



## A Conflict Between Two Evolutionary Levels in Trees

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Due to the lack of germ line segregation in plants, it is possible to consider plant evolution (but not the evolution of most animals) as being composed of *two* evolutionary levels:

1. Intra-organism, in which the replicating unit is a part of the tree (e.g. a branch), reproduction is asexual, mutations are somatic, and selection operates only upon traits relevant to vegetative growth.
2. Inter-organism, in which the replicating unit is the whole tree, reproduction is sexual, and selection operates upon all the traits.

In this work, a case of a *conflict* between these two levels is studied. The dynamics of a mutation, which is advantageous on the branch level but harmful for the whole tree, are discussed for a one-locus two-allele model. Several cases are considered: dominant, partially dominant, and haploid. Necessary and sufficient conditions for fixation of such a mutation are found. The model predicts that as the longevity of a tree species increases, the trees are expected to be more strongly shifted from their optimal growth-to-reproduction ratio towards growth, and resource allocation between branches and other tree parts is expected to be shifted in favor of the branches. Traditional approach, considering the second level only, is justified as a limit case for short longevity.

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### Introduction

#### SOMATIC MUTATIONS IN PLANTS

The relevance of Weismann's (1904) classical doctrine of germ line segregation to plants had been questioned a long time ago (e.g. Sutherland & Atkinson, 1986; Klekowski, 1984). According to Weismann, mutational events should be divided into two classes, depending on the kind of cell in which they occur. While somatic mutations can be expressed, but not inherited, germ cell mutations can be both expressed and

inherited. Germ cell mutations are, therefore, the only means for an evolutionary change.

This fundamental distinction is valid in many animals, where germ cells are produced by undifferentiated cells that are set aside in the reproducing organs during most of the organism's lifetime. In plants, the case is different. In most plants, gametes are produced by cell lineages that are undifferentiated, but go through mitotic divisions and somatic mutations throughout the life span of the plant (Klekowski, 1988). These somatic mutations can be a means for an evolutionary change.

Several plant characteristics allow the accumulation of somatic mutations, and make them

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a more significant factor in plant evolution:

- Plants have a relatively long life span and a large number of cells in a clone—somatic mutations are more effective as the plant lives longer or grows larger. The relevant somatic *mutation rate* per individual per generation is expected (and in specific cases has been empirically shown, see for example Klekowski & Godfrey, 1989) to be considerably high.
- The complete yearly regeneration of the plant buds increases the probability that a somatic mutation, which has occurred in the individual, will be expressed in the plant *population*.
- Highly important for our model is the fact that a plant is composed of many repeating units—branches (Whitham & Slobodchikoff, 1981). While animals are composed of different systems (at the organ level of organization) which have to cooperate in order to survive and reproduce via a single reproductive bottleneck, the equivalent units in plants are able to reproduce independently (Tuomi & Vuorisalo, 1989). Thus, they are capable of *competition*. As a result, a single plant may be considered an intermediate entity between an organism and a population.

#### TWO EVOLUTIONARY LEVELS

As a result of competition between similar units of a plant, a mutation in a bud will not necessarily be inherited in the same frequency in which it occurs. If the mutation has an effect on vegetative growth rate (which might be either harmful or advantageous), the mutant branch will go through somatic selection. Therefore, while dealing with sufficiently long-living plants, there are at least two relevant evolutionary levels, differing in their replicating units, replication processes and selection powers:

*Intra-organism level*—the selected unit is a branch, reproduction is asexual, and only the traits relevant for vegetative growth are being selected.

*Inter-organism level*—the selected unit is the whole plant, reproduction is sexual, and all the traits are selected.

For sufficiently long-living plants, evolution may be a combination of these two levels, and its dynamics are prone to be complicated. A mutation may, of course, have a similar effect on both levels, but this is not necessarily the case. A mutation may be neutral in one level, while having a selective value in the other. An interesting situation occurs when a mutation has opposite effects in these two levels.

Of the nine possible combinations, three are of special interest.

#### *Case I. A Neutral–negative Mutation*

One of the negative aspects of somatic mutations (from the plant's point of view) includes free accumulation of mutations that are neutral on the branch's level, but negative for the whole plant, as the plant grows older (Klekowski, 1988). Recessive mutations at any locus, and dominant mutations in loci relevant "only" for roots, stem development or the sexual reproduction, are included in this category. Genetic load was measured empirically, and shown to be significantly larger in old trees compared to young ones of the same species (Aizen & Dove, 1995), and in long-living plants compared to non-related short-living ones (Klekowski & Godfrey, 1989).

#### *Case II: A Positive–positive Mutation*

In the case of a mutation that is advantageous in both levels, such as resistance to a certain parasite, higher growth rate for a tree in a dense forest, etc., a plant may have a considerably greater chance to make use of it than an animal of similar life span (Whitham & Slobodchikoff, 1981; Gill, 1986). Assume that such a mutation has occurred in an apical initial, resulting in a completely or partially mutant meristem.

The mutation may be amplified with the help of the tree's hormone system. The mutated bud generates a mutated branch, which grows faster than other branches, has more leaves, and produces more oxin. It becomes a "sink" and draws more cytokinin. As a result it grows even faster, splits, and sheds other branches. Its chances to be lost by natural pruning are reduced, as it becomes more apically dominant. In the next reproduction season this branch will have relatively more flowers and seeds, which (at least partly)

will carry the mutation. In the next tree generation, the mutation will be expressed in the population at a higher frequency than its occurrence.

By this mechanism “acquired traits” may be inherited—in the sense that environment exerts selection on the random mutations within the plant, and determines which of them will be preferably inherited by the next generation. The single plant may thus be genetically adapted to environmental changes. Even subpopulations of branches within the plant may be differently adapted to different microenvironments, such as the northern or southern side of the tree. This mechanism may be of special importance in the context of plants and herbivores co-evolution (see Gill *et al.*, 1995, for review), because plants often live much longer than plant-eaters. A single mutation can have a drastic positive effect on fitness in the case of host and parasite gene-for-gene complementarity (Keen, 1990), and vegetative amplification may be significant.

