\\ \hat{F}_p &= \frac{\theta\gamma}{\mu + \delta} - \frac{\omega\gamma}{\gamma\beta - (\mu + \delta)\psi}.\end{aligned}\right\} \tag{A 8}$$

Substituting this steady state in the differential equation for the non-senescent type we find

$$\frac{dF_p}{dt} = \left\{-\frac{\alpha\theta\gamma}{\mu + \delta} + \frac{\alpha\omega\gamma}{\gamma\beta - \psi(\mu + \delta)} + \delta\right\}F_p, \tag{A 9}$$

and the term within brackets justifies criterion (19).

The internal steady state can be calculated from

$$\left.\begin{aligned} &\theta-\omega\hat{R}-\frac{\beta\hat{R}}{1+\psi\hat{R}}\hat{F}_{\mathrm{a}}-\frac{\beta\hat{R}}{1+\psi\hat{R}}\hat{F}_{\mathrm{p}}=0,\\ &g\frac{\beta\hat{R}}{1+\psi\hat{R}}-\mu-\alpha\hat{F}_{\mathrm{p}}=0,\\ &g\frac{\beta\hat{R}}{1+\psi\hat{R}}-\mu+\alpha\hat{F}_{\mathrm{a}}-\delta=0. \end{aligned}\right\} \tag{A 10}$$

Lengthy but straightforward calculations show that the internal steady state is given by

$$\left.\begin{aligned} &\hat{R}=\frac{\theta\psi-\omega-\dfrac{\delta\beta}{\alpha}+\sqrt{\left[\left(\theta\psi-\omega-\dfrac{\delta\beta}{\alpha}\right)^2+4\omega\psi\theta\right]}}{2\omega\psi},\\ &\hat{F}_{\mathrm{a}}=\frac{1}{\alpha}\left\{\delta+\mu-\gamma\frac{\beta\hat{R}}{1+\psi\hat{R}}\right\},\quad \hat{F}_{\mathrm{p}}=\frac{1}{\alpha}\left\{\gamma\frac{\beta\hat{R}}{1+\psi\hat{R}}-\mu\right\}. \end{aligned}\right\} \tag{A 11}$$

References

Collins, R. A. & Saville, B. J. (1990). Independent transfer of mitochondrial chromosomes and plasmids during unstable vegetative fusion in *Neurospora*. *Nature* **345**, 177–179.

Court, D. A., Griffiths, A. J. F., Kraus, S. R., Russell, P. J. & Bertrand, H. (1991). A new senescence-inducing mitochondrial linear plasmid in field isolated *Neurospora crassa* strains from India. *Current Genetics* **19**, 129–137.

DeAngelis, D. L. (1992). *Dynamics of Nutrient Cycling and Food Webs*. London: Chapman & Hall.

Debets, F., Yang, X. & Griffiths, A. J. F. (1994). Vegetative incompatibility in *Neurospora*: its effect on horizontal transfer of mitochondrial plasmids and senescence in natural populations. *Current Genetics* **26**, 113–119.

Debets, F., Yang, X. & Griffiths, A. J. F. (1995). The dynamics of mitochondrial plasmids in a Hawaiian population of *Neurospora intermedia*. *Current Genetics* **29**, 44–49.

Griffiths, A. J. F. (1992). Fungal senescence. *Annual Review of Genetics* **26**, 351–372.

Griffiths, A. J. F. (1995). Natural plasmids of filamentous fungi. *Microbiological Reviews* **59**, 673–685.

Griffiths, A. J. F. & Bertrand, H. (1984). Unstable cytoplasms in Hawaiian strains of *Neurospora intermedia*. *Current Genetics* **8**, 387–398.

Griffiths, A. J. F., Kraus, S. R., Barton, R., Court, D. A. & Bertrand, H. (1990). Heterokaryotic transmission of senescence plasmid DNA in *Neurospora*. *Current Genetics* **17**, 139–145.

Kirkwood, T. B. L. & Holliday, R. (1979). The evolution of ageing and longevity. *Proceedings of the Royal Society of London*, *Series B* **205**, 531–546.

Kirkwood, T. B. & Rose, M. R. (1991). Evolution of senescence: late survival sacrificed for reproduction. *Philosophical Transactions of the Royal Society London*, *Series B* **332**, 15–24.

Lipsitch, M., Nowak, M. A., Ebert, D. & May, R. M. (1995). The population dynamics of vertically and horizontally transmitted parasites. *Proceedings of the Royal Society of London*, *Series B* **260**, 321–327.

Mylyk, O. M. (1976). Heteromorphism for heterokaryon incompatibility genes in natural populations of *Neurospora crassa*. *Genetics* **83**, 275–284.

Williams, G. C. (1957). Pleotropy, natural selection, and the evolution of senescence. *Evolution* **11**, 398–411.

Yang, X. & Griffiths, A. J. F. (1993). Plasmid suppressors active in the sexual cycle of *Neurospora intermedia*. *Genetics* **135**, 993–1002.