### *Case III: A Positive–negative Mutation*

These two different cases naturally invoke new questions, concerning the evolutionary implications of “conflict mutations”, having a positive effect in one level and a negative effect in the other. A mutation increasing the growth rate of the branch, due to overutilization of the tree resources, is one example from this group. This egoistic mutation can be spread in the single tree by the same mechanism as the positive–positive mutation. Therefore, it will be expressed in its seeds with frequency above the mutation rate, while its effect on the whole tree and tree population is completely negative—the tree carrying it is shifted from its optimal resource allocation. Similar, but not identical in its effect, is a mutation for a higher growth rate of the branch, due to reduced number of flowers in the very same branch. The branch carrying that mutation is not considered an egoist, since it pays the cost itself. It is the *branch's* inter-tree function which is harmed, while its intra-tree function is benefited. This sort of mutations will be discussed in this paper.

The opposite case of a negative–positive mutation can be demonstrated by the back mutations of the last few examples. This sort of mutation

will be eliminated within each tree, but the seeds produced meanwhile will have a higher fitness, or a larger number. A seemingly identical mutation, of more flowers and less vegetative growth, occurring in an optimally allocated tree, is simply negative–negative.

The case of a negative–negative mutation, such as a dominant mutation for chlorophyll deficiency, is not complicated. The mutation will be expressed in seeds below the mutation rate; therefore, it can be eliminated more easily. If the mutation is negative even in the single cell level, a more efficient elimination is possible. Competition occurs even within the apical meristem itself (Klekowski & Kazarinova-Fukshansky, 1984; Otto & Orive, 1995; Otto & Hastings, 1998), and the mutation can be eliminated with a negligible cost to the branch or to the tree. This work will concentrate on two of the possible evolutionary levels—branch and tree.

### WHAT IS BETWEEN A TREE AND A COLONY OF APHIDS?

Considering a tree as a clone necessarily raises the question, what is the difference between such a clone and the elaborately discussed (Eshel, 1972; Cohen & Eshel, 1976; Wilson, 1980; Fagerstrom, 1992, and references therein) clones of aphids, daphnies, etc? Specifically, why is it impossible to apply a simple case of the founder effect (with a founding group of size one) to trees?

One major difference stems from the fact that the mitotically reproducing unit in a tree is not an independent unit, but a module. The competing branches share a common trunk and roots system. Therefore, it is very hard to describe trees' life cycle simply as a meristem–meristem cycle, which can be adequate to describe the evolution of many organisms that are clonal in a stronger sense, such as buttercups, lemna, etc. (Fagerstrom, 1992). Conflicts may appear between the branches and the roots or the trunk—for example overutilization of resources by the branches, costly parasite resistance, or overgrowth making the trunk more likely to collapse. Such conflicts cannot appear in a system of independent units reproducing sexually or asexually, as is the classical clone.

Another difference is the tree being a structured organism. In addition to the specific structure

of each species, a single stem has a limited carrying capacity. Thus, branches cannot reproduce (asexually) indefinitely, and geometric growth seems highly unrealistic after the developmental stage. As a result, the effect of somatic mutations can be smaller (Michod, 1997), while competition between the branches might be more severe. Certain trees (e.g. aspen) are also classical clones, in the sense that the whole tree can reproduce asexually, indefinitely, to create new stems and roots systems. In that case, the usual clonal conflict exists between the sexual and vegetative systems, but within each tree similar vegetative units compete over the right to produce the asexual descendants. The relevant tissue (that can be branches or roots, depending on the specific mechanism of asexual reproduction) is still in conflict with the other vegetative tissues.

Last, comparing a tree and a colony of aphids, there exists a difference in the colony founding stage: a colony of aphids, even when established by a single mother, begins with a considerable number of fertilized eggs, differing *gametically*. A tree starts as a seed, which is exactly one zygote. Therefore, *somatic mutations* are the only origin for variability within a tree. The choice of founding stage (a parent founder with many fertilized eggs or a descendant founder of one cell, determining whether the colony is composed of a single clone or more) may be influenced by several factors. While beginning in a single cell probably affords easier differentiation and development, beginning with a group has the important advantage of improved resistance to parasites, due to higher variability. Technical problems (fertilization, seeds spreading, etc.) may also be considered.

### The General Model

Consider a large population of long-living trees of constant size. New trees germinate exclusively in places emptied by the death of old trees. Pollination and seed spreading occur randomly. The age at death is an exponential random variable, and the probability to die every year,  $\rho$ , is equal for all tree types, independent of the mutations discussed. Each tree grows vegetatively within a constant volume, having  $n$  branches of equal size competing with each

other. For simplicity,  $n$  is assumed to be constant. One or more branches die every year and are replaced by others. Once a year the tree produces seeds that compete with the rest of the seeds on the patches emptied that year. For the sake of simplicity, we assume that a young tree becomes a full-size tree before the first reproduction season. That assumption ignores the stage of competition within the tree during development (Michod, 1997; Otto & Orive, 1995). In the context of conflict mutations of the sort discussed here, a mutation occurring during the tree's development may spread more efficiently, and thus a full-size tree growing from a non-mutant seed is not limited to the options of zero or one mutant branch considered here. This would shift the results further toward the intra-organism level.

Dynamics of gene frequencies in this forest are considered in relation to a specific class of "conflict mutations" i.e. mutations having different effects on different evolutionary levels. Each mutation has an advantage in the vegetative level—the branch in which it occurs grows faster, and a cost in the whole tree level. Since the mutant branches grow faster, the mutation will spread in the tree. At least,  $n + 1$  tree types are therefore considered, differing in the number of mutant branches per tree.

We shall assume that each branch has a single genotype. This is an accurate description only for plants having a single initial cell dominating each meristem (as do most vascular cryptogams; Klekowski *et al.*, 1984), but not for most trees. When each meristem is composed of a group of mitotically active initials there are in fact many branch types, differing in the frequency of mutant cells in their meristems. A single mutant cell in a multi-celled meristem has three possible fates: it may become extinct, be fixed in a meristem, or stay at frequencies between zero and one forever. Restricting our attention to mutations having no frequency-dependent effect at the cell level, the last possibility seems highly unlikely. In all other cases, a chimeric meristem finally generates homogenous meristems, by mechanisms of stochastic subsampling or differential proliferation. As the number of founding initials in a new meristem is not large, the time required for fixation or extinction would usually not be long.

Therefore, the dynamics of a tree with multi-celled meristems should not be qualitatively different from those of a “tree” with single-celled meristems (Fagerstrom *et al.*, 1998), while the last is much easier to analyse. We shall thus model only the evolution of single-celled meristems, in itself a model for the evolution of multi-celled meristems. The parameter  $m$ , indicating mutation rate, would represent the rate of appearance of a homogenous mutant meristem. If, however, even a branch with a partially mutant meristem grows faster than one with a homogenous non-mutant meristem, then fixation probability (in a meristem) increases and these two rates would not be too different.

Different sorts of costs can be considered in the inter-organism level:

(1) The mutant branch (and a mutant tree germinating from its seeds) has a lower amount of flowers than the non-mutant branch. Any mutation increasing the resource allocation to somatic growth within the branch will have an effect of that sort.

(2) The fitness of the whole tree (i.e. the number of its seeds, its life span, or its size) is a decreasing function of the relative frequency of mutant branches. Each branch has the same number of seeds. This is a classic egoistic mutation, an example of which can be seen in the case of overutilization of tree resources by the mutant branch.

(3) The seeds of a mutant branch have a lower chance to germinate. This may happen, for example, if their root-to-leaves allocation ratio is lower than the optimum.

In each of the cases the relative success of the mutant,  $\omega$  (be it a seed, a branch, or a tree) is a decreasing function of the improvement in the branch's growth rate. As the results in the three cases appear not to be qualitatively different, only the first case will be presented here.

If tree size is not constant, the effect of mutation on the two levels is somewhat mixed. Mutations of the first and third types might also enable the tree to be larger, due to higher vegetative growth rate, and thus have an extra inter-tree advantage. This will enable the mutation to spread more easily, as its overall inter-tree drawback is reduced. The

effect of variable tree size on an egoist mutation, on the other hand, depends on the specific mutation. Specific egoistic mutations result in reduced amount of seeds, smaller size, or a combination of these effects. In order to present the conflict between the two evolutionary levels in a clear form, we shall present mutations which are purely negative at the inter-tree level and purely positive at the intra-tree level, while tree size is kept constant. In nature, many conflict mutations probably have multiple (and sometimes contrasting) effects, even at the very same level.

Had the vegetative advantage of the mutant branch been very small, random processes would have taken an important role, and a different model should have been considered. Restricting our attention to the simpler case, the mutation is assumed to grant its branch a notable vegetative advantage.

### The Haploid Case

In the simplest case, we consider a “conflict mutation” which has an advantage in the vegetative level—the branch in which it occurs grows faster, and a cost in reproduction—the branch has fewer flowers (case 1), or its seeds have a lower chance to germinate (case 3). These cases are identical in a haploid model. The results for an “egoistic branch” (case 2) in the haploid model are qualitatively similar, and will not be presented here.

Denote by  $P_i$  the frequency of trees with  $i$  mutant branches (out of  $n$  branches for the whole tree).

First, we consider the relatively simple case in which a tree of type  $P_i$  has a probability  $q_i$  to become a tree of type  $P_{i-1}$  in the next year, and a probability zero to become a tree of any other type (i.e. the mutation spreading rate is limited to one branch a year at the most).  $q_0 = m$ , the rate of appearance of mutant meristems per tree per year.

Let  $\omega$  be the relative reproductive success of the mutant, and  $y$  the frequency of mutants among all seeds produced in the forest:

$$y = \frac{\omega \sum_{k=0}^n k P_k}{\sum_{k=0}^n [\omega k P_k + (n - k) P_k]} \\ = \frac{\omega \sum_{k=0}^n k P_k}{n - (1 - \omega) \sum_{k=0}^n k P_k}. \quad (1)$$

Note that a proportion  $\rho$  of all trees will be replaced in the next generation. Of these, a proportion  $y$  will be of mutant seeds, and thus of  $n$  mutant branches. A proportion  $1 - y$  will be of non-mutant seeds, having 0 mutant branches. The rest of the trees, say  $1 - \rho$  of them, will be old. Since old trees with  $k < n$  mutant branches have a probability  $q_k$  to become trees of  $k + 1$  mutant branches, we get the recursion

$$\begin{aligned}
 P'_0 &= P_0(1 - \rho)(1 - q_0) + \rho(1 - y), \\
 P'_1 &= P_1(1 - \rho)(1 - q_1) + P_0q_0(1 - \rho), \\
 &\vdots \\
 P'_k &= P_k(1 - \rho)(1 - q_k) + P_{k-1}q_{k-1}(1 - \rho), \\
 &\vdots \\
 P'_n &= P_n(1 - \rho) + P_{n-1}q_{n-1}(1 - \rho) + \rho y. \quad (2)
 \end{aligned}$$

At equilibrium,

$$\begin{aligned}
 P_0 &= \frac{\rho}{\rho + m(1 - \rho)}(1 - y), \\
 &\vdots \\
 P_k &= \frac{q_{k-1}(1 - \rho)}{\rho + q_k(1 - \rho)}P_{k-1}, \\
 &\vdots \\
 P_n &= \frac{q_{n-1}(1 - \rho)}{\rho}P_{n-1} + y \quad \text{when } q_n = 0.
 \end{aligned}$$

We get

$$\begin{aligned}
 P_k &= P_0 \prod_{i=1}^k \frac{(1 - \rho)q_{i-1}}{\rho + (1 - \rho)q_i} \\
 &= (1 - y) \frac{\rho}{\rho + m(1 - \rho)} \prod_{i=1}^k \frac{(1 - \rho)q_{i-1}}{\rho + (1 - \rho)q_i}, \\
 &k = 1, \dots, n - 1,
 \end{aligned}$$

and where  $q_n = 0$

$$P_n = (1 - y) \frac{\rho}{\rho + m(1 - \rho)} \prod_{i=1}^n \frac{(1 - \rho)q_{i-1}}{\rho + (1 - \rho)q_i} + y$$

The frequency of mutant branches at equilibrium is thus

$$\frac{\sum_{k=0}^n kP_k}{n} = M(1 - y) + y, \quad (3)$$

where

$$M = \frac{\rho}{n(\rho + m - m\rho)} \sum_{k=1}^n \left[ k \prod_{i=1}^k \frac{q_{i-1}(1 - \rho)}{\rho + q_i - \rho q_i} \right]. \quad (4)$$

Using eqns (1) and (3), we get

$$y = \frac{\omega[M(1 - y) + y]}{1 - (1 - \omega)[M(1 - y) + y]} \quad (5)$$

or

$$\begin{aligned}
 &y^2[M(1 - \omega) - (1 - \omega)] \\
 &+ y[1 - (1 - \omega)M + \omega M - \omega] - \omega M = 0.
 \end{aligned}$$

Solving eqn (5) yields two possible fixed points

$$y = 1 \quad (\text{fixation}), \quad \text{and}$$

$$y = \frac{\omega M}{(1 - M)(1 - \omega)} \quad (\text{polymorphism}). \quad (6)$$

At polymorphism

$$\begin{aligned}
 \sum_{k=0}^n \frac{k}{n} P_k &= M(1 - y) + y \\
 &= M \left( 1 - \frac{\omega M}{(1 - M)(1 - \omega)} \right) \\
 &\quad + \frac{\omega M}{(1 - M)(1 - \omega)} = \frac{M}{1 - \omega}.
 \end{aligned}$$

This is equivalent to the classic mutation selection balance, with an *amplified mutation rate*  $M$  (as a result of vegetative competition), due to which not only the frequency of mutants cannot be neglected, but the mutation may even be fixed in the population.  $M$  depends exclusively on

$\rho$  and  $q_i$ , and it can be easily shown that  $\rho \rightarrow 1 \Rightarrow M \rightarrow 0$ , while  $\rho \rightarrow 0 \Rightarrow M \rightarrow 1$ .

We see that fixation is stable if and only if

$$\omega \geq 1 - M. \tag{7}$$

Note, however, that the solutions of eqn (6) characterize the one-dimensional parameter  $y$  of a fixed point of the  $(n + 1)$ -dimensional dynamics (2) and (3). The stability of the fixed points determining the value of  $y$  is hard to analyse, and is therefore checked by simulations. The relative vegetative success of the mutant branch is denoted by  $1 + a$ ,  $0 \leq a \leq 1$ , and the relation between  $q_i$  and  $a$  is assumed to be

$$q_i = \frac{i(1 + a)}{i(1 + a) + 1(n - i)}, \quad i = 1, \dots, n - 1. \tag{8}$$

The equations were run to equilibrium in the computer for a wide range of parameters. As was shown by these simulations, the polymorphic equilibrium is always stable if it exists, and fixation is stable if and only if no polymorphism exists.

*Numeric results.* Assume that  $\omega = 1 - ca$ , where  $c$  is the cost ratio—the ratio between the reproductive cost and the vegetative growth advantage

of the mutation. Equilibrium value is an increasing function of average tree age ( $1/\rho$ ), and a decreasing function of cost ratio ( $c$ ), as shown in Fig. 1.

**Remark 1**—*A symmetric model.* In our model, we have considered, for simplicity, unidirectional mutation. That simplification is justified by the fact that backward mutation cannot spread in the tree. On the contrary, it is gradually being eliminated. Straightforward calculations indicate that its effect on the results is negligible.

**Remark 2**—*Higher spreading rates.* In a more general case, the mutation may spread more than one branch a year—a tree of type  $j$  has a probability  $q_{jl}$  to become a tree of type  $l$  ( $j < l$ ) in one year. The recursion equations in this case become

$$\begin{aligned} P'_0 &= P_0(1 - \rho) \left( 1 - \sum_{j=1}^n q_{0j} \right) + \rho(1 - y), \\ &\vdots \\ P'_k &= P_k(1 - \rho) \left( 1 - \sum_{j=k-1}^n q_{kj} \right) + \sum_{i=0}^{k-1} P_i q_{ik}(1 - \rho), \\ &\vdots \\ P'_n &= P_n(1 - \rho) + \sum_{j=0}^{n-1} P_j q_{jn} + \rho y. \end{aligned} \tag{9}$$

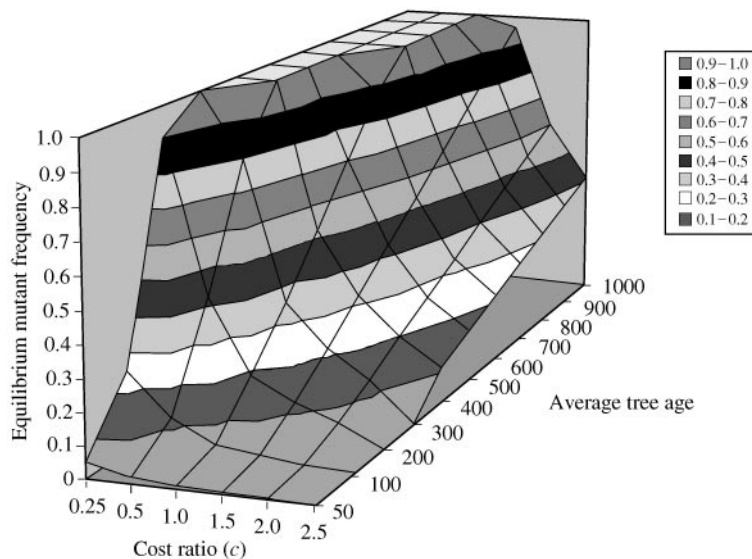


FIG. 1. Equilibrium values as a function of average tree age ( $1/\rho$ ) and cost ratio ( $c$ ), assuming  $\omega = 1 - ca$ . Equilibrium values are computed for  $n = 0.0005$ ,  $a = 0.2m$  is taken to be  $5 \times 10^{-4}$  considering a basic mutation rate of  $10^{-6}$  (which seems a rather low estimate, see Witham & Slobodchikoff, 1981), 50 branches per tree and 10 mitotic divisions per year.

In such a case, it can be shown (see the appendix) that the frequency of mutant branches at equilibrium becomes

$$\frac{\sum_{k=0}^n kP_k}{n} = M_1(1 - y) + y. \tag{10}$$

$M_1$  being a constant calculated from the  $q_i$ 's. This turns out to be equivalent to eqn (3), with a different estimation of the amplified mutation rate.

In the following parts, we shall concentrate on the simplest case of one branch per year at the most.

### The Diploid Case

In this part a single locus with genotypes AA, Aa and aa is considered. A is the mutant allele of a positive-negative mutation of the kind discussed in the first part. In the sexual case there is a difference between the dynamics of two sorts of mutations that are equal in the asexual case, depending on the specific stage in the life cycle in which the cost is paid. In case the seeds have a smaller chance to germinate, the cost is paid *after* segregation and fertilization, while if the mutant branch has fewer flowers, the cost is paid *before* segregation occurs. The second case is presented here.

Quite interestingly, the cases of complete and incomplete dominance yield qualitatively different results. Hence, they are discussed separately.

#### COMPLETE DOMINANCE

New trees are assumed to be of genotype aa (the wild type), Aa or AA. Assuming a relatively low mutation rate each way, the effect of somatically neutral mutations (from Aa to AA vice versa) and somatically deleterious mutations (from Aa to aa), is ignored. We consider, however, the somatically advantageous mutation from aa to Aa. Hence, as before,  $n - 1$  additional tree types of  $k$  branches of genotype Aa and  $n - k$  branches of genotype aa are considered.

We denote by  $P_0$  the frequency of trees of genotype aa (wild type),  $P_i$ ,  $i = 1, \dots, n$  the frequency of trees in which  $i$  of their branches are of genotype Aa while the rest are of genotype aa, i.e. trees in which the mutation is spreading, and

$P_{AA}$  the frequency of trees of genotype AA, which have germinated from AA seeds. Let  $y_0$ ,  $y_1$  and  $y_2$  be the frequencies of seeds of genotypes aa, Aa and AA, respectively. The seed frequencies depend directly on the frequency of the mutant allele— $y$ :  $y_0 = (1 - y)^2$ ,  $y_1 = 2y(1 - y)$ , and  $y_2 = y^2$ .

The recursion equations in this case, in analogy to eqn (2), can be written as

$$\begin{aligned} P'_0 &= P_0(1 - \rho)(1 - m) + \rho y_0, \\ &\vdots \\ P'_k &= P_k(1 - \rho)(1 - q_k) + P_{k-1}q_{k-1}(1 - \rho), \\ &\vdots \\ P'_n &= P_n(1 - \rho) + P_{n-1}q_{n-1}(1 - \rho) + \rho y_1, \\ P'_{AA} &= P_{AA}(1 - \rho) + \rho y_2. \end{aligned} \tag{11}$$

Let  $\omega$  be the relative amount of flowers of the mutant branch. Half of the gametes produced on heterozygote branches are of type A, and the rest of type a:

$$y' = \frac{\omega \sum_{k=0}^n (k/n) P_k + 2\omega P_H}{2\omega \sum_{k=0}^n (k/n) P_k + 2\omega P_H + 2 \sum_{k=0}^n ((n - k)/n) P_k}. \tag{12}$$

At equilibrium

$$\frac{\sum_{k=0}^n kP_k}{n} = My_0 + y_1. \tag{13}$$

Applying eqn (13) and  $\sum_{k=0}^n P_k + P_H = 1$  to eqn (12), we get

$$\begin{aligned} y &= \frac{\omega(My_0 + y_1 + 2P_{AA})}{2[1 - (1 - \omega)(My_0 + y_1 + P_{AA})]} \\ &= \frac{\omega[M(1 - y)^2 + 2y]}{2[1 - (1 - \omega)(M(1 - y)^2 + 2y - y^2)]}. \end{aligned} \tag{14}$$

The equilibria are

$$y = 1$$

and

$$y = \frac{\omega M}{2(1 - M)(1 - \omega)}. \tag{15}$$

Not surprisingly, the polymorphic equilibrium point is exactly half of its haploid equivalent [eqn (6)].

It was shown by simulation that equilibrium is stable whenever it exists, and fixation is stable in all other cases. The condition for stability of fixation of this dominant mutation is therefore:

$$\frac{\omega}{2 - \omega} \geq 1 - M. \tag{16}$$

Comparing this result with its equivalent in the haploid model (1.7) shows that the condition for fixation of such a dominant mutation in a diploid population is stronger than the condition for fixation of an identical mutation in a haploid population for any  $0 < \omega < 1$ , and equal to it in the cases  $\omega = 1$  and  $0$ . In the first case, the mutation is fixed in both populations for any vegetative advantage higher than zero, and in the second it cannot be fixed in either of them.

PARTIAL DOMINANCE

In this part, the traits of the heterozygote (including both the relative vegetative success and the relative reproductive success) are assumed to have intermediate values, between the traits of the two homozygotes. The absolute advantage and disadvantage of the mutation are assumed to be related in the same way, i.e.  $w_i = 1 - ca_i$ , with the same cost ratio  $c$ , for all allele combinations.

This assumption does not enable us to simplify the model in the former manner, of only one additional tree type. Since the mutant homozygote branch has a vegetative advantage over the heterozygote one, and since the heterozygote is vegetatively superior to the wild-type

section, we have seen that, at least when a haploid case is considered, the evolutionary effect of that class of conflict mutations becomes really important only when  $\rho$  is small, whereas the whole model is relevant only when  $a$  is not very small. Namely, the effect is strong when the expected lifetime of the tree is long and the spread of a new mutation is relatively fast. Restricting our attention to such a situation, the relatively small chance that a new mutation from Aa to AA has occurred between the occurrence of the first heterozygote mutation and its taking over of the entire tree can be ignored. Instead, we assume that the second mutation can occur only *after* the first mutation has taken over the tree. Therefore, we have only two spreading phases, and  $2n$  tree types.

Let  $R_i$  be the frequency of trees such that  $i$  of their branches are of genotype AA and the rest are of genotype Aa,  $t_i$  the probability of a tree of type  $R_i$  to become a tree of type  $R_{i-1}$  in one year. Denote by  $\omega_1, \omega_2$  the amount of flowers of Aa and AA branches, respectively, relative to the wild type aa.

The recursion equations in this case are

$$\begin{aligned} P'_0 &= P_0(1 - \rho)(1 - m) + \rho y_0, \\ &\vdots \\ P'_n &= P_n(1 - \rho)(1 - m) + q_{n-1}(1 - \rho)P_{n-1} + \rho y_1, \\ R'_1 &= R_1(1 - \rho)(1 - t_1) + mP_n(1 - \rho), \\ &\vdots \\ R'_k &= R_k(1 - \rho)(1 - t_k) + t_{k-1}R_{k-1}(1 - \rho), \\ &\vdots \\ R'_n &= R_n(1 - \rho) + t_{n-1}R_{n-1}(1 - \rho) + \rho y_2, \end{aligned} \tag{17}$$

$$y' = \frac{\omega_1 [\sum_{k=0}^n kP_k + \sum_{k=1}^n (n - k)R_k] + 2\omega_2 \sum_{k=1}^n kR_k}{2\omega_1 [\sum_{k=0}^n kP_k + \sum_{k=1}^n (n - k)R_k] + 2\omega_2 \sum_{k=1}^n kR_k + 2 \sum_{k=0}^n (n - k)P_k}. \tag{18}$$

homozygote, there are three possible phases of spreading within the tree. The Aa genotype spreads within the aa tree, the AA spreads within the Aa tree, and the AA may also spread within the aa tree, if it had appeared before the aa was completely eliminated by the Aa. In the previous

Defining

$$\tilde{M} = \frac{\rho}{n(\rho + m - m\rho)} \sum_{k=1}^n \left[ k \prod_{i=1}^k \frac{t_{i-1}(1 - \rho)}{\rho + t_i - \rho t_i} \right]. \tag{19}$$

representing the amplified mutation rate of the second mutation,

$$D = \frac{\rho}{n(\rho + m - m\rho)} \times \left( n + \sum_{k=1}^n \left[ (n-k) \prod_{i=1}^k \frac{q_{i-1}(1-\rho)}{\rho + q_i - \rho q_i} \right] \right)$$

and

$$m_n = \frac{\rho}{(\rho + m - m\rho)} \prod_{i=1}^n \frac{q_{i-1}(1-\rho)}{\rho + q_i - \rho q_i},$$

eqn (18) becomes

$$y' = \frac{\omega_1 [1 - \tilde{M}(m_n y_0 + y_1) - y_2 - D y_0] + 2\omega_2 [\tilde{M}(m_n y_0 + y_1) + y_2]}{2\omega_1 [1 - \tilde{M}(m_n y_0 + y_1) - y_2 - D y_0] + 2\omega_2 [\tilde{M}(m_n y_0 + y_1) + y_2] + 2D y_0}. \tag{20}$$

At equilibrium, this third-order polynomial has three solutions, one of which is always one. The other two solutions are not necessarily within the parameter range. It has been shown by simulations that when the other two solutions are out of the parameter range, fixation is stable. If there is only one polymorphic solution within the parameter range, it has been shown that only the polymorphism is stable. If there are three different solutions within the parameter range, both fixation and the lower polymorphism are shown to be stable, while the intermediate is not (Fig. 2). Sufficient and necessary conditions for the existence of the different solutions were found with the aid of the Maple mathematical engine. Together with the simulations, it results in the following two conditions:

Fixation is stable if and only if

$$\frac{\omega_2}{\omega_1} \geq (1 - \tilde{M}). \tag{21}$$

Whenever eqn (21) is not satisfied, a single polymorphic solution exists, and only the polymorphism is stable. In addition, the case of partial dominance, unlike the former models, allows the existence of three equilibrium points. Assume,

for example, eqn (18) and

$$t_i = \begin{cases} m, & i = 0, \\ \frac{i(1 + a_2)}{i(1 + a_2) + (1 + a_1)(n - i)}, & i = 1, \dots, n - 1. \end{cases} \tag{22}$$

It is shown that a range of  $c$  for which there are three equilibria exists if and only if

$$(a_2 - a_1)(1 - 4\tilde{M}^2 + 2\tilde{M} + \tilde{M}m_n - D) < a_1 2\tilde{M}D. \tag{23}$$

In this case, the two extremes are stable, while the intermediate is not (Fig. 2). As a result, the final equilibrium depends on the initiation conditions. When eqn (23) is not met, i.e. when a single stable equilibrium exists, the equilibrium frequency of the mutants increases with average tree age, and decreases with  $c$  (Fig. 3), similar to the haploid case.

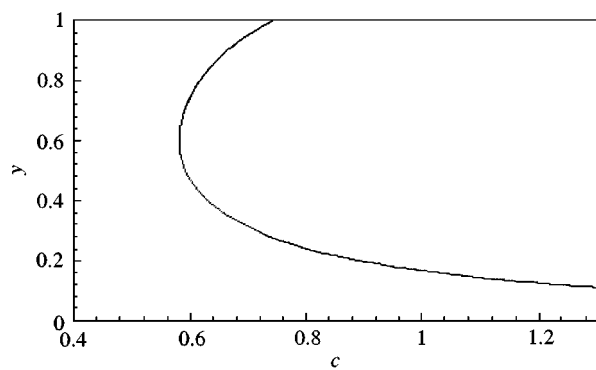


FIG. 2. Equilibrium solutions of mutant frequency as a function of cost ratio,  $c$ , in a diploid model with partial dominance. Taking  $a_1 = 0.15$  and  $a_2 = 0.2$ , condition (23) is fulfilled, and three different equilibria exist. The lower polymorphic solution is always stable, while the intermediate is always unstable. Fixation is stable when there are no other solutions, or when three solutions exist within parameter range. That is fixation is stable for any  $c$  smaller than  $c_1$ , the value of  $c$  for which the intermediate solution crosses one. This corresponds to condition (21).

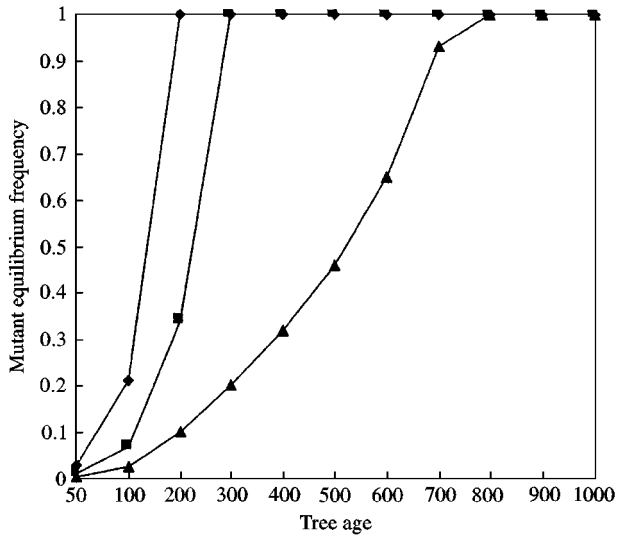


FIG. 3. Mutant frequency at equilibrium as a function of tree age, in a diploid model with partial dominance, is shown for three different values of the cost ratio,  $c$ . The absolute vegetative advantages of Aa and AA are  $a_1 = 0.1$  and  $a_2 = 0.2$ . Similar to the haploid case, equilibrium mutant frequency is an increasing function of tree age, and a decreasing function of the cost ratio: (—○—) 0.5; (—■—) 1; (—▲—) 2.

The condition for stability of fixation of a partially dominant mutation (21) depends only on the second mutation, Aa to AA. It is equivalent to condition (7) for the stability of fixation of an identical mutation (from  $a$ , having the vegetative success  $1 + a_1$  and the reproductive success  $\omega_1$ , to A, having the vegetative success  $1 + a_2$  and the reproductive success  $\omega_2$ ), in the haploid model.

Quite interestingly, conditions for fixation stability of the mutation are often weaker in the case of partial dominance than in that of complete dominance (see Fig. 4(a), (b)). Using eqns (7) and (21) we see that when

$$a_1 > a_2 \frac{M - \tilde{M}}{M(1 - \tilde{M})}, \quad (24)$$

the conditions for fixation of a partially dominant mutation in a diploid model are even weaker than the parallel conditions in a haploid model. Having assumed the transition probabilities  $q_i$  and  $t_i$  as defined in eqns (8) and (22), respectively, and recalling the definitions (4) and (19) of  $M$  and  $\tilde{M}$ , respectively, it is easily shown that condition (24) is satisfied for a wide range of

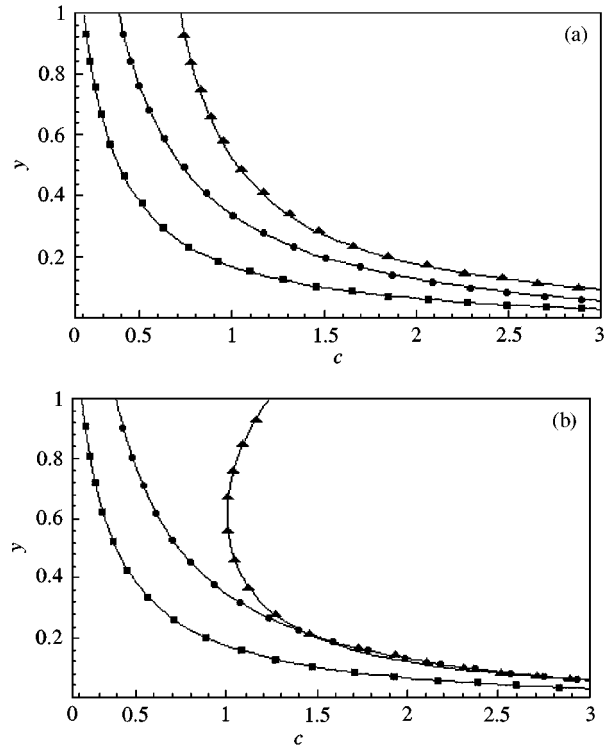


FIG. 4. Equilibrium solutions of mutant frequencies as a function of cost ratio  $c$  are shown for three cases: haploid (circles), dominant diploid (squares), and partially dominant diploid (triangles). Solutions are computed for an average tree of 300 years. (a)  $a_1 = 0.1$ ,  $a_2 = 0.2$ . A single stable equilibrium exists in each case. Mutant frequency is higher in the partially dominant case than in the haploid case, and in the haploid than in the dominant diploid. (b)  $a_1 = 0.15$ ,  $a_2 = 0.2$ . Two stable equilibria exist in the partially dominant case. The partially dominant is found in the highest frequency part of the time, but the value of the lower equilibrium point (which is more relevant) is very close to that of the haploid, and is slightly exceeded by it for certain values of  $c$ .

parameters. Moreover, when fixation is impossible, the equilibrium value of the partially dominant mutation is often higher than the asexual case, in itself twice the dominant equilibrium value (see Fig. 4). These results can be explained by the improved spreading ability of the partially dominant mutation, since the intermediate phenotype creates a relatively convenient pathway from a wild-type homozygote to a mutant homozygote within the lifetime of the single organism, assuming that it is long enough.

*Numeric results.* All calculations were done assuming that  $q_i$  and  $t_i$  are determined uniquely by the vegetative advantages  $a$ ,  $a_1$ ,  $a_2$  and the

number of branches in a tree,  $n$ , as suggested in examples (8) and (22). Equilibrium values were found for the case  $n = 50$ , and  $m$ , the mutation rate per tree per year, equals 0.0005.

### Discussion

In this work, we studied the dynamics of somatic mutations granting the branch carrying them a vegetative advantage, but reducing the fitness of the whole tree due to a shift from the optimal resource allocation. This shift may affect the balance between growth and reproduction within the branch, preferring vegetative growth, or the balance between the different tree tissues, in favor of the mutant branches.

A relatively simple model is introduced here, where each tree has an equal number of branches, all of them of the same size and apical dominance, going through somatic mutation, reproduction, and selection. The mutant branches have a higher probability to replace other branches, but less flowers. The first section dealt with the simplest case of asexual reproduction, and later sections studied the effect of sexual reproduction in one locus and random fertilization, with complete or incomplete dominance.

The dynamics of the somatic change at each tree is given by the mutation rate  $m$  per tree (which is usually low, and yet several orders of magnitude higher than the mutation rate per cell), and by a number of parameters describing the vegetative advantage of the mutation at each stage of its spreading. We have seen that the combined effect of these factors on the genetic dynamics of the population can be characterized by a single parameter  $M$  (in the case of asexual reproduction, or sexual reproduction with complete dominance), or by two parameters,  $M$  and  $\tilde{M}$ , in the case of partial dominance. We have shown that the genetic dynamics of the whole population is equivalent to that determined by selection powers between the individuals, in addition to a unidirectional mutation rate  $M$  from the wild type to the mutant in the first case, or  $M$  from the wild type to the heterozygote mutant, and  $\tilde{M}$  from the heterozygote mutant to the homozygote mutant in the second. The last quantity is an analog of the normal mutation rate, differing from it in one consequential

manner—it is *not* negligibly small. As a result, *mutation–selection balance* gains a new meaning. It has been shown that a positive–negative mutation of significant effect may be found in a *stable polymorphism* (which is not necessarily close to zero), with no need of overdominance.

Moreover, a *fixation* of a purely negative mutation in the inter-organism level is possible within the range of a reasonable mutation rate per cell per year. Consequently, this simple two-level one-locus system is not always adaptive. Several tree characteristics, such as the amount of flowers or the growth rate, are *not necessarily the result of an optimization process at the whole tree level*. The shift from optimum is expected to be stronger as the organism grows bigger or has a longer life span. The prediction that investment in intra-tree functions should increase with age is consistent with previous work on within-organism change (Michod, 1999).

An extreme kind of intra-organism evolution can be expected in a substantial group of plants (including for example most pine trees), in which there is a strong control of the upper initial. The main branch produces most of the gametes and hormonally depresses the other branches. In these cases, the effect of the somatic mutation will be determined in a “double or nothing” manner. If it grants its branch a significant advantage it will grow faster and conquer the tree, i.e. become the main branch itself. If, on the other hand, the mutation’s advantage is not drastic enough, the mutation would almost surely not be inherited.

As demonstrated in the third part, very simple genetic situations (one locus, two alleles, partial dominance) in single-level dynamics may have unexpected results in a system of two evolutionary levels. Fixation of partially dominant mutation can be easier than that of a completely dominant one, and two stable equilibria may be obtained by a partially dominant mutation. It seems that single-level results cannot be automatically generalized to two-level systems without reexamination.

### Biological Predictions

Due to the lack of experimental data, especially in relation to the parameter  $c$  (the ratio

between the vegetative benefit and the reproductive cost) and to the actual genetic mechanism governing the relevant traits, it is not yet possible to have accurate quantitative predictions. Assuming that  $c$  is not very large and that one locus is a reasonable approximation, several general predictions can be made.

#### VEGETATIVE INVESTMENTS

Resource allocation between different vegetative tree parts in long-living trees is expected to be shifted from its optimum, in favor of the branches. This favoritism of the branches over, for example, the roots, reflects a fundamental asymmetry within the tree. While the roots go through somatic reproduction, mutation and selection, in complete analogy to branches, the *flowers* grow exclusively on the branches. As a result, the relative part of the branches out of the tree's biomass might increase with the average age of the tree species, while the fitness is expected to decrease.

In addition to resource allocation, accumulation of harmful mutations over generations is more likely to occur in loci affecting exclusively the roots and reproductive system than in loci relevant for the branches. The reason for this is that a considerable group of harmful mutations in the branches, impairing vegetative growth, is eliminated by somatic selection (Otto & Hastings, 1998). Unlike the case of the roots, this somatic selection is expressed in terms of gene frequencies in future generations. Thus, the branches' genetic load is expected to be smaller than both the reproductive load and the non-branch vegetative load. Quite paradoxically, while harmful somatic mutations concerning the roots are expected to accumulate in the branches (and therefore in the seeds) as the tree grows older, they are expected to be eliminated by somatic selection in the roots themselves at the same time. The roots of the tree (and the roots of a young tree grown directly from the roots) are expected, for example, to be more resistant to root parasites than the roots of a young tree germinating from the seeds. The difference is expected to increase with average tree age.

#### REPRODUCTIVE EFFORT

A smaller reproductive effort is expected to be found in long-living trees, compared to their short-living relatives. Optimal allocation of energy between reproduction and growth has often been discussed (King & Roughgarden, 1982; Kozlowsky & Wiegert, 1986; Cohen & Dukas, 1990), but the actual values may be shifted from optimum to prefer vegetative growth in long-living trees. The amount of flowers and especially fruits in long-living trees is expected to be considerably smaller than that of short-living trees. However, it might be difficult to isolate that effect from the former, as one of the common indications of a decrease in fitness is a reduced amount of seeds.

A certain supporting evidence can already be found in the fact that long-lived species tend to have a smaller number of seeds per mass unit, and lower seed/ovule and fruit/flower ratios (Charlesworth, 1989; Wiens, 1984; Sutherland, 1986). Yet, this relatively general phenomenon can be explained in several different ways, including resource allocation for the following years, uncertainty of pollination and genetic load due to mutation accumulation (Klekowsky & Godfrey, 1989).

#### INTRA-POPULATION VARIANCE

A difference might be found between young and old trees in the same population, in relation to the conflict mutations discussed. In the case of polymorphism, older trees are expected to be more strongly shifted from the optimum (better represented by trees of short life span). That is, had optimal resource allocation been independent of age, both reproductive effort and the relative investment in vegetative non-branch tissues (roots, stem, etc.) would have been expected to decrease with age. The first is opposite to the result of several life-history models (Cohen, 1971; Kozlowski & Uchmanski, 1987; Roff, 1992, and references therein), based on optimization of organism fitness at the inter-organism level only, predicting an increase in reproductive effort with age. The overall effect of age on reproductive investment is probably determined by both inter-organism optimization and intra-organism dynamics, so that the qualitative result depends on

the specific quantities, and thus is hard to predict, given our scarce knowledge of parameter values. The effect is not necessarily similar, even in its direction, for different tree species. However, while experimental evidence showing a decline in reproductive investment with age clearly supports our model, the opposite result (no significant difference or even an increase with age) does not falsify it, as the reproductive effort can still be below the single-level optimum.

It should be noted that polymorphism is not the only possible outcome. The parameter  $c$  being small enough, and life span long enough, a conflict mutation may be *fixed* in the population. Variance within the population will not be found in relation to that mutation.

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## APPENDIX

Let us define

$$Q_k = \sum_{i=k-1}^n q_{ki}, \quad k = 0, \dots, n-1,$$

$$Q_n = 0.$$

By recursion

$$A(0) = \frac{\rho}{\rho + Q_0 - \rho Q_0},$$

$$A(k) = \sum_{i=0}^{k-1} \frac{q_{ik} A(i)}{\rho + Q_k - \rho Q_k}.$$

The equilibrium is simplified:

$$P_0 = \frac{\rho}{\rho + Q_0(1 - \rho)} (1 - y) = A(0)(1 - y),$$

$$P_1 = \frac{q_{01}}{\rho + Q_1(1 - \rho)} P_0 = A(1)(1 - y),$$

⋮

$$P_k = \frac{\sum_{i=0}^{k-1} P_i q_{ik}}{\rho + Q_k(1 - \rho)} = A(k)(1 - y),$$

⋮

$$P_n = A(n)(1 - y) + \rho y.$$

Defining

$$M_1 = \sum_{k=0}^n \frac{k}{n} A(k),$$

the frequency of mutant branches at equilibrium becomes

$$\frac{\sum_{k=0}^n k P_k}{n} = M_1(1 - y) + y.$$

This is eqn (3) again, with different coefficients